

REESTABLISHMENT OF A KEYSTONE SPECIES: INITIAL OUTCOMES AND
ECOSYSTEM RESPONSES

by

Sarah L. Hale

Copyright © Sarah L. Hale 2017

A Dissertation Submitted to the Faculty of the
SCHOOL OF NATURAL RESOURCES AND THE ENVIRONMENT

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2017

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Sarah Hale titled: Reestablishment of a Keystone Species: Initial Outcomes and Ecosystem Responses and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Date: 2 June 2017
John L. Koprowski

Date: 2 June 2017
R. William Mannan

Date: 2 June 2017
Steven R. Archer

Date: 2 June 2017
Cecil Schwalbe

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Date: 2 June 2017
Dissertation Director: John L. Koprowski

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of the requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Sarah L. Hale

ACKNOWLEDGMENTS

First, I would like to thank my advisor, Dr. John L. Koprowski, who has readily and happily provided encouragement, guidance, and support throughout my project, despite the hour of the day or night! During my time as a PhD student, I have been pushed to grow more than I thought possible as a scientist, scholar, and teacher, which would not have been possible without John.

Thank you to Annise Degonzague, Kirsten Fulgham, Alejandro Grajal-Puche Allyson Hawkins, Levi Heffelfinger, Theresa Huckleberry, Raquel Irigoyen-Au, Chad Ketcham, Darin Kopp, Ariana La Porte, Jorge Marin, Luis Martinez, Maxwell Mazzella, Rachel More-Hla, Rocio Ojeda, James Pitts, Brianna Rico, Allison Rosengren, Kaitlin Ruggirello, Samantha Springs, Stephanie Snedecker, Michael Taylor, Clarissa Teixeira, and Kaycie Waters for your assistance both in the field and in the lab, and thank you to the Koprowski Conservation lab for providing a wonderful support group. A special thank you to, Samuel Abercrombie, Maria Altemus, Kendell Bennett, Brian Blais, Colin Brocka, Allie Burnett, Hsiang Ling Chen, Jonathan Derbridge, Sandy Doumas, Kirsten Fulgham, Emily Goldstein, Vicki Greer, Nate Gwinn, Kira Hefty, Timothy Jessen, Shari Ketcham, Allyssa Kilanowski, Maxwell Mazzella, Melissa Merrick, Marina Morandini, Karen Munroe, Geoffry Palmer, Rosa Palmer, Shambhu Paudel, Erin Posthumus, Seafha Ramos, Nicolas Ramos-Lara, Amanda Veals, Ivan Mauricio Vela-Vargas, and Stuart Wells for your invaluable feedback on abstracts, papers, posters, reports, and presentations, as well as for your friendship. All of your constructive criticism has helped hone my writing and presentation skills more than I thought possible.

I would like to thank the School of Natural Resources and the Environment for fostering an atmosphere of cohesion and encouragement among undergraduate students, graduate students, and faculty, for the captivating courses that challenged me to alter my ways of thinking, and for the support provided throughout my graduate career at the University of Arizona. Additionally, I would like to thank my committee members Steve Archer, Bill Mannan, and Cecil Schwalbe for their enthusiastic input, guidance, and feedback throughout this project.

My research project would not have been possible without the financial support of the Arizona Center for Nature Conservation-Phoenix Zoo, the Arizona Game and Fish Department, the Bureau of Land Management, the National Fish and Wildlife Foundation, the University of Arizona, and T & E Inc.

Finally, I would like to thank those in my personal life that have supported me and seen me through this journey. To my parents, John and Laura Hale, my brother, James Hale, my sister-in-law, Julie Hale, my mother and father in-law Mike and Laura Mazzella, and my best friend KC Mullin, thank you for always being there for me, believing in me, and never questioning my desire to travel this crazy path. To my non-human family, Paisley, Pandora, Mu, and Dory Hale-Mazzella, thank you for your emotional support both in the field and at home. Finally, to my wonderful husband, Maxwell Mazzella. Not only did you agree to embark upon this journey with me 6 years ago, but you actually agreed to marry me during it! I could not have accomplished all that I have without your unwavering love, support, encouragement, and unpaid labor.

TABLE OF CONTENTS

LIST OF TABLES	7
LIST OF FIGURES	9
ABSTRACT.....	12
INTRODUCTION	13
PRESENT STUDY	15
LITERATURE CITED	16
APPENDIX A: HOW DO KEYSTONE SPECIES REINTRODUCTIONS INFLUENCE ECOSYSTEM-LEVEL PROCESSES?	18
Author Contributions	19
Abstract	19
Introduction	20
Methods	22
Results	23
Discussion	24
Literature Cited	27
Figure Legends	33
Tables	34
Figures	37
APPENDIX B: Active management contributes to successful reintroduction of a keystone species: black-tailed prairie dog (<i>Cynomys ludovicianus</i>) return to grasslands	43
Abstract	45
Introduction	46
Methods	48
<i>Study area</i>	48
<i>Colony area</i>	51
<i>Trapping and marking</i>	51
<i>Observation</i>	52
<i>Analysis</i>	53
Results	54
<i>Colony area</i>	54
<i>General characteristics and dispersal</i>	54
<i>Population trends</i>	55
<i>Reproduction and juvenile survival</i>	56
Discussion	57
<i>Colony area</i>	57
<i>General characteristics and dispersal</i>	58
<i>Population trends</i>	59
<i>Reproduction and juvenile survival</i>	60
<i>Conclusions and management implications</i>	61
Acknowledgments	62
References	63
Figures	70
APPENDIX C: Return of a keystone species does not immediately affect diversity of small mammals	74
Abstract	76

Introduction.....	77
Methods.....	80
<i>Study area</i>	<i>80</i>
<i>Small mammal trapping.....</i>	<i>81</i>
<i>Analysis</i>	<i>83</i>
Results	84
<i>Species diversity.....</i>	<i>84</i>
<i>Species evenness and richness</i>	<i>85</i>
<i>Small mammal abundance</i>	<i>87</i>
<i>Guild characteristics.....</i>	<i>88</i>
Discussion.....	91
Acknowledgments	95
References	96
Figures.....	102
APPENDIX D: Could the reintroduction of a small, native herbivore regulate woody plant encroachment?	105
Abstract.....	107
Introduction.....	109
Methods.....	111
<i>Study site</i>	<i>111</i>
<i>Woody plant surveys</i>	<i>112</i>
<i>Exclosure experiments</i>	<i>113</i>
<i>Simulated sapling experiments</i>	<i>113</i>
<i>Data analysis</i>	<i>115</i>
Results	116
<i>Woody plant surveys</i>	<i>116</i>
<i>Exclosure experiments</i>	<i>116</i>
<i>Simulated sapling experiments</i>	<i>117</i>
Discussion.....	118
Acknowledgments	123
Literature Cited	124
Figure Legends.....	131
Figures.....	132

LIST OF TABLES

TABLE A1. Publications assessing ecosystem-level effects of keystone species reintroductions with description of keystone taxon of interest and aspects assessed in study.	34
TABLE A2. Focal taxa of publications discussing keystone species and reintroduction, reestablishment, or translocation in order from most common to least common.	35
TABLE B1. Mean (\pm SE) annual (calendar year) temperature and total annual precipitation during our study on black-tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies at the Las Cienegas National Conservation Area, Pima County, Arizona.	50
TABLE B2. Change in area (ha) of reestablished black-tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies between 2011 and 2016 at the Las Cienegas National Conservation Area, Pima County, Arizona.	54
TABLE B3. Yearly and overall growth rates (λ) for colonies A, B, and C between 2011 and 2015 at reestablished black-tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies at Las Cienegas National Conservation Area, Pima County, Arizona. $\lambda > 1$ indicates an increase, $0 < \lambda < 1$ indicates a decrease, $\lambda = 1$ indicates stability, and $\lambda = 0$ indicates extirpation.	56
TABLE B4. Total juvenile black-tailed prairie dogs (<i>Cynomys ludovicianus</i>) emerging and litter sizes (juveniles/lactating female) at Las Cienegas National Conservation Area, Pima County, Arizona between 2011 and 2015.	57
TABLE C1. Summary of studies that use small mammal abundance, richness, or diversity as an estimate of biodiversity on prairie dog (<i>Cynomys</i> spp.) colonies in the United States and Mexico.	79
TABLE C2. Candidate generalized linear models used to determine main effects on Shannon-Wiener species diversity of small mammals on black-tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies, on colony peripheries, and off of colonies.	85
TABLE C3. Candidate generalized linear models used to determine main effects on evenness of small mammals on black-tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies, on colony peripheries, and off of colonies.	86

TABLE C4. Candidate generalized linear models used to determine main effects on richness of small mammal species on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies.

.....87

TABLE C5. Candidate generalized linear models used to determine main effects on the relative abundance of small mammals on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies.

.....88

LIST OF FIGURES

FIGURE A1. Publication topics and their relative proportion of 69 publications on keystone species reintroductions. Values above each bar indicate number of publications.	37
FIGURE A2. Number of publications on keystone species reintroductions in relation to year (1995 to 2016). Dotted lines represent 95% confidence intervals.	38
FIGURE A3. Categories of keystone species (after Mills et al. 1993) studied in publications, and the relative proportion of each category focused on. Some studies did not have a focal keystone species (n < 69).	39
FIGURE A4. Groups of taxa to which focal keystone species belong, and the proportion of studies that examine a keystone species within each group. Some studies did not have a focal keystone species (n < 69).	40
FIGURE A5. Countries in which studies were conducted, and their relative proportions of studies which occurred in specific geographic locales. Some studies did not have specific locales (e.g. literature reviews; n < 69).	41
FIGURE A6. Ecosystems inhabited by focal keystone species of study, and the relative proportion of publications focusing on specific ecosystems. Some studies examined more than one ecosystem (n > 69).	42
FIGURE B1. Location of Las Cienegas National Conservation Area, Pima County, Arizona (A, inset) and black-tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies (A), and boundaries of colonies A (C), B (B), and D (D) between 2011 and 2016. Colony C failed and is not pictured.	70
FIGURE B2. Area (ha) trends over time at black-tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies A (a), B (b), C (c), and D (d) at Las Cienegas National Conservation Area, Pima County, Arizona. Asterisks indicate years in which managers mowed colonies in August or September.	71
FIGURE B3. Population trends at black tailed prairie dog (<i>Cynomys ludovicianus</i>) colonies A (a), B (b), and C (c) between 2011 and 2015 at Las Cienegas National Conservation Area, Pima County, Arizona. Dotted lines indicate the linear regression trends for each population.	72

FIGURE B4. Juvenile black-tailed prairie dog (*Cynomys ludovicianus*) survival vs the proportion of mothers that were yearlings at Las Cienegas National Conservation Area, Pima County, Arizona. Dotted lines represent 95% confidence intervals.

.....73

FIGURE C1. Example of web design for trapping small mammals on and surrounding black-tailed prairie dog (*Cynomys ludovicianus*) colonies, Pima County, Arizona. Only one transect shown. Idealized for a colony with a 100 m radius. Transects originated at the center of each colony, and extended for 500 m. Traps from 0-80 m are considered within the ‘on colony’ colony zone; those from 100-180 m are considered within the ‘colony periphery’ colony zone; those from 200-500 m were considered within the ‘off colony’ colony zone. Trapping stations used in all years are represented by *; additional stations introduced in 2013 are represented by X. Trapping stations included in each colony zone varied by colony and year, but zones are simplified in figure.

.....102

FIGURE C2. Mean (\pm SE; pooled across colonies and sample dates) small mammal Shannon-Wiener species diversity (a), species richness (b), and abundance (c) on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies 2012-2014. Differing letters indicate significant differences ($\alpha = 0.05$).

.....103

FIGURE C3. Small mammal guild abundance (as % of total abundance) on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies, Pima County, Arizona. Larger size indicates greater abundance and smaller size indicates lesser abundance.

.....104

FIGURE D1: Transect design for simulated sapling experiments. Transects originated at the center of the black-tailed prairie dog (*Cynomys ludovicianus*) colony and extended 100 m past the colony boundary. Saplings were spaced evenly along the on-colony transect segments (total distance varied per transect) and at 10 m intervals along the off-colony segment.

.....132

FIGURE D2: Density (a) and percent cover of shrubs (b) (*Prosopis velutina* and *Acacia greggii*) on and off of black-tailed prairie dog (*Cynomys ludovicianus*) colonies and on the control colony. Differing letters represent significant differences.

.....133

FIGURE D3: Mean (\pm SE; untransformed data) change in canopy area/30 days of shrubs inside and outside of exclosures placed on black-tailed prairie dog (*Cynomys ludovicianus*) colonies and on a control (a), and off of colonies (b). Differing letters represent significant differences within each panel.

.....134

FIGURE D4: Mean (\pm SE) percentage of “simulated” *Prosopis velutina* and *Larrea tridentata* saplings disturbed (a), degree of disturbance (b), and degree of disturbance by trial (pooled

across species; c) on, on peripheries, and off of active black-tailed prairie dog (*Cynomys ludovicianus*) colonies and the control site. Differing letters indicate significant differences.

.....135

FIGURE D5: Mean percentage of simulated *Prosopis velutina* and *Larrea tridentata* “saplings” disturbed along transects originating at the centers (position = 0) of active black-tailed prairie dog (*Cynomys ludovicianus*) colonies and a control colony. Transects varied in length, depending upon the size of the colony, so positions are relativised such that 1-8 represent on colony, 9-11 represent the colony periphery, and 12-20 the off colony locations (see Fig. 1).

.....136

ABSTRACT

The keystone species concept was introduced in 1969 in reference to top-down regulation of communities by predators, but has expanded to include myriad species at different trophic levels. Keystone species play disproportionately large, important roles in their ecosystems, but human-wildlife conflicts often drive population declines. A prominent example of anthropogenically driven keystone species decline is the prairie dog (*Cynomys* spp.) in North America. Prairie dogs were once widespread, but were considered pests and eradicated throughout much of the North American west; however, prairie dogs are keystone species that maintain the organization and diversity of their ecosystem, thus their removal can have a cascade of effects on the environment. Population declines have resulted in the necessity of keystone species reintroductions, however, studies of such reintroductions are rare. Managers have reintroduced prairie dogs as a grassland conservation tool, but often do not monitor populations intensively enough following reintroduction to accurately determine success. Furthermore, most studies of keystone species do not assess ecosystem-level effects of reestablishment. I studied four recently reestablished black-tailed prairie dog (*Cynomys ludovicianus*) colonies to determine if the reestablishment effort was successful, and if keystone roles were resumed after a prolonged absence. I found that two of three prairie dog populations monitored for demography grew, and three of four prairie dog colonies expanded in area. Furthermore, I found that reestablished black-tailed prairie dogs did not immediately influence small mammal diversity, richness, or abundance, but prairie dogs did resume their keystone role of regulating woody plant growth on colonies.

INTRODUCTION

The keystone species concept was first introduced in reference to the regulatory role that top predators, *Pisaster ochraceous* and *Charonia* spp., played in an intertidal ecosystem (Paine 1966, Paine 1969). Since its introduction, the keystone species concept has expanded to encompass a suite of species at different trophic levels (Mills et al. 1993, Power et al. 1996). Keystone species are critical in maintaining the organization and diversity of their communities, have effects that are disproportionately large relative to their abundance, and functionally cannot be replaced (Mills et al. 1993, Power et al. 1996, Kotliar et al. 2000, Delibes-Mateos et al. 2011).

The black-tailed prairie dog (BTPD; *Cynomys ludovicianus*) has been commonly described as a keystone species in grassland ecosystems (Miller et al. 1994, Hoogland 1995, Kotliar et al. 1999, Kotliar et al. 2006). BTPDs physically alter their environment by burrowing, foraging, and maintaining short vegetation on their colonies, which provides habitat and shelter for other species, creates macropores for water percolation, facilitates soil turnover, provides young nutritious plant shoots for grazers, creates fire breaks in grasslands, and may prevent woody plant encroachment (Archer et al. 1987, Kotliar et al. 1999, Underwood and Van Pelt 2000). BTPDs have been considered pests despite their important role in the grassland ecosystem, and many state and federally funded campaigns were executed beginning in the early 1900s to eradicate the BTPD range-wide. Eradication programs in Arizona resulted in extirpation of the BTPD by 1960 (Underwood and Van Pelt 2000); however, in 2008, the Arizona Game and Fish Department began reestablishing the BTPD to its historical range in southeastern Arizona.

Many studies of the keystone species concept have only investigated areas occupied by the focal species or the effects of their removal on the ecosystem, which leaves an important

question unanswered: What are the effects of the return of a keystone species to an environment after a prolonged absence? The reestablishment of BTPDs to Arizona provided me with a unique opportunity to not only assess whether BTPDs could persist after reintroduction, but to also measure ecosystem-level responses to BTPD reestablishment.

I present a study that documents population dynamics and colony expansion of four recently reestablished BTPD colonies over a five year period, and assesses the ability of a keystone species to resume their role after a 50 year absence. I test hypotheses about effects of keystone species on associated biodiversity, and whether or not they may act as a management tool for a problem common in degraded rangelands, woody encroachment.

PRESENT STUDY

My dissertation includes four manuscripts formatted for submission to peer-reviewed journals. The first manuscript, intended for submission to *Restoration Ecology* (Appendix A), “How do keystone species reintroductions influence ecosystem-level processes?” is a literature review that highlights the dearth of information on ecosystem-level effects of keystone species reintroductions. The second manuscript, intended for submission to *PLoS ONE* (Appendix B), “Active management contributes to successful reintroduction of a keystone species: black-tailed prairie dog (*Cynomys ludovicianus*) return to grasslands” documents the population dynamics and monitors colony area change of four recently reestablished black-tailed prairie dog colonies. The third manuscript, also intended for submission to *PLoS ONE* (Appendix C), “Return of a keystone species does not immediately affect diversity of small mammals” tests the hypothesis that prairie dogs increase small mammal diversity on colony peripheries by creating edge environments. The third manuscript, intended for submission to *Ecological Applications* (Appendix D), “Could the Reintroduction of a Small, Native Herbivore Regulate Woody Plant Encroachment?” tests the hypothesis that prairie dogs regulate woody encroachment by felling visual obstructions on their colonies. Methods, results, and conclusions of this study are presented in the appended manuscripts.

LITERATURE CITED

- Delibes-Mateos M, Smith AT, Slobodchikoff CN, Swenson JE. 2011. The paradox of keystone species persecuted as pests: a call for the conservation of abundant small mammals in their native range. *Biological Conservation* 144: 1335-1346.
- Hoogland JL. 1995. The black-tailed prairie dog. Social life of a burrowing mammal. Chicago: University of Chicago Press.
- Kotliar NB. 2000. Application of the new keystone-species concept to prairie dogs: how well does it work? *Conservation Biology* 14: 1715-1721.
- Kotliar NB, Baker BW, Whicker AD. 1999. A critical review of assumptions about the prairie dog as a keystone species. *Environmental Management* 24: 177-192.
- Kotliar NB, Miller BJ, Reading RP, Clark TW. 2006. The prairie dog as a keystone species. In: Hoogland JL, editor. *Conservation of the black-tailed prairie dog: saving North America's western grasslands*. Washington, DC: Island Press. pp. 53-64
- Miller B, Ceballos G, Reading R. 1994. The prairie dog and biotic diversity. *Conservation Biology* 8: 677-681.
- Mills LS, Soulé ME, Doak DF. 1993. The keystone-species concept in ecology and conservation. *Bioscience* 43: 219-224.
- Paine RT. 1966. Food web complexity and species diversity. *The American Naturalist* 100: 65-75.
- Paine RT. 1969. A note on trophic complexity and community stability. *The American Naturalist* 103: 91-93.

Power ME, Tilman D, Estes JA, Menge BA, Bond WJ, Mills LS, Daily G, Castilla JC,

Lubchenco J, Paine RT. 1996. Challenges in the quest for keystones. *Bioscience* 46: 609-620.

Underwood JG, Van Pelt WE. 2000. A proposal to reestablish the black-tailed prairie dog

(*Cynomys ludovicianus*) to southern Arizona. Nongame and Endangered Wildlife Program Draft Technical Report. Phoenix: Arizona Game and Fish Department.

APPENDIX A: HOW DO KEYSTONE SPECIES REINTRODUCTIONS INFLUENCE ECOSYSTEM-LEVEL PROCESSES?

Sarah L. Hale and John L. Koprowski

(In the format of *Restoration Ecology*)

How do keystone species reintroductions influence ecosystem-level processes?

Running Head: Do keystone reintroductions affect ecosystems?

SARAH L. HALE (corresponding author), shale16@gmail.com. *School of Natural Resources and the Environment, University of Arizona, 1064 E. Lowell Street, Tucson, AZ 85721, USA.*

JOHN L. KOPROWSKI, *School of Natural Resources and the Environment, University of Arizona, 1064 E. Lowell Street, Tucson, AZ 85721, USA.*

AUTHOR CONTRIBUTIONS

SLH carried out the literature review, analyzed data, and wrote the manuscript. JLK contributed new analyses and edited the manuscript.

ABSTRACT.

The keystone species concept was introduced in 1969 in reference to top-down regulation of communities by predators, but has expanded to include myriad species at different trophic levels. Keystone species play disproportionately large, important roles in their ecosystems, but human-wildlife conflicts often drive population declines. Population declines have resulted in the necessity of keystone species reintroductions, however, studies of such reintroductions are rare. We conducted a literature review and found only 30 peer-reviewed journal articles that assessed reintroduced populations of keystone species, and only 11 of these assessed ecosystem-level effects following reintroduction. Nine of 11 publications assessing ecosystem-level effects found evidence of resumption of keystone roles; however, these publications focus on a narrow range of species. We highlight the deficit of peer-reviewed literature on keystone species reintroductions, and draw attention to the need for assessment of ecosystem-level effects so that the presence, extent, and rate of ecosystem restoration driven by keystone species can be better understood.

KEY WORDS ecosystem-level effects, keystone species concept, literature review

INTRODUCTION

The keystone species concept was first introduced in 1969 as an explanation of the disproportionately large top-down influence purple sea stars (*Pisaster ochraceus*) and sea snails (*Charonia* spp.) imposed on their communities (Paine 1966; Paine 1969). While originally focused on top predators, the keystone species concept has evolved to include myriad species at different trophic levels (Mills et al. 1993; Power et al. 1996). The current and most broadly accepted definition of keystone species can be summarized as such: species that maintain the organization, stability, and function of their communities, and have disproportionately large, inimitable impacts on their ecosystems (Mills et al. 1993; Power et al. 1996; Kotliar et al. 2000; Delibes-Mateos et al. 2011). Gray wolves (*Canis lupus*), sea otters (*Enhydra lutris*), kangaroo rats (*Dipodomys* spp.) and prairie dogs (*Cynomys* spp.) are common examples of keystone species in their ecosystems. Wolves prevent ungulate overpopulation, and in doing so prevent over-browsing of vegetation (McLaren & Peterson 1994), and provide scavengers with carrion in winters (Wilmsers et al. 2003). Sea otters consume sea urchins (*Strongylocentrotus* spp.), thereby maintain the integrity of the kelp forest's community structure (Mills et al. 1993). Kangaroo rats and prairie dogs modify their habitat, thus influencing other species and ecosystem processes (e.g. nutrient cycling; Whicker & Detling 1988; Krogh et al. 2002), and serve as an important prey source for many avian and terrestrial carnivores (Kotliar et al. 1999).

Although keystone species provide essential services to their ecosystems, anthropogenic factors often drive declines in keystone species' populations (Delibes-Mateos et al. 2011). Sea otters were overexploited in the early 20th century for the fur trade, which led to their near extinction (Ravalli 2009), gray wolves in the United States were intensively hunted following

European settlement due to negative depiction in folklore, and frequent livestock depredation resulting from market hunters overharvesting native prey (Fritts et al. 2010), and prairie dogs have been eliminated from most of their former range in North America due to habitat loss and perceived pest status by ranchers (Hoogland 1995). In addition to anthropogenic factors, keystone species declines may be driven by natural processes. For example, recent increases in orca (*Orcinus orca*) predation on sea otters have resulted in population declines (Estes et al. 2004), and prairie dog populations are often extirpated following outbreaks of sylvatic plague (*Yersinia pestis*; Cully et al. 2006).

Keystone species affect a multitude of other species and processes in their ecosystems, hence their removal, either naturally or anthropogenically, can have a cascade of effects (Delibes-Mateos et al. 2011). For example, in Alaska, after sea otter populations declined, previously dense kelp forests upon which many fish and invertebrate species relied (Schiel & Foster 2015) were denuded by sea urchins, the preferred prey of sea otters (Estes et al. 2004). Additionally, the removal of a top predator, the largemouth bass (*Micropterus salmoides*), from a Michigan lake allowed an increase in zooplanktivorous fish that had previously been consumed by bass, which resulted in a decrease in zooplankton (*Daphnia* spp.) that maintained water clarity (Mittelbach et al. 1995) and suppressed eutrophication (Sarnelle 1992). Finally, declines in prairie dog populations in Mexico have resulted in shrub invasion (Ceballos et al. 2010) and desertification of previously occupied prairie dog colonies via soil compaction, increased erosion, reduced water infiltration, reduced soil carbon storage capacity, and reduced herbaceous biomass (Martínez-Estévez et al. 2013).

Anthropogenically driven population declines have resulted in a need for keystone species restoration. One common method of restoration is translocation, the movement of living

organisms from one area with free release in another (IUCN 1987). Translocation has three forms: (1) Introduction: intended or unintended movement of an organism out of its native range; (2) Reintroduction: intended movement of an organism into native range from which it has been extirpated; and (3): Restocking: movement of members of a species to augment the number of individuals in an original habitat (IUCN 1987). Managers have increasingly used keystone species translocations as a tool for conservation benefits, such as restoration of important processes to ecosystems (IUCN/SSC 2013; Cortés-Avizanda et al. 2015; Plein et al. 2016).

Literature reviews that focus on keystone species typically address the function of species in their ecosystems (Kotliar et al. 1999; Janiszewski et al. 2014), the definition of keystone species (Mills et al. 1993; Kotliar 2000; Mouquet et al. 2012), or methods of reintroduction (Truett et al. 2001). Because keystone species restoration is proposed as a conservation tool (Cortés-Avizanda et al. 2015; Plein et al. 2016), we desired to review and synthesize the extent, efficacy, and success of keystone species reintroductions. Furthermore, we sought to ascertain the current state of knowledge of the ecosystem-level effects of keystone species reintroductions.

METHODS

We conducted our literature review using a topic search in the Web of Science database because of the breadth of scientific fields and dates encompassed (Falagas et al. 2008). We imposed no restrictions on time period except an end date of 2016, and began with a general search for the exact term “keystone species,” then narrowed our search to focus on reintroduction of keystone species. We only used terms that referred to the movement of species within their native range, so we conducted literature searches with the following combinations of terms: keystone and restor*, keystone and translocat*, keystone and reintro*, keystone and re-intro*, keystone and reest*, keystone and re-est*, keystone and re est*, keystone and restock*, and

keystone and re-stock*. Asterisks were used in Web of Science to represent words with multiple forms (e.g. reintro* includes reintroduce, reintroduced, reintroducing, and reintroduction). When we located publications discussing reintroduction, reestablishment, or translocation of keystone species, we entered title, year of publication, focal taxa, location of study, if the focal taxa were reintroduced, and focus of study (e.g. population dynamics, behavior, etc.) into a database. We excluded publications that focused on species substitutions, movement of species outside of their native range, and invasive species.

We used JMP® Version 12 to perform statistical analyses. We performed linear regression with year as the explanatory variable and number of publications as the dependent variable to determine if number of publications changed over time.

RESULTS

We found 1178 publications that include the term “keystone species” in the topic. Among these, only 69 discussed reintroductions. Of the publications that discussed keystone species and reintroduction, 30 focused on populations that had been reintroduced to an area, and 11 assessed ecosystem-level effects of such reintroductions (Fig. 1, Table 1). Publication dates ranged from 1995 to 2016, with between one and eight publications per year, and the number of publications increased with year ($F_{1,19} = 75.71$, $R^2 = 0.81$, $P < 0.001$; Fig. 2). Forty-seven different focal taxa were studied in the 69 publications on keystone species reintroductions; however, only 12 taxa were the subject of more than one publication (Table 2). Four of five categories of keystone species (keystone predator, keystone prey, keystone plant, keystone link, keystone modifier; Mills et al. 1993) were represented in this literature, but most publications (35%) focused on keystone modifiers (Figure 3). Over half of the publications (54%) focused on mammals (Figure 4) and the majority (42%) focused on keystone species found in the United

States (Figure 5). Keystone species inhabiting 21 ecosystems were addressed in the 69 publications, but 50% focused on species in just four ecosystems (forest, riparian, grassland, and Mediterranean; Figure 6). Of the 11 publications focusing on ecosystem-level effects of reintroduced keystone species, nine assessed effects of keystone species on other species, and two assessed effects on ecosystem processes such as soil characteristics and hydrological processes. Nine of the 11 publications found evidence of the resumption of keystone functions, one found negative effects on prey species, and one found no effect. Additionally, studies assessing ecosystem-level effects were conducted 14.40 ± 5.60 (mean \pm SE) years following reintroduction of the focal keystone species. Studies documenting resumption of keystone roles were conducted 10.3 ± 3.97 (mean \pm SE; range = 1 to 36 y) years following reintroduction of the focal keystone species.

