COLUMBIA BASIN PYGMY RABBIT REINTRODUCTION AND GENETIC MANAGEMENT PLAN 2019
Washington Department of Fish and Wildlife

Addendum to
Washington State Recovery Plan for the Pygmy Rabbit (1995)

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ACKNOWLEDGEMENTS

This 2019 Columbia Basin pygmy rabbit reintroduction and genetic management plan is an update of the 2011 reintroduction and genetic management plan (Becker 2011). This plan summarizes information developed by a number of people involved with captive and semi-wild breeding and conservation of the Columbia Basin pygmy rabbit, including: Hannah Anderson, Penny Becker, Michelle Eames, Stephanie DeMay, Heather Fuller, Katie Haman, Ilai Keren, Peter Lancaster, Kristin Mansfield, Matt Monda, Stacey Nerkowski, Janet Rachlow, Michael Schroeder, David Shepherdson, Lisa Shipley, Matt Vander Haegan, David Volsen, Lisette Waits, JoAnn Wisniewski, and Brian Zinke.

Photo credit for the cover: Brian Zinke.

EXECUTIVE SUMMARY

This document summarizes pygmy rabbit (*Brachylagus idahoensis*) reintroduction activities in central Washington since 2011, outlines fundamental principles guiding decision-making, and presents the Washington Department of Fish and Wildlife's reintroduction strategy for the next several years. It is a consolidated update of the 2011 Reintroduction and Genetic Management Plan (Becker et al. 2011). A comprehensive outline of strategies and tasks needed to attain population viability of the Columbia Basin (CB) pygmy rabbit are provided in the Washington State Recovery Plan (WDFW 1995). A periodic status review of the CB pygmy rabbit and the factors affecting its status was recently completed (Hayes 2018). Based on the recommendation in that document, the Washington Fish and Wildlife Commission maintained the state endangered classification.

In 2011, the reintroduction strategy transitioned to breeding pygmy rabbits in on-site breeding enclosures in shrub-steppe habitat and augmenting captive-bred CB rabbits with wild rabbits translocated from beyond the Columbia Basin in order to provide adequate numbers of rabbits for releases and to improve genetic diversity. Sagebrush Flat (Douglas County) was identified as the highest priority site for reintroductions. Two permanent breeding enclosures were established on WDFW's SBF Wildlife Area within the Sagebrush Flat Recovery Area. From 2011 to 2013, 112 adult pygmy rabbits were captured from out of state populations in Oregon, Nevada, Utah, and Wyoming and translocated to semi-wild breeding enclosures at SBF and mixed with captive-bred CB pygmy rabbits. A third and fourth permanent breeding enclosure were established in the Beezley Hills Recovery Area and Burton Draw Recovery Area. From 2011 to 2019, 2,246 kits were produced in semi-wild breeding enclosures and 1,518 rabbits were released at Sagebrush Flat. In the Beezley Hills Recovery Area, a release of 479 rabbits failed to establish a population in 2015 but reintroductions were later resumed in this recovery area with the release of 50 rabbits in 2017-2019. In the Burton Draw Recovery Area, 38 rabbits were released in 2018-2019.

Non-invasive genetic monitoring of fecal DNA was used to evaluate demographic and genetic status of reintroduced populations (DeMay et al. 2016, DeMay et al. 2017). For rabbits released from 2012 to 2014, apparent survival (winter detections) was 39%, 13% and 10%, respectively (DeMay et al. 2017). In the Sagebrush Flat Recovery Area, the percentage of wild-born rabbits increased from 9% in the early years of releases to between 88 and 100% in later years. Heterozygosity, a measure of genetic diversity, averaged 0.747 (range 0.701 – 0.789) from 2012-2019, remaining stable within this period. Currently, the genetic diversity of both the enclosure and wild populations in the Columbia Basin are similar to values observed in Great Basin populations. Average percent composition of Columbia Basin ancestry within individual rabbits declined from 70% in the fully captive pygmy rabbits (USFWS 2012) to 22-24% (range 20.8% to 28.7%) in the semi-wild population in 2019. The remaining genetic composition averages about 67% from the collective Great Basin population (NV/OR/ID), 10% from the WY/ UT (northern) population, and <1% from the UT population (southern). The transition to on-site, semi-permanent, large breeding enclosures to support reintroduction efforts was successful in producing adequate numbers of kits to support large-scale releases through 2016.

While the pygmy rabbit recovery effort has had many successes, including early signs indicating establishment of a wild population at SBF, the program experienced some setbacks beginning in 2015. In 2015, a disease outbreak of coccidia occurred in all four breeding enclosures and resulted in an associated decline in adult survival, kit production, and survival of released kits. Habitat conditions within breeding enclosures also declined. Both high rabbit densities and long durations of occupancy of rabbits in enclosures likely contributed to degraded habitat conditions by decreasing natural forage and increasing

invasive weeds as a result of soil disturbance, soil compaction, and seeds introduced from supplemental food. Further, in 2017 the largest and most productive breeding enclosure at Beezley Hills, along with nearly half the semi-wild breeding population, was lost from a wildfire and resulted in suspension of additional releases that year. Over the next two years, overwinter mortality was high and productivity was low which resulted in low numbers of kits available for releases. To address degraded habitat conditions and disease prevalence in enclosures the project transitioned to a smaller, mobile breeding enclosure design that would allow relocating the enclosures to new locations in shrub-steppe every 2-3 years. On-going risk to the SBF population from wildfire prompted resuming reintroductions to establish new populations in other recovery areas.

Given that kit production in the permanent breeding enclosures was insufficient to support releases or sustain rabbit populations within the enclosures, the Pygmy Rabbit Science Team supported the capture of a small number (20-30) of rabbits (kits/adults) from the wild SBF population for translocation to mobile breeding enclosures and/or temporary release pens in BH and BD Recovery Areas. In the summer of 2018, 19 kits were captured from the wild SBF population and translocated to the single mobile breeding enclosure at BH and to temporary release pens at BH and BD Recovery Areas. Results from implementing the new mobile breeding enclosures were positive with increased adult survival and kit production, declining coccidia and parasite levels, and significantly reduced labor required for maintenance. However, record snowfall in February and snowmelt in late March of 2019 prevented access to field sites to construct additional mobile breeding enclosures at BH and BD Recovery Areas, or the capture of 20-30 adults from the wild SBF population for translocation to the two mobile breeding enclosures before the breeding season began in March. Attempts to capture kits from the wild SBF population in the summer of 2019 resulted in low trapping success. Two additional mobile breeding enclosures were completed in the fall of 2019 and constructed on-site at BH and BD Recovery Areas. Additional attempts to capture a small number of kits from the wild SBF population in the fall of 2019 for translocation to BH and BD Recovery Areas resulted in no rabbits captured. Findings from the summer and fall trapping in 2019 at the wild SBF population indicated that this population had experienced reduced survival or productivity and therefore was unsuitable as a source of rabbits for in-state translocation in 2020.