DISCUSSION

Anthropogenic movement of organisms has taken place for millennia, but conservation based reintroductions, especially of keystone species (Cortés-Avizanda et al. 2015), are a relatively new conservation practice (Seddon et al. 2007). Early reintroduction efforts often resulted in failure due to lack of planning, so managers and researchers have applied more rigorous scientific approaches in preparation for and implementation of reintroductions (Shier 2015). The necessity of science-based approaches can be illustrated by two attempts to reintroduce black-tailed prairie dogs (*Cynomys ludovicianus*) to southeastern Arizona. The first reintroduction was attempted in 1972, but was unsuccessful ostensibly due to disagreement about release sites and methods (Brown et al. 1974) that resulted in prairie dogs being released on the landscape without site preparation (D. E. Brown, 2012, Arizona State University, Tempe, AZ, personal communication). The next effort to reintroduce black-tailed prairie dogs in 2008 was

based on extensive research into habitat requirements and suitable sites for reintroduction (Coates 2005), involved collaboration among many stakeholders, and followed thorough guidelines for site preparation, procurement of founder individuals, and release of animals onto the landscape (Underwood and Van Pelt 2000). The scientific rigor applied to the second attempt at black-tailed prairie dog reintroduction proved effective, as the reintroduction effort has resulted in a sustained population of black-tailed prairie dogs within their former range (Hale 2017).

Prior to 1995, peer-reviewed articles focused on keystone species reintroduction were absent from the literature, likely due to the novelty of reintroduction biology (Seddon et al. 2007). Over time, however, the number of publications on keystone species reintroductions has increased with the necessity of reintroduction as a conservation tool (Shier 2015) and the desire for more research-based approaches to reintroductions (Seddon et al. 2007). Although articles discussing keystone species reintroduction have become more common ($n = 69$), most focus on restoration recommendations, environmental needs, and behavior of existing or theoretical populations, whereas few studies have assessed reintroduced populations. Studies that do assess reintroduced populations of keystone species most often focus on population dynamics, which provide valuable information to managers about the success or failure of reintroduction and allow early detection of problems (Long et al. 2006, Hale 2017), but assessment of ecosystem-level effects after keystone species reintroductions is lacking. Our literature search only returned 11 publications that assessed the ecosystem-level effects of the reintroduction of keystone species, and of those, three (27%) focused on beavers (*Castor* spp). After reintroduction, beavers resume several keystone functions, such as influencing hydrological processes and space use of bats (Ciechanowski et al. 2011; Law et al. 2014), but there is a dearth of information on

how other keystone species affect their ecosystems following reintroduction, as only 12 taxa have been investigated, and most are only the subject of a single study.

Keystone species *in situ* substantially influence their ecosystems (Whicker & Detling 1988; Mills et al. 1993; McLaren & Peterson 1994; Hoogland 1995; Kotliar et al. 1999; Wilmers et al. 2003; Cosentino et al. 2014), but it is unknown if, when, and to what extent keystone species can resume their roles following reintroduction, especially after prolonged absence. Managers often justify keystone reintroductions based on the anticipated or assumed benefits to the ecosystem (Underwood & Van Pelt 2000; Stringer & Gaywood 2016), but actual ecosystem responses to keystone species reintroductions are poorly understood and rarely assessed, indicated by only 0.9% of keystone species publications that focus on ecosystem-level effects of reintroductions. Furthermore, nine publications discussed evidence of the resumption of keystone roles, but on average, detection of ecosystem responses required more than a decade. Lags in ecosystem-level responses to keystone species reintroductions indicates that certain aspects of keystone functions may resume at different rates, so delayed responses may not be detected in the duration of study, and may be interpreted as lack of response. For example, prairie dogs physically modify their environments by burrowing, which turns soil and cycles nutrients (Whicker & Detling 1988). After reintroduction, prairie dogs would likely resume their role of nutrient cycling immediately through burrowing activities, but the influence on the biotic community (e.g. small mammals and vegetation) may not be manifested in the short-term (Davidson et al. 1999), which could be interpreted as prairie dogs' inability to resume their keystone role. It is important to not only understand potential ecosystem-level outcomes prior to implementation of keystone species reintroduction as a management tool, but also the timeline of occurrence so that effects may be accurately assessed and interpreted.

Our review highlights the deficit of peer-reviewed articles that assess ecosystem-level consequences of keystone species reintroductions. While studies of population dynamics of reintroduced keystone species are important to inform managers about the success of reintroductions, more studies must focus on ecosystem-level effects of reintroductions (Robert 2015) so that the presence, extent, and rate of ecosystem restoration driven by keystone species can be understood. Studies of ecosystem-level effects will better inform managers as to whether keystone species can resume their roles following reintroduction, and will provide new insights into ecosystem management or restoration through the reintroduction of a single species.

LITERATURE CITED

- Brown DE, Todd RL, Levy SH (1974) Proposal for the reintroduction of the black-tailed prairie dog into Arizona, Project W-53-R-24. Arizona Small Game Investigations, Arizona Game and Fish Department
- Ceballos G, Davidson A, List R, Pacheco J, Manzano-Fischer P, Santos-Barrera G, Cruzado J (2010) Rapid decline of a grassland system and its ecological and conservation implications. PLoS ONE 5: e8562
- Ciechanowski M, Kubic W, Rynkiewicz A, Zwolicki A (2011) Reintroduction of beavers *Castor fiber* may improve habitat quality for vespertilionid bats foraging in small river valleys. European Journal of Wildlife Research 57: 737-747
- Coates CA (2005) Suitability of potential habitat for the extirpated Arizona black-tailed prairie dog. M.S. Thesis, University of Arizona, Tucson
- Cortés-Avizanda A, Colomer MÀ, Margalida A, Ceballos O, Donazar JA (2015) Modeling the consequences of the demise and potential recovery of a keystone-species: wild rabbits and avian scavengers in Mediterranean landscapes. Scientific Reports 5: 17033

- Cosentino BJ, Schooley RL, Bestelmeyer BT, Kelly JF, Coffman JM (2014) Constraints and timelags for recovery of a keystone species (*Dipodomys spectabilis*) after landscape restoration. *Landscape Ecology* 29: 665-675
- Cully JF, Biggins DE, Seerey DB (2006) Conservation of prairie dogs in areas with plague. Pages 157-168 In: Hoogland JL (editor) *Conservation of the black-tailed prairie dog: saving North America's western grasslands*. Island Press, Washington DC
- Davidson AD, Parmenter RR, Gose JR (1999) Responses of small mammals and vegetation to a reintroduction of Gunnison's prairie dogs. *Journal of Mammalogy* 80: 1311-1324
- Delibes-Mateos M, Smith AT, Slobodchikoff CN, Swenson JE (2011) The paradox of keystone species persecuted as pests: a call for the conservation of abundant small mammals in their native range. *Biological Conservation* 144: 1335-1346
- Estes JA, Danner EM, Doak DF, Konar B, Springer AM, Steinberg PD, Tinker MT, Williams TM (2004) Complex trophic interactions in kelp forest ecosystems. *Bulletin of Marine Science* 74: 621-638
- Falagas ME, Pitsouni EI, Malietzis GA, Pappas G (2008) Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. *The FASEB Journal* 22: 338-342
- Fariñas-Franco JM, Allcock L, Smyth D, Roberts D (2013) Community convergence and recruitment of keystone species as performance indicators of artificial reefs. *Journal of Sea Research* 78: 59-74
- Fritts SH, Stephenson RO, Hayes RD, Boitani L (2010) Wolves and Humans. Pages 289-316 In: Mech LD, Boitani L (eds) *Wolves: behavior, ecology, and conservation*. University of Chicago Press, Chicago, Illinois

- Fulgham KM, Koprowski JL (2016) Kangaroo rat foraging in proximity to a colony of reintroduced black-tailed prairie dogs. *Southwestern Naturalist* 61: 194-202
- Hale SL (2017) Active management contributes to successful reintroduction of a keystone species: black-tailed prairie dog (*Cynomys ludovicianus*) return to grasslands. PhD Dissertation, University of Arizona, Tucson
- Hoogland JL (1995) The black-tailed prairie dog: social life of a burrowing mammal. University of Chicago Press, Chicago, Illinois
- IUCN (1987) Position statement on translocations of living organisms: introductions, reintroductions, and re-stocking. International Union for Conservation of Nature and Natural Resources Council, Gland, Switzerland
- IUCN/SSC (1998) Guidelines for reintroductions. IUCN Species Survival Commission, Gland, Switzerland
- IUCN/SSC (2013) Guidelines for reintroductions and other conservation translocations. IUCN Species Survival Commission, Gland, Switzerland
- Janiszewski P, Hanzal V, Misiukiewicz W (2014) The Eurasian beaver (*Castor fiber*) as a keystone species – a literature review. *Baltic Forestry* 20: 277-286
- Kotliar NB (2000) Application of the new keystone-species concept to prairie dogs: how well does it work? *Conservation Biology* 14: 1715-1721
- Kotliar NB, Baker BW, Whicker AD (1999) A critical review of assumptions about the prairie dog as a keystone species. *Environmental Management* 24: 177-192
- Kowalczyk R, Taberlet P, Coissac E, Valentini A, Miquel C, Kamiński T, Wójcik JM (2011) Influence of management practices on large herbivore diet-case of European bison in Białowieża Primeval Forest (Poland). *Forest Ecology and Management* 261: 821-828

- Krogh SN, Zeisset MS, Jackson E, Whitford WG (2002) Presence/absence of a keystone species as an indicator of rangeland health. *Journal of Arid Environments* 50: 513-519
- Law A, Jones KC, Willby NJ (2014) Medium vs. short-term effects of herbivory by Eurasian beaver on aquatic vegetation. *Aquatic Botany* 116: 27-34
- Le Floc'H E, Neffati M, Chaieb M, Floret C, Pontanier R (1999) Rehabilitation experiment at Menzel Habib, southern Tunisia. *Arid Soil Research and Rehabilitation* 13: 357-368
- Long D, Bly-Honness K, Truett JC, Seery DB (2006) Establishment of new prairie dog colonies by translocation. Pages 188-209 In: Hoogland JL (ed) *Conservation of the black-tailed prairie dog: saving North America's western grasslands*. Island Press, Washington, D.C.
- Lovari S, Boesi R, Minder I, Mucci N, Randi E, Dematteis A, Ale SB (2009) Restoring a keystone predator may endanger a prey species in a human-altered ecosystem: the return of the snow leopard to Sagarmatha National Park. *Animal Conservation* 12: 559-570
- Martínez-Estévez L, Balvanera P, Pacheco J, Ceballos G (2013) Prairie dog decline reduces the supply of ecosystem services and leads to desertification of semiarid grasslands. *PLoS ONE* 8: e75229
- McLaren BE, Peterson RO (1994) Wolves, moose, and tree rings on Isle Royale. *Science* 266: 1555-1558
- Mills LS, Soulé ME, Doak DF (1993) The keystone-species concept in ecology and conservation. *Bioscience* 43: 219-224
- Mittelbach GG, Turner AM, Hall DJ, Rettig JE, Osenberg CW (1995) Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. *Ecology* 76: 2347-2360

- Mouquet N, Gravel D, Massol F, Calcagno V (2012) Extending the concept of keystone species to communities and ecosystems. *Ecology Letters* 16: 1-8
- Paine RT (1966) Food web complexity and species diversity. *The American Naturalist* 100: 65-75
- Paine RT (1969) A note on trophic complexity and community stability. *The American Naturalist* 103: 91-93
- Plein M, Bode M, Moir ML, Vesk PA (2016) Translocation strategies for multiple species depend on interspecific interaction type. *Ecological Applications* 26: 1186-1197
- Power ME, Tilman D, Estes JA, Menge BA, Bond WJ, Mills LS, Daily G, Castilla JC, Lubchenco J, Paine RT (1996) Challenges in the quest for keystones. *Bioscience* 46: 609-620
- Prober SM, Lunt ID (2009) Restoration of *Themeda australis* swards suppresses soil nitrate and enhances ecological resistance to invasion by exotic annuals. *Biological Invasions* 11: 171-181
- Puttock A, Graham HA, Cunliffe AM (2017) Eurasian beaver activity increases water storage, attenuates flow and mitigates diffuse pollution from intensively-managed grasslands. *Science of the Total Environment* 576: 430-443
- Ravalli R (2009) The near extinction and reemergence of the pacific sea otter, 1850-1938. *The Pacific Northwest Quarterly* 100: 181-191
- Robert A, Colas B, Guigon I, Kerbiriou C, Mihoub JB, Saint-Jalme M, Sarrazin F (2015) Reintroducing reintroductions into the conservation arena. *Animal Conservation* 18: 413-414

- Sarnelle O (1992) Nutrient enrichment and grazer effects on phytoplankton in lakes. *Ecology* 73: 551-560
- Schiel DR, Foster MS (2015) The biology and ecology of giant kelp forests. University of California Press, Oakland, California
- Seddon PJ, Armstrong DP, Maloney RF (2007) Developing the science of reintroduction biology. *Conservation Biology* 21: 303-312
- Shier DM (2015) Developing a standard for evaluating reintroduction success using IUCN Red List indices. *Animal Conservation* 18: 411-412
- Stringer AP, Gaywood MJ (2016) The impacts of beavers *Castor* spp. on biodiversity and the ecological basis for their reintroduction to Scotland, UK. *Mammal Review* 46: 270-283
- Truett JC, Dullum JALD, Matchett MR, Owens E, Seery D (2001) Translocating prairie dogs: a review. *Wildlife Society Bulletin* 29: 863-872
- Underwood JG, Van Pelt WE (2000) A proposal to reestablish the black-tailed prairie dog (*Cynomys ludovicianus*) to southern Arizona. Arizona Game and Fish Department, Nongame and Endangered Wildlife Program Draft Technical Report, Phoenix, Arizona
- Whicker AD, Detling JK (1988) Ecological consequences of prairie dog disturbances. *Bioscience* 38: 778-785
- Wilmers CC, Crabtree RL, Smith DW, Murphy KM, Getz WM (2003) Trophic facilitation by introduced top predators: grey wolf subsidies to scavengers in Yellowstone National Park. *Journal of Animal Ecology* 72: 909-916

Figure Legends

Figure 1. Publication topics and their relative proportion of 69 publications on keystone species reintroductions. Values above each bar indicate number of publications.

Figure 2. Number of publications on keystone species reintroductions in relation to year (1995 to 2016). Dotted lines represent 95% confidence intervals.

Figure 3. Categories of keystone species (after Mills et al. 1993) studied in publications, and the relative proportion of each category focused on. Some studies did not have a focal keystone species ($n < 69$).

Figure 4. Groups of taxa to which focal keystone species belong, and the proportion of studies that examine a keystone species within each group. Some studies did not have a focal keystone species ($n < 69$).

Figure 5. Countries in which studies were conducted, and their relative proportions of studies which occurred in specific geographic locales. Some studies did not have specific locales (e.g. literature reviews; $n < 69$).

Figure 6. Ecosystems inhabited by focal keystone species of study, and the relative proportion of publications focusing on specific ecosystems. Some studies examined more than one ecosystem ($n > 69$).

Tables

Table 1. Publications assessing ecosystem-level effects of keystone species reintroductions with description of keystone taxon of interest and aspects assessed in study.

Authors	Year	Focal Keystone Taxon	Subject
Mittelbach et al.	1995	<i>Micropterus salmoides</i>	Effects of removal and reintroduction
Le Floch et al.	1999	<i>Plantago albicans</i> , <i>Stipa lagascae</i> , <i>Cenchrus ciliaris</i> , <i>Rhanterium suaveolens</i>	Ecosystem restoration
Wilmers et al.	2003	<i>Canis lupus</i>	Provision of carrion to scavengers
Prober and Lunt	2009	<i>Themeda australis</i>	Effects on soil nitrate and exotic invasions
Lovari et al.	2009	<i>Uncia uncia</i>	Effects on prey populations
Ciechanowski et al.	2011	<i>Castor</i> spp.	Effects on vespertilionid bats
Kowalczyk et al.	2011	<i>Bison bonasus</i>	Effects on treestand
Fariñas-Franco et al.	2013	<i>Modiolus modiolus</i>	Effects on community succession
Law et al.	2014	<i>Castor</i> spp.	Effects on macrophytes
Fulgham and Koprowski	2016	<i>Cynomys ludovicianus</i>	Effects on <i>D. spectabilis</i> foraging
Puttock et al.	2017	<i>Castor</i> spp.	Effects on hydrological processes

Table 2. Focal taxa of publications discussing keystone species and reintroduction, reestablishment, or translocation in order from most common to least common.

Focal Taxon	Publications (#)	Common Name	Group
<i>Castor</i> spp.	9	Beaver	Mammal
<i>Oryctolagus cuniculus</i>	7	European Rabbit	Mammal
<i>Cynomys</i>	4	Prairie Dog	Mammal
<i>Canis lupus</i>	3	Gray Wolf	Mammal
<i>Panthera leo</i>	3	African Lion	Mammal
<i>Acropora cervicornis</i>	2	Staghorn Coral	Coral
<i>Castanea dentata</i>	2	American Chestnut	Tree
<i>Enhydra lutris</i>	2	Sea Otter	Mammal
<i>Ficus</i> spp.	2	Ficus Tree	Tree
<i>Pinus albicaulis</i>	2	Whitebark Pine	Tree
<i>Salvelinus namaycsh</i>	2	Lake Trout	Fish
<i>Pelecanoides urinatrix</i>	1	Common Diving-petrel	Bird
<i>Micropterus salmoides</i>	1	Largemouth Bass	Fish
<i>Sander vitreus</i>	1	Walleye	Fish
<i>Aristida stricta</i>	1	Pineland Threeawn	Grass
<i>Cenchrus ciliaris</i>	1	Buffelgrass	Grass
<i>Stipa lagascae</i>	1	Alatham (Algeria)	Grass
<i>Themeda australis</i>	1	Kangaroo Grass	Grass
<i>Coelostomidia zealandica</i>	1	Great Giant Scale	Insect
<i>Bison bison</i>	1	American Bison	Mammal
<i>Bison bonasus</i>	1	European Bison	Mammal
<i>Canidae</i>	1	Wild Canids	Mammal
<i>Canis lupus dingo</i>	1	Dingo	Mammal
<i>Crocuta crocuta</i>	1	Spotted Hyena	Mammal
<i>Dipodomys spectabilis</i>	1	Banner-tailed Kangaroo Rat	Mammal
<i>Equus ferus</i>	1	Horse	Mammal
<i>Uncia uncia</i>	1	Snow Leopard	Mammal

<i>Crassostrea virginica</i>	1	Oyster	Mollusk
<i>Modiolus modiolus</i>	1	Horse mussel	Mollusk
<i>Bryum pseudotriquetrum</i>	1	Bryum Moss	Moss
<i>Campylium stellatum</i>	1	Star Campylium Moss	Moss
<i>Sphagnum</i>	1	Sphagnum Moss	Moss
<i>Sphagnum warnstorffii</i>	1	Warnstorff's Peat Moss	Moss
<i>Tomenthypnum nitens</i>	1	Tomenthypnum Moss	Moss
<i>Gopherus polyphemus</i>	1	Gopher Tortoise	Reptile
<i>Carex</i> spp.	1	Sedges	Sedge
<i>Gahnia radula</i>	1	Thatch Saw Sedge	Sedge
<i>Lepidosperma concavum</i>	1	Sandhill Swordsedge	Sedge
<i>Lepidosperma laterale</i>	1	Variable Swordsedge	Sedge
<i>Ceroxylon echinulatum</i>	1	Palm	Tree
<i>Pinus chiapensis</i>	1	Chiapas Pine	Tree
<i>Pinus elliottii</i>	1	Slash Pine	Tree
<i>Acacia</i> spp.	1	Acacia Shrub	Woody Plant/Shrub
<i>Artemisia tridentata wyomingensis</i>	1	Wyoming Big Sagebrush	Woody Plant/Shrub
<i>Banksia attenuata</i>	1	Candlestick Banksia	Woody Plant/Shrub
<i>Plantago albicans</i>	1	Plantain	Woody Plant/Shrub
<i>Rhanterium suaveolens</i>	1	Arfadja	Woody Plant/Shrub