Reintroductions necessary to support establishment of new populations at BH and BD require obtaining pygmy rabbits from out-of-state sources in 2020. The Department is in communication with other state agencies on the availability of rabbits for translocation to central Washington in March of 2020. Pygmy rabbit populations in the Great Basin (California, Nevada, Oregon, Idaho, and Montana) are the highest priority region for genetic or demographic rescue to the CB population, followed by northern Utah/Wyoming region, and lastly the southern Utah region.

INTRODUCTION

This document summarizes pygmy rabbit (*Brachylagus idahoensis*) reintroduction activities in central Washington since 2011, outlines fundamental principles guiding decision-making, and presents the Washington Department of Fish and Wildlife's reintroduction strategy for the next several years. It is a consolidated update of the 2011 Reintroduction and Genetic Management Plan (Becker et al. 2011). A comprehensive outline of strategies and tasks needed to attain population viability of the Columbia Basin (CB) pygmy rabbit are provided in the Washington State Recovery Plan (WDFW 1995). A periodic status review of the CB pygmy rabbit and the factors affecting its status was recently completed (Hayes 2018). Based on the recommendation in that document, the Washington Fish and Wildlife Commission maintained the state endangered classification.

The CB pygmy rabbit is genetically distinct from other pygmy rabbit populations throughout its species range (Warheit 2001). The CB distinct population segment (DPS) of the pygmy rabbit was emergency listed under the U.S. Endangered Species Act as endangered in 2001, with a final rule maintaining federal endangered status in 2003 (USFWS 2003). An updated DPS assessment was completed in 2010 (USFWS 2010) and a federal recovery plan for the Columbia Basin DPS was finalized in 2012 (USFWS 2012).

Beginning in 2011, the entire captive population of pygmy rabbits was transitioned to controlled field breeding enclosures within their former range. From 2011 to 2019, releases of 2,035 rabbits to the wild have resulted in a population estimated at 150-250 adults at Sagebrush Flat and a small number of rabbits in the wild (<30 adults each) in both the Beezley Hills and Burton Draw Recovery Areas as of 2019.

FUNDAMENTAL PRINCIPLES GUIDING REINTRODUCTIONS

The goal of the recovery program for the CB pygmy rabbit is to increase the number, distribution and security of free-ranging populations to ensure a high probability of the population's long-term persistence within its historical distribution so that it may eventually be delisted (WDFW 1995, USFWS 2012). As such, recovery actions with respect to reintroductions require consideration of both: 1) ensuring adequate reproductive output in breeding enclosures to support large-scale releases of kits to the wild, and 2) maintaining as much of the unique genetic characteristics of the CB population as possible. The reintroduction plan was amended in 2011 because of the need for both genetic and demographic rescue to maintain the CB population in Washington (Becker et al. 2011). The amended reintroduction strategy addressed the demographic and genetic obstacles encountered during the captive breeding program by transitioning to managed breeding in on-site enclosures in shrub-steppe where captive -bred rabbits were mixed with translocated wild rabbits from other source states outside the Columbia Basin. The Department continues to engage in a collaborative effort with the USFWS and the Pygmy Rabbit Science Team in providing guidance to the Department on balancing demographic and genetic considerations with reintroductions. Principles that guide reintroductions are prioritized as follows:

1. Establish multiple pygmy rabbit populations of sufficient size, number, and distribution to minimize risks from demographic, environmental, and genetic stochasticity and natural catastrophes (e.g., wildfires) and to ensure long-term population viability of this population segment in Washington.
    a. Continue recovery actions for the Sagebrush Flat to ensure a self-sustaining population.
    b. Build on recent reintroductions at Beezley Hills (BH) and Burton Draw (Dormaier, BD) to establish pygmy rabbit populations at these two new sites.

2. Maintain access to pygmy rabbits by maintaining semi-wild breeding enclosures to produce sufficient numbers for large-scale releases to the wild.
3. Manage the genetic diversity of this population segment in Washington.
    a. Maintain the unique Columbia Basin ancestry in pygmy rabbit populations in Washington.

NATURAL HISTORY

Comprehensive reviews of the status of the Columbia Basin pygmy rabbit and potential threats to the population are provided in status assessments by the U.S. Fish and Wildlife Service (USFWS 2010) and Washington Department of Fish and Wildlife (Hayes 2018).

The pygmy rabbit is the smallest rabbit species in North America. Adults weigh 375 to about 500 g (0.83-1.1 lb), and measure 23.5-29.5 cm (9.3-11.6 in) in length with females tending to be slightly larger than males (Gahr 1993, USFWS 2003). Overall pelage color is slate-gray, tipped with brown; legs, chest and nape are a tawny cinnamon brown; the ventral surface is buff; and the edges of their ears are pale buff. A single annual molt occurs, usually in mid to late summer. The pygmy rabbit is distinguished from other rabbit species within its range by its relatively small size; small hindfoot; short, rounded ears, and short tail that's buff in color rather than white on the underside (WDFW 1995, Chapman and Litvaitis 2003, USFWS 2003).

Pygmy rabbits are found in shrub-steppe habitat within the Temperate Desert Ecoregion in western North America as described by Bailey (1998). This includes the Columbia Basin of Washington and the Columbia Plateau and Great Basin of Oregon, Idaho, Montana, Wyoming, Utah, California, and Nevada of the United States. Nearly the entire historical distribution of the Columbia Basin pygmy rabbit overlaps the big sagebrush (*Artemisia tridentata*) – bluebunch wheatgrass (*Pseudoroegneria spicata*) zonal habitat type (Daubenmire 1988).

Pygmy rabbits are sagebrush obligates (Heady and Laudré 2005). Pygmy rabbits have a patchy distribution and are typically found in areas of tall, dense sagebrush (*Artemisia* spp.) and deep, sandy-loam soils (Green and Flinders 1980, Weiss and Verts 1984, Gahr 1993, Katzner and Parker 1997, Larrucea and Brussard 2008). Dense stands of sagebrush provide pygmy rabbits with year round food and cover (Green and Flinders 1980, Weiss and Verts 1984, Schmalz et al. 2014), while native, perennial grasses and forbs provide an important food source beginning in spring and especially in summer and fall (Green and Flinders 1980, Thines et al. 2004, Schmalz et al. 2014). Deep, loose soil allows pygmy rabbits to construct burrows for shelter and to give birth to their young (Janson 1946, Rachlow et al. 2005).