Figures

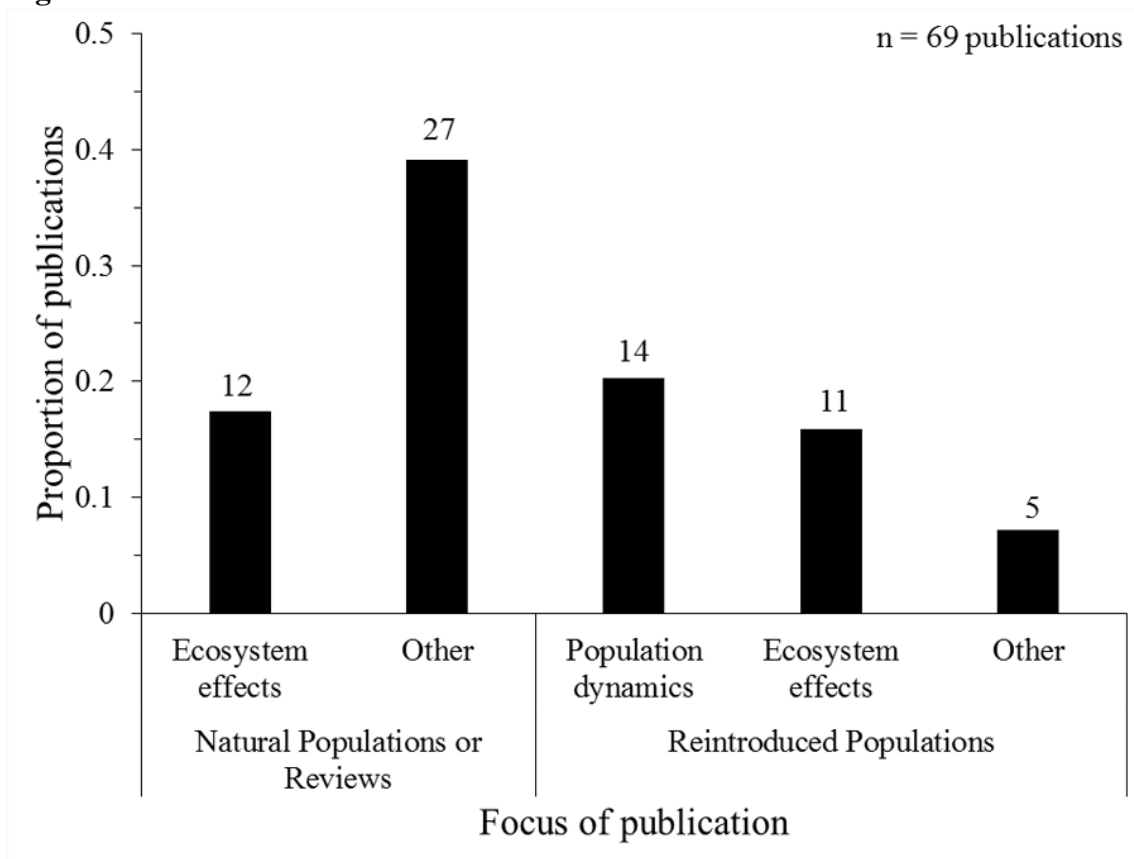


Figure 1

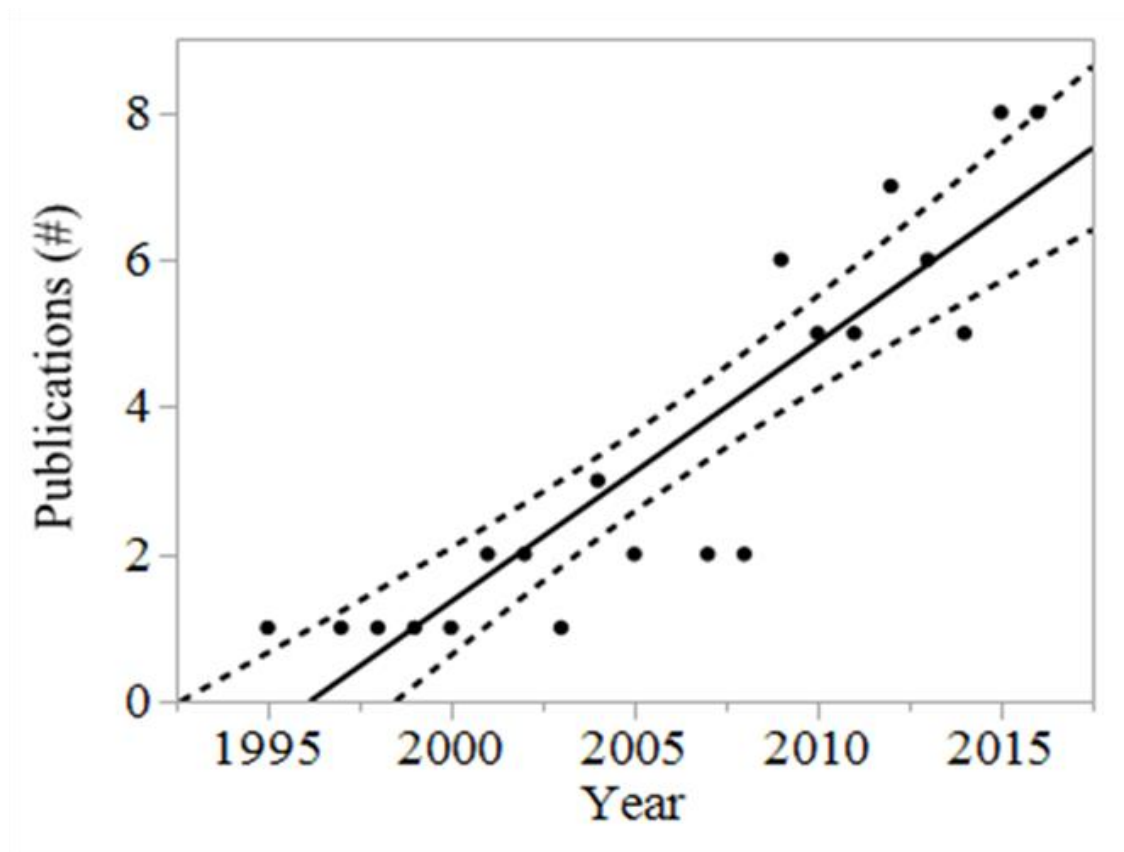


Figure 2

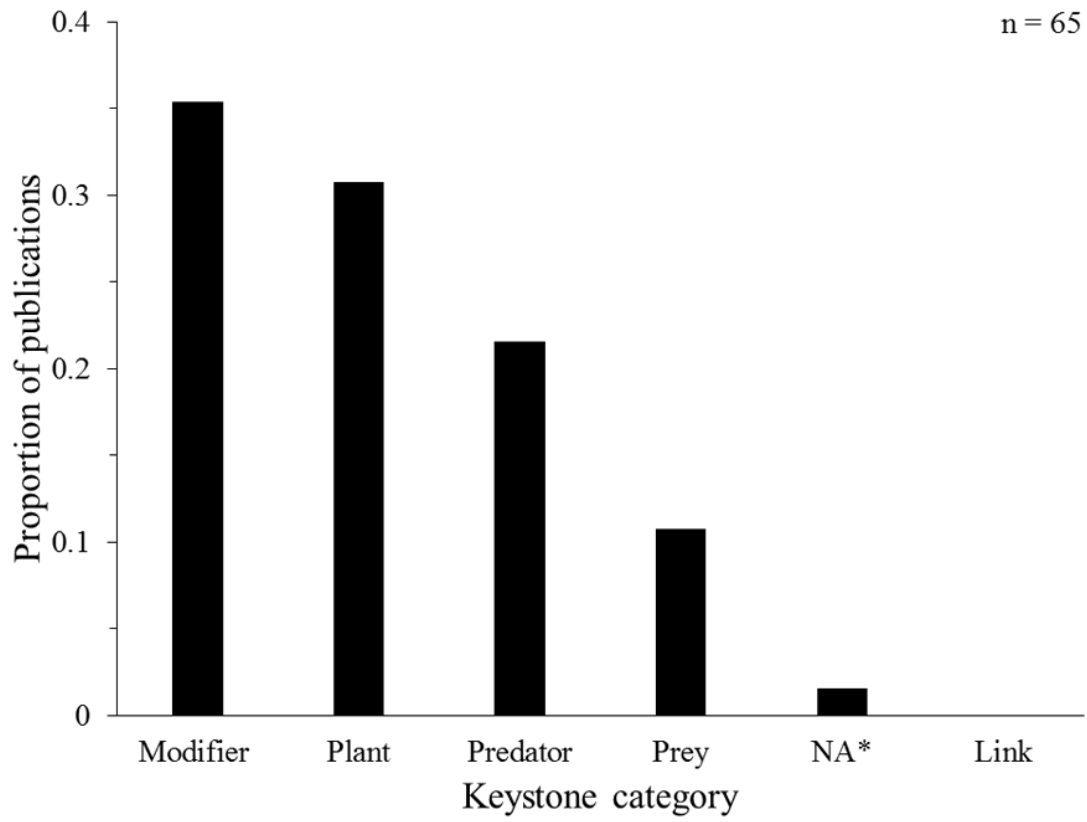


Figure 3

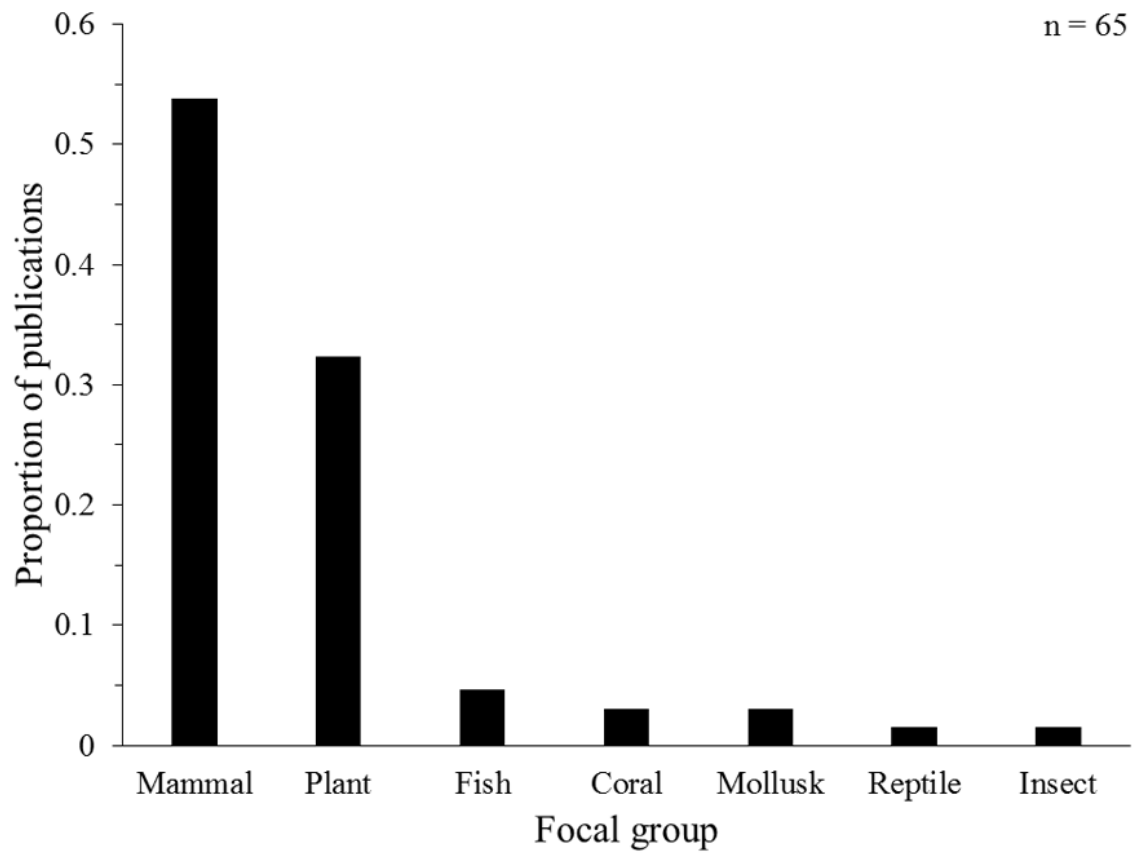


Figure 4

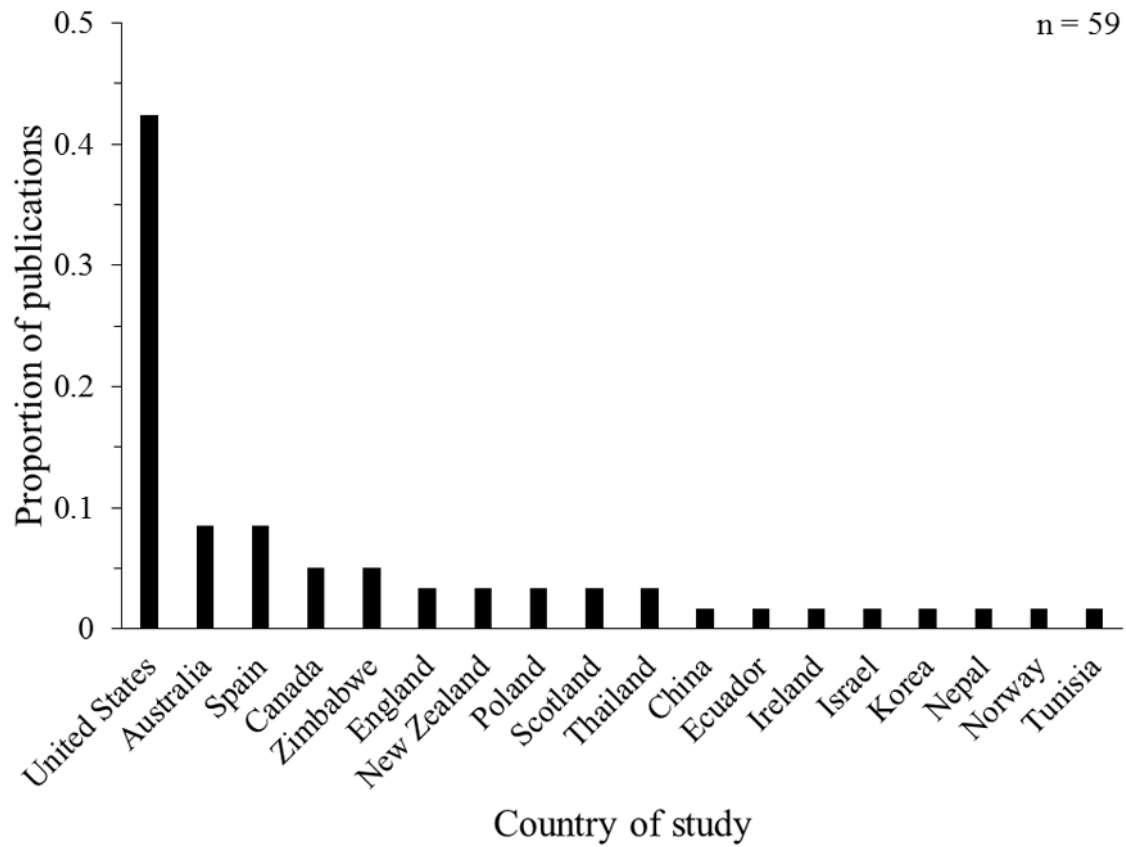


Figure 5

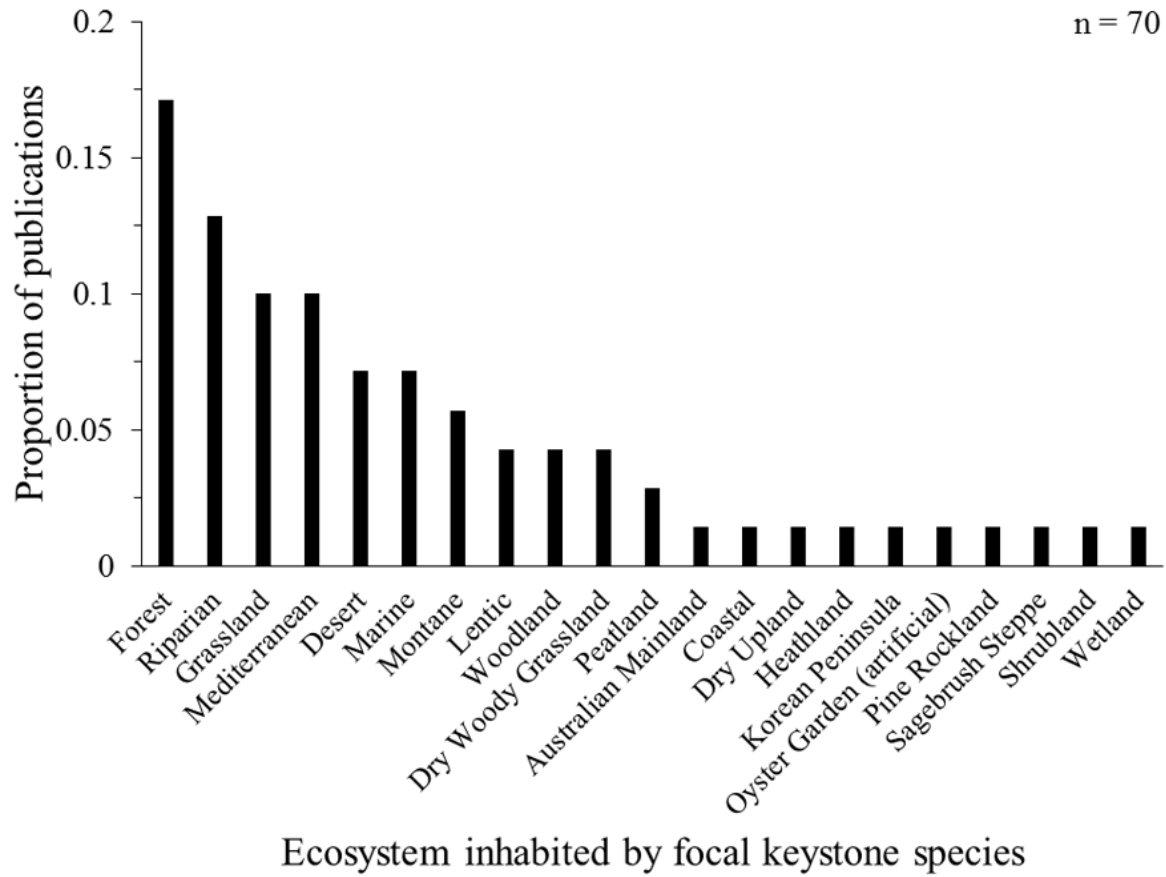


Figure 6

APPENDIX B: ACTIVE MANAGEMENT CONTRIBUTES TO SUCCESSFUL
REINTRODUCTION OF A KEYSTONE SPECIES: BLACK-TAILED PRAIRIE DOG
(*CYNOMYS LUDOVICIANUS*) RETURN TO GRASSLANDS

Sarah L. Hale and John L. Koprowski

(In the format of *PLoS ONE*)

Active management contributes to successful reintroduction of a keystone species: black-tailed
prairie dog (*Cynomys ludovicianus*) return to grasslands

Sarah L. Hale^{1*} and John L. Koprowski¹

¹School of Natural Resources and the Environment, University of Arizona, Tucson, AZ, United States of America

*Corresponding author

E-mail: shale@email.arizona.edu (SLH)

Abstract

Anthropogenic factors pose many threats to wildlife, especially in cases of human-wildlife conflict when species are perceived as threats or pests. A prominent example of anthropogenically-driven species decline is the prairie dog (Cynomys spp.) in North America. Prairie dogs were once widespread, but were considered pests and were eradicated throughout much of the North American west. Prairie dogs are keystone species that maintain the organization and diversity of their ecosystem, thus their removal can have a cascade of effects on the environment. Managers have reintroduced prairie dogs as a grassland conservation tool, but often do not monitor populations intensively enough following reintroduction to accurately determine success. We combined repeated visual counts with application of visible markers to individuals at three recently reestablished black-tailed prairie dog (Cynomys ludovicianus) colonies in southeastern Arizona. We communicated problems to managers as they were detected so that they could be addressed. Additionally, we assessed population growth, reproduction, and overall success of the reintroduction effort, and repeatedly mapped colony boundaries to determine how colony area changed over time. We found that two of three prairie dog populations monitored for demography grew, that three of four prairie dog colonies expanded in area, and that mature woody plants (Prosopis velutina) acted as barriers to colony expansion. Furthermore, we found that supplemental feeding may have contributed to increased population growth. Our study highlights the importance of multi-year intensive monitoring of reintroduced populations, and the necessity of in situ collaboration between researchers and management agencies to enhance the success of species reintroductions.

Introduction

Anthropogenic factors impose many risks and constraints to wildlife such as habitat loss and fragmentation [1, 2], ecological and evolutionary traps [3], disturbance from noise and light pollution [4, 5, 6], and overexploitation [2]. Additionally, human-wildlife conflicts often lead to the destruction of wildlife habitat or elimination of species perceived as a threat or pest [7, 8]. For example, large carnivores are often removed by humans in response to perceived risks of livestock depredation [9].

A prominent example of human-wildlife conflict driving a species decline is the attempted eradication of prairie dogs (*Cynomys* spp.), large bodied ground dwelling squirrels, in North America. Prairie dogs historically were widely distributed across the North American west [10]; however, as humans began to settle in areas occupied by prairie dogs, development and agriculture resulted in habitat loss, and prairie dogs were systematically eliminated throughout much of their range due to their perceived pest status [11, 12, 13]. Despite evidence to the contrary [13], prairie dogs are viewed as competitors with livestock for forage, thus have been actively removed due to the perceived economic threat [12]. Targeted eradication efforts and land conversion have reduced prairie dog populations to 2% of historical numbers, and have left only 1% of the prairie dogs' former range occupied [12, 13, 14].

The black-tailed prairie dog (BTPD – *C. ludovicianus*) is a keystone species in grassland ecosystems [13, 15, 16, 17]. Keystone species maintain biodiversity and ecosystem functions [18]; therefore, their removal can have significant consequences. Sea otters (*Enhydra lutris*), for example, are considered keystone species in nearshore communities [19]. Abundant sea otter populations supported dense kelp forests in the Aleutian archipelago, but after otters became sparse, so too did kelp forests [20]. Prairie dogs in grasslands provide burrows for other animals

such as burrowing owls (*Athene cunicularia*) and rattlesnakes (*Crotalus* spp.), physically turn soil during burrow excavation, and serve as a food source for raptors and terrestrial carnivores [12, 16, 21]. Prairie dogs also contribute to grassland heterogeneity by increasing species richness and diversity of vegetation, creating fire breaks through maintenance of short vegetation on colonies, and preventing woody encroachment [16, 21, 22, 23]. Additionally, at least 9 vertebrate species rely on prairie dogs to varying degrees [16]. Declines in black-footed ferret (*Mustela nigripes*), mountain plover (*Charadrius montanus*), and burrowing owl populations are correlated with prairie dog decreases, and prairie dogs are known to influence the local distribution of six other species (ferruginous hawks [*Buteo regalis*], golden eagles [*Aquila chrysaetos*], swift foxes [*Vulpes velox*], horned larks [*Eremophila alpestris*], deer mice [*Peromyscus maniculatus*], and grasshopper mice [*Onychomys* spp.]) [16].

Widespread declines in biodiversity driven by anthropogenic factors [1] have created a need for species restoration through reintroduction. Reintroduction refers to return of a species to former range from which it had been extirpated by anthropogenic or natural factors [24]. Reintroduction is successful when the result is a self-sustaining population [25]. Because of the vital ecosystem services that prairie dogs provide, prairie dog reintroductions have been a tool for grassland conservation throughout the prairie dog's former range in North America [26, 27, 28, 29]. Measuring the success of a prairie dog reintroduction effort involves periodic monitoring of populations, but intensive monitoring is seldom conducted following reintroduction [28].

Although intensive monitoring of reintroduced populations is infrequent, several long term studies of established prairie dog populations have used visible temporary markers (e.g. fur dye) to identify individuals combined with daily observation to accurately estimate population

sizes [15, 30, 31]. Such intensive monitoring also allows documentation of rarely observed events such as predation, dispersal, and infanticide [15]. Marking and intensive monitoring does not typically occur following prairie dog reintroduction efforts, but would facilitate detection and mitigation of problems such as dispersal from or depredation at the reintroduction site [26].

BTPD were recently reintroduced into their native range in southern Arizona, USA, which provides a unique opportunity to monitor populations post-reintroduction. Between 2011 and 2015, we monitored abundance, reproduction, and survival, and tracked colony boundary expansion at four BTPD colonies in southeastern Arizona. We sought to determine whether reintroduction efforts resulted in self-sustaining populations, and to better understand factors that may negatively affect early stages of reintroductions. Our goals were to assess the influence of founder population size, composition, and management interventions on population growth rate and colony boundary expansion. We provide information and management recommendations useful to future reestablishment efforts.

Methods

Study area

Las Cienegas National Conservation Area (hereafter Las Cienegas) is a working cattle ranch managed by the Bureau of Land Management. Las Cienegas is a 17,000 ha mosaic of five rare ecosystems of the southwestern United States (cottonwood [*Populus fremontii*]-willow [*Salix gooddingii*, *S. taxifolia*] riparian forest, marshland, mesquite [*Prosopis velutina*] bosque, sacaton [*Sporobolus wrightii*] floodplain, and semidesert grassland) [32, 33], and is located 72 km southeast of Tucson, AZ. Mean annual precipitation (MAP) is 405 mm, mostly falling during the summer monsoon months (July-September); mean annual temperature (MAT) is 15.7° C [34]. During our study (2011-2015) annual precipitation and temperature (\pm SE) averaged 303

± 45 mm and $16.2 \pm 0.14^\circ$ C (Empire Remote Area Weather Station; QEMA3, <http://mesowest.utah.edu>). In all years of our study, temperature was above-average, and in all years except 2015, mean annual precipitation was below-average (Table 1).

Grasslands at Las Cienegas are located between 1300 and 1500m in elevation, and soils are primarily gravelly, sandy, and clay loam [34]. Semidesert grasslands in Arizona historically supported BTPDs, which were extirpated by 1960 [21]. The Arizona Game and Fish Department began reintroducing BTPDs at Las Cienegas in 2008 from populations in New Mexico, USA and Sonora, Mexico.

Colonies were situated on sites with similar soils (fine, deep, well-drained), slopes (0-15%), and elevations (1367-1412 m). Site preparation by the Arizona Game and Fish Department consisted of shrub removal, mowing of tall grass, and installation of 25 artificial burrows on a 4 ha plot [see 35 for details]. One colony was initiated per year starting in 2008 and ending in 2011. Throughout our study, three of the four colonies remained occupied by BTPD; however, one colony (hereafter referred to as the “control colony”) failed and was unoccupied during 2014 and 2015. This “control colony” received the same initial treatment as the other colonies, but lacked prairie dogs throughout the majority of our study period (fewer than 10 prairie dogs were present in May 2013 and the colony was vacant by October 2013).

Study colonies will be referred to as colony A (established in 2008), B (established in 2010), C (established in 2012). When populations declined below 20 individuals, colonies A and B received additional BTPDs from colonies in New Mexico or Mexico. Colony C was initially populated with 30 individuals (10 yearlings/adults and 20 juveniles) from colony B in 2012. Another colony was established at Las Cienegas in 2009 (hereafter referred to as colony D), but

individuals were not monitored. Colonies A, B, and D were continuously occupied throughout our study, but colony C was only occupied between September of 2012 and 2013.

During our study, several management interventions occurred. In June, 2011, the AZGFD began supplemental feeding with Mazuri® ADF 16 herbivore diet, provided by the Arizona Center for Nature Conservation-Phoenix Zoo. Supplemental feeding was initiated in February and ended in August in all subsequent years. Mowing was conducted when short (< 30 cm) vegetation height, and thus the ability to visually detect predators, could not be maintained by BTPDs. When mowing was necessary, vegetation on colonies was mowed to a height of 8 cm. Colonies A and B were mowed in August and September 2011, colony C in September 2012, and colony D in September 2011 and 2013. Finally, in 2012, 2013, and 2014, the Bureau of Land Management uprooted and stacked mature mesquite (*P. velutina*) plants > approximately 3 m in height from areas surrounding colonies B and C (200-500 m from colony boundaries), and during 2014 and 2015 stacks were gradually removed.

Table 1. Mean (\pm SE) annual (calendar year) temperature and total annual precipitation during our study on black-tailed prairie dog (*Cynomys ludovicianus*) colonies at the Las Cienegas National Conservation Area, Pima County, Arizona.

Year	Mean Temperature (°C)	Total Annual Precipitation (mm)
2011	15.9 \pm 0.11	307
2012	16.5 \pm 0.10	158
2013	16.0 \pm 0.11	267
2014	16.6 \pm 0.10	355
2015	16.1 \pm 0.10	429
Mean During Study (2011-2015)	16.2 \pm 0.14	303 \pm 45.2
Mean Long Term (1895-2014)	15.7	405

Colony area

In 2011, 2012, 2013, 2014, and 2016, we recorded the location of colony boundaries as polygons with a handheld global positioning system (GPS; GeoExplorer 2005 series Geo XT and Geo XM; Trimble Inc., Sunnyvale, California; Garmin Etrex Legend Cx; Garmin International, Inc., Olathe, Kansas). We combined two common methods of mapping colonies: following the ‘clip-zone,’ where vegetation clipped by prairie dogs meets taller, un-clipped vegetation, and locating the outermost active burrows [36]. We used ArcGIS 10.3.1 (ESRI, Redlands, California, USA) to map colony boundaries and determine colony area (ha).

Trapping and marking.

From February through October 2011, March through September 2012 and 2013, and March through October 2014 and 2015, we trapped BTPDs with Tomahawk live traps (12.7 x 12.7 x 40.6 cm single-door, 15.2 x 15.2 x 48.3 cm single-door, and 15.2 x 15.2 x 61.0 cm double-door; model numbers 201, 202, and 203, Tomahawk Live Trap Co., Hazelhurst, Wisconsin). Traps were baited with whole oats and peanut butter prior to sunrise and monitored with binoculars throughout the day from blinds (1.2 x 1.2 x 1.5 m) atop 2.1 m high platforms (Vertex Quad-Pod and blind combo; Big Game Treestands, Windom, Minnesota). Traps were closed during the hottest period of the day (approximately 12:00 -15:30) when ambient temperatures typically exceeded 35° C.

We moved captured BTPDs into the shade prior to processing individuals. We transferred animals from live traps into cone shaped canvas handling bags [37], measured body mass with Pesola scales (1000 g and 2500 g; Pesola AG, Schindellegi, Switzerland), assessed reproductive condition, affixed ear tags (Monel #1005-1; National Band and Tag Co., Newport, Kentucky), inserted passive integrated transponder (PIT) tags under the skin between shoulder

blades (HPT9: 9 mm 134.2 kHz ISO FDX-B; Biomark, Boise, Idaho), and marked fur with black Nyanzol D dye [15] (Greenville Colorants, LLC., New Brunswick, New Jersey). If animals were already marked upon capture, we followed the same procedure without application of semi-permanent markers (ear and PIT tags). Identification of previously marked individuals allowed us to determine survival and document dispersal events, with dispersal distance considered the minimum straight line distance between emigration and immigration colonies. We released animals at the site of capture. Animal handling methods followed the American Society of Mammalogists guidelines for the care and use of mammals [38] and were approved by the University of Arizona Institutional Animal Care and Use Committee (protocol 11-251).

Observation

We used the scan sampling method [39] to conduct observations for over 1469 h. Between May and August of 2011-2014, we monitored BTPD from blinds at colonies A, B, and C. In 2011, we conducted observations for 12 h/day, five days/week. In 2012 and 2015, we observed colonies at least once per month for 4 h/day, and in 2013 and 2014, we observed at least once/week for 4h/day. We began observation prior to sunrise to reduce disturbance to the colony and to detect BTPD as they emerged from burrows. During each observation period, we recorded individually marked BTPDs. When juveniles began to emerge, we recorded lactating females, and counted the total number of juveniles per colony (because multiple lactating females often shared burrows and juveniles were mobile, we could not assign specific juveniles to specific mothers). During each observation period in 2011, $90.5 \pm 2.0\%$ (mean \pm SE) of non-juvenile (\geq one-year-old) BTPDs at colony A and $94.3 \pm 1.8\%$ of non-juvenile BTPDs at colony B were detected (calculated as [individuals observed/number known alive] *100), indicating that our counts were accurate. Fates of individual BTPDs were unknown except in rare cases when

mortality events were documented, but due to high detectability, individuals not observed for \geq two observation periods were assumed to no longer occupy the colony of interest (either due to mortality or dispersal).

Analysis

We determined population size at each colony by marking individuals and conducting repeated observations at colonies A, B, and C. We used the initial population size (prior to juvenile emergence) each field season to calculate yearly and overall population growth rates ($\lambda = N_{t+1}/N_t$) for each colony. We used linear regressions to explore population trends at each colony, with population size as the dependent variable, and Julian date as the main effect. Each year had a different number of census occasions (e.g. 45 in 2011 vs 4 in 2012) at different time intervals, so we chose four main temporal events to represent in our regression: population size at the beginning of the field season, population size prior to juvenile emergence, population size immediately following juvenile emergence, and population size at the end of the field season. In addition to the four temporal events, special events (augmentation or removal of individuals from a colony) were included.

We used the maximum number of juveniles counted each year (N_t), and the number of yearlings trapped the following year (N_{t+1}) to calculate yearly juvenile survival rates ($l_x = N_{t+1}/N_t$) at each colony. Additionally, we conducted a simple linear regression with juvenile survival rate as the dependent variable, and the proportion of mothers that were yearlings as the explanatory variable. To investigate trends in colony area, we performed linear regressions with colony area as the dependent variable and year as the explanatory variable.

Results

Colony area

Colony boundaries fluctuated annually (Figure 1), but between 2011 and 2016, colonies A, B, and D increased in area. Colony C decreased in area to zero as all individuals disappeared (Table 2). Most notably, Colony B grew approximately 240% between 2014 and 2016. (Figure 2).

Table 2. Change in area (ha) of reestablished black-tailed prairie dog (*Cynomys ludovicianus*) colonies between 2011 and 2016 at the Las Cienegas National Conservation Area, Pima County, Arizona.

Colony	Starting Area (ha)	Ending Area (ha)	Change in Area (ha)	Change in Area (%)
A	1.95	2.67	+0.72	+36.92
B	3.51	11.93	+8.42	+239.89
C	3.96	0.00	-3.96	-100.00
D	2.71	3.82	+1.11	+40.96

Figure 1. Location of Las Cienegas National Conservation Area, Pima County, Arizona (a, inset) and black-tailed prairie dog (*Cynomys ludovicianus*) colonies (A), and boundaries of colonies A (C), B (B), and D (D) between 2011 and 2016. Colony C failed and is not pictured.

Figure 2. Area (ha) trends over time at black-tailed prairie dog (*Cynomys ludovicianus*) colonies A (a), B (b), C (c), and D (d) at Las Cienegas National Conservation Area, Pima County, Arizona. Asterisks indicate years in which managers mowed colonies in August or September.

General characteristics and dispersal

We captured and marked 564 individual BTPDs. At colonies A and B (continuously occupied throughout our study), the mean sex ratio (females/males, \pm SE) of trapped adult and

yearling individuals was 1.5 ± 0.2 and 1.6 ± 0.2 , respectively. Adult mass differed between sexes, with males being heavier (1038 ± 11.1 g) than females (916 ± 9.1 g; Student's *t*-test, $P < 0.001$). Adult mass did not differ between colonies A and B (Student's *t*-test, $P = 0.923$). Sex and colony had no interactive effect ($F_{1,824} = 0.18$, $P = 0.667$) on mass. Mean (\pm SE) population density was 16.4 ± 3.3 BTPD/ha at colony A, and 16.8 ± 3.2 BTPD/ha at colony B. We documented three mortality events through observation, and six intercolony dispersal events through trapping. Mortality events consisted of two depredations by coyote (*Canis latrans*), and one depredation by red-tailed hawk (*Buteo jamaicensis*). Of six emigrants, three were yearling males (mass = 1073 ± 122.5 g), one was a male of unknown age (adult or yearling; mass = 1350 g), and two were adult females (mass = 785 ± 5.0 g). Male emigrants were 1% above average mass, and female emigrants were 15% below average mass of individuals at our study colonies. Mean distance traveled during dispersal was 3.0 ± 0.8 km, with males traveling 2.4 ± 1.1 km and females traveling 4.2 ± 0.0 km. No statistical difference was detected between sexes (Student's *t*-test, $P = 0.191$).

Population trends

Our intensive monitoring, high detectability ($92.4 \pm 1.9\%$; mean \pm SE across colonies A and B), and ability to trap and mark the majority of each population ($95.0 \pm 4.3\%$ [mean \pm SE; calculated as (individuals marked/number known alive) * 100] at colony A, $89.8 \pm 4.8\%$ at colony B, and 100% at colony C [all individuals were marked prior to being moved to the colony]) allowed us to accurately determine population size at each colony. Colonies A and B experienced positive growth in 2011-2015 (Colony A: slope = 0.01 individuals/Julian Day, $R^2 = 0.47$, $P < 0.001$; Colony B: slope = 0.02 individuals/Julian Day, $R^2 = 0.41$, $P = 0.001$), while colony C experienced negative growth (slope = -0.03 individuals/Julian Day, $R^2 = 0.86$, $P =$

0.023) as it was established and extirpated within one year ($\lambda = 0$; Table 3). Intra-annual patterns were observed in populations at all colonies. Populations at colonies A and B followed similar intra-annual trends, with the lowest and highest population counts per season immediately prior to (with the exception of colony B in 2013) and following juvenile emergence (Figure 3a and 3b). The population at colony C declined following establishment, had a slight increase following juvenile emergence, then declined to zero (Figure 3c).

Table 3. Annual and overall growth rates (λ) for colonies A, B, and C between 2011 and 2015 at black-tailed prairie dog (*Cynomys ludovicianus*) reintroduction sites at Las Cienegas National Conservation Area, Pima County, Arizona. $\lambda > 1$ indicates an increase, $0 < \lambda < 1$ indicates a decrease, $\lambda = 1$ indicates stability, and $\lambda = 0$ indicates extirpation.

Time Period	Growth Rate (λ)		
	Colony A	Colony B	Colony C
2011-2012*	1.73	0.76	n/a ⁺
2012-2013	2.68 ⁺⁺	2.79	0.23
2013-2014	0.67	0.89	0
2014-2015	1.47	1.70	n/a
2011-2015	4.55	2.11	0

*Colony A and B were augmented in 2011 with 30 and 20 BTPD, respectively

⁺Colony C was not in existence until September 2012, and was extirpated by the end of 2013

⁺⁺Excludes the 10 adults/yearlings captured in 2012 and transferred to Colony C

Figure 3. Population trends at black tailed prairie dog (*Cynomys ludovicianus*) colonies A (a), B (b), and C (c) between 2011 and 2015 at Las Cienegas National Conservation Area, Pima County, Arizona. Dotted lines indicate the linear regression trends for each population.