Pygmy rabbits begin breeding after their first winter (Gahr 1993, Shai-Braun and Hackländer 2016). Breeding is highly synchronous and promiscuous. Male reproductive activity begins in January, peaks in March, and declines in June. Females can be pregnant from late February through June and nurse young from March through September. Most females become pregnant and produce kits (Elias et al. 2006, DeMay et al. 2016). About 2-3 weeks after mating, but a week before giving birth, the female excavates a single-entrance natal burrow at a location separate from the residential burrow system. The natal burrow terminates at a nest chamber that the female lines with grass and fur (Rachlow et al. 2005, Elias et al. 2006). After a gestation period of about 24 days the female opens the entrance to the natal burrow and gives birth to her young that are born with their eyes closed and with little fur (Elias et al. 2006). In some parts of the species' range, females may have up to three litters per year and average six young per litter

(Green 1978; Wilde 1978). Females in the semi-captive population at Sagebrush Flat had an average of 2.5 litters per year (range 1 - 5) and an average of 7.5 kits per breeding females per year (DeMay et al. 2016). After giving birth, females cover the entrance to the natal burrow with soil, presumably to conceal the location and protect against predators. Females open the natal burrow prior to nursing the young once or twice daily. After nursing, young remain in the natal burrow. Juveniles permanently emerge from the natal burrow two weeks after birth and are first observed in March (DeMay et al. 2016). Nursing ceases by about two weeks post-emergence (Elias et al. 2006).

Median natal dispersal distances are three times farther for juvenile females (2.9 km, range = 0.02-11.9 km [1.8 mi, range = 0.01-7.4 mi]) than juvenile males (1.0 km, range = 0.03-6.5 km [0.6 mi, range = 0.01-4.0 mi]) in Idaho (Estes-Zumpf and Rachlow 2009). Juveniles monitored with telemetry at Sagebrush Flat remained close (mean 204 m, range 0 m -1.5 km [669 ft, range 0 ft-0.93 mi]) to release sites but this method likely underestimated dispersal based on evidence from fecal DNA (DeMay et al. 2017).

Pygmy rabbits generally live less than three years (Sanchez 2007, Zeoli et al. 2008) though survival rates can be highly variable among study sites, years, and sexes (Sanchez 2007, Crawford et al. 2010, Price et al. 2010). In east-central Idaho, juvenile mortality was 69.2% and 88.5% for male and females, respectively, with the highest mortality occurring within the first two months of emergence from natal burrows (Estes-Zumpf and Rachlow 2009). Annual adult survival of males and females in Idaho ranged from 0.07 to 0.45 (Sanchez 2007) and 0.003 to 0.173 in Oregon and Nevada (Crawford et al. 2010).

BREEDING AND GENETIC MANAGEMENT OF PYGMY RABBITS

This section is a summary of activities, methods, and results for breeding and genetic management of the semi-wild pygmy rabbit population within the breeding enclosures from 2011 to 2019. Becker et al. (2011) outlined the strategy and justification for transitioning the remaining CB pygmy rabbits from captivity to semi-wild enclosures within their former range and the translocation of wild pygmy rabbits from Great Basin populations to provide adequate numbers of breeding adults and improve genetic diversity. The purpose of the semi-wild breeding population has remained the same since 2011, to produce adequate numbers of juveniles (kits) for release into the wild for population establishment.

Semi-wild Breeding Program and Out-of-State Translocations

From 2011 to 2013, 112 adult pygmy rabbits were captured from Great Basin populations and translocated to the semi-wild breeding enclosures. Source populations included Oregon (24 rabbits), Nevada (34), Utah (24), and Wyoming (30). These translocated rabbits joined the captive-born CB/Idaho cross pygmy rabbits in multiple large breeding enclosures. This approach was very successful, as the number of kits born within the breeding enclosures averaged 415 kits/year from 2012-2016 (range 150-794) and easily achieved reintroduction objectives of releasing large numbers of kits into the wild while retaining some to sustain breeding enclosure population. The size of this breeding population varied annually, ranging from 20-190 adults.

DeMay et al. (2016) analyzed the breeding effort dynamics within the breeding enclosures. Overall productivity was good, with an average of 7 kits/female and 2.5 litters/female documented. Breeding success was variable between different enclosures and breeding seasons, ranging from 4.7 – 14.1 kits/female. Factors associated with higher female productivity were lower density of breeding females (5-10 vs 16-22 adults/ha) and higher genetic diversity. The decline in female reproductive output with increasing rabbit density might have been attributed to increased competition among females for natal burrow sites or increased stress of breeding females (DeMay et al. 2016).

Genetic diversity of kits produced in the breeding enclosures, measured as heterozygosity index (proportion of loci that have multiple alleles, 0 being least diverse where the population is fixed at a single allele and 1 being most diverse) has averaged 0.747 (range 0.701 – 0.789) from 2012-2019, remaining stable within this period (S. Nerkowski, *unpubl. data*). The current genetic diversity of both the enclosure and wild populations in the Columbia Basin are similar to values observed in populations within the Great Basin and nearly double the diversity observed in the last pure CB population (Warheit 2001).

Current Status of Semi-wild Breeding Program

Maintaining a sustainable and genetically healthy semi-wild breeding pygmy rabbit population will always be challenging as they are not immune to biological limitations or stochastic events. The semi-wild breeding program experienced several significant problems from 2015-2018. In 2015, we observed a severe coccidia outbreak within the entire breeding enclosure population. This parasitic protozoan is unique to CB pygmy rabbits and is particularly lethal to kits (Duszynski et al. 2005, Becker et al. 2011). Following this outbreak, we observed extremely low survival of released kits and a 74% decline in kit production in the 2016 breeding season.

The most significant single event was the devastating loss of the largest and most productive breeding enclosure (BH) along with nearly half the semi-wild breeding population from the 2017 Sutherland Canyon wildfire. A minimum 85 pygmy rabbits were killed in the fire, while 32 were rescued and relocated to the remaining breeding enclosure sites. Mark-recapture census efforts of the remaining breeding enclosure population were 76 rabbits (range 61-94 ± 2.8 SE), as of October 2017 (Gallie and Zinke 2018). Most of these rabbits were kits, which we typically observe 60% annual survival when retained as future breeders in the enclosures.

The enclosure experienced unusually high over-winter mortality of rabbits during the winter of 2017-18. Only 20% of the rabbits observed in October 2017 were found during the 2018 breeding season. The remaining breeders then displayed the lowest productivity yet observed in the breeding enclosures, with 2.4 kits/female (WDFW *unpubl. data*). We concluded the low survival and productivity were the culmination of multiple independent factors rather than a single event. Nearly half the documented rabbits in October 2017 were rescued from the fire. While they appeared to look healthy in the following months after the fire, we believe lingering effects (smoke inhalation, stress) contributed to higher mortality over the winter. The poor productivity was likely the result of continued coccidia infection, site degradation of the enclosure habitat, or possibly general fitness loss in the enclosure lineage. The decline in productivity of the enclosures appeared to be a gradual one even before the stochastic events. Female productivity was highest the first year a breeding enclosure was in use and subsequently declined in future years of operation (Gallie and Zinke 2018).