Reproduction and juvenile survival

Between 2011 and 2015, 121 juveniles emerged aboveground at colony A, and 342 juveniles emerged at colony B (Table 4). Mean litter size (calculated as number of juveniles per lactating female; excluding colony A in 2011) was 2.9 ± 0.6 . Juvenile survival on colonies A

and B was not affected by year ($F_{1,5} = 1.13$, $P = 0.348$) or colony (Student's t -test, $P = 0.793$), but was negatively correlated with the proportion of mothers that were yearlings (slope = -0.63, $R^2 = 0.68$, $P = 0.043$; Figure 4). Lactation occurred in $79.0 \pm 0.1\%$ of yearlings observed over the 2012-2015 period.

Table 4. Total juvenile black-tailed prairie dogs (*Cynomys ludovicianus*) emerging and litter sizes (juveniles/lactating female) at Las Cienegas National Conservation Area, Pima County, Arizona between 2011 and 2015.

Colony	Year	Total Juveniles	Juveniles/Female	Juveniles/Lactating Female
A	2011	3	0.33	3*
A	2012	47	4.27	4.27
A	2013	21	0.75	1.40
A	2014	50	2.63	2.94
B	2011	1	0.04	0.06
B	2012	70	3.50	5.00
B	2013	47	1.34	1.57
B	2014	75	3.13	3.95
B	2015	149	3.55	3.82

*lactating females were not counted in 2011 at colony A, but at minimum, one adult lactated.

Figure 4. Juvenile black-tailed prairie dog (*Cynomys ludovicianus*) survival vs the proportion of mothers that were yearlings at Las Cienegas National Conservation Area, Pima County, Arizona. Dotted lines represent 95% confidence intervals.

Discussion

Colony area

Colony area increased between 2011 and 2016 at colonies A, B, and D, whereas colony area decreased to zero for colony C, which was occupied for only 1 year. Mowing likely was not the only factor that contributed to overall colony expansion, because colony area decreased at the 3 occupied colonies after mowing (2012-2013 at colony A and D, 2011-2013 at colony B). After 2013 mowing at colony D, BTPD maintained short vegetation in the mowed area throughout

2014, and expanded the colony boundary by 2016. Most notably, colony B nearly tripled in area between 2013 and 2016, which coincided with the removal of adjacent woody plants and debris by managers and wood collectors. Furthermore, colony D was bordered on the south and west sides with mature stands of mesquite, and on the north side by a 5 m hill. Most expansion of colony D occurred to the east, into areas where woody plants were less dense (personal observation). Vegetation [40] and artificial visual barriers [41] have not inhibited prairie dog colony expansion in certain circumstances; however, patterns observed at colonies B and D suggest that mature woody vegetation (shrubs > approximately 3 m in height) inhibits colony expansion, confirming an influence of certain types of visual barriers on colony expansion [42, 43, 44]. The addition or removal of visual barriers could therefore act as an effective, non-lethal, management tool to regulate or direct colony expansion. When colony reestablishment is a management goal, the removal of visual barriers (e.g. mature stands of woody plants) could facilitate colony growth, and also allow managers to control the direction into which a colony expands [42]. Furthermore, the addition of visual barriers (e.g. planting shrubs or trees) could prevent colony expansion onto areas where land owners/managers may be opposed to prairie dogs.

General characteristics and dispersal

Population demographics at our study colonies were typical of BTPD (see [15, 45, 46]). The adult sex ratio was female biased, male-biased sexual dimorphism in body size was present, and population density was within the known range (8-68 individuals/ha; [45]). Average litter size (emergent juveniles/litter) was close to the mean for BTPDs reintroduced to another xeric region (3.3 juveniles/litter [47]) as well as the mean in more mesic regions (3.1 juveniles/litter; [46]). Dispersal distance was above average (2.4 km [48]), but below the maximum (6.0 km

[46]) reported in the literature. Relative to all BTPD at Las Cienegas Conservation Area, dispersing males were slightly (1%) above average body mass, but dispersing females were 15% below average mass. Female BTPDs are philopatric, meaning they tend to remain in their natal coterie (family group) throughout life; thus, to avoid inbreeding, males disperse either within (intracolony) or between (intercolony) colonies [15]. Females also disperse, but reasons vary from eviction to disappearance of close kin [15, 49]. Female dispersal/emigration often indicates poor conditions, such as reduced food availability, and might explain why female dispersers were below average weight at our study site.

Population trends

Population size at two reintroduced BTPD colonies (colonies A and B) at Las Cienegas National Conservation Area expanded between 2011 and 2015. Populations showed natural fluctuations each season, but generally grew in a linear fashion. We speculate that intensive *in situ* monitoring, and close collaboration between researchers and managers contributed partially to the success of colonies A and B. For example, in 2011, two important management practices, supplemental feeding and population augmentation, were implemented based on researcher observations. As in most years of our study, 2011 was characterized by below-average precipitation and above-average temperatures. We noticed that BTPD foraged far (~ 10 m or more) from burrows, which ostensibly left them vulnerable to predators. The population decreased from 40 individuals to 25 within one month, so we speculated that the combination of drought and predation was driving this decline. We communicated our observations to AZGFD personnel, and supplemental feeding was initiated to potentially reduce predation risk by keeping BTPD closer to burrows. Additionally, BTPD colonies were augmented with individuals from established colonies in New Mexico and Mexico (colony A's population had declined to 10

individuals prior to augmentation); colony A was augmented with 30 individuals and colony B was augmented with 20 individuals in 2011. Supplemental feeding appeared to stabilize populations, and this practice was continued in all years of our study (beginning during breeding season).

In contrast to colonies A and B, colony C was uninhabited within a single year after its reestablishment. Managers suggest releasing 60-100 prairie dogs onto new colony sites [28], but only 30 individuals were moved from colony B to colony C. Furthermore, 20 of the 30 individuals released at colony C were juveniles, which typically have lower survival rates, are less likely to reproduce, rarely excavate new burrows [28], and are more likely to disperse [15]. The combination of the small number and inexperience of individuals released at colony C likely led to extirpation of the nascent population.

Reproduction and juvenile survival

Reproduction at colonies A and B followed similar patterns, with extremely low reproduction in 2011 (three juveniles emerged at colony A, one at colony B), followed by greater reproduction in subsequent years. Supplemental feeding beginning in the breeding seasons of 2012-2015 likely contributed to greater reproduction at each colony. Supplemental feeding also likely contributed to increased reproduction by yearling females. Typically, only 35% of yearling female BTPDs copulate, and only 43% of those that do copulate will wean litters [50]. In contrast, most yearlings at our study colonies reproduced (approximately 80%). Among yearlings, heavier individuals are more likely to reproduce [15], so the high rate of yearling reproduction was likely attributed to supplemental feeding. Although yearlings at our study colonies reproduced more often than would be expected based on literature reports, juvenile survival was low ($l_x = 0.2 \pm 0.1$) in years with a high proportion of yearling mothers. In many

mammals, maternal experience increases the chance of successfully weaning offspring [51], so inexperience could explain the low juvenile survival rates observed at our colony sites where most mothers were yearlings.

Conclusions and management implications

The reintroduction of BTPDs to Arizona at Las Cienegas National Conservation Area appears to have been successful in three of four cases. Population sizes at two of the three intensively studied colonies have increased over time, and three of four colonies have increased in area. Population augmentation was necessary in 2011 at two colonies, and with the implementation of supplemental feeding, populations increased in subsequent years.

Alternatively, Gunnison's prairie dogs (*Cynomys gunnisoni*) reintroduced to the southern periphery of their range were not provided supplemental food regularly, and populations required augmentation in four subsequent years [29]. Supplemental feeding may be especially effective after translocation in drought prone areas because it may reduce predation, enhance reproduction, and prevent or mitigate population crashes [47]. While supplemental feeding appears to be a viable short-term option to provide stable food resources, management of surrounding vegetation is necessary for establishment of natural food resources, and long-term success [28].

Management of vegetation is also necessary to provide habitat for BTPD. BTPDs are less likely to colonize areas in which vegetation obstructs their vision [43], and mature stands of woody plants appear to have hindered BTPD colony expansion at our study site; thus, thinning of vegetation surrounding colonies may facilitate colony growth. Alternatively, the targeted removal or addition of woody stands and other visual barriers could function as a non-lethal management tool to control direction and extent of colony expansion.

Our study highlights the importance of multi-year intensive monitoring for successful reestablishment of BTPD populations. Furthermore, we demonstrate the necessity of *in situ* collaboration between researchers and agency personnel to better detect and address potential problems. Our findings provide valuable information that may be applied to future reestablishment efforts, especially in arid regions and on range peripheries where reestablishment may be difficult [29].

Acknowledgments

Thank you to Holly Hicks, Karen Simms, Tim Snow, Ian Tomlinson, and Bill Van Pelt for their tireless efforts to reintroduce the black-tailed prairie dog to Arizona. Thank you to Annise Degonzague, Alejandro Grajal-Puche Allyson Hawkins, Levi Heffelfinger, Raquel Irigoyen-Au, Chad Ketcham, Darin Kopp, Ariana La Porte, Jorge Marin, Luis Martinez, Maxwell Mazzella, Rachel More-Hla, Rocio Ojeda, James Pitts, Brianna Rico, Allison Rosengren, Kaitlyn Ruggirello, Stephanie Snedecker, and Clarissa Teixeira for assistance with data collection and entry. Thank you to Melissa Merrick for creating maps. Work was funded by the National Fish and Wildlife Foundation, the Bureau of Land Management, T & E, Inc, and Arizona Center for Nature Conservation.

References

- [1] Wilson EO. Biodiversity. Washington DC: National Academy Press; 1988.
- [2] Millennium Ecosystem Assessment. Ecosystems and human well-being: synthesis.
Washington, DC: Island Press; 2005.
- [3] Robertson BA, Rehage JS, Sih A. Ecological novelty and the emergence of evolutionary traps. *Trends Ecol Evol.* 2013;28: 552-560.
- [4] Salmon M. Protecting sea turtles from artificial night lighting at Florida's oceanic beaches.
In: Rich C, Longcore T, editors. Ecological consequences of artificial night lighting.
Washington DC: Island Press; 2013. pp. 141-168.
- [5] Chen HL, Koprowski JL. Animal occurrence and space use change in the landscape of anthropogenic noise. *Biol Conserv.* 2015;192: 315-322.
- [6] Shannon G, McKenna MF, Angeloni LM, Crooks KR, Fristrup KM, Brown E, et al. A synthesis of two decades of research documenting the effects of noise on wildlife. *Biol Rev.* 2015;91: 982-1005.
- [7] Treves A, Wallace RB, White S. Participatory planning of interventions to mitigate human-wildlife conflicts. *Conserv Biol.* 2009;23: 1577-1587.
- [8] Delibes-Mateos M, Smith AT, Slobodchikoff CN, Swenson JE. The paradox of keystone species persecuted as pests: a call for the conservation of abundant small mammals in their native range. *Biol Conserv.* 2011;144: 1335-1346.
- [9] Kulbhushansingh RS, Bhatnagar YV, Redpath S, Mishra C. People, predators and perceptions: patterns of livestock depredation by snow leopards and wolves. *J Appl Ecol.* 2013;50: 550-560.
- [10] Hall RE. The mammals of North America, Vol 1. 2nd ed. Caldwell: Blackburn Press; 2001.

- [11] Summers CA, Linder RL. Food habits of the black-tailed prairie dog in western South Dakota. *J Range Manage.* 1978;31: 134-136.
- [12] Whicker AD, Detling JK. Ecological consequences of prairie dog disturbances. *BioScience.* 1988;38: 778-785.
- [13] Miller B, Ceballos G, Reading R. The prairie dog and biotic diversity. *Conserv Biology.* 1994;8: 677-681.
- [14] Luce RJ, Manes R, Van Pelt B. A multi-state plan to conserve prairie dogs. In: Hoogland JL, editor. *Conservation of the black-tailed prairie dog: saving North America's western grasslands.* Washington, DC: Island Press; 2006. pp. 210-217.
- [15] Hoogland JL. *The black-tailed prairie dog. Social life of a burrowing mammal.* Chicago: University of Chicago Press; 1995.
- [16] Kotliar NB, Baker BW, Whicker AD, Plumb G. A critical review of assumptions about the prairie dog as a keystone species. *Environ Manage.* 1999;24: 177-192.
- [17] Kotliar NB, Miller BJ, Reading RP, Clark TW. The prairie dog as a keystone species. In: Hoogland JL, editor. *Conservation of the black-tailed prairie dog: saving North America's western grasslands.* Washington, DC: Island Press; 2006. pp. 53-64
- [18] Mills LS, Soulé ME, Doak DF. The keystone-species concept in ecology and conservation. *Bioscience.* 1993;43: 219-224.
- [19] Estes JA, Palmisano JF. Sea otters: their role in structuring nearshore communities. *Science.* 1974;185: 1058-1060.

- [20] Reisewitz SE, Estes JA, Simenstad CA. Indirect food web interactions: sea otters and kelp forest fishes in the Aleutian archipelago. *Oecologia*. 2006;146: 623-631.
- [21] Underwood JG, Van Pelt WE. A proposal to reestablish the black-tailed prairie dog (*Cynomys ludovicianus*) to southern Arizona. Nongame and Endangered Wildlife Program Draft Technical Report. Phoenix: Arizona Game and Fish Department; 2000.
- [22] Archer SR, Garrett MG, Detling JK. Rates of vegetation change associated with prairie dog (*Cynomys ludovicianus*) grazing in North American mixed-grass prairie. *Vegetatio*. 1987;72: 159-166.
- [23] Weltzin JF, Archer SR, Heitschmidt RK. Small-mammal regulation of vegetation structure in a temperate savanna. *Ecology*. 1997;78: 751-763.
- [24] IUCN/SSC. Guidelines for reintroductions and other conservation translocations. Gland, Switzerland: IUCN Species Survival Commission; 2013.
- [25] Griffith B, Scott JM, Carpenter JW, Reed C. Translocation as a species conservation tool: status and strategy. *Science*. 1989;245: 477-480.
- [26] Truett JC, Dullum JALD, Matchett MR, Owens E, Seery D. Translocating prairie dogs: a review. *Wild Soc Bull*. 2001;29: 863-872.
- [27] Shier DM. Social and ecological influences on the survival skills of black-tailed prairie dogs: a role for behavior in conservation. Ph.D. dissertation, University of California at Davis. 2004. Available from: http://tesf.org/wordpress/wp-content/uploads/2014/01/small_shier_2004.pdf

- [28] Long D, Bly-Honness K, Truett JC, Seery DB. Establishment of new prairie dog colonies by translocation. In: Hoogland JL, editor. Conservation of the black-tailed prairie dog: saving North America's western grasslands. Washington, DC: Island Press; 2006. pp. 188-209.
- [29] Davidson AD, Friggens MT, Shoemaker KT, Hayes CL, Erz J, Duran R. Population dynamics of reintroduced Gunnison's prairie dogs in the southern portion of their range. *J Wildl Manage.* 2014;78: 429-439.
- [30] Hoogland JL. Why do Gunnison's prairie dogs give anti-predator calls? *Anim Behav.* 1996;51: 871-880.
- [31] Hoogland JL, Hale SL, Kirk AD, Sui YD. Individual variation in vigilance among white-tailed prairie dogs (*Cynomys leucurus*). *Southwest Nat.* 2013;58: 279-285.
- [32] Gori D, Schussman H. State of the Las Cienegas National Conservation Area. Part I. Condition and trend of the desert grassland and watershed. Prepared by The Nature Conservancy of Arizona; 2005. 63 pp.
- [33] Bodner GS, Simms K. State of the Las Cienegas National Conservation Area. Part 3. Condition and trend of riparian target species, vegetation, and channel geomorphology. Prepared by The Nature Conservancy of Arizona; 2008. 69pp.
- [34] Bodner GS, Robles MD. Enduring a decade of drought: patterns and drivers of vegetation change in a semi-arid grassland. *J Arid Environ.* 2017;136:1-14.
- [35] Hale SL, Koprowski JL, Hicks H. Review of black-tailed prairie dog reintroduction strategies and site selection: Arizona reintroduction. In: Gottfried GJ, Folliott PF, Gebow BS, Eskew LG, Collins LC, editors. Proceedings--Merging science and management in a rapidly changing world: biodiversity and management of the Madrean Archipelago III, 1-5

May 2012, Tucson, Arizona, USA. U.S. Department of Agriculture, Fort Collins, Colorado, USA. RMRS-P-67. 2013; pp. 310-315.

- [36] Biggins DE, Sidle JG, Seery DB, Ernst AE. Estimating the abundance of prairie dogs. In: Hoogland JL, editor. Conservation of the black-tailed prairie dog: saving North America's western grasslands. Washington, DC: Island Press; 2006. pp. 94-107.
- [37] Koprowski JL. Handling tree squirrels with a safe and efficient restraint. Wild Soc Bull. 2002;30: 101-103.
- [38] Sikes RS, the Animal Care and Use Committee of the American Society of Mammalogists. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J Mammal. 2016;97: 663-688.
- [39] Altmann J. Observational study of behavior: sampling methods. Behaviour. 1974;49: 227-267.
- [40] Eddy Z. Efficacy of native grassland barriers at limiting prairie dog dispersal in Logan county, Kansas. M.A. Thesis, Kansas State University. 2011. Available from: <http://krex.k-state.edu/dspace/handle/2097/12055>
- [41] Merriman JW, Zwank PJ, Boal CW, Bashore TL. Efficacy of visual barriers in reducing black-tailed prairie dog colony expansion. Wild Soc Bull. 2004;32: 1316-1320.
- [42] Franklin WL, Garrett MG. Nonlethal control of prairie dog colony expansion with visual barriers. Wild Soc Bull. 1989;17: 426-430.

- [43] Terrall DF. Use of natural vegetative barriers to limit black-tailed prairie dog town expansion in western South Dakota. M.Sc. Thesis, South Dakota State University. 2006. Available from: <http://openprairie.sdstate.edu/etd/575/>
- [44] Gray MB. Evaluation of barriers to black-tailed prairie dog (*Cynomys ludovicianus*) colony expansion, Bad River Ranches, South Dakota. M.Sc. Thesis, South Dakota State University. 2009. Available from: <http://openprairie.sdstate.edu/etd/441/>
- [45] Hoogland JL. Social behavior of prairie dogs. In: Hoogland JL, editor. Conservation of the black-tailed prairie dog: saving North America's western grasslands. Washington, DC: Island Press; 2006. pp. 7-26.
- [46] Hoogland JL. Demography and population dynamics of prairie dogs. In: Hoogland JL, editor. Conservation of the black-tailed prairie dog: saving North America's western grasslands. Washington, DC: Island Press; 2006. pp. 27-52.
- [47] Facka AN, Roemer GW, Mathis VL, Kam M, Geffen E. Drought leads to collapse of black-tailed prairie dog populations reintroduced to the Chihuahuan desert. J Wildl Manage. 2010;74: 1752-1762.
- [48] Garrett MG, Franklin WL. Behavioral ecology of dispersal in the black-tailed prairie dog. J Mammal. 1988;69: 236-250.
- [49] Hoogland JL. Prairie dogs disperse when all close kin have disappeared. Science. 2013;339: 1205-1207.
- [50] Hoogland JL. Black-tailed, Gunnison's, and Utah prairie dogs reproduce slowly. J Mammal. 2001;82: 917-927.

[51] Erlandsson R, Meijer T, Wagenius S, Angerbjörn A. Indirect effects of prey fluctuation on survival of juvenile arctic fox (*Vulpes lagopus*): a matter of maternal experience and litter attendance. Can J Zool. 2017;95:239-246.

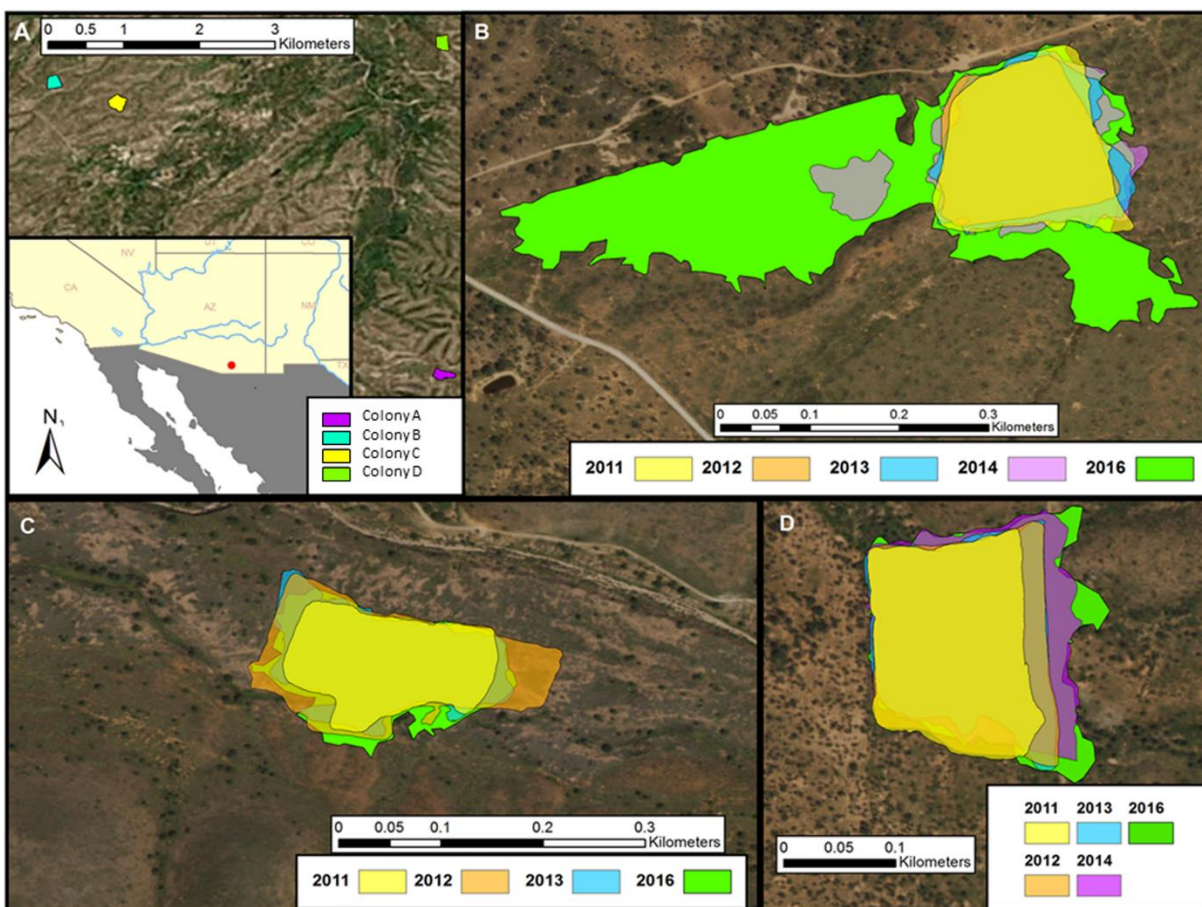


Figure 1

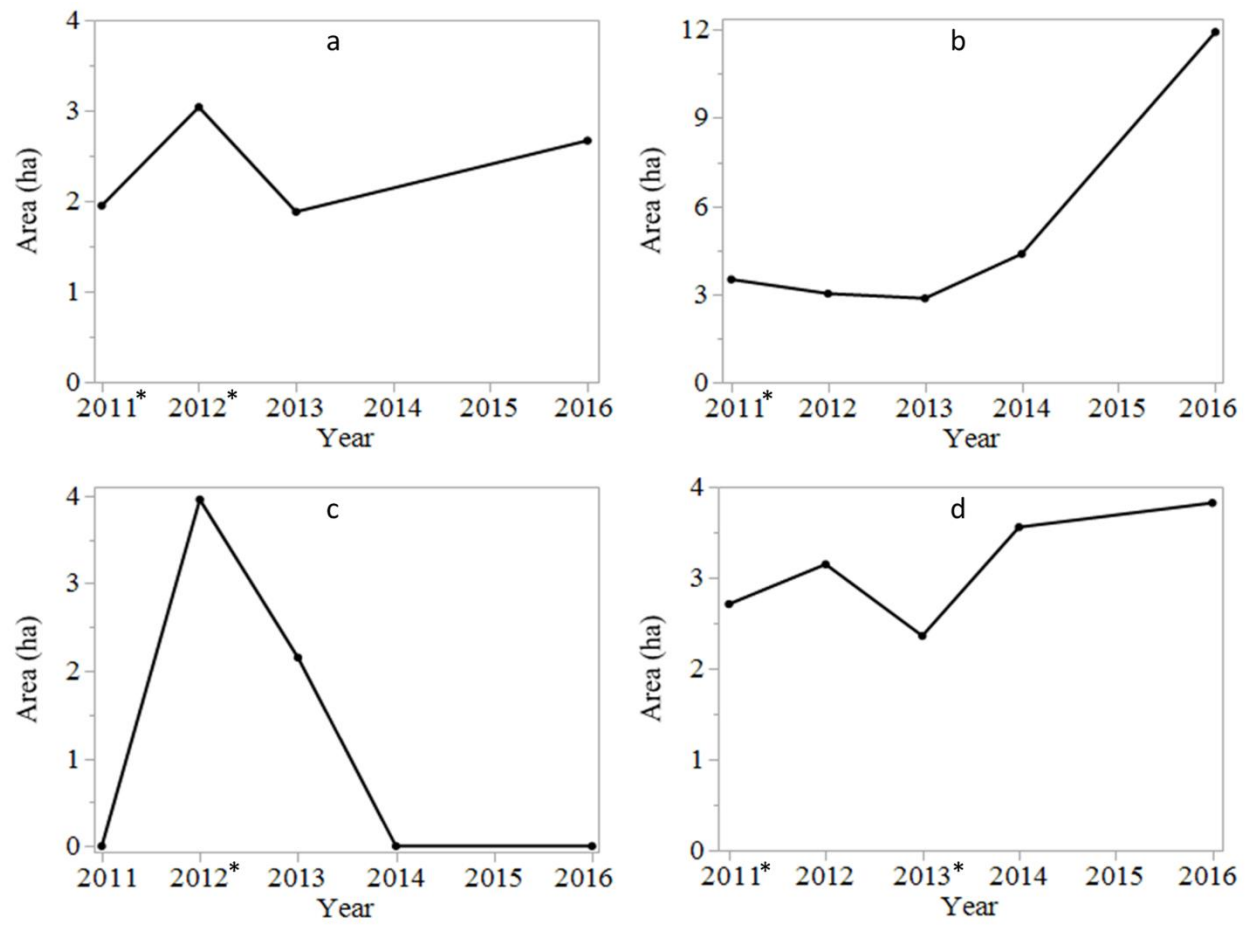
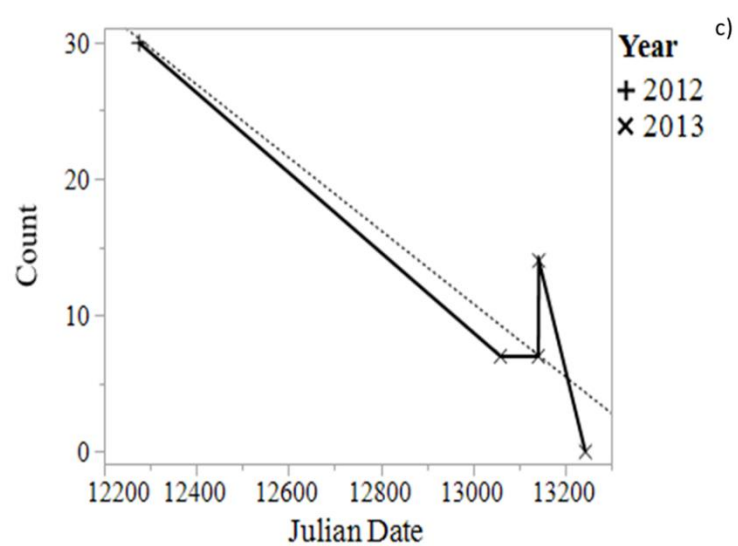
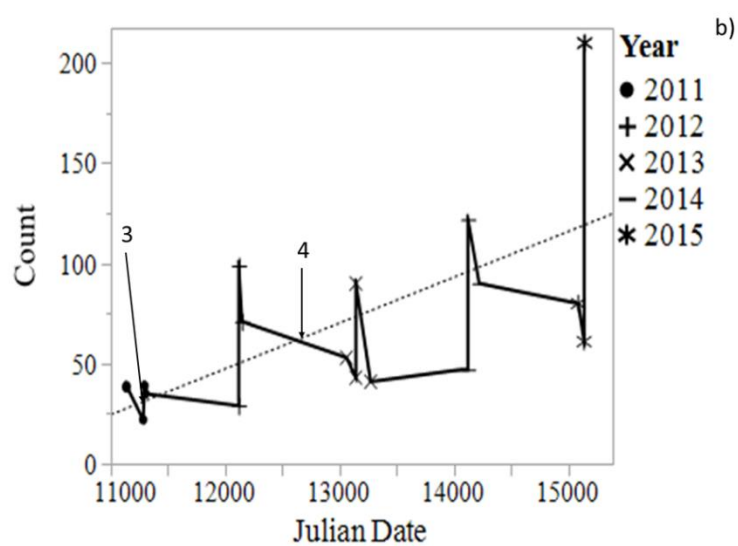
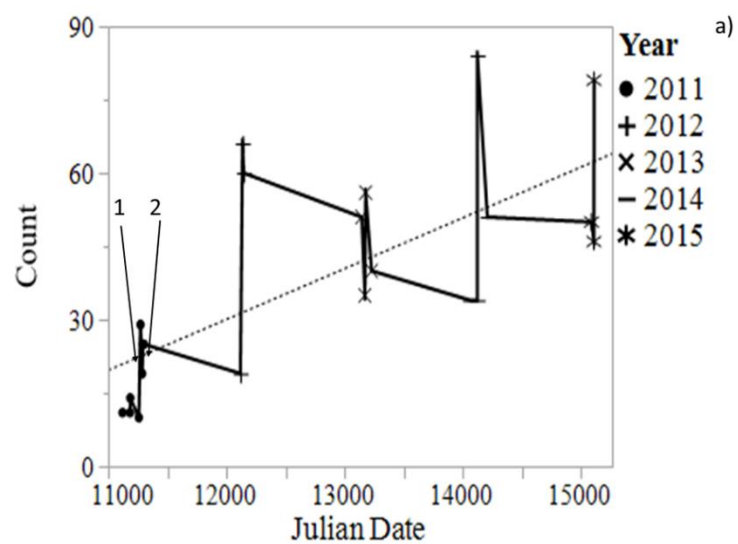


Figure 2



¹Population was augmented with 20 additional prairie dogs on 25 September 2011. The point prior to this indicates the population size prior to augmentation, and the point following indicates the population size following augmentation.

²Population was augmented with 10 additional prairie dogs on 15 October 2011. The point prior to this indicates the population size prior to augmentation, and the point following indicates the population size following augmentation.

³Population was augmented with 20 additional prairie dogs on 15 October 2011. The point prior to this indicates the population size prior to augmentation, and the point following indicates the population size following augmentation.

⁴30 prairie dogs were taken from this colony to start colony C on 24 September 2013

Figure 3.

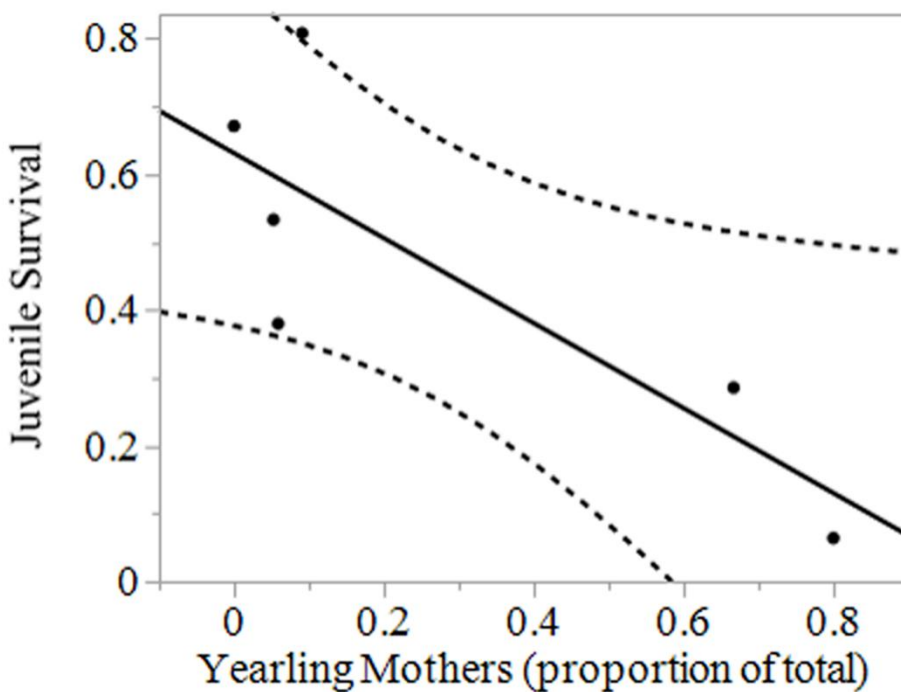


Figure 4.

APPENDIX C: RETURN OF A KEYSTONE SPECIES DOES NOT IMMEDIATELY
AFFECT DIVERSITY OF SMALL MAMMALS

Sarah L. Hale and John L. Koprowski

(In the format of *PLoS ONE*)

Return of a keystone species does not immediately affect diversity of small mammals.

Short Title: Keystone species do not immediately affect diversity

Sarah L. Hale^{1*} and John L. Koprowski¹

¹School of Natural Resources and the Environment, University of Arizona, Tucson, AZ, United States of America

*Corresponding author

E-mail: shale@email.arizona.edu (SLH)

Abstract

Biodiversity can be important for ecosystem functioning. Anthropogenic factors have led to a rapid decline in biodiversity, which can have amplified effects if keystone species are eliminated. Keystone species are important to maintain the organization and diversity of their communities, and one well-documented keystone herbivore is the prairie dog (Cynomys spp.). Many scientists hypothesize that prairie dogs increase small mammal diversity on prairie dog colonies through their structural alterations, but most compare small mammal diversity on colonies to that of adjacent unoccupied areas, and do not take time-lags into account. We sought to quantify diversity of small mammals over three consecutive years on colonies, off of colonies, and on colony peripheries, and used recently reestablished prairie dog colonies of known ages as our model system. We found that small mammal diversity was lowest on colonies, and higher but comparable on colony peripheries and off of colonies. Results suggest that small mammal diversity on colonies < 6 y of age may be suppressed rather than enhanced, perhaps reflecting the small size of newly established colonies. Additionally, many studies of small mammal diversity on prairie dog colonies find conflicting evidence about where diversity is greatest, but time lags in response time may contribute to inconsistencies in the literature. Our study indicates that small mammal communities may require longer time periods and large areas of occupation by keystone species to respond to reintroductions.

Introduction

Biodiversity refers not only to the variety of life and associated genetic diversity but also to the variety of ecological roles within an ecosystem [1, 2]. Biodiversity is potentially important for ecosystem functioning, and also provides many beneficial ecosystem services to humans [2], such as air and water purification, carbon sequestration, and nutrient cycling [3]. Despite such benefits, anthropogenic factors have driven a rapid decline in biodiversity [1, 2], which can have cascading effects if critical ecosystem functions and services are lost. The loss of keystone species fulfilling these critical roles will have disruptive effects. Keystone species are important in maintaining organization and diversity of their communities, and play unique and disproportionately large roles that cannot be replicated by other species [4, 5, 6]. Originally, the keystone species concept referred to the top-down role of *Pisaster ochraceus* in maintaining biodiversity in an intertidal community [7, 8]. Subsequently, the keystone concept has expanded to include a broader suite of species and trophic levels [4], and myriad studies have sought to quantify keystone species' effects on biodiversity. For example, plateau pikas (*Ochotona curzoniae*) maintain biodiversity on the Tibetan plateau [9], European rabbits (*Oryctolagus cuniculus*) are associated with greater raptor species richness on the Iberian Peninsula [10], and beavers (*Castor fiber* and *Castor canadensis*) have a positive influence on biodiversity [11].

Greater biodiversity in grassland systems enhances primary productivity, ecosystem stability [12, 13], and ecosystem resilience [14], so the loss of species may decrease an ecosystem's ability to recover from disturbances. Keystone species are often cited as drivers of increased biodiversity [9, 10, 11]. A common model system used to investigate how keystone species affect grassland biodiversity are prairie dog (*Cynomys* spp.) colonies. Prairie dogs are keystone species in the grassland ecosystem, and often are associated with an increase in

biodiversity on and around colonies through increased diversity of plant species, provision of habitat and nesting sites for other species through burrowing and vegetation alteration, and acting as food for avian and terrestrial carnivores [15, 16]. Studies of how prairie dogs affect biodiversity have examined the diversity of several taxa, such as birds [17, 18, 19] and arthropods [20], but the most commonly used measures are small mammal abundance, richness, or diversity (Table 1).

Small mammals play a variety of roles in grassland ecosystems, provide important ecosystem functions such as seed predation and dispersal [21], and have the potential to substantially alter vegetative communities [22, 23]. Prairie dogs directly influence vegetative composition on their colonies through foraging activities, colony maintenance, and burrow excavation [24], but also have the potential to indirectly influence vegetation on and surrounding colonies via small mammal communities. Small mammal abundance, richness, and diversity as measures of biodiversity typically only compare areas occupied or unoccupied by prairie dogs, and occur on colonies of varying ages, so an understanding of initial responses and potential time lags of small mammal communities to ecosystem restoration (i.e., prairie dog reestablishment) is lacking. Responses of communities to ecological changes such as restoration often exhibit delays or lag times [25, 26, 27], so if responses of biodiversity to habitat alteration by prairie dogs is delayed, lag times may explain conflicting findings among studies.

For our study, we used newly reestablished black-tailed prairie dog (*Cynomys ludovicianus*) colonies as our model system to assess the initial influence of a keystone species on biodiversity. We hypothesized that recently reestablished prairie dog colonies would influence small mammal species diversity, richness, and abundance on and around colonies by creating previously absent ecotones. We included the periphery of prairie dog colonies in our

sampling design because peripheries (i.e. ecotones or edges) of vegetation types and structures are often associated with greater small mammal diversity [28]. Accordingly, we predicted that if prairie dogs were influencing biodiversity as one of their keystone roles, the greatest diversity of small mammals would occur on the periphery of colonies. We also predicted that small mammal diversity would increase with colony age, and certain guilds (specifically kangaroo rats [*Dipodomys* spp.] and grasshopper mice [*Onychomys* spp.; 29, 30, 31, 32]) of small mammals would be preferentially associated with colonies. We addressed our hypothesis by live-trapping small mammals on colonies, on colony peripheries, and off of colonies at three newly established prairie dog colonies over three years.

Table 1. Summary of studies that use small mammal abundance, richness, or diversity as an estimate of biodiversity on prairie dog (*Cynomys* spp.) colonies in the United States and Mexico.

Citation	Year	Sampling Design	Location	Duration*	Measure	Results
40	1982	On ^a vs Off ^b	OK, USA	2 years	Abundance	Greater on colonies
17	1986	On vs Off	SD, USA	2 years	Abundance	Greater on colonies
					Richness	Greater off colonies
49	1999	On vs Off	CH, MX	1 year	Diversity	Greater on colonies
31	1999	On vs Off ^c	NM, USA	1 year	Abundance	No difference
41	2004	On vs Off	OK, USA	3 years	Richness	No difference
50	2006	On vs Off	CO, USA	3 years	Diversity	Greater on colonies
51	2007	On vs Off vs Vacant ^d	AZ, USA	2 years	Abundance	No difference
					Richness	No difference
30	2007	On vs off vs Vacant	CO, USA	1 year	Abundance	No difference
					Richness	No difference
52	2009	On vs Off vs Poisoned ^e	TX, USA	2 years	Abundance	No difference
32	2010	On vs Off	TX, USA	2 years	Diversity	No difference 2002
						Greater off colonies 2003

*Duration of small mammal trapping portion of study

^aRefers to on prairie dog colonies

^bRefers to locations off of prairie dog colonies

^cBefore and after introduction of prairie dogs

^dRefers to vacant prairie dog colonies

^eRefers to poisoned, vacant, prairie dog colonies

Methods

Study Area

Las Cienegas National Conservation Area (hereafter Las Cienegas) is a working cattle ranch managed by the Bureau of Land Management. Las Cienegas is a 17,000 ha mosaic of five rare ecosystems of the southwestern United States (cottonwood [*Populus fremontii*]-willow [*Salix gooddingii*, *S. taxifolia*] riparian forest, marshland, mesquite [*Prosopis velutina*] bosque, sacaton [*Sporobolus wrightii*] floodplain, and semidesert grassland) [33, 34], and is located 72 km southeast of Tucson, AZ. Mean annual precipitation (MAP) is 405 mm, mostly falling during the summer monsoon months (July-September); mean annual temperature (MAT) is 15.7° C [35]. During our study (2012-2014) annual precipitation and temperature (\pm SE) averaged 260 \pm 57 mm and 16.4 \pm 0.18 ° C (Empire Remote Area Weather Station; QEMA3, <http://mesowest.utah.edu>). In all years of our study, temperature was above-average and precipitation was below-average.

Grasslands at Las Cienegas are located between 1300 and 1500m in elevation, and soils are primarily gravely, sandy, and clay loam [35]. Semidesert grasslands in Arizona historically supported BTPDs, which were extirpated by 1960 [36]. The Arizona Game and Fish Department began reintroducing BTPDs at Las Cienegas in 2008 from populations in New Mexico, USA and Sonora, Mexico.

Colonies were situated on sites with similar soils (fine, deep, well-drained), slopes (0-15%), and elevations (1367-1412 m). Site preparation by the Arizona Game and Fish Department consisted of shrub removal, mowing of tall grass, and installation of 25 artificial

burrows on a 4 ha plot [see 37 for details]. One colony was initiated per year starting in 2008 and ending in 2011. Throughout our study, three of the four colonies remained occupied by BTPD; however, one colony (hereafter referred to as the “control colony”) failed and was unoccupied during 2014 and 2015. This “control colony” received the same initial treatment as the other colonies, but lacked prairie dogs throughout the majority of our study period (fewer than 10 prairie dogs were present in May 2013 and the colony was vacant by October 2013).

We trapped small mammals on and surrounding three of the four reestablished BTPD colonies at Las Cienegas. Colonies will be referred to as colony A (established 2008), colony B (established 2010), and colony C (established 2012). Colonies A and B were continuously occupied by prairie dogs throughout our study, and colony C was initiated in 2013, but failed and was vacant by 2014. Colonies averaged 3.00 ± 0.65 (mean \pm SE) years in age, and 2.67 ± 0.23 ha in area.

Small Mammal Trapping

We used folding aluminum Sherman Live Traps (7.5 x 9 x 23 cm, H. B. Sherman Traps, Inc., Tallahassee, FL) set in a web design [38] to trap small mammals on and around each prairie dog colony. Webs consisted of one trap at the center of each colony (determined by locating the point equidistant from the north and south sides, and where it intersected the point equidistant from the east and west sides), and eight transects extending from the center for 500m in each cardinal and inter-cardinal direction. In 2012, traps were placed every 20m for the first 100m, and then every 100m thereafter on each transect for a total of 73 traps per web. In 2013 and 2014, transects were the same as in 2012, except we placed traps every 20m for the first 200m in order to better sample the colony periphery, for a total of 105 traps per web. We defined 3 colony zones: on colony (traps that were located on colonies, where vegetation was clipped by prairie

dogs), colony periphery (traps that were located in the 100m after tall, unclipped vegetation began), and off colony (traps that were located past the colony periphery zone out to 500m; Figure 1). Colony boundaries were delineated as the junction of short, prairie-dog grazed grass with tall, un-clipped grass where burrow entrances also became absent.

Fig 1. Example of web design for trapping small mammals on and surrounding black-tailed prairie dog (*Cynomys ludovicianus*) colonies, Pima County, Arizona. Only one transect shown. Idealized for a colony with a 100 m radius. Transects originated at the center of each colony, and extended for 500 m. Traps from 0-80 m are considered within the ‘on colony’ colony zone; those from 100-180 m are considered within the ‘colony periphery’ colony zone; those from 200-500 m were considered within the ‘off colony’ colony zone. Trapping stations used in all years are represented by *; additional stations introduced in 2013 are represented by X. Trapping stations included in each colony zone varied by colony and year, but zones are simplified in figure.

For each sampling session, we trapped for a maximum of four consecutive nights. At each colony, each year, we carried out three trapping sessions per season (once per month for three consecutive months within May - September; 12 nights per year). Traps were baited with whole oats and peanut butter at sunset, left open overnight, and checked at sunrise. We did not trap on nights of thunderstorms or during full moons when small mammal activity is known to decrease [39]. We identified small mammals and marked individuals with unique ear tags (Monel #1005-1, National Band and Tag Co., Newport, KY) or ear punches (2 mm, Roboz Surgical Instrument Co., Inc., Gaithersburg, MD) in cases when ears were too small for tags.

We trapped and processed small mammals under all relevant regulations (Arizona Game and Fish Department scientific collecting license # SP696903). Animal handling methods followed American Society of Mammalogists guidelines for the care and use of mammals [40] and were approved by the University of Arizona Institutional Animal Care and Use Committee (protocol 11-251). No threatened or endangered species were sampled.

Analysis

We used the catch-per-unit-effort method [41] to calculate relative abundance (number of individuals/100 trapping nights) of each species or genus of small mammals in each colony zone (on colony, colony periphery, off colony) per year at each colony. We then used relative abundance estimates to calculate Shannon-Wiener diversity indices and Pielou's evenness index. We used JMP®, Version 12 [42] for statistical analysis of data. For species diversity, richness, abundance, and evenness, we performed generalized linear models for a set of candidate models incorporating additive and interactive effects of year of study (hereafter referred to as "year"), colony zone, and colony, then performed analysis of variance (ANOVA) on the top model (the model with the lowest AICc value) for each estimate. For Shannon-Wiener species diversity, we performed a two-way ANOVA on our top model with colony zone and year as main effects. For species richness, we performed a three-way ANOVA with colony zone, year, and colony as main effects. A second model had similar AICc values, so we performed a two-way ANOVA with colony zone and year as our main effects. We averaged richness estimates between these two models, but P-values for Tukey's hsd tests are reported from our top model. For small mammal abundance, we performed a three-way analysis of variance of our top model with colony, colony zone, and year as our main effects. For evenness, our null model was the top model, so no ANOVA was performed.

Additionally, we used relative abundance estimates to determine the proportion of total captures that small mammal guilds (kangaroo rats, pocket mice [*Perognathus* spp., *Chaetodipus* spp.], deer mice [*Peromyscus* spp.], grasshopper mice, pygmy mice [*Baiomys taylorii*], cotton rats [*Sigmodon* spp.], white-throated woodrats [*Neotoma albigula*], and harvest mice [*Reithrodontomys* spp.]) represented per year in each colony zone. We then normalized data

with an arcsine transformation, and performed an ANOVA with year as the main effect, and proportion of total captures per colony zone as the dependent variable for each guild within each colony zone. We also performed an ANOVA with colony zone as the main effect, and relative abundance of small mammals as the dependent variable, and an ANOVA with guild as the main effect, and relative abundance as the dependent variable for each colony zone. Finally, for guilds with $n > 5$ individuals for each colony zone, we performed a one-way ANOVA with colony zone as the main effect and body mass (g) as the dependent variable.

Results

Species Diversity

Over 9823 trap nights, we captured a total of 14 species of small mammals, 1376 unique individuals, and had 1374 recaptures. For Shannon-Wiener species diversity, our top candidate model incorporated the additive effects of year and colony (Table 2). Colony zone (i.e. on colony, colony periphery, off colony; $F_{2,26} = 12.12$, $P < 0.001$) and year ($F_{2,26} = 15.74$, $P < 0.001$) influenced species diversity significantly. Regardless of year, Shannon-Wiener species diversity pooled across colonies (mean \pm SE) was lower on prairie dog colonies (1.51 ± 0.07) than on colony peripheries (1.82 ± 0.07 ; Tukey's hsd test, $P = 0.022$) or off of colonies (2.03 ± 0.07 ; Tukey's hsd test, $P < 0.001$), and was comparable at colony peripheries and off of colony locations (Tukey's hsd test, $P = 0.133$; Figure 2a). Regardless of colony zone, species diversity was lower in 2012 (1.45 ± 0.07) than in 2013 (2.00 ± 0.07 ; Tukey's hsd test, $P < 0.001$) or 2014 (1.91 ± 0.07 ; Tukey's hsd test, $P < 0.001$). Species diversity did not differ in 2013 or 2014 (Tukey's hsd test, $P = 0.676$).

Table 2. Candidate generalized linear models used to determine main effects on Shannon-Wiener species diversity of small mammals on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies.

Model for Species Diversity	AICc	Δ_i	w_i
Y = b_0 + Year + Colony Zone	6.27	0.00	0.87
Y = b_0 + Year + Colony + Colony Zone	10.63	4.36	0.10
Y = b_0 + Year + Colony Zone + Year* Colony Zone	12.92	6.65	0.03
Y = b_0 + Year	19.95	13.68	0.00
Y = b_0 + Colony Zone	23.87	17.60	0.00
Y = b_0 + Year + Colony	24.75	18.48	0.00
Y = b_0 (Null Model)	28.65	22.38	0.00
Y = b_0 + Colony Zone + Colony	28.89	22.62	0.00
Y = b_0 + Colony	33.04	26.77	0.00
Y = b_0 + Year + Colony + Year*Colony	39.34	33.07	0.00
Y = b_0 + Colony + Colony Zone + Colony* Colony Zone	45.70	39.43	0.00
Y = b_0 + Year + Colony + Colony Zone + Year*Colony* Colony Zone	62.70	56.43	0.00

Figure 2. Mean (\pm SE; pooled across colonies and sample dates) small mammal Shannon-Wiener species diversity (a), species richness (b), and abundance (c) on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies 2012-2014. Differing letters indicate significant differences ($\alpha = 0.05$).

Species Evenness and Richness

None of the variables explain the variance in evenness (how similar each species was in proportion) between years, colony zones, or colonies, as our top candidate model did not differ from the null model (Table 3). Our top candidate model for species richness incorporated additive effects of year, colony zone, and colony on species richness, but our second top model was close in AICc value ($\Delta_i < 2$), so estimates were averaged between the top 2 models (Table 4). In all years at all colonies, richness was greater off of colonies (11 ± 1) than on colony peripheries (9 ± 1 ; Tukey's hsd test, $P = 0.011$) or on colonies (6 ± 1 ; Tukey's hsd test, $P < 0.001$), and species richness was greater on colony peripheries than on colonies (Tukey's hsd

test, $P = 0.001$; Figure 2b). Species richness differed among colonies in all colony zones and years. Species richness at colony A (7 ± 1) was lower than richness at colony B (9 ± 1 , Tukey's hsd test, $P = 0.038$), but did not differ from richness at colony 2012 (9 ± 1). Species richness at colony 2012 also did not differ from richness at colony 2010 (Tukey's hsd test, $P = 0.655$). Pooled across all zones and colonies, species richness was higher in 2013 (10 ± 1) and 2014 (10 ± 1) than in 2012 (6 ± 1 ; Tukey's hsd test 2013-2012, $P < 0.001$; Tukey's hsd test 2014-2012, $P.001 < 0$). Species richness did not differ between 2013 and 2014 (Tukey's hsd test, $P = 0.989$).

Table 3. Candidate generalized linear models used to determine main effects on evenness of small mammals on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies.

Model for Evenness	AICc	Δ_i	w_i
$Y = b_0$ (Null Model)	-77.87	0.00	0.23
$Y = b_0 + \text{Year}$	-77.87	0.00	0.23
$Y = b_0 + \text{Year} + \text{Colony Zone}$	-77.72	0.15	0.21
$Y = b_0 + \text{Colony Zone}$	-76.79	1.08	0.13
$Y = b_0 + \text{Year} + \text{Colony}$	-75.53	2.34	0.07
$Y = b_0 + \text{Colony}$	-75.10	2.77	0.06
$Y = b_0 + \text{Year} + \text{Colony} + \text{Colony Zone}$	-73.94	3.93	0.03
$Y = b_0 + \text{Colony Zone} + \text{Colony}$	-73.42	4.45	0.03
$Y = b_0 + \text{Year} + \text{Colony Zone} + \text{Year*Colony Zone}$	-63.52	14.35	0.00
$Y = b_0 + \text{Year} + \text{Colony} + \text{Year*Colony}$	-59.31	18.56	0.00
$Y = b_0 + \text{Colony} + \text{Colony Zone} + \text{Colony*Colony Zone}$	-59.03	18.84	0.00
$Y = b_0 + \text{Year} + \text{Colony} + \text{Colony Zone} + \text{Year*Colony*Colony Zone}$	-40.19	37.68	0.00

Table 4. Candidate generalized linear models used to determine main effects on richness of small mammal species on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies.

Model for Species Richness	AICc	Δ_i	w_i
$Y = b_0 + \text{Year} + \text{Colony} + \text{Colony Zone}$	117.74	0.00	0.57
$Y = b_0 + \text{Year} + \text{Colony Zone}$	118.37	0.63	0.41
$Y = b_0 + \text{Year} + \text{Colony Zone} + \text{Year} * \text{Colony Zone}$	124.75	7.01	0.02
$Y = b_0 + \text{Colony Zone}$	139.28	21.54	0.00
$Y = b_0 + \text{Year}$	141.71	23.97	0.00
$Y = b_0 + \text{Colony Zone} + \text{Colony}$	142.89	25.15	0.00
$Y = b_0 + \text{Year} + \text{Colony}$	145.57	27.83	0.00
$Y = b_0$ (Null Model)	148.77	31.03	0.00
$Y = b_0 + \text{Colony}$	152.51	34.77	0.00
$Y = b_0 + \text{Colony} + \text{Colony Zone} + \text{Colony} * \text{Colony Zone}$	159.62	41.88	0.00
$Y = b_0 + \text{Year} + \text{Colony} + \text{Year} * \text{Colony}$	162.67	44.93	0.00
$Y = b_0 + \text{Year} + \text{Colony} + \text{Colony Zone} + \text{Year} * \text{Colony} * \text{Colony Zone}$	167.42	49.68	0.00

Small Mammal Abundance

Our top candidate model for small mammal relative abundance (individuals/100 trap nights) incorporated the additive effects of colony, year, and colony zone on relative abundance (Table 5). Year ($F_{2,26} = 20.52$, $P < 0.001$), colony ($F_{2,26} = 9.53$, $P = 0.001$), and colony zone ($F_{2,26} = 65.56$, $P < 0.001$) were each significant. Pooled across colony and colony zones, abundance was lower in 2012 (10.08 ± 1.25), than in 2013 (17.45 ± 1.25 ; Tukey's hsd test, $P = 0.001$) or 2014 (21.20 ± 1.25 ; Tukey's hsd test, $P < 0.001$), and abundance did not differ between 2013 and 2014 (Tukey's hsd test, $P = 0.110$). Regardless of year or colony zone, abundance was lower on one colony (12.12 ± 1.25) than the other two (16.84 ± 1.25 ; Tukey's hsd test, $P = 0.038$; 19.76 ± 1.25 ; Tukey's hsd test, $P < 0.001$), which did not differ (Tukey's hsd test, $P = 0.246$). Regardless of year or colony, abundance was lowest on prairie dog colonies (6.14 ± 1.25), at an intermediate level on colony peripheries (16.22 ± 1.25 ; Tukey's hsd test on colony-colony periphery, $P < 0.001$; Tukey's hsd test colony periphery-off colony, $P < 0.001$), and

greatest off of colonies (26.36 ± 2.86 ; Tukey's hsd test on colony-off colony, $P < 0.001$; Figure 2c).

Table 5. Candidate generalized linear models used to determine main effects on the relative abundance of small mammals on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies.

Model for Abundance	AICc	Δ_i	w_i
$Y = b_0 + \text{Year} + \text{Colony} + \text{Colony Zone}$	163.82	0.00	0.99
$Y = b_0 + \text{Year} + \text{Trap Zone}$	174.09	10.27	0.01
$Y = b_0 + \text{Colony Zone} + \text{Colony}$	186.15	22.33	0.00
$Y = b_0 + \text{Colony Zone}$	187.10	23.28	0.00
$Y = b_0 + \text{Year} + \text{Colony Zone} + \text{Year*Colony Zone}$	187.33	23.51	0.00
$Y = b_0 + \text{Colony} + \text{Colony Zone} + \text{Colony*Colony Zone}$	200.97	37.15	0.00
$Y = b_0 + \text{Year}$	207.45	43.63	0.00
$Y = b_0$ (Null Model)	207.97	44.15	0.00
$Y = b_0 + \text{Year} + \text{Colony}$	210.63	46.81	0.00
$Y = b_0 + \text{Colony}$	210.73	46.91	0.00
$Y = b_0 + \text{Year} + \text{Colony} + \text{Year*Colony}$	226.95	63.13	0.00
$Y = b_0 + \text{Year} + \text{Colony} + \text{Colony Zone} + \text{Year*Colony*Colony Zone}$	252.54	88.72	0.00

Guild Characteristics

Abundance of small mammals varied by guild in each colony zone (On Colony: $F_{7,71} = 6.24$, $P < 0.001$; Colony Periphery: $F_{8,72} = 4.94$, $P < 0.001$; Off Colony: $F_{7,71} = 3.76$, $P = 0.002$; Figure 3). Within the on colony zone, the relative abundance of kangaroo rats (2.95 ± 1.03) was greater than grasshopper mice (0.88 ± 0.14 ; Tukey's hsd test, $P = 0.014$), harvest mice (0.25 ± 0.12 ; Tukey's hsd test, $P < 0.001$), deer mice (0.74 ± 0.29 ; Tukey's hsd test, $P = 0.006$), pygmy mice (0.10 ± 0.10 ; Tukey's hsd test, $P < 0.001$), and woodrats (0.06 ± 0.04 ; Tukey's hsd test, $P < 0.001$).

0.001), but did not differ from pocket mice (1.62 ± 0.38 ; Tukey's hsd test, $P = 0.310$). Relative abundance of all guilds excluding kangaroo rats was statistically equivalent (Tukey's hsd test, $P > 0.111$ for all comparisons). Within the colony periphery zone, relative abundance of kangaroo rats (6.41 ± 1.88) was greater than pocket mice (2.20 ± 0.59 ; Tukey's hsd test, $P = 0.012$), harvest mice (1.90 ± 0.58 ; Tukey's hsd test, $P = 0.005$), deer mice (1.42 ± 0.38 ; Tukey's hsd test, $P = 0.001$), pygmy mice (1.34 ± 0.50 ; Tukey's hsd test, $P = 0.001$), cotton rats (0.74 ± 0.29 ; Tukey's hsd test, $P < 0.001$), and woodrats (0.43 ± 0.11 ; Tukey's hsd test, $P < 0.001$), but did not differ from grasshopper mice (2.89 ± 0.66 ; Tukey's hsd test, $P = 0.07$). Relative abundance of all guilds except for kangaroo rats did not differ (Tukey's hsd test, $P > 0.431$ for all comparisons). Within the off colony zone, the relative abundance of kangaroo rats (6.17 ± 2.23) was greater than pygmy mice (1.42 ± 0.41 ; Tukey's hsd test, $P = 0.016$), woodrats (1.39 ± 0.61 ; Tukey's hsd test, $P = 0.014$), and cotton rats (0.61 ± 0.22 ; Tukey's hsd test, $P = 0.002$), but did not differ from grasshopper mice (4.14 ± 0.70 ; Tukey's hsd test, $P = 0.793$), pocket mice (4.10 ± 0.27 ; Tukey's hsd test, $P = 0.776$), harvest mice (2.98 ± 0.85 ; Tukey's hsd test, $P = 0.264$), or deer mice (2.63 ± 0.52 ; Tukey's hsd test, $P = 0.156$). Relative abundance of all guilds except for kangaroo rats did not differ (Tukey's hsd test, $P > 0.160$ for all comparisons).