To begin rebuilding the enclosure breeding population, we translocated pygmy rabbits from the wild SBF population into the replacement BH breeding enclosure. Seven kits were captured in the summer of 2018 and placed into the enclosure. Results of this strategy were positive with increased adult survival and kit production (10 kits/female) and lower coccidia and parasite levels (WDFW *unpubl. data*). Following this successful trial, we looked to capture additional pygmy rabbits from the SBF population and transfer them into two additional breeding enclosures in 2019. We were unable to accomplish this due to poor weather in the early spring and subsequently abandoned this strategy when poor trapping success indicated an apparent decline had occurred in the SBF population.

Genetic Management of Columbia Basin Ancestry

Genetic monitoring of the breeding enclosure and wild population within the Columbia Basin initially utilized microsatellite markers and in 2016 transitioned to SNPs (single nucleotide polymorphisms). The goal was to document the CB ancestry within these groups and how it changes over time. The semi-wild breeding enclosures facilitated free mate choice among adults, which limited the ability of project managers to directly influence the CB ancestry in the resulting offspring. CB ancestry estimates were calculated from the parentage analysis and percent captive (CB/ID) ancestry. Overall genetic ancestry was estimated using a Bayesian clustering software (Structure 2.3.4) where founding rabbits were grouped into four genetic clusters: (1) Washington (captive CB/Idaho), (2) Nevada/Oregon, (3) northern Utah/Wyoming, and (4) southern Utah (DeMay et al. 2016). In 2018, we obtained tissue samples of pure CB pygmy rabbits and were able to update our reference populations, which allowed us to genetically isolate CB ancestry. The Idaho ancestry that had previously been grouped in the captive ancestry (average of 4.4%) was now in the same grouping as the Nevada/Oregon cluster.

Since 2016 (three breeding seasons following the last translocation of out of state rabbits), all offspring produced in the enclosures and wild-born rabbits contain a portion of CB ancestry (9.70 – 54.60%) (Nerkowski, *unpubl. data*). The average percent composition of CB ancestry represented within individual pygmy rabbits declined 60% between 2011 and 2016 (S. Nerkowski, *unpubl. data*). Percent captive ancestry in enclosure rabbits averaged 23.1% (range 11.52 – 40.33%) during 2016-2018 and CB ancestry averaged 20.18% (range 12.16 – 28.26%) in 2018-2019. In the established wild populations, the percent captive ancestry (CB/ID) averaged 27.16% (range 11.82 – 54.60 %) from 2015-2017 and CB ancestry averaged 21.81% (range 9.70 – 53.84%).

Initial results following the translocation of out of state pygmy rabbits found males with >50% CB ancestry had higher reproductive output than the other ancestry groups. Males with higher northern Utah/Wyoming ancestry and females with high levels of Nevada/Oregon ancestry had the lowest reproductive output (DeMay et al. 2016). As of 2019, the predominant ancestry in both the enclosures and wild populations represent the Nevada/Oregon/Idaho ancestry (~67%), followed by the Columbia Basin, and Wyoming/northern Utah ancestry (~14%).

RADSeq (Restriction site associated DNA sequencing) was used to generate the first genome-wide set of SNP markers for the species (Ali et al. 2016). Analysis of the SNPs revealed four genetic clades among pygmy rabbit populations (congruent with the microsatellite findings): (1) Columbia Basin, (2) Great Basin (Nevada/Oregon/Idaho/Montana/California), (3) Wyoming/northern Utah, and (4) southern Utah (Figure 1, Table 1). Current analyses are identifying adaptive loci and private alleles for each of the regions and development of a SNP panel and to evaluate their possible effects on fitness.

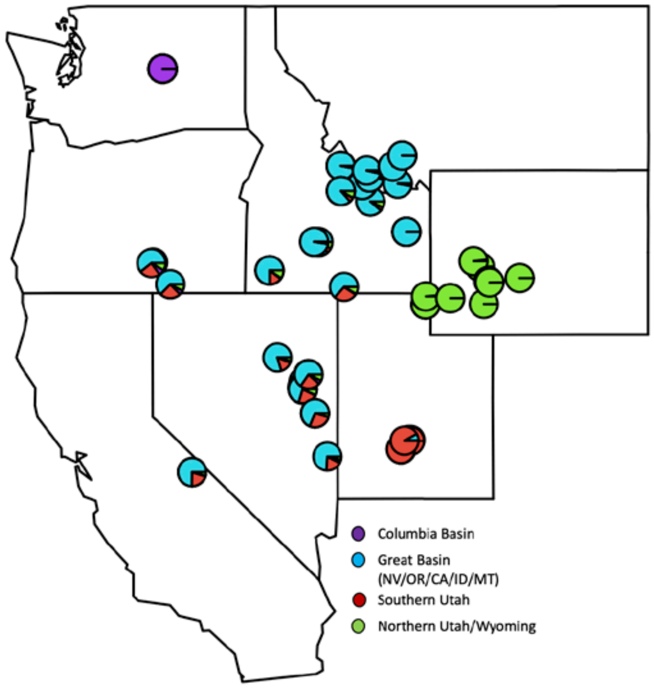

Figure 1. Location of the four genetic clades (Columbia Basin, Great Basin, southern Utah, and northern Utah/Wyoming) of the pygmy rabbit identified through SNP analysis. Pie charts represent the proportion of each of the four genetic clades.

Table 1. F_{ST} values between each of four identified pygmy rabbit clades using 12,084 SNPs.

	WY/N-UT	UT-S	Columbia Basin
Great Basin (NV/OR/ID/MT/CA)	0.106	0.111	0.251
WY/N-UT		0.202	0.351
UT-S			0.365

REINTRODUCTIONS BY RECOVERY AREA: 2011-2019

The following section provides a synopsis of reintroduction activities and results for each Recovery Area. Pygmy rabbits have been reintroduced in all three Recovery Areas (Table 2, Figure 2), beginning with the release of the last 42 kits born in captivity in 2011. Since then, 1,969 enclosure-born pygmy rabbits have been released. The number of pygmy rabbits released was highly variable among years reflecting variability in the size and productivity of the semi-wild breeding population. The vast majority of these pygmy rabbits were kits, except for 165 adults released in 2014 and 2015 to reduce crowding in the enclosures. During 2018 and 2019, small-scale translocations of wild born kits between Recovery Areas were conducted, with 24 wild kits captured in the SBF Recovery Area and released in the BH and BD Recovery Areas. Kits were released primarily from April-August, except for 2014 and 2015 when releases occurred from March through November.

Table 2. Number of kits born in breeding enclosures and released into the wild among three Recovery Areas, central Washington, 2011-2019.

	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Kits Produced	0	150	381	794	590	157	122	17	35	2246
Rabbits Released	42	104	272	830	579	120	26	27	35	2035
Sagebrush Flat	42	104	272	830	150	120	0	0	0	1,518
Beezley Hills	0	0	0	0	429	0	26	10	14	479
Burton Draw	0	0	0	0	0	0	0	17	21	38

Recovery Areas

Becker et al. (2011) details the selection process for identifying areas suitable for reintroductions (formally known as Recovery Emphasis Areas- REAs). The general criteria included: 1) previous known occurrence of pygmy rabbits, 2) access and compatible land management, and 3) landscape with enough suitable habitat available to support a viable pygmy rabbit population. SBF (Douglas County) and BH (Grant County) were ranked as the number one and two priority reintroduction areas, respectively, by the Science Advisory Group and documented in the federal recovery plan (USFWS 2012). The third reintroduction site (BD- Douglas County) was added after completion of the federal recovery plan.

Reintroductions occurred from 2011-2016 in SBF and then began at BH in 2015. In 2018, reintroductions began at Burton Draw, occurring sooner than anticipated. Wildfires had destroyed nearly half of the shrub-steppe habitat in the BH Recovery Area from 2010 to 2018. In response to wildfire risk, releases efforts were divided between BH and BD beginning in 2018 to reduce potential loss from these catastrophic events.

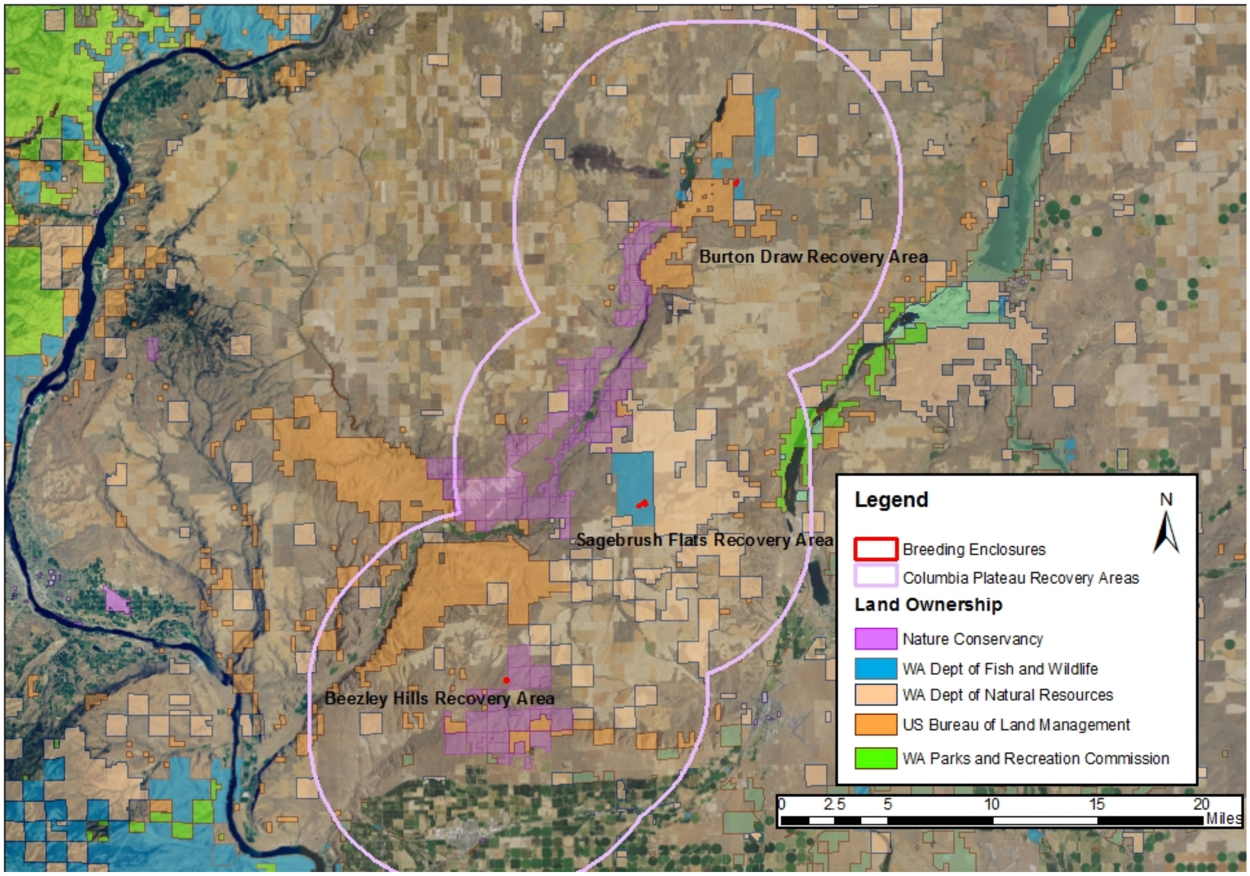

Figure 2. Recovery Areas for the CB pygmy rabbit in Grant and Douglas Counties of the Columbia Basin, Washington.

Reintroduction Activities at Sagebrush Flat: 2011-2016

The priority area for reintroduction, SBF (Figure 3) was the location of the last native population of CB pygmy rabbits. We utilized several release methods including soft-release pens, hard releases with artificial burrows, and hard releases with natural burrows. Soft-release pens (Appendix A) were used during 2011-12. The increasing number of kits being released necessitated a transition to a hard-release method using artificial burrows (Appendix A) that was initially tested in 2012 and then became the primary method through 2015. In 2016, hard releases began to use inactive pygmy rabbit burrow systems instead of artificial burrows.