Figure 3. Small mammal guild abundance (as % of total abundance) on black-tailed prairie dog (*Cynomys ludovicianus*) colonies, on colony peripheries, and off of colonies, Pima County, Arizona. Larger size indicates greater abundance and smaller size indicates lesser abundance.

Differences in proportion of captures per colony zone were detected among years for two guilds of small mammals. Year affected the proportion of captures of harvest mice on colonies ($F_{2,8} = 6.37$, $P = 0.033$) on colony peripheries ($F_{2,8} = 9.98$, $P = 0.012$), and off of colonies ($F_{2,8} = 12.88$, $P = 0.007$), and white-throated woodrats on colony peripheries ($F_{2,8} = 28.42$, $P < 0.001$). On colonies, harvest mice made up a greater proportion of captures in 2014 (0.29 ± 0.06) than in

2012 (0.00 ± 0.00 ; Tukey's hsd test, $P = 0.031$), but did not differ from 2013 (0.08 ± 0.08 ; Tukey's hsd test, $P = 0.105$). The proportion of captures that were harvest mice also did not differ between 2012 and 2013 (Tukey's hsd test, $P = 0.613$). On colony peripheries, harvest mice made up a greater proportion of captures in 2013 (0.35 ± 0.10) and 2014 (0.44 ± 0.08) than in 2012 (0.00 ± 0.00 ; Tukey's hsd test 2012-2013, $P = 0.033$; Tukey's hsd test 2012-2014, $P = 0.013$), and did not differ between 2013 and 2014 (Tukey's hsd test, $P = 0.721$). The same pattern was observed off of colonies for harvest mice and on colony peripheries for woodrats. Off of colonies, harvest mice made up a greater proportion of captures in 2013 (0.45 ± 0.04) and 2014 (0.39 ± 0.08) than in 2012 (0.05 ± 0.05 ; Tukey's hsd test 2012-2013, $P = 0.008$; Tukey's hsd test 2012-2014, $P = 0.016$), and did not differ between 2013 and 2014 (Tukey's hsd test, $P = 0.807$). On colony peripheries, woodrats made up a greater proportion of captures in 2013 (0.20 ± 0.06) and 2014 (0.16 ± 0.02) than in 2012 (0.00 ± 0.00 ; Tukey's hsd test 2012-2013, $P < 0.001$; Tukey's hsd test 2012-2014, $P = 0.003$), and did not differ between 2013 and 2014 (Tukey's hsd test, $P = 0.334$).

Body mass varied by colony zone for kangaroo rats ($F_{2, 457} = 5.13$, $P = 0.006$) and grasshopper mice ($F_{2, 211} = 2.96$, $P = 0.05$). Kangaroo rats were heavier (g) on colonies (52.74 ± 2.59) than off of colonies (46.52 ± 0.73 ; Tukey's hsd test, $P = 0.004$), and were at an intermediate weight on colony peripheries (47.91 ± 1.18 ; Tukey's hsd test on colony-colony periphery, $P = 0.05$; Tukey's hsd test off colony-colony periphery, $P = 0.623$). Grasshopper mice were heavier off of colonies (26.18 ± 0.46) than on colonies (23.04 ± 1.65 ; Tukey's hsd test, $P = 0.044$), and were at an intermediate weight on colony peripheries (25.38 ± 0.74 ; Tukey's hsd test on colony-colony periphery, $P = 0.211$; Tukey's hsd test off colony-colony periphery, $P = 0.630$).

Discussion

Small mammal species diversity (Shannon-Wiener index) was lowest on prairie dog colonies, and did not differ between colony peripheries or off of colonies. Across time, species diversity increased initially and remained stable in the following years. Increases were likely due to natural fluctuations of small mammal populations and not the presence of prairie dogs because increases occurred across all colony zones. Species richness and relative abundance increased across colony zones (on colony, colony periphery, off colony), but evenness remained the same across time and colony zone. Lower diversity, richness, and abundance on colonies was expected due to lack of vegetative cover and associations of small mammal predators with prairie dog colonies (e.g. burrowing owls [*Athene cunicularia*] and rattlesnakes [*Crotalus* spp.]) [43], but a greater diversity, richness, and abundance was expected on colony peripheries. Edge environments typically foster greater diversity [44] due to the proximity of vegetation communities (e.g. forest next to field) and harbor species from each, so we anticipated that the peripheries of prairie dog colonies would act in a similar manner, but we did not find this during our study.

Several small mammal species (e.g. kangaroo rats and grasshopper mice) are known to be associated with prairie dog colonies [31, 32, 45, 46], because of the availability of burrows and reduced grass cover that facilitate foraging [29, 31, 32], but we did not find evidence of colony zone influence on guilds. However, at the individual level, colony zone influenced body mass for kangaroo rats and grasshopper mice. At our study site kangaroo rats were not associated with any specific colony zone, but individuals captured on prairie dog colonies were heavier than individuals captured off of colonies, indicating that even though it is potentially more risky to forage on prairie dog colonies [47], better quality or a higher abundance of resources may be

available to individuals that assume the risk. Conversely, grasshopper mice were lighter on prairie dog colonies than off of colonies, suggesting that resources at our study site were better quality or more abundant off of colonies than on colonies. Differences in body mass among individuals indicate that although prairie dog colonies were not influencing the measured small mammal community characteristics, the quality of individuals using colonies was affected, which may lead to differential survival and reproduction within guilds over time.

Results did not support our initial hypotheses that small mammal diversity would be greatest on colony peripheries, and that certain small mammal guilds would be associated with colonies; however, our results demonstrate interesting patterns that were not expected, such as lower abundance and richness on colony peripheries than off of colonies. Furthermore, we provided valuable baseline data for future studies as we measured small mammal diversity early on in the establishment of prairie dog colonies, and repeat studies will likely take place in 5-10 years at our study site. Lack of prairie dog influence on small mammals during the first one to six years following their reintroduction does not necessarily indicate prairie dogs are not a keystone species, because they have immediate impacts on other aspects of their ecosystem such as woody plant growth [48, Appendix D] and soil characteristics [49]. Prairie dog colonies at our study site were very young and small in area, so may not have been established long enough, or impacted a large enough area to influence small mammals ([32] considered 8.8 ha colonies to be small). Another study examining small mammal responses to a reintroduced population of prairie dogs found no influence in the year following reintroduction [31], so small mammal populations may not immediately respond to the reintroduction of prairie dogs. Furthermore, because prairie dog colonies at our study site were relatively small in area, small mammals may not have encountered them during normal foraging activities. For example, the home range size

of two of the smallest and two of the largest small mammals captured, *P. flavus*, *B. taylori*, *N. albigula*, and *D. spectabilis*, are 1100m² [50], 45-729m² [51], 161-486m² [52], and 412-717m² [53], respectively, so home ranges may not have included prairie dog colonies. In addition to colony size, colony age may have been too young for differences to be detected between colony peripheries and off colony areas. Lag times often exist following ecological changes [26, 27], so small mammal populations at our study site may not have had enough time to respond to the presence of prairie dogs during our study for differences to be detected; however, differences in the quality (i.e. body mass) of kangaroo rats and grasshopper mice on and off of colonies suggests that over time certain guilds may begin to have differential survival and reproduction across colony zones (on colony, colony periphery, off colony).

Biodiversity estimates on prairie dog colonies based on small mammal diversity, richness, or abundance as an estimate have been relatively inconsistent. Researchers have found greater small mammal abundance [45, 17] and diversity [54, 55] on prairie dog colonies, greater richness [17] and diversity [32] off of prairie dog colonies, and no difference in richness, abundance, or diversity on or off of prairie dog colonies [30, 31, 32, 46, 56, 57]. Discrepancies may be explained by lagged responses going undetected during the time period encompassed by studies (1.88 ± 0.23 years; mean \pm SE). If the response of small mammals to prairie dog addition or removal is delayed, researchers may not have detected differences between sampling areas due to lags in response time. For example, in two arid grasslands, small mammal abundance did not differ among prairie dog colonies, off colony areas, and areas where prairie dogs had been removed naturally (by plague) or by anthropogenic means (by poisoning); however, researchers only measured differences \geq two years post-plague and one to two years post-poisoning [56, 57], so small mammals may not have had time to respond to changes prior to study.

Time lags occur frequently after ecological disturbances or restoration, and can be observed in the responses of several different taxa in arid systems. Habitat restoration of a Chihuahuan desert grassland resulted in increased density of banner-tailed kangaroo rats (*Dipodomys spectabilis*), but responses required a decade to be detected [27]. Lizard community composition in the same system changed following habitat restoration, but also exhibited a delayed response [26]. Additionally, cattle exclusion from a desertified shrubland resulted in perennial grass recovery, but recovery took over 40 years to be detected [25]. The regularity of time lags following ecological changes indicates that some biotic communities require longer response periods following ecosystem restoration, especially in arid regions.

Biodiversity is potentially necessary for ecosystem functioning, and provides ecosystem services beneficial to human health [2, 3], thus understanding how to preserve biodiversity is of paramount importance; however, researchers must be able to accurately compare diversity among ecosystems, which may be complicated if time lags are not considered. Keystone species play disproportionately large roles in their ecosystems, and their removal may result in cascading consequences to ecosystem function and health. For example, kangaroo rats demonstrably maintain Chihuahuan desert shrubland stability [58]. Following kangaroo rat removal, shrubland changed to desert grassland with increases in tall grasses and grassland associated rodents, and decreases in bare ground, resulting in fewer seed eating birds [58]. The removal of a keystone guild resulted in a cascade of changes to the ecosystem, including the conversion from one ecotype to another, highlighting the importance of keystone species in maintaining their ecosystems. The protection and conservation of keystone species will likely lead to an overall preservation of ecosystems and associated biodiversity, but similarly to responses

following habitat restoration [26, 27], time lags may occur in response to ecological changes imposed by keystone species.

Acknowledgments

Thank you to Annise Degonzague, Allyson Hawkins, Levi Heffelfinger, Raquel Irigoyen-Au, Jorge Marin, Maxwell Mazzella, Rachel More-Hla, Rocio Ojeda, James Pitts, Brianna Rico, Allison Rosengren, and Michael Taylor for assistance with small mammal trapping. Work was funded by the National Fish and Wildlife Foundation, the Bureau of Land Management, T & E, Inc, and Arizona Center for Nature Conservation.

References

- [1] Wilson DE, Nichols JD, Rudran R, Southwell C. Introduction. In: Wilson DE, Cole FR, Nichols JD, Rudran R, Foster MS, editors. Measuring and monitoring biological diversity: standard methods for mammals. Washington and London: Smithsonian Institution Press; 1996. pp. 1.
- [2] Hooper DU, Chapin FS III, Ewel JJ, Hector A, Inchausti P, Lavorel S, et al. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr.* 2005;75: 3-35.
- [3] Singh SP. Balancing the approaches of environmental conservation by considering ecosystem services as well as biodiversity. *Curr Sci.* 2002;82: 1331-1335.
- [4] Mills LS, Soulé ME, Doak DF. The keystone-species concept in ecology and conservation. *Bioscience.* 1993;43: 219-224.
- [5] Power ME, Tilman D, Estes JA, Menge BA, Bond WJ, Mills LS, et al. Challenges in the quest for keystones. *Bioscience.* 1996;46: 609-620.
- [6] Kotliar NB. Application of the new keystone-species concept to prairie dogs: how well does it work? *Conserv Biol.* 2000;14: 1715-1721.
- [7] Paine RT. Food web complexity and species diversity. *Am Nat.* 1966;100: 65-75.
- [8] Paine RT. A note on trophic complexity and community stability. *Am Nat.* 1969;103: 91-93.
- [9] Smith A, Foggin JM. The plateau pika (*Ochotona curzoniae*) is a keystone species for biodiversity on the Tibetan plateau. *Anim Conserv.* 1999;2: 235-240.
- [10] Delibes-Mateos M, Redpath SM, Angulo E, Ferreras P, Villafuerte R. Rabbits as a keystone species in southern Europe. *Biol Conserv.* 2007;137: 149-156.
- [11] Stringer AP, Gaywood MJ. The impacts of beavers *Castor* spp. on biodiversity and the ecological basis for their reintroduction to Scotland, UK. *Mamm Rev.* 2016;46: 270-283.

- [12] Tilman D, Wedin D, Knops J. Productivity and sustainability influence by biodiversity in grassland ecosystems. *Nature*. 1996;379: 718-720.
- [13] Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, et al. Plant diversity and productivity experiments in European grasslands. *Science*. 1999;286: 1123-1127.
- [14] Peterson G, Allen CR, Holling CS. Ecological resilience, biodiversity, and scale. *Ecosystems*. 1998;1: 6-18.
- [15] Miller B, Ceballos G, Reading R. The prairie dog and biotic diversity. *Conserv Biology*. 1994;8: 677-681.
- [16] Kotliar NB, Baker BW, Whicker AD. A critical review of assumptions about the prairie dog as a keystone species. *Environ Manage*. 1999;24: 177-192.
- [17] Agnew W, Uresk DW, Hansen RM. Flora and fauna associated with prairie dog colonies and adjacent ungrazed mixed grass prairie in western South Dakota. *J Range Manage*. 1986;39: 135-139.
- [18] Barko VA, Shaw JH, Leslie DM Jr. Birds associated with black-tailed prairie dog colonies in southern shortgrass prairie. *Southwest Nat*. 1999;44: 484-489.
- [19] Augustine DJ, Baker BW. Associations of grassland bird communities with black-tailed prairie dogs in the North American Great Plains. *Conserv Biology*. 2013;27: 324-334.
- [20] Duval BD, Whitford WG. Reintroduced prairie dog colonies change arthropod communities and enhance burrowing owl foraging resources. *Immediate Sci Ecol*. 2012;1: 12-23.
- [21] Williams PA, Karl BJ, Bannister P, Lee WG. Small mammals as potential seed dispersers in New Zealand. *Austral Ecol*. 2000;25: 523-532.

- [22] Batzli GO, Pitelka FA. Influence of meadow mouse populations on California grassland. Ecology. 1970;51: 1027-1039.
- [23] Howe HF, Brown JS. Effects of rodent granivory on experimental forb communities. Ecol Appl. 2000;10: 917-924.
- [24] Whicker AD, Detling JK. Ecological consequences of prairie dog disturbances. Bioscience. 1988;38: 778-785.
- [25] Valone TJ, Sauter P. Effects of long-term cattle exclosure on vegetation and rodents at a desertified arid grassland site. J Arid Environ. 2005;61: 161-170.
- [26] Cosentino BJ, Schooley RL, Bestelmeyer BT, Coffman JM. Response of lizard community structure to desert grassland restoration mediated by a keystone rodent. Biodivers Conserv. 2013;22: 921-935.
- [27] Cosentino BJ, Schooley RL, Bestelmeyer BT, Kelly JF, Coffman JM. Constraints and time lags for recovery of a keystone species (*Dipodomys spectabilis*) after landscape restoration. Landsc Ecol. 2014;29: 665-675.
- [28] Lidicker WZ Jr. Responses of mammals to habitat edges: an overview. Landsc Ecol. 1999;14: 333-343.
- [29] Curtin, CG. Interactions between cattle, prairie dogs, and small mammals in a desert grassland. In: Curtin CG, editor. Emergent outcomes of the interplay of climate, fire and grazing in a desert grassland. Desert plants. Volume 24. Tucson, Arizona: University of Arizona Press for Boyce Thompson Southwestern Arboretum; 2008. pp. 29-38.
- [30] Stapp P. Rodent communities in active and inactive colonies of black-tailed prairie dogs in shortgrass steppe. J Mammal. 2007;88: 241-249.

- [31] Davidson AD, Parmenter RR, Gosz JR. Responses of small mammals and vegetation to a reintroduction of Gunnison's prairie dogs. *J Mammal*. 1999;80: 1311-1324.
- [32] Pruett AL, Boal CW, Wallace MC, Whitlaw HA, Ray JD. Small mammals associated with colonies of black-tailed prairie dogs (*Cynomys ludovicianus*) in the southern high plains. *Southwest Nat*. 2010;55: 50-56.
- [33] Gori D, Schussman H. State of the Las Cienegas National Conservation Area. Part I. Condition and trend of the desert grassland and watershed. Prepared by The Nature Conservancy of Arizona; 2005. 63 pp.
- [34] Bodner GS, Simms K. State of the Las Cienegas National Conservation Area. Part 3. Condition and trend of riparian target species, vegetation, and channel geomorphology. Prepared by The Nature Conservancy of Arizona; 2008. 69pp.
- [35] Bodner GS, Robles MD. Enduring a decade of drought: patterns and drivers of vegetation change in a semi-arid grassland. *J Arid Environ*. 2017;136:1-14.
- [36] Underwood JG, Van Pelt WE. A proposal to reestablish the black-tailed prairie dog (*Cynomys ludovicianus*) to southern Arizona. Nongame and Endangered Wildlife Program Draft Technical Report. Phoenix: Arizona Game and Fish Department; 2000.
- [37] Hale SL, Koprowski JL, Hicks H. Review of black-tailed prairie dog reintroduction strategies and site selection: Arizona reintroduction. In: Gottfried GJ, Folliott PF, Gebow BS, Eskew LG, Collins LC, editors. *Proceedings--Merging science and management in a rapidly changing world: biodiversity and management of the Madrean Archipelago III*, 1-5 May 2012, Tucson, Arizona, USA. U.S. Department of Agriculture, Fort Collins, Colorado, USA. RMRS-P-67. 2013; pp. 310-315.

- [38] Anderson DR, Burnham KP, White GC, Otis DL. Density estimation of small-mammal populations using a trapping web and distance sampling methods. *Ecology* 1983;64: 674-680.
- [39] Justice KE. Nocturnalism in three species of desert rodents. Ph.D. Dissertation, The University of Arizona. 1960. Available from: <http://hdl.handle.net/10150/284386>
- [40] Sikes RS. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *J Mammal*. 2016;97: 663-688.
- [41] Lancia RA, Beshir JW. Removal methods. In: Wilson DE, FR Cole, JD Nichols, R Rudran, MS Foster, editors. *Measuring and monitoring biological diversity: standard methods for mammals*. Washington and London: Smithsonian Institution Press; 1996. pp. 200-217.
- [42] JMP®, Version 12. SAS Institute Inc., Cary, NC, 1989-2007.
- [43] Williston SW. The prairie dog, owl and rattlesnake. *Am Nat*. 1878;12: 203-208.
- [44] Harris L. Edge effects and conservation of biotic diversity. *Conserv Biology*. 1988;2: 330-332.
- [45] O’Meilia MF, Knopf FL, Lewis JC. Some consequences of competition between prairie dogs and beef cattle. *J Range Manage*. 1982;35: 580-585.
- [46] Lomolino MV, Smith GA. Terrestrial vertebrate communities at black-tailed prairie dog (*Cynomys ludovicianus*) towns. *Biol Conserv*. 2004;115: 89-100.
- [47] Fulgham KM, Koprowski JL. Kangaroo rat foraging in proximity to a colony of reintroduced black-tailed prairie dogs. *Southwest Nat*. 2016;61: 194-202.
- [48] Weltzin JF, Archer SR, Heitschmidt RK. Small-mammal regulation of vegetation structure in a temperate savanna. *Ecology*. 1997;78: 751-763.

- [49] Barth CJ, Liebig MA, Hendrickson JR, Sedivec KK, Halvorson G. Soil change induced by prairie dogs across three ecological sites. *Soil Sci. Soc. Am J.* 2014;78: 2054-2060.
- [50] Best TL, Skupski MP. *Perognathus flavus*. *Mammal Species*. 1994;471: 1-10.
- [51] Eshelman BD, Cameron GN. *Baiomys taylori*. *Mammal Species*. 1987;285: 1-7.
- [52] Macêdo RH, Mares MA. *Neotoma albigula*. *Mammal Species*. 1988;310: 1-7.
- [53] Best TL. 1988. *Dipodomys spectabilis*. *Mammal Species*. 1988;311: 1-10.
- [54] Ceballos G, Pacheco J, List R. Influence of prairie dogs (*Cynomys ludovicianus*) on habitat heterogeneity and mammalian diversity in Mexico. *J Arid Environ.* 1999;41: 161-172.
- [55] Shipley BK, Reading RP. A comparison of herpetofauna and small mammal diversity on black-tailed prairie dog (*Cynomys ludovicianus*) colonies and non-colonized grasslands in Colorado. *J Arid Environ.* 2006;66: 27-41.
- [56] Bartz SE, Drickamer LC, Kearsley MJC. Response of plant and rodent communities to removal of prairie dogs (*Cynomys gunnisoni*) in Arizona. *J Arid Environ.* 2007;68: 422-437.
- [57] McCaffrey RE, Wallace MC, Ray JD. Small mammals and ground-dwelling invertebrates associated with active and controlled colonies of black-tailed prairie dogs (*Cynomys ludovicianus*). *Southwest Nat.* 2009;54: 300-306.
- [58] Brown JH, Heske EJ. Control of a desert-grassland transition by a keystone rodent guild. *Science*. 1990;250: 1705-1707.

Figures

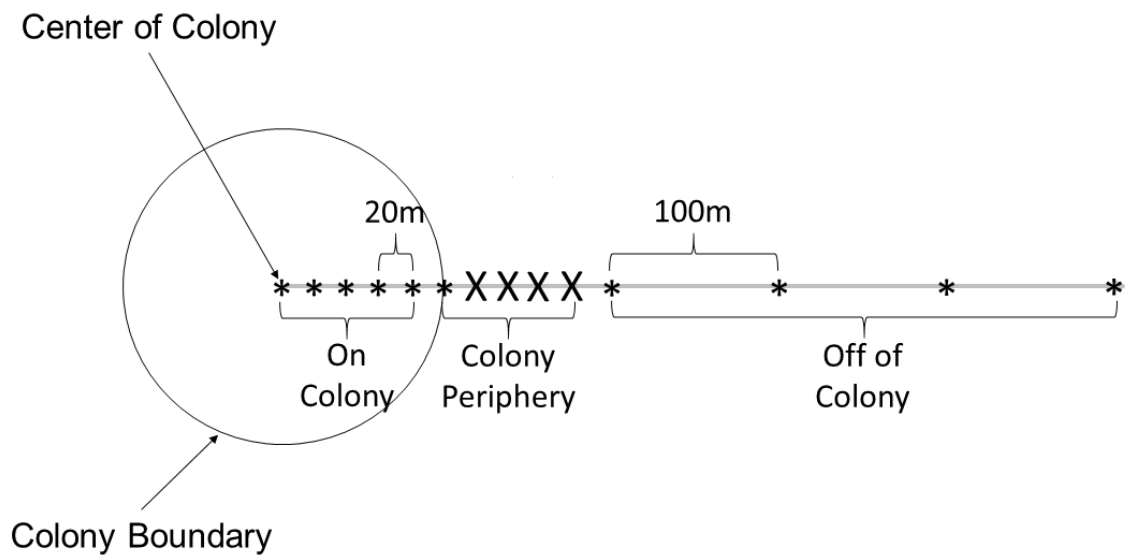


Figure 1

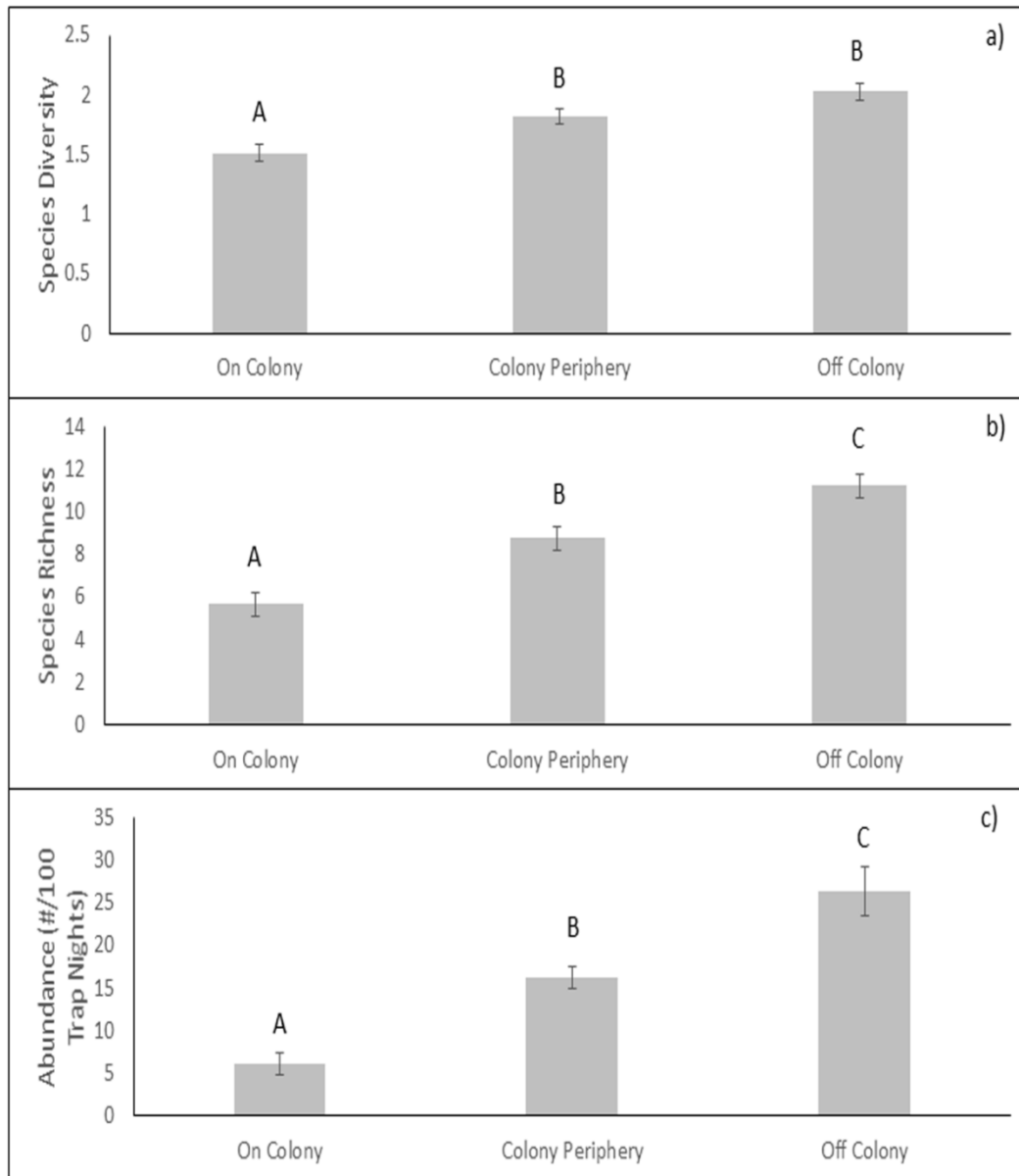


Figure 2

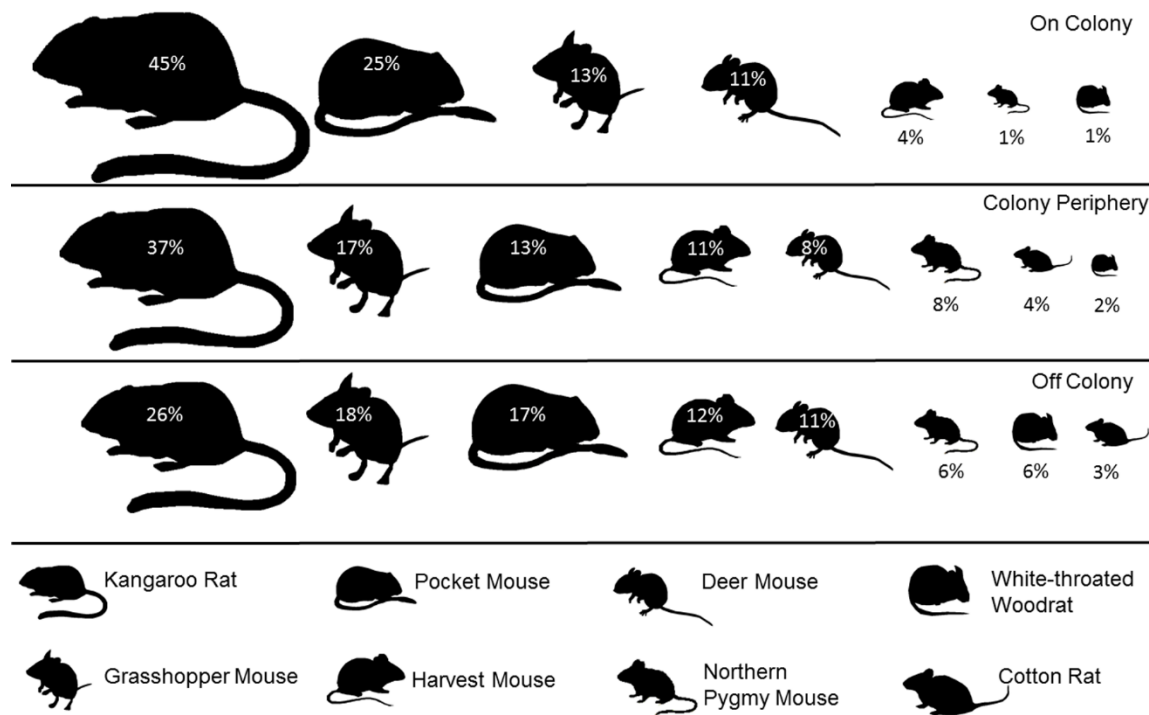


Figure 3

APPENDIX D: COULD THE REINTRODUCTION OF A SMALL, NATIVE HERBIVORE
REGULATE WOODY PLANT ENCROACHMENT?