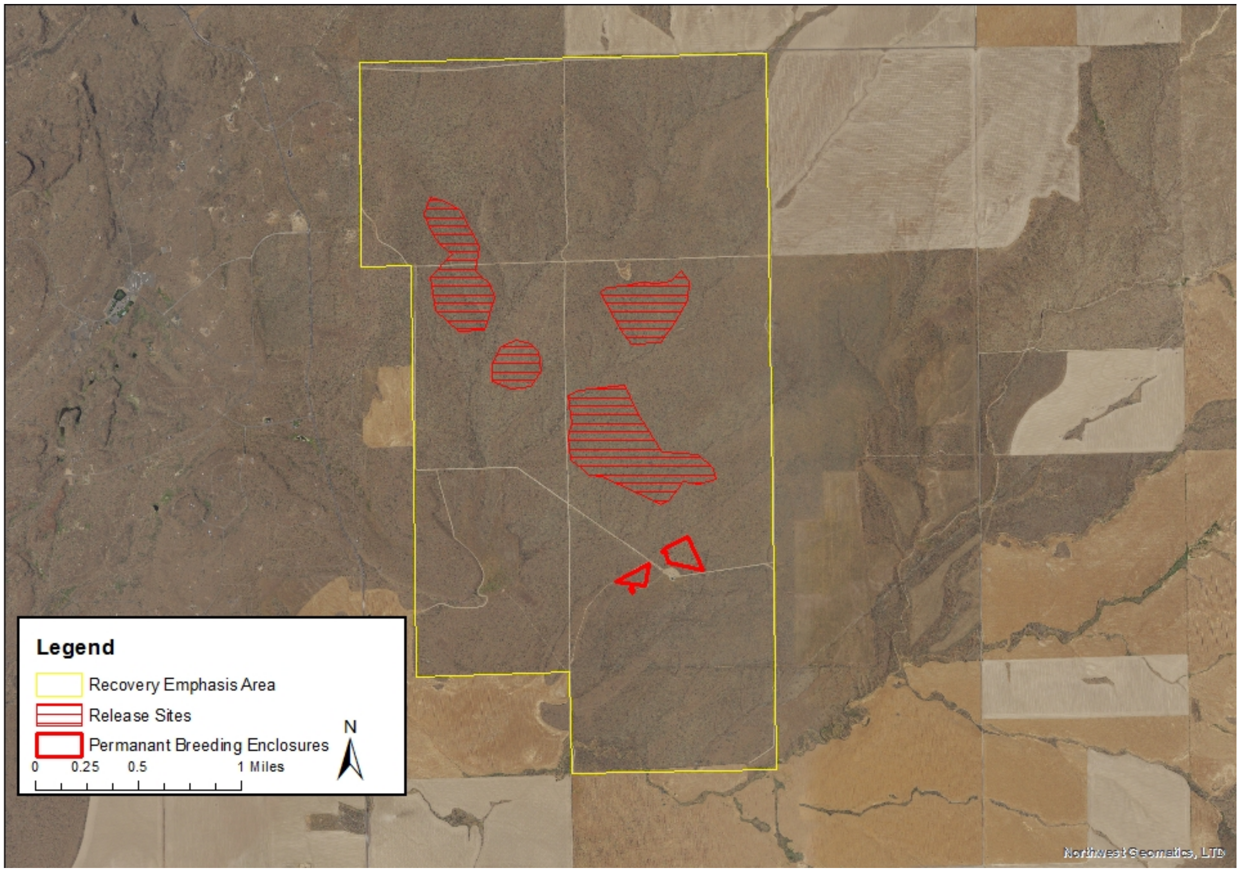

Figure 3. Sagebrush Flat Recovery Emphasis Area (owned by WDFW). Release site polygons used from 2011-2016 and included soft release pens, artificial burrows, natural burrow methods. Permanent breeding enclosures established in 2011.

There was significant annual variation in post-release kit survival (Table 3). Post-release survival of kits was approximately 10% using soft-release pens and ranged from 37% to <1% (averaging 15%) with hard releases using artificial burrows. This variation suggests numerous factors beyond release method affect post-release kit survival. Survival of kits released at inactive pygmy rabbit burrows was only 4%.

Wild reproduction on the release area was first documented in 2012. Initially, the proportion of wild born pygmy rabbits detected on surveys was low, comprising <10% of all free ranging pygmy rabbits on the release area from 2011-2015 (Table 3). This began to change following 2015, when most of the pygmy rabbits detected during winter surveys were wild born. Since the winter of 2016-2017, every pygmy rabbit detected on SBF has been wild born.

The SBF population, indexed by winter burrow counts and the minimum number of individuals identified using noninvasive genetic analyses, increased rapidly beginning in 2016 (Table 3). The rapid increase in the SBF population was correlated with the proportion of breeding adults being wild born and a distribution shift from the native shrubsteppe habitat to adjacent fields enrolled into the Conservation Reserve Program (CRP). These fields provide excellent pygmy rabbit habitat with deep soils and early to mid-seral stage shrubsteppe community (20-30 years old). Currently, 80-90% of the wild population at SBF occurs in CRP field habitat (Table 4).

Table 3. Summary data from winter burrow surveys and genetic analysis of fecal pellets of CB pygmy rabbits among three Recovery Areas in central Washington from 2012 to 2019.

	Winter Survey Year						
	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	2018/19
Sagebrush Flat RA							
# of Active Burrows	52	64	174	88	146	336	306
% of Released Kits Detected (est. survival rate)	37	11	10	<1	4	-	-
# of Genetically Identified Pygmy Rabbits	45	44	91	17	62	175	140
% of Identified Pygmy Rabbits being Wild-Born	9	9	3	88	92	100	100
Beezley Hills RA							
# of Active Burrows							6
% of Released Kits Detected (est. survival rate)							30
# of Genetically Identified Pygmy Rabbits							4
% of Identified Pygmy Rabbits being Wild-Born							25
Burton Draw RA							
# of Active Burrows							11
% of Released Kits Detected (est. survival rate)							35
# of Genetically Identified Pygmy Rabbits							6
% of Identified Pygmy Rabbits being Wild-Born							0

Table 4. Summary data from winter burrow surveys of CB pygmy rabbits on amount of occupied habitat and distribution on CRP lands among three Recovery Areas in central Washington, from 2012 to 2019.

	Winter Survey Year						
	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	2018/19
Sagebrush Flat RA							
Active Burrows	52	64	174	88	146	336	306
% of Active Burrows Occuring in CRP Habitat	15.4	12.5	20.1	76.1	72	79	90
Sq. Km of Occupied Habitat	6.4	6.7	10.9	12.3	20.1	23.6	19.8
Beezley Hills RA							
Active Burrows							6
% of Active Burrows Occuring in CRP Habitat							100
Sq. Km of Occupied Habitat							1
Burton Draw RA							
Active Burrows							11
% of Active Burrows Occuring in CRP Habitat							0
Sq. Km of Occupied Habitat							1

Reintroduction Activities at Beezley Hills: 2015-2019

Reintroduction efforts began at BH in 2015 (Figure 4). With the large number of kits produced in breeding enclosures and increasing size of the SBF population, the Science Advisory Team advised establishing a second population. In 2015, 429 pygmy rabbits (mostly kits) were released in BH using the hard release method with artificial burrows. Incredibly, post-release surveys failed to document a single active burrow or surviving pygmy rabbit. As a result, release efforts were suspended in 2016 until the factors contributing to the poor survival were understood and appropriate corrective measures implemented. Pygmy rabbits released at SBF during 2015 also experienced very poor survivorship, therefore, the unsuccessful reintroduction attempt at BH in 2015 could be attributed to factors present in both release areas. In 2015, a coccidia outbreak within the breeding enclosures occurred and would likely have contributed to post-release mortality of all kits released that year. Additionally, much of eastern Washington experienced a significant drought in 2015, which could have contributed to additional stress on pygmy rabbits transitioning to the wild.

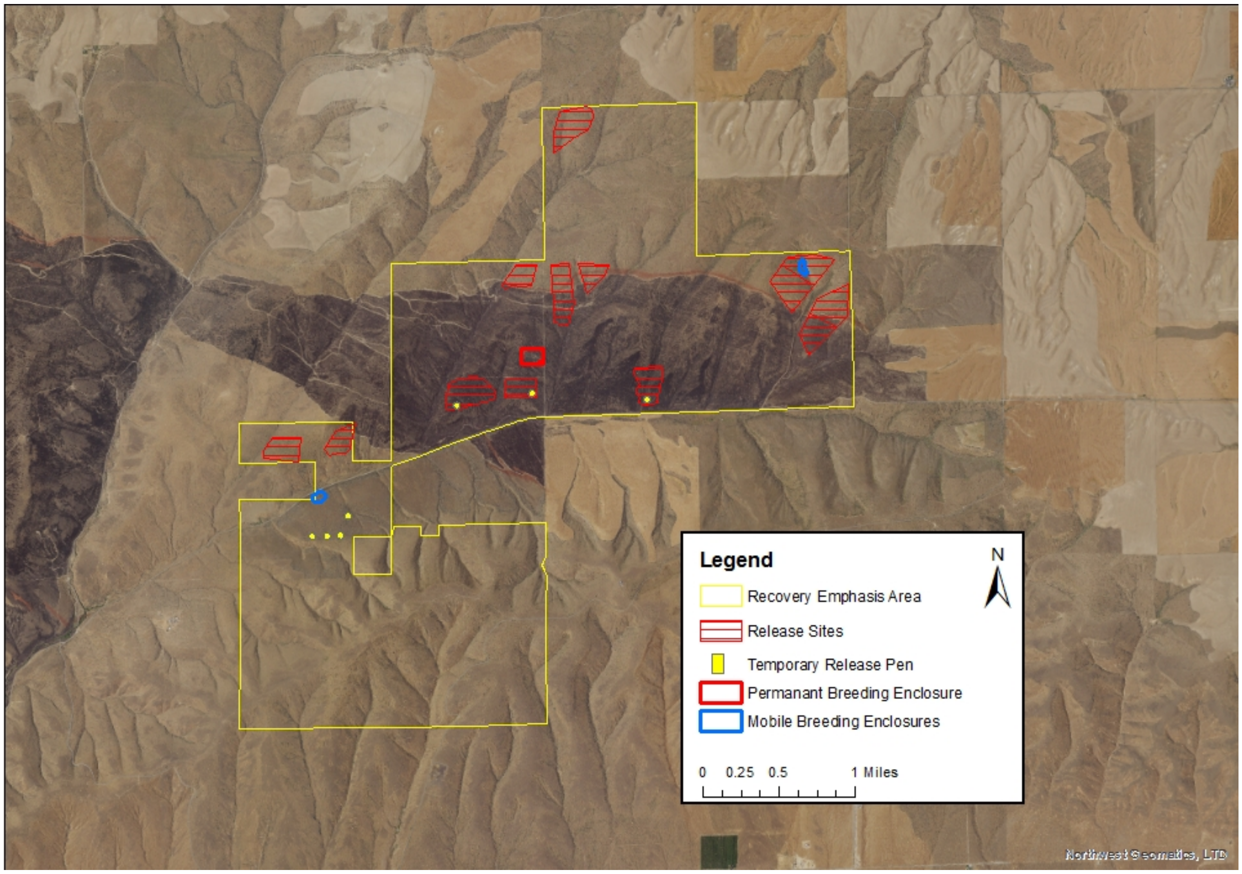

Figure 4. Beezley Hills Recovery Emphasis Area (owned by The Nature Conservancy and private landowner). Release site polygons used in 2015 for artificial burrows. Temporary release pen locations were used 2017-2019. Permanent breeding enclosure established in 2013 and mobile breeding enclosures established in 2017 and 2019. The 2017 Sutherland Canyon fire scar can be seen in black, destroyed all infrastructure within its perimeter.

In 2017, release efforts resumed at BH utilizing temporary release pens (Appendix A). An adaptation to the soft release technique, these pens were about 1-acre in size and their purpose was to limit initial dispersal and predation, providing an improved acclimation period for kits to begin burrow development. In 2017, 26 kits were released into three temporary pens but subsequently died during the Sutherland Canyon Fire (Figure 4). As a result, release efforts were suspended for the remainder of 2017 due to the combined loss of the of breeding enclosure population and designated release areas.

Despite all the losses of shrub-steppe habitat in BH from wildfires, portions of suitable habitat remained unburned within the recovery area. Reintroductions resumed at BH in 2018 utilizing temporary release pens and continued in 2019. Releases in 2018-2019 comprised both enclosure born kits and translocation of wild born kits from the wild SBF population. Survival rate of wild born kits was higher (50%) than that of enclosure born kits (20%). Initial results with temporary release pens are encouraging as total post-release kit survivorship increased to 33% for the 2018 release effort.

Reintroduction Activities at Burton Draw: 2018-2019

Following the catastrophic losses from the 2017 Sutherland Canyon fire, there was greater recognition of the wildfire threat to pygmy rabbit reintroduction. To address wildfire risk, the Science Advisory Team supported the approach of releases occurring in multiple Recovery Areas simultaneously. Despite the low numbers of kits available for release in recent years, distributing released kits between BD and BH ensures some protection against catastrophic loss from wildfire. Release efforts at BD began in 2018 and continued into 2019 (Figure 5) using temporary release pens. Similar to the approach at BH, a combination of enclosure born kits and wild born kits from the SBF population were used.

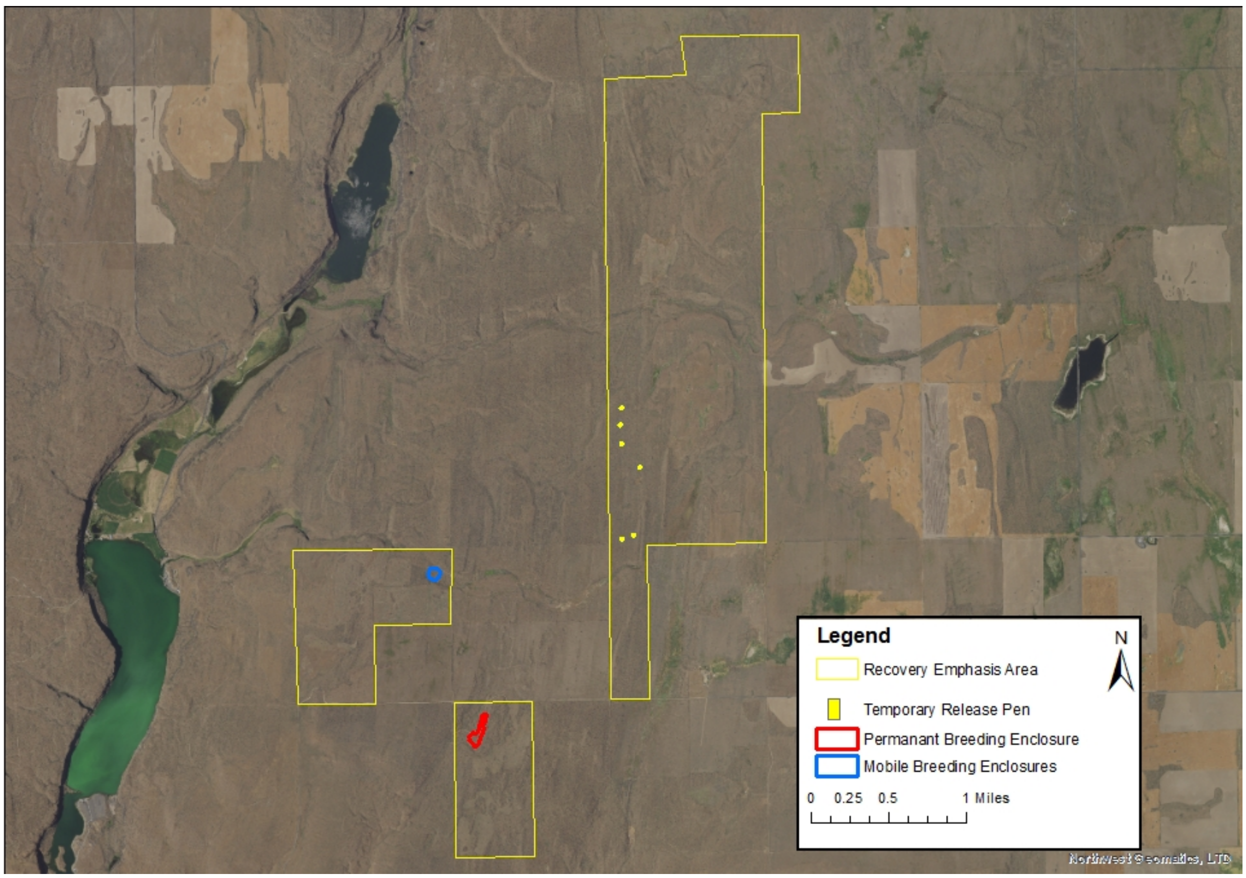

Figure 5. Burton Draw Recovery Emphasis Area (owned by WDFW). Temporary release pen locations were used 2018-2019. Permanent breeding enclosure established in 2012 and mobile breeding enclosure established in 2019.

ADAPTIVE MANAGEMENT OF METHODS AND STRATEGIES

The following section is a summary of all the methods, evaluation and results, adaptive management strategies, and guidelines for selected components of the reintroduction effort since 2011. Additional background information on these can be found in Becker et al. (2011) and Hayes (2018).

Semi-wild Breeding Population

The purpose of the semi-wild breeding population is to produce adequate numbers of juveniles (kits) for release into the wild for population establishment. To achieve this, we need to utilize methods and strategies that contribute to a sustainable and genetically healthy rabbit population. The following are general guidelines for the semi-wild breeding program.

Minimize habituation and fitness loss. Any form of captivity or human influence can reduce animal fitness (Snyder et al. 1996; McPhee 2003; Williams and Hoffman 2009), which is a challenge for animals destined for release into the wild. Infrastructure should allow as much natural behavior as possible, balancing the need for enhancing their survival and reproduction without habitation. Retention time of individual pygmy rabbits within breeding enclosures should be minimized if they are to be released into the wild. To minimize fitness loss in the semi-wild population over time, periodically (2-3 years) we may need to supplement wild pygmy rabbits from within Washington or Great Basin source populations.

Husbandry. Even with semi-wild conditions, pygmy rabbits within enclosure will require some level of human care, provisions, or treatments. However, given the eventual goal of releasing most of the pygmy rabbits into the wild, the level of accommodations should be kept to a minimum needed to enhance survival and reproduction but not impact their ability to survive in the wild.

Population size. This will be limited by the available space within the enclosures. Zeoli et al. (2008) used demographic data from the captive pygmy rabbit population to model population dynamics and provide guidance on appropriate breeding population size and harvest (for release) rate. Managing the number of breeding adults at or below carrying capacity of the enclosure habitat will minimize crowding stress, reduce disease threat, and prevent over-utilization of vegetation. DeMay et al. (2016) found that as female density increased, productivity decreased and suggested maintaining density at 5-10 adults/ha, which still yielded high kit production (7-10 kits/female).

Recruitment of wild pygmy rabbits. Introduction of wild pygmy rabbits to the semi-wild breeding population may be needed periodically. Wild pygmy rabbits should only be added to the breeding population to enhance the number of available breeders, improve reproductive fitness, increase genetic diversity, or alter the genetic composition. High genetic diversity within individual pygmy rabbits was linked to both increased reproductive fitness and survivorship in the wild post-release (DeMay et al. 2016, DeMay et al. 2017).

Retention of enclosure-born pygmy rabbits. To sustain the semi-wild population and kit production, some kits will need to be retained in the enclosures as future breeders. Initially, kits with the highest % CB genes were retained, but this should not be the sole consideration. Demographics, level of relatedness, and genetic diversity should all be weighed when selecting kits for retention as future breeders.

Breeding Enclosures

Establishing permanent large breeding enclosures facilitated the transition of captive pygmy rabbits to their former native habitat, allowed for semi-wild breeding and behavior, and protected this source population from predation. Breeding enclosure design is provided in Appendix A. Multiple breeding enclosures were established in each Recovery Area to manage risk from environmental stochastic events, such as disease and wildfire. In 2011, a large (10 acre) and small (6 acre) breeding enclosure were established on the Sagebrush Flat Unit of the Sagebrush Flat Wildlife Area, owned by WDFW. In 2012, a third breeding enclosure (6 acre) was established on the Dormaier Unit of the Sagebrush Flat Wildlife Area, located 16 miles from the SBF Unit. In 2013, a fourth breeding enclosure (10 acres) was established on private land within Beezley Hills, approximately 10 and 26 miles south of the SBF and Dormaier Units, respectively.

There was a significant amount of site modification within enclosures to ensure the survival of the transferred captive pygmy rabbits. Artificial burrows (Appendix A) were excavated in mounded topography containing sagebrush and the burrows were covered with netting for raptor protection. Rabbits in enclosures were maintained on a combination of natural forage, supplemental feed, and water. Feeding stations were established and stocked with commercial rabbit pellets and fresh greens (iceberg lettuce, and alfalfa), and were replenished weekly. An irrigation system within enclosures ensured adequate natural forage and a water source. The semi-wild breeding enclosure design was successful at limiting rabbit mortality from predation, enhancing rabbit survival and kit production, and sustaining releases to the wild.

While all these modifications were introduced to ensure rabbit survival, some of them developed unintended consequences. Soil disturbance from the installation and maintenance of enclosure infrastructure and artificial burrows allowed for the establishment of invasive weeds. These infestations began to spread