Sarah L. Hale, John L. Koprowski, and Steven R. Archer

(In the format of *Ecological Applications*)

Running Head: Native Herbivores Regulate Woody Encroachment

Title: Could the Reintroduction of a Small, Native Herbivore Regulate Woody Plant Encroachment?

Sarah L. Hale¹, John L. Koprowski², and Steven R. Archer³

*University of Arizona, School of Natural Resources and the Environment, 1064 E. Lowell St.,
P.O. Box 210137, Tucson, AZ 85721, USA.*

Abstract

Tree and shrub proliferation has been widespread in grasslands and savannas worldwide, and has altered ecosystem function and wildlife habitat. Several causes have been proposed for the ‘woody encroachment’ phenomenon. The widespread removal of a native keystone herbivore in North America, the prairie dog (Cynomys spp.), is one potential contributing factor that has received relatively little attention. Because woody encroachment has coincided with the eradication of prairie dogs across much of their former range, we hypothesized that prairie dogs would have historically suppressed woody plant growth on their colonies, and act as a “browse trap” in grassland systems through their systematic clipping of tall vegetation. We tested this hypothesis by conducting transect surveys for woody plants and experimentally manipulating their accessibility to black-tailed prairie dogs (C. ludovicianus) on and around four recently reestablished colonies in southeastern Arizona, USA. Mean \pm SE shrub densities (primarily Prosopis velutina and Acacia greggii) were highest off of colonies (161 ± 17.1 plants ha^{-1}). However, while woody plants were common on colonies (76 ± 11.9 plants ha^{-1}) they were suppressed in stature (height and canopy area on colonies = 29 ± 5.3 cm and 0.3 ± 0.1 m²/plant compared to 78 ± 4.2 cm and 1.2 ± 0.1 m²/plant off of colony sites). Furthermore, the aboveground growth (canopy expansion) of shrubs protected by exclosures was greater than that of shrubs left exposed to prairie dogs on colonies. Large (~ 1.1 cm diameter) branches collected from adult shrubs on nearby areas and used to simulate ‘saplings’ were mutilated within three days of ‘planting’ on colonies, but were untouched off the colonies. This was true for both a deciduous N₂-fixing shrub (P. velutina) and an evergreen non N₂-fixing shrub (creosote bush, Larrea tridentata). Our data indicate that while prairie dogs do not necessarily extirpate woody plants, they suppress their abundance, stature and growth and prevent them from achieving

dominance. Prairie dogs represent an enigma in keystone conservation. Whereas the reintroduction of large or charismatic keystone species (e.g. gray wolves [Canis lupus] and sea otters [Enhydra lutris]) are conducted to restore critical ecological function, small keystone herbivores such as prairie dogs are traditionally viewed as pests and are more typically targeted for eradication. Our findings indicate that the reintroduction of these negatively perceived small herbivores could function as a tool to suppress woody encroachment and in so doing help maintain habitat for other grassland endemics while simultaneously promoting biological diversity and other ecosystem services across the prairie dog's former range.

Key Words: Black-tailed prairie dog, browse trap, ecosystem engineer, exclosure, grassland, keystone species

Introduction

“Woody encroachment”, the proliferation of tree and shrub species in grasslands and savannas, is a phenomenon affecting arid and semiarid grasslands and savannas worldwide. Shifts from grass to woody plant dominance affects a variety of ecological processes and the provision of numerous ecosystem goods and service (Eldridge et al. 2011, Archer and Predick 2014). Increases in atmospheric CO₂, and changes in climate, fire regimes, and livestock grazing regimes favoring woody plants have been proposed as causal factors; however the direct and indirect effects of intensification of livestock grazing over the past century appears to be a primary driver (Archer et al. 2017).

Coinciding with the intensification of livestock grazing in North America has been the widespread eradication of the prairie dog (*Cynomys* spp.), a rodent traditionally viewed as an impediment to livestock production (Vermeire et al. 2004). Although prairie dogs are now recognized as contributing to the maintenance of grassland ecosystems (Hoogland 1995, Kotliar et al. 1999) the consequences of their removal has received little attention in the context of regulating grass-woody plant interactions. In Africa, herbivores of varying sizes mediate woody plant abundance through browsing activities (e.g. elephants [*Loxodonta* spp.], dik-diks [*Madoqua* spp.], impala [*Aepyceros melampus*]; Sankaran et al. 2013). Browsing maintains woody vegetation at a small stature, thereby creating a “browse trap” that also contributes to a “fire trap” (Bond and Keeley 2005, Staver et al. 2009, Sankaran et al. 2013). Woody plants caught in a fire or browse trap are unable to achieve dominance due to repeated disturbance, so do not reach adult stature or dominance until the suppressing pressure is removed. We suggest that prairie dogs in North America historically created and maintained browse traps that suppressed woody vegetation, and their eradication would have released already-established

woody plants permitting them to reach adult stature in grasslands throughout the prairie dog's former range.

Prairie dogs were once widely distributed across the North American west (Hall 2001), however, were deemed a range pest in competition with livestock, leading to widespread eradication efforts beginning in the early 1900s (Summers and Linder 1978). Historic population sizes are hard to pin-point (Vermeire et al. 2004), but at the most conservative estimate, active eradication, habitat loss, and sylvatic plague (*Yersinia pestis*; Luce et al. 2006) appear to have reduced prairie dog populations to 2% of historic numbers (Whicker and Detling 1988, Miller et al. 1994), and range occupancy to $\leq 1\%$ (Luce et al. 2006). Prairie dogs are grazers, but systematically gnaw, clip, and girdle other vegetation to maximize predator detection, thereby maintaining herbaceous and woody plants on their colonies at small stature (< 30 cm; Hoogland 1995, Weltzin et al. 1997).

Woody encroachment proliferation has necessitated the implementation of “brush management” techniques involving heavy machinery, herbicides, and prescribed fire (Hamilton et al. 2004, Collins et al. 2015). Such techniques are typically expensive with short-lived effects, and hence are seldom cost-effective (Archer et al. 2011). We sought to investigate whether black-tailed prairie dogs (*Cynomys ludovicianus*; hereafter BTPD) might constitute a natural, potentially long-term, sustainable solution for managing woody plant encroachment and proliferation in grasslands. We hypothesized that BTPD would have historically suppressed woody plant growth on their colonies, and act as a “browse trap” in grassland systems through their systematic clipping of tall vegetation. We predicted that woody plants on newly established BTPD colonies would be smaller and less abundant than those off-colony, that shrubs left

unprotected on BTPD colonies would have slower growth rates than protected shrubs, and that disturbance to “simulated saplings” would be greater on colonies than off colonies.

From a broader perspective, our study is also intended to determine whether the reintroduction of a keystone herbivore following a prolonged absence could restore the browse trap that would have historically excluded woody plants or reduced their height in grassland systems. Studies involving keystone species typically seek to assess ecosystem effects while the species of interest is present or absent from the landscape (e.g. Reisewitz et al. 2006). Our study is one of the first to investigate consequences of the reintroduction of a keystone species, and the extent to which that reintroduction might be justified as part of an integrated, comprehensive plan to more effectively address ecosystem management challenges in drylands.

Methods

Study site

Las Cienegas National Conservation Area (hereafter Las Cienegas) is a working cattle ranch managed by the Bureau of Land Management. Las Cienegas is a 17,000 ha mosaic of five rare ecosystems of the southwestern United States (cottonwood [*Populus fremontii*]-willow [*Salix gooddingii*, *S. taxifolia*] riparian forest, marshland, mesquite [*Prosopis velutina*] bosque, sacaton [*Sporobolus wrightii*] floodplain, and semidesert grassland; Gori and Schussman 2005, Bodner and Simms 2008), and is located 72 km southeast of Tucson, AZ. Mean annual precipitation is 405 mm, mostly falling during the summer monsoon months (July-September); mean annual temperature is 15.7° C (Bodner and Robles 2017). During our study (2013-2015) annual precipitation and temperature (\pm SE) averaged 350 ± 46.9 mm $16.2 \pm 0.2^\circ$ C (Empire Remote Area Weather Station; QEMA3, <http://mesowest.utah.edu>). Grasslands at Las Cienegas

are located between 1300 and 1500m in elevation, and soils are primarily gravely, sandy, and clay loam (Bodner and Robles 2017). Semidesert grasslands in Arizona historically supported BTPDs, which were extirpated by 1960 (Underwood and Van Pelt 2000). The Arizona Game and Fish Department began reintroducing BTPDs at Las Cienegas in 2008 from populations in New Mexico, USA and Sonora, Mexico. We conducted experiments on four of these colonies.

Colonies were situated on sites with similar soils (fine, deep, well-drained), slopes (0-15%), and elevations (1367-1412 m). Site preparation by the Arizona Game and Fish Department consisted of shrub removal, mowing of tall grass, and installation of 25 artificial burrows on a 4 ha plot (see Hale et al. 2013 for details). One colony was initiated per year starting in 2008 and ending in 2011. Throughout our study, three of the four colonies remained occupied by BTPD; however, one colony (hereafter referred to as the “control colony”) failed and was unoccupied during 2014 and 2015. This “control colony” received the same initial treatment as the other colonies, but lacked prairie dogs throughout the majority of our study period (fewer than 10 prairie dogs were present in May 2013 and the colony was vacant by October 2013).

Woody plant surveys

We surveyed woody plants on each colony in May of 2013 and 2014. Surveys consisted of eight belt transects (5 m width) originating at the center of each colony and extending 100 m beyond the colony perimeter. Colony boundaries were delineated as the junction of short, prairie dog-grazed grass with tall, un-clipped grass and an absence of burrows. Shrubs originating within the belt were measured for height and canopy dimensions (longest axis and the widest point perpendicular to the longest axis) and the presence and degree of disturbance in the form of clipping, chewing, or girdling of their stems was noted on a scale of 0-3 (0 = no disturbance, 1 =

few stems disturbed, 2 = many stems disturbed, 3 = majority of stems disturbed). Shrub canopy area (CA, m²) was computed as $CA = \pi ab$, where a and b equal the radii of the orthogonal axes. Shrub canopy cover (%) on belt transects was calculated as the total CA of plants encountered/total area of transect x 100.

Exclosure experiments

Among the woody plants encountered in our 2013 survey, a random subset of both dominant species ($n = 40$) were tagged for monitoring on each colony (typically $n = 20$ on-colony, 20 off-colony). Exclosures were installed around half of the plants at each on-off colony location, with the other half remaining unprotected. Garden fencing (mesh size 5.1 cm x 7.6 cm) was used to exclude prairie dogs only for half of the exclosures, and hardware cloth (mesh size 0.64 cm x 0.64 cm) was used for the remainder of the exclosures ($n = 5$) exclude all small mammals on the sites (e.g. *Baiomys taylori*, *Chaetodipus* spp., *Dipodomys* spp., *Neotoma* spp., *Onychomys* spp., *Perognathus* spp., *Peromyscus* spp., *Reithrodontomys* spp., *Sigmodon* spp.). Exclosures were in place for ca. 3 months (mid-May through August) in 2013 and 2014 and for ca. 5 months (mid-May through late October) in 2015. Plants were measured at the time of exclosure installation and again at the time of exclosure removal. The same plants were followed each year; if a plant measured on the previous year had died, the nearest live individual of the same species was used as a replacement. Growth is expressed as change in canopy area (CA, cm²) standardized to a monthly basis (30 days).

Simulated Sapling Experiments

We removed foliated branches [average (\pm SE) length = 47 ± 0.6 cm; diameter = 1.1 ± 0.02 cm] from mature shrubs in nearby off-colony areas, and inserted them ~10 cm into the ground

along four transects that originated at the center of each colony and extended 100 m beyond the colony boundary in randomized directions. These ‘simulated saplings’ were intended to assess how BTPD would react to the growth of additional woody plants on their colonies. The ‘saplings’ ($n=10$) were evenly spaced along transect segments on the colonies (the spacing distance varied for each transect segment, depending on its absolute length), and spaced at 10 m intervals along the off-colony transect segments ($n=10$). Three simulated saplings on each transect were considered ‘periphery’: the sapling installed on the colony boundary, and the closest sapling inside and outside of the colony boundary (Fig. 1). We used two woody species: velvet mesquite (*Prosopis velutina*; hereafter ‘mesquite’), a native shrub common on and around the BTPD colonies at our study site, and creosote bush (*Larrea tridentate*; hereafter ‘creosote’), also a native shrub, but one that did not occur in or around the colonies on the Las Cienegas. Both species are abundant in the Sonoran Desert. Mesquite is a thorny, N₂-fixing deciduous shrub (Fabaceae), whereas Creosote is non-thorny, non-N₂-fixing evergreen shrub (Zygophyllaceae) that dominates many landscapes in each of the three North American hot deserts. Saplings were measured for height (cm) and the number of secondary stems from the main stem were recorded (typically several for mesquite and none for creosote). After 3 days of exposure to prairie dogs, the ‘saplings’ were re-measured and the presence of damage (i.e. chewing, clipping, or girdling) was recorded. Damage was quantified as ‘degree of disturbance’ for each ‘sapling’ and consisted of the proportion of total stems disturbed on mesquite ‘saplings’ and the relative proportion of height change of creosote ‘saplings’ since there were rarely secondary stems present. The experiment was conducted with mesquite in May 2014 and repeated in late July/early August 2014. The experiment was conducted with creosote ‘saplings’ in early September 2015, and repeated in late September 2015.

Data analysis

Statistical analyses were performed in JMP[®], Version 12 (SAS Institute Inc.). For cover and density of transects, we performed a full factorial three-way analysis of variance (ANOVA) with year (2013, 2014), location (on-colony, on control, off-colony), and species (*P. velutina*, *A. greggii*) as main effects, and density or log transformed canopy area (CA) as the dependent variables. Shrub canopy growth (cm²/month) in exclosure experiments, was log transformed prior to performing a full factorial two-way ANOVA with exclosure mesh size (only prairie dogs excluded or all small mammals excluded) and location as main effects. No differences were detected between exclosures of differing sizes, so exclosures were pooled for subsequent analyses. For each year and location (i.e. on colony 2013, on colony 2014, on colony 2015, colony periphery 2013, colony periphery 2014, colony periphery 2015, off colony 2013, off colony 2014, off colony 2015), we performed two way Student's *t*-tests with exclosure and colony as main effects, and change in canopy area as the dependent variable. Outliers ($n = 8$ of 188 data points for % canopy cover on transects, $n = 7$ of 188 data points for density on transects, and $n = 10$ of 161 canopy expansion data points) were removed from analysis based on examination of histograms and residual plots. A full factorial three-way ANOVA was performed for the simulated sapling experiments, with trial number (1st or 2nd), species (mesquite or creosote), and location (on-colony, colony periphery, off-colony, on-control, control periphery, off-control) as independent variables, and proportion of total plants disturbed or degree of disturbance as the dependent variables. Transects were considered the experimental unit ($n = 4$ per colony for each iteration of the experiment).

When significant differences were indicated by ANOVA ($\alpha \leq 0.05$), differences among means were evaluated using Tukey's honest significant difference (hsd) tests (where statistical

significance was set at 0.05 divided by the total number of comparisons in each test). In cases where no interactions occurred between effects having only 2 levels, Student's *t*-tests were performed on each effect with $\alpha \leq 0.05$. Statistical analyses were conducted on transformed data, but means and SEs reported in the results are based on untransformed data.

Results

Woody plant surveys

Shrubs encountered on and around all colonies consisted of two species: *P. velutina* and *A. greggii*. Mean (\pm SE; plants ha⁻¹) shrub density was influenced by location ($F_{2,180} = 9.04$, $P < 0.001$), and was greater off of colonies (128 ± 14.2) than on colonies (71 ± 9.4 ; Tukeys hsd test, $P = 0.003$) or the control site (53 ± 11.3 ; Tukeys hsd test, $P < 0.001$), and was statistically equivalent on colonies and the control site (Tukey's hsd test, $P = 0.429$; Fig. 2a). Mean (\pm SE; %) canopy cover was influenced by the interaction of year, location, and species ($F_{2,179} = 10.82$, $P < 0.001$), with mesquite off of colonies in 2014 having significantly greater canopy cover than in all other combinations of year, location and species, but all other comparisons were statistically equivalent (Tukey's hsd test, $P > 0.080$; Fig. 2b)

Height of shrubs was greater off colonies (77.8 ± 4.2 cm) than on colonies (29.1 ± 5.3 cm; Tukey's hsd, $P < 0.001$) and on the control colony (39.1 ± 9.1 cm; Tukey's hsd, $P < 0.001$), and height did not differ between shrubs on colonies or on the control (Tukey's hsd, $P = 0.608$). The frequency of disturbance was greatest for shrubs on colonies (85% of 329 plants) compared to 35% ($n = 91$) on the control colony and only 9% ($n = 355$) in off-colony locations.

Exclosure experiments

Shrub mortality during our study was low: only four of the 153 mesquite shrubs tagged on and off colonies died. Survival of mesquite did not differ on colonies ($96 \pm 1.0\%$) and off of colonies ($100 \pm 1.0\%$; Student's t -test, $P = 0.07$), or inside ($97 \pm 1.0\%$) and outside of exclosures ($99 \pm 1.0\%$; Student's t -test, $P = 0.525$).

Changes in shrub canopy area (CA = growth) were statistically equivalent in exclosures of differing mesh size (Student's t -test: $t = 1.97$, $P = 0.371$, so these data were pooled for subsequent analyses. Mean (\pm SE; m^2/month) growth of shrubs protected by exclosures was greater than that of unprotected individuals on colonies in all years (2013: inside = 0.08 ± 0.01 , outside = 0.04 ± 0.01 , Student's t -test, $P = 0.009$; 2014: inside = 0.04 ± 0.01 , outside = -0.00 ± 0.01 , Student's t -test, $P = 0.003$; 2015: inside = 0.02 ± 0.01 , outside = 0.01 ± 0.01 , Student's t -test, $P = 0.002$; Fig. 4a). Growth was comparable inside and outside of exclosures on the control site in all years (Student's t -test 2013: $P = 0.371$, 2014: $P = 0.196$, 2015: $P = 0.592$; Fig. 4a). Off the colonies, the growth of protected plants (0.07 ± 0.03) was greater than that of unprotected plants (-0.00 ± 0.04 ; Student's t -test, $P = 0.012$) in 2013, but growth was statistically equivalent in 2014 (Student's t -test, $P = 0.219$) and 2015 (Student's t -test, $P = 0.537$; Fig. 4b). Mean (\pm SE; m^2/month) growth of shrubs was also affected by colony, but only in off-colony zones in 2015 ($F_{3,70} = 5.30$, $P = 0.003$). Shrubs (pooled across exclosure presence) in the off-colony zone of colony C (0.11 ± 0.06) grew at a greater rate than those in the off-colony zone of colony D (-0.09 ± 0.06).

Simulated sapling experiments

Colony had no effect on proportion of saplings disturbed or degree of disturbance, so data were pooled across colonies for analyses. Neither experimental trial number (1st or 2nd; $F_{1,191} = 0.28$, $P = 0.599$) nor shrub species (creosote or mesquite; $F_{1,191} = 0.49$, $P = 0.486$) influenced the

proportion of saplings disturbed, but location (on-colony, colony periphery, or off-colony, on-control, control periphery, off-control) did ($F_{1,191} = 167.17$, $P < 0.001$). The proportion of saplings disturbed was greatest on colonies ($89 \pm 2.0\%$), followed by colony peripheries ($38 \pm 2.0\%$; Tukey's hsd, $P < 0.001$); disturbances in all other locations were essentially nil (0 to 2%; Fig. 5a). Spatially, the proportion of saplings disturbed was consistently high among on colony and control transect positions, decreasing sharply at the colony periphery locations, and was consistently low for off-colony positions (Fig. 6).

Shrub species (*P. velutina*, *L. tridentata*) X location ($F_{5,191} = 7.30$, $P < 0.001$) and trial number X location ($F_{5,191} = 4.93$, $P < 0.001$) interactions significantly influenced degree of disturbance to saplings. Levels of disturbance were consistently greatest on colonies, intermediate on colony edges, and least off of colonies and on control sites (Fig. 5a and 5b). Degree of disturbance only differed between species on colony, with mesquite being disturbed to a greater degree (0.67 ± 0.03) than creosote (0.50 ± 0.03 ; Tukey's hsd, $P = 0.004$; Fig. 5b). Similarly, degree of disturbance only differed between experimental trials on colony, with saplings being disturbed to a greater degree during trial 2 (0.69 ± 0.03) than trial 1 (0.48 ± 0.03 ; Tukey's hsd, $P < 0.001$; Fig. 5c).

Discussion

Prairie dogs typically maintain short vegetation on their colonies to aid in visual detection of predators (Hoogland 1995) through the systematic clipping and felling of plants. Accordingly, shrubs surveyed on active colonies were less dense and had less canopy cover than those on the off-colony sites, which suggests that BTPD suppressed the growth of shrubs on colonies. Additionally, shrubs on colonies were disturbed more frequently than those located on colony peripheries, off of colonies, or at the control colony, as indicated by observational

(surveys) and experimental (exclosures and simulated saplings) results, and experienced the greatest degree of disturbance as indicated by our simulated sapling experiments. Interestingly, the presence of BTPDs did not affect shrub survival, but was correlated with reduced shrub growth, demonstrating that shrubs were persistent even under heavy levels of herbivory, and the removal of BTPDs could release shrubs from the browse trap. *P. velutina* and *A. greggii* have the ability to regenerate from roots following removal of aboveground biomass (Cross and Wiedemann 1997, Bovey 2016), so BTPD at Las Cienegas likely act to prevent new shoots from reaching mature stature, as indicated by the suppression of unprotected plants and simulated saplings on colonies. No differences existed in growth between shrubs in exclosures of differing mesh sizes, suggesting that BTPD, and not other small rodents, were regulating shrubs. This is consistent with another study showing that small rodents on BTPD colonies do not influence rates of shrub seed disappearance (Weltzin et al. 1997). Furthermore, we directly observed prairie dogs chewing and clipping simulated saplings, so are confident that disturbance to plants on colonies was caused by BTPDs and not other small mammals. Off of colonies (with the exception of 2013) and on the control site, change in canopy area of plants inside and outside of exclosures were statistically equivalent, so exclosures were not creating microhabitats or directly influencing plant growth.

A combination of factors have facilitated woody encroachment in the North American west; however, reduction of pressure from small, native herbivores, likely has been a major factor (Weltzin et al. 1997). Woody plants have extensive root systems allowing access to deep water stores (Scott et al. 2006), but roots also aid in re-sprouting following loss of aboveground biomass (Scholes and Archer 1997). Prairie dogs historically suppressed recovery of aboveground biomass through clipping activities during colony maintenance (Weltzin et al.

1997), but prairie dog removal eliminated this constraint on woody growth. Furthermore, native grazers, such as bison (*Bison bison*) and non-native grazers, such as domestic cattle (*Bos taurus*) have diets that consist primarily of grasses and forbs (Meagher 1986, Beck and Peek 2005) so likely do not function to reduce woody plants after seedling stage. This would be particularly so for unpalatable woody species such as mesquite and creosote bush (Campbell and Taylor 2006, Brock et al. 2014, Bovey 2016). Thus, in the absence of prairie dogs, woody plants experience reduced clipping/browsing pressure and are able to reach large stature, even in the presence of other grazers.

Once woody plants begin to dominate an area, grazing management alone is not enough to inhibit woody encroachment and additional methods must be used (Archer et al. 2011). Historically, management of woody plants, known as brush management, focused on benefits to livestock and livestock handlers such as increased forage production, reduced threats to livestock (i.e. predators and pest insects), and increased ease of animal handling (Archer et al. 2011). More recently, brush managers have realized the importance of interdisciplinary management for purposes other than livestock production, so have incorporated other goals such as overall ecosystem function into decision making efforts (Campbell and Taylor 2006, Archer et al. 2011). Brush management often results in increased herbaceous plant growth, but other proposed benefits, such as increased ground water recharge, streamflow, and habitat for wildlife, are not widely documented (Archer et al. 2011). Furthermore, methods used for brush management often involve herbicide applications, mechanical treatments such as cutting or chaining, and prescribed burning (Collins et al. 2015), all of which are costly, can have detrimental effects on the environment, and require repeated treatments to prevent woody plants from reestablishing dominance following initial removal (Archer et al. 2011). Brush management results in several

benefits to grassland ecosystems, and is the only option to reduce woody encroachment in some localities; however, in other areas, many similar outcomes can be achieved by native keystone species, such as prairie dogs. Prairie dogs provide habitat for other wildlife species, and create macropores that reduce runoff and facilitate groundwater recharge through burrow excavation (Kotliar et al. 1999, Kotliar et al. 2006). Additionally, although herbaceous, non-woody, vegetation communities did not differ between recently reestablished Gunnison's prairie dog (*Cynomys gunnisoni*) colonies and control sites in New Mexico (Davidson et al. 1999), empirical evidence supports the notion that prairie dogs maintain shrubs at small stature because woody plants on active colonies are often of smaller stature and occur in lower densities than in adjacent areas, and woody plants appear to 'invade' colonies once prairie dogs vacate (Weltzin et al. 1997, Ceballos et al. 2010). Prairie dogs also suppress woody plant growth on existing colonies (Weltzin et al. 1997; Ponce-Guevara 2016), which supports the idea that prairie dogs act as a browse trap where colonies have long been established. Likewise, we determined that prairie dogs do begin to effectively suppress shrub growth immediately after their reintroduction, and thus affect successful establishment of woody species within and immediately adjacent to colonies. Although prairie dogs may not be capable of felling large, mature woody plants, they are a viable option for preventing regeneration from roots following their removal.

Our study indicates that prairie dogs do have immediate effects on vegetation following their reintroduction, and restore the browse trap in grassland systems. Our sapling experiments suggest that prairie dogs are not limited by previous experience with woody species, as they felled both a species they encountered regularly (*P. velutina*) as well as a species they had never encountered (*L. tridentata*). This indicates that BTPD can restore the browse trap even when source and reintroduction sites differ in endemic woody species. Reintroduction of prairie dogs

would restore an important constraint (i.e. the systematic clipping of woody vegetation) that historically may have kept shrubs from encroaching into North American grasslands (Weltzin et al. 1997). This suggests that prairie dog reintroduction within areas of their former range could simultaneously function as a natural, less costly, and long-term option for reducing woody encroachment and restoring degraded grasslands in the North American west.

A coincidence between the elimination of small native grazers and woody encroachment is not restricted to North America. Plains vizcacha (*Lagostomus maximus*) in Argentina exhibit similarities to prairie dogs not only in their ecology and behavior, but also in their pest status, which has led to their elimination from parts of the landscape (Jackson et al. 1996). Likewise, plateau pikas (*Ochotona curzoniae*) in China play a keystone role in their ecosystem analogous to that of BTPDs, but have also been subjected to eradication efforts (Smith and Foggin 1998, Delibes-Mateos et al. 2011, Fahong, et al. 2012). Similarly to what has occurred in North America, elimination of perceived pests has coincided with woody encroachment across formerly occupied areas (Brandt et al. 2013, González-Roglich et al. 2015). This coincidence suggests that small native grazers around the world can actively suppress woody plant growth, and conservation and reintroduction of these keystone species may be an effective strategy to mitigate woody encroachment and the loss of critical grassland habitat. Additionally, reintroduction of small native grazers may extend the longevity of brush management treatments, and thereby promote the restoration of grassland habitats and the plants and animals endemic to them (similarly to targeted browsing with non-native species such as goats; Campbell and Taylor 2006).

The conservation and reintroduction of large or charismatic keystone species (e.g. gray wolves [*Canis lupus*] and sea otters [*Enhydra lutris*]) can restore critical ecological function to

ecosystems (Estes and Palmisano 1974, Callan et al. 2013); however, small keystone herbivores are often viewed as pests that are subjected to eradication rather than conservation (Delibes-Mateos et al. 2011). An understanding of the roles such keystone species play in ecosystems is well-known (Delibes-Mateos et al. 2011); however, the ecological implications following reintroduction of small, keystone herbivores have not been widely investigated. We add to this understanding, and highlight the ability of small keystone herbivores to address significant ecological issues, such as woody encroachment, immediately following reintroduction. Our results along with trends observed worldwide (Jackson et al. 1996, Smith and Foggin 1998) indicate the important role small keystone herbivores play in maintaining healthy ecosystems, and the substantial ecological consequences that can result from their removal (Weltzin et al. 1997, Brandt et al. 2013, González-Roglich et al. 2015). The conservation of such species and reintroduction to areas after extirpation have the potential to aid in restoring degraded ecosystems worldwide.

Acknowledgments

Work was funded by the National Fish and Wildlife Federation, the Arizona Center for Nature Conservation, the Arizona Game & Fish Department, T & E, Inc., the Bureau of Land Management and Arizona Agricultural Experiment Station projects ARZT-1360540-H12-199 and ARZT-1390040-M12-205. Kirsten Fulgham, Paisley and Pandora Hale, Allyson Hawkins, Levi Heffelfinger, Theresa Huckleberry, Max Mazzella, Rachel More-Hla, Rocio Ojeda, Samantha Springs, Michael Taylor, and Kaycie Waters assisted with field experiments and data collection. Patricia King (King's Anvil Ranch) allowed collection of creosote from her property for simulated sapling experiments.

Literature Cited

- Archer, S. R. and K. Predick. 2014. An ecosystem services perspective on brush management: research priorities for competing land use objectives. *Journal of Ecology* **102**:1394–1407.
- Archer, S. R., E. M. Andersen, K. I. Predick, S. Schwinning, R. J. Steidl, and S. R. Woods. 2017. Woody plant encroachment: causes and consequences. In: Briske, D. D. editor. 2017. Rangeland systems: processes, management and challenges. Springer, New York, New York, USA. (In Press)
- Archer, S. R., K. W. Davies, T. E. Fullbright, K. C. McDaniel, B. P. Wilcox, and K. I. Predick. 2011. Brush management as a rangeland conservation strategy: a critical evaluation. Pages 105-170 in Briske, D. D. editor. 2011. Conservation benefits of rangeland practices: assessment, recommendations, and knowledge gaps. United States Department of Agriculture, Natural Resources Conservation Service.
- Asner G. P., A. J. Elmore, L. P. Olander, R. E. Martin, and A. T. Harris. 2004. Grazing systems, ecosystem responses, and global change. *Annual Review of Environment and Resources* **29**:261-299.
- Bailey R. G. 1996. Ecosystem Geography. Springer, New York, New York, USA.
- Beck J. L. and J. M. Peek. 2005. Diet composition, forage selection, and potential for forage competition among elk, deer, and livestock on aspen-sagebrush summer range. *Rangeland Ecology and Management* **58**:135–147.

- Bodner, G. S. and K. Simms. 2008. State of the Las Cienegas National Conservation Area. Part 3. Condition and trend of riparian target species, vegetation, and channel geomorphology. Prepared by The Nature Conservancy of Arizona. 69pp.
- Bodner, G. S. and M. D. Robles. 2017. Enduring a decade of drought: patterns and drivers of vegetation change in a semi-arid grassland. *Journal of Arid Environments* **136**:1–14.
- Bond, W. J. and J. E. Keeley. 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution* **20**:387–394.
- Bovey, R. W. 2016. Mesquite. Texas A&M University Press, College Station, Texas, USA.
- Brandt, J. S., M. A. Haynes, T. Kuemmerle, D. M. Waller, and V. C. Radeloff. 2013. Regime shift on the roof of the world: alpine meadows converting to shrublands in the southern Himalayas. *Biological Conservation* **158**:116–127.
- Brock, J., B. Brandau, D. Arthun, A. L. Humphrey, G. Dominguez, and A. Jacobs. 2014. Long-term results of tebuthiuron herbicide treatment on creosote bush (*Larrea tridentate*) in southeast Arizona, USA. *Journal of Arid Environments* **110**:44–46.
- Callan, R., N. P. Nibbelink, T. P. Rooney, J. E. Wiedenhoft, and A. P. Wydeven. 2013. Recolonizing wolves trigger a trophic cascade in Wisconsin (USA). *Journal of Ecology* **101**:837–845.
- Campbell, E. and C. A. Taylor. 2006. Targeted grazing to manage weedy brush and trees. Pages 77–88 in Launchbaugh, K. editor. Targeted grazing: a natural approach to vegetation management and landscape enhancement. American Sheep Industry Association.

- Ceballos G., A. Davidson, R. List, J. Pacheco, P. Manzano-Fischer, G. Santos-Barrera, and J. Cruzado. 2010. Rapid decline of a grassland system and its ecological and conservation implications. *PLoS ONE* **5**:e8562.
- Collins, C. D. H., M. A. Kautz, R. Tiller, S. Lohani, G. Ponce-Campos, J. Hottenstein, and L. J. Metz. 2015. Development of an integrated multiplatform approach for assessing brush management conservation efforts in semiarid rangelands. *Journal of Applied Remote Sensing* **9**:096057.
- Cross, B. T. and H. T. Wiedemann. 1997. Control of catclaw acacia and mimosa by grubbing. *Applied Engineering in Agriculture* **13**:291–293.
- Davidson, A. D., R. R. Parmenter, and J. R. Gosz. 1999. Responses of small mammals and vegetation to a reintroduction of Gunnison's prairie dogs. *Journal of Mammalogy* **80**:1311–1324.
- Delibes-Mateos, M., A. T. Smith, C. N. Slobodchikoff, and J. E. Swenson. 2011. The paradox of keystone species persecuted as pests: a call for the conservation of abundant small mammals in their native range. *Biological Conservation* **144**:1335–1346.
- Eldridge D. J., M. A. Bowker, F. T. Maestre, E. Roger, J. F. Reynolds, and W. G. Whitford. 2011. Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. *Ecology Letters* **14**:709–722.
- Estes, J. A. and J. F. Palmisano. 1974. Sea otters: their role in structuring nearshore communities. *Science* **185**:1058–1060.

- Fahong, Y., S. Li, W. C. Kilpatrick, P. M. McGuire, K. He, and W. Wei. 2012. Biogeographical study of plateau pikas *Ochotona curzoniae* (Lagomorpha, Ochotonidae). *Zoological Science* **29**:518–526.
- González-Roglich, M., J. J. Swenson, D. Villarreal, E. G. Jobbágy, and R. B. Jackson. 2015. Woody plant-cover dynamics in Argentine savannas from the 1880s to 2000s: the interplay of encroachment and agriculture conversion at varying scales. *Ecosystems* **18**:481–492.
- Gori, D. and H. Schussman. 2005. State of the Las Cienegas National Conservation Area. Part I. Condition and trend of the desert grassland and watershed. The Nature Conservancy of Arizona. 63 pp.
- Grover, H. D. and H. B. Musick. 1990. Shrubland encroachment in southern New Mexico U.S.A.: an analysis of desertification processes in the American southwest. *Climatic Change* **17**:305–330.
- Hale, S. L., J. L. Koprowski, and H. Hicks. 2013. Review of black-tailed prairie dog reintroduction strategies and site selection: Arizona reintroduction. Pages 310–315 in Gottfried, G. J.; P. F. Folliott, B. S. Gebow, L. G. Eskew, and L. C. Collins, compilers. Proceedings--Merging science and management in a rapidly changing world: biodiversity and management of the Madrean Archipelago III, 1-5 May 2012, Tucson, Arizona, USA. U.S. Department of Agriculture, Fort Collins, Colorado, USA. RMRS-P-67.
- Hall, R. E. 2001. The mammals of North America, Volume 1, Second edition. The Blackburn Press, Caldwell, New Jersey, USA.

- Hamilton, W.T., A. McGinty, D. N. Ueckert, C. W. Hanselka, and M. R. Lee. 2004. Brush management: past, present, future. Texas A&M University Press, College Station, Texas, USA.
- Hoogland, J. L. 1995. The black-tailed prairie dog. Social life of a burrowing mammal. University of Chicago Press, Chicago, Illinois, USA.
- Jackson, J. E., L. C. Branch, and D. Villarreal. 1996. *Lagostomus maximus*. *Mammalian Species* **543**:1–6.
- JMP®, Version 12. SAS Institute Inc., Cary, NC, 1989-2007.
- Kotliar, N. B., B. W. Baker, A. D. Whicker, and G. Plumb. 1999. A critical review of assumptions about the prairie dog as a keystone species. *Environmental Management* **24**:177–192.
- Kotliar, N. B., B. J. Miller, R. P. Reading, and T. W. Clark. 2006. The prairie dog as a keystone species. Pages 53–64 in Hoogland, J. L. editor. Conservation of the black-tailed prairie dog: saving North America's western grasslands. Island Press, Washington, D.C., USA.
- Luce, R. J., R. Manes, and B. Van Pelt. 2006. A multi-state plan to conserve prairie dogs. Pages 210-217 in Hoogland, J. L. editor. Conservation of the black-tailed prairie dog: saving North America's western grasslands. Island Press, Washington, D.C., USA.
- Meagher, M. 1986. Bison bison. *Mammalian Species* **266**:1–8.
- Miller, B., G. Ceballos, and R. Reading. 1994. The prairie dog and biotic diversity. *Conservation Biology* **8**:677–681.

- Ponce-Guevara, E., A. Davidson, R. Sierra-Corona, and G. Ceballos. 2016. Interactive effects of black-tailed prairie dogs and cattle on shrub encroachment in a desert grassland ecosystem. *PLoS ONE* **11**:e0154748
- Reisewitz S. E., J. A. Estes, and C. A. Simenstad. 2006. Indirect food web interactions: sea otters and kelp forest fishes in the Aleutian archipelago. *Oecologia* **146**:623–631.
- Sankaran, M., D. J. Augustine, and J. Ratnam. 2013. Native ungulates of diverse body sizes collectively regulate long-term woody plant demography and structure of a semi-arid savanna. *Journal of Ecology* **101**:1389–1399.
- Scholes R. J. and S. R. Archer. 1997. Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics* **28**:517–544.
- Scott R. L., T. E. Huxman, D. G. Williams, and D. C. Goodrich. 2006. Ecohydrological impacts of woody-plant encroachment: seasonal patterns of water and carbon dioxide exchange within a semiarid riparian environment. *Global Change Biology* **12**:311–324.
- Smith, A. T. and J. M. Foggin. 1999. The plateau pika (*Ochotona curzoniae*) is a keystone species for biodiversity on the Tibetan plateau. *Animal Conservation* **2**:235–240.
- Staver, A. C., W. J. Bond, W. D. Stock, S. J. van Rensburg, and M. S. Waldram. 2009. Browsing and fire interact to suppress tree density in an African savanna. *Ecological Applications* **19**:1909–1919.
- Summers, C. A. and R. L. Linder. 1978. Food habits of the black-tailed prairie dog in western South Dakota. *Journal of Range Management* **31**:134–136.

- Underwood, J. G. and W. E. Van Pelt. 2000. A proposal to reestablish the black-tailed prairie dog (*Cynomys ludovicianus*) to southern Arizona. Nongame and Endangered Wildlife Program Draft Technical Report. Arizona Game and Fish Department. Phoenix, Arizona, USA.
- Vermeire, L. T., R. K. Heitschmidt, P. S. Johnson, and B. F. Sowell. 2004. The prairie dog story: do we have it right? *Bioscience* **54**:689-695.
- Weltzin, J. F., S. R. Archer, and R. K. Heitschmidt. 1997. Small-mammal regulation of vegetation structure in a temperate savanna. *Ecology* **78**:751–763.
- Whicker, A. D. and J. K. Detling. 1988. Ecological consequences of prairie dog disturbances. *BioScience* **38**:778–785.

Figure 1: Transect design for simulated sapling experiments. Transects originated at the center of the black-tailed prairie dog (*Cynomys ludovicianus*) colony and extended 100 m past the colony boundary. Saplings were spaced evenly along the on-colony transect segments (total distance varied per transect) and at 10 m intervals along the off-colony segment.

Figure 2: Density (a) and percent cover of shrubs (b) (*Prosopis velutina* and *Acacia greggii*) on and off of black-tailed prairie dog (*Cynomys ludovicianus*) colonies and on the control colony. Differing letters represent significant differences.

Figure 3: Mean (\pm SE; untransformed data) change in canopy area/30 days of shrubs inside and outside of exclosures placed on black-tailed prairie dog (*Cynomys ludovicianus*) colonies and on a control (a), and off of colonies (b). Differing letters represent significant differences within each panel.

Figure 4: Mean (\pm SE) percentage of “simulated” *Prosopis velutina* and *Larrea tridentata* saplings disturbed (a), degree of disturbance (b), and degree of disturbance by trial (pooled across species; c) on, on peripheries, and off of active black-tailed prairie dog (*Cynomys ludovicianus*) colonies and the control site. Differing letters indicate significant differences.

Figure 5: Mean percentage of simulated *Prosopis velutina* and *Larrea tridentata* “saplings” disturbed along transects originating at the centers (position = 0) of active black-tailed prairie dog (*Cynomys ludovicianus*) colonies and a control colony. Transects varied in length, depending upon the size of the colony, so positions are relativised such that 1-8 represent on colony, 9-11 represent the colony periphery, and 12-20 the off colony locations (see Fig. 1).

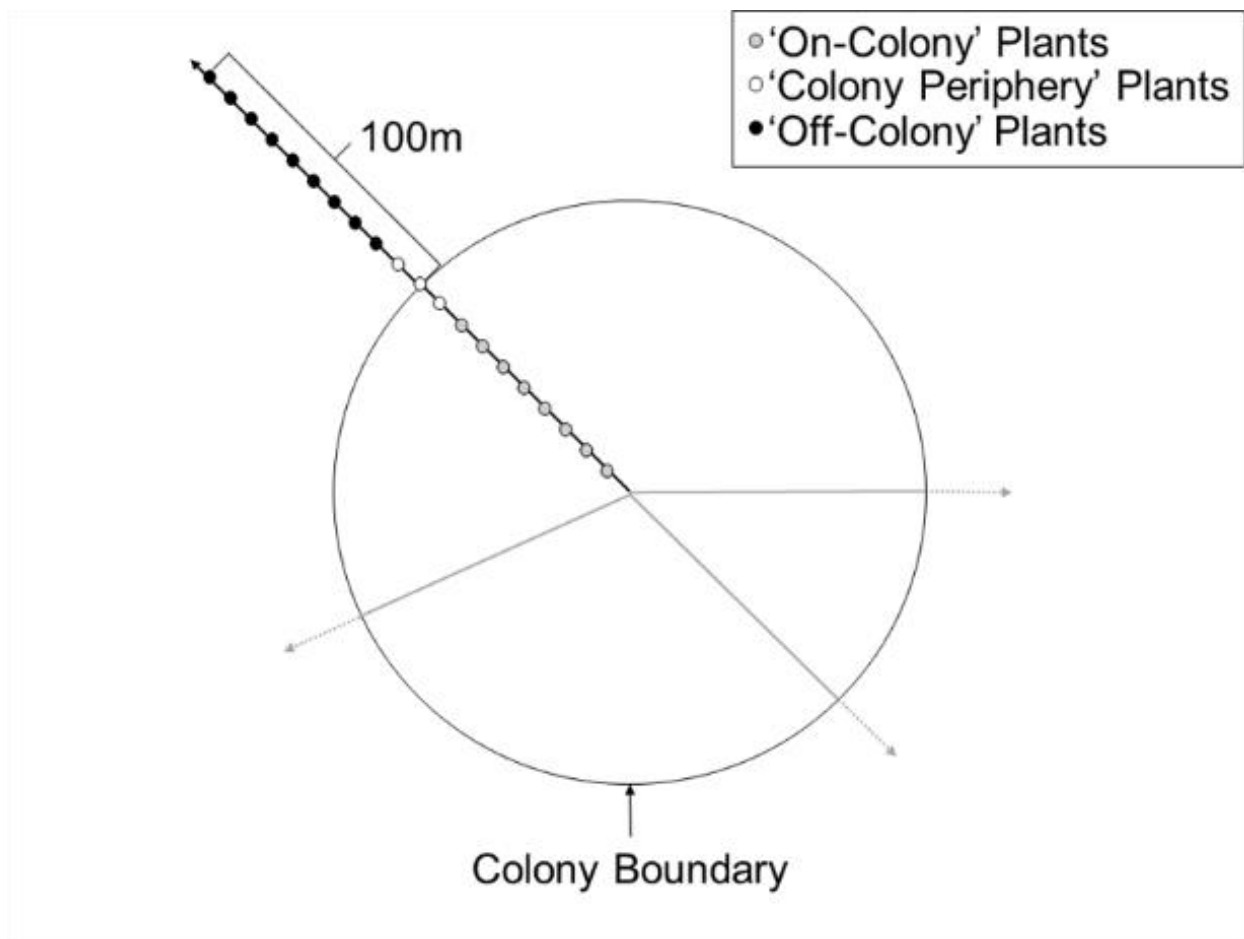


Figure 1.

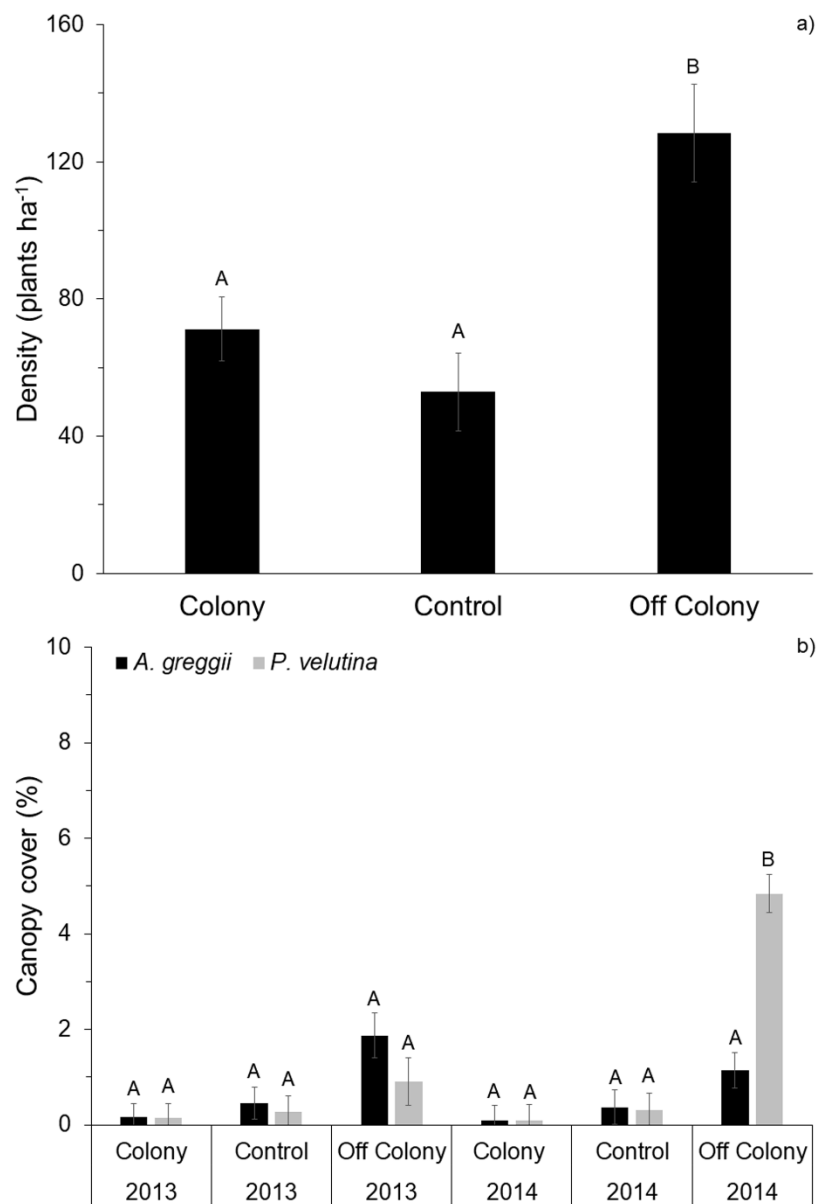


Figure 2.

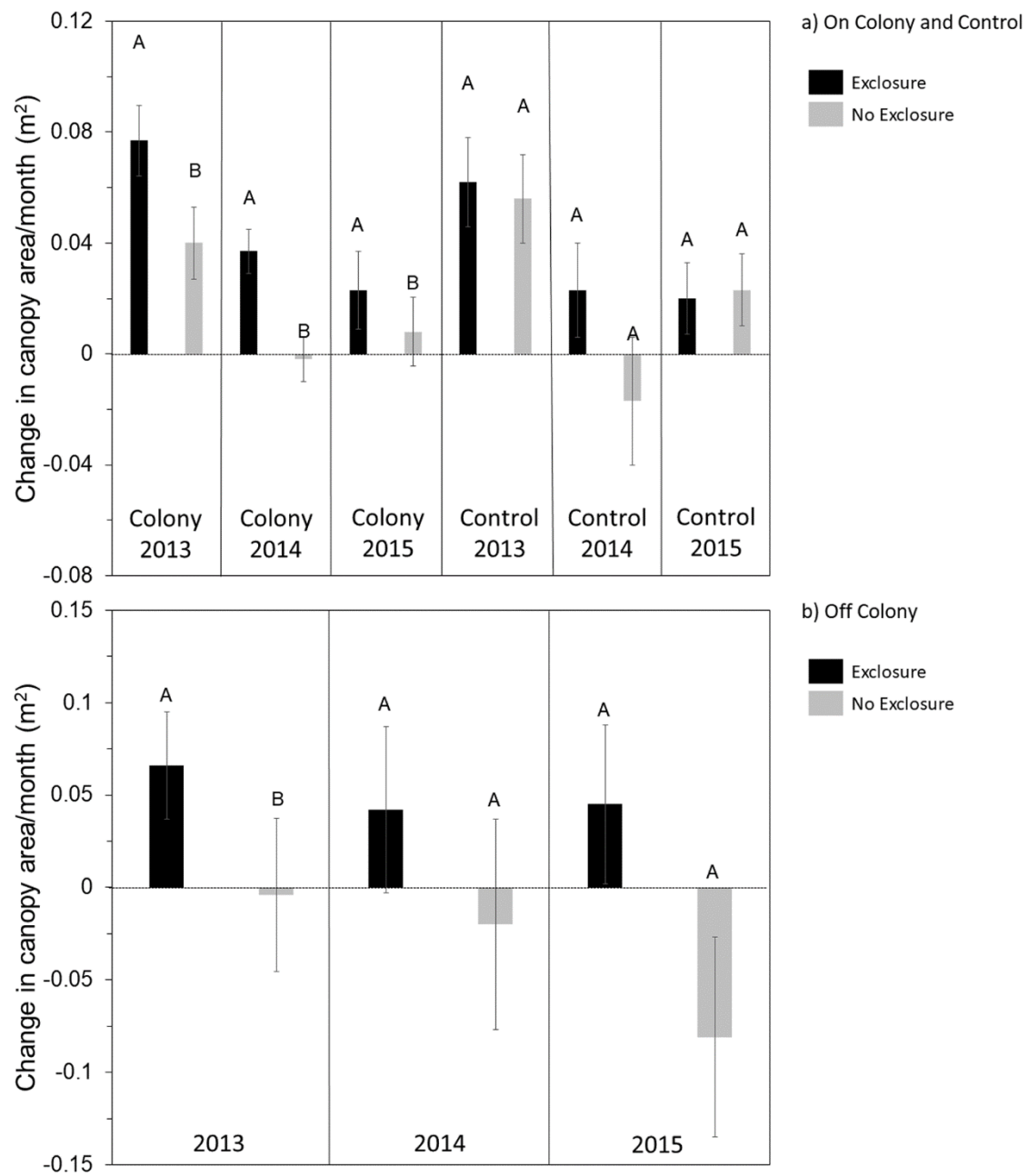


Figure 3.

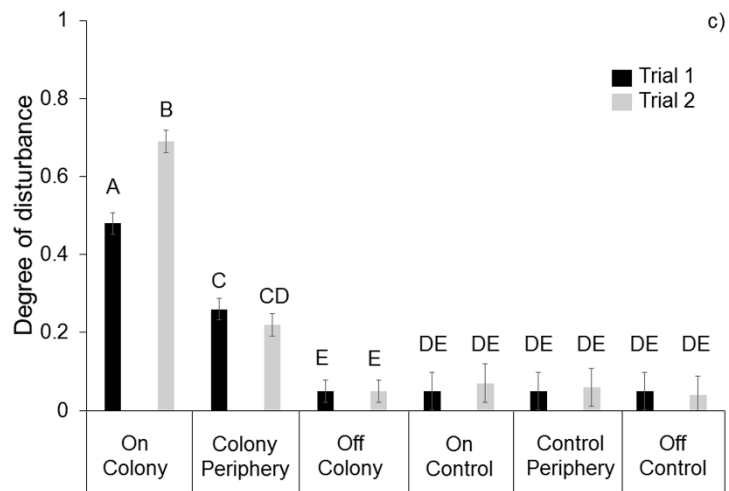
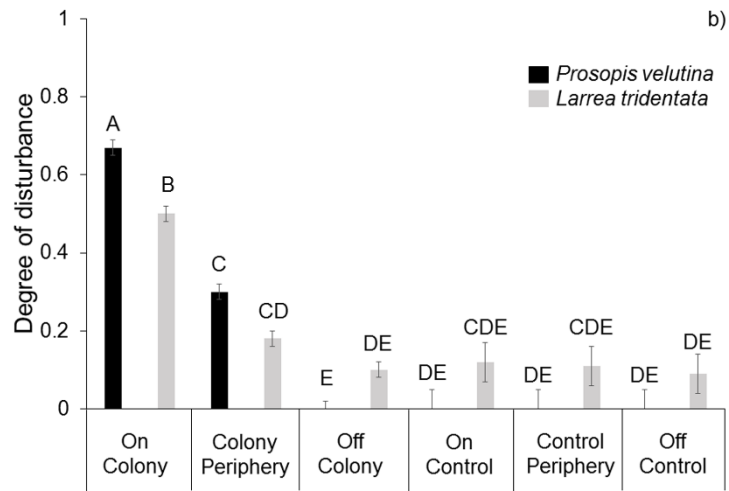
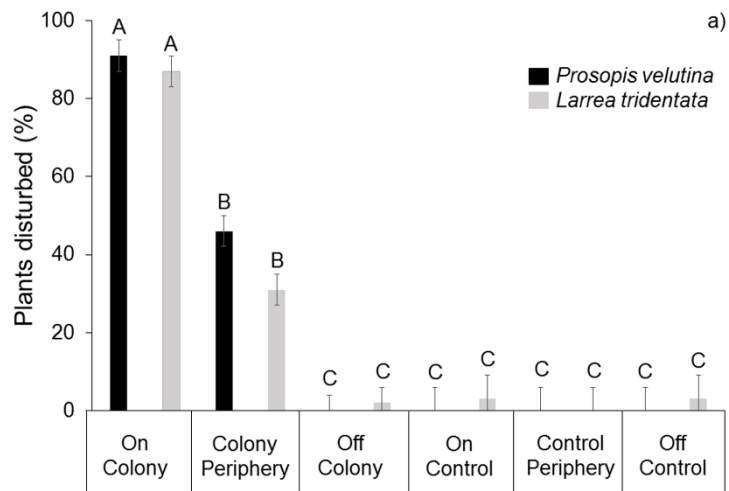


Figure 4.

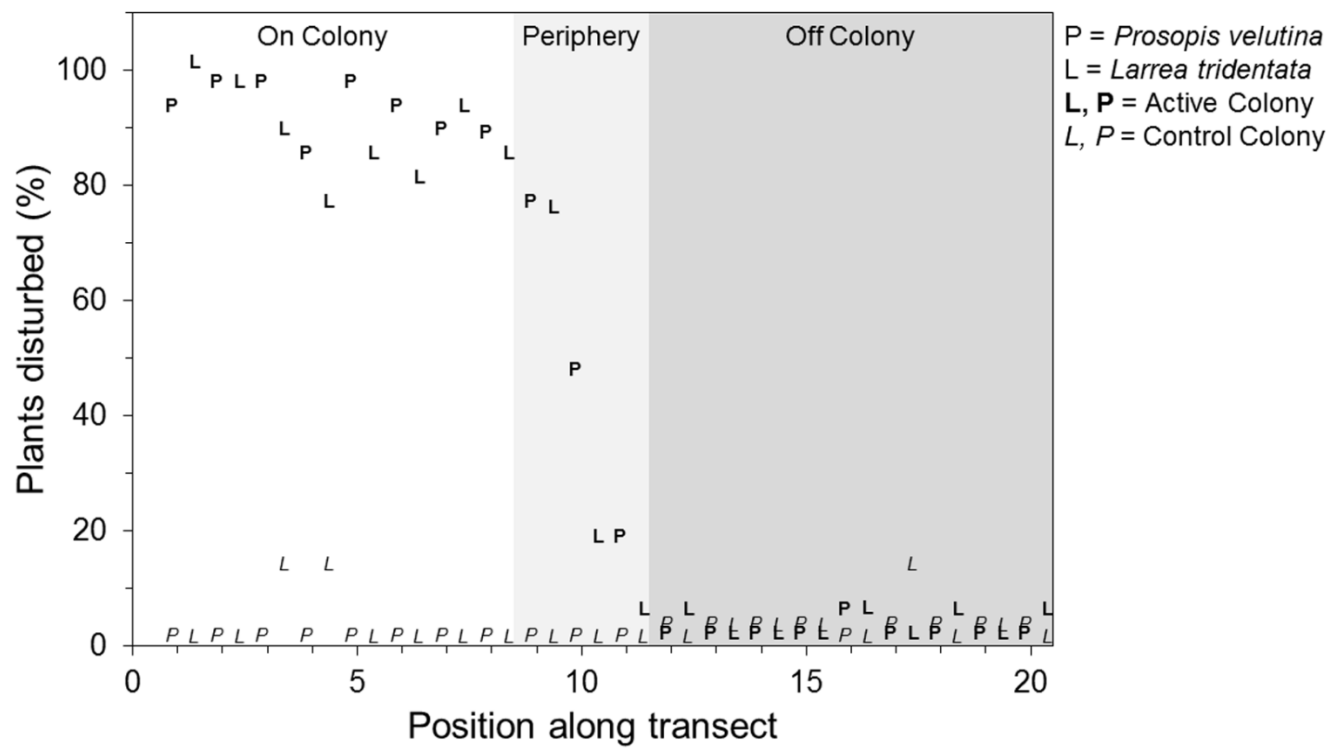


Figure 5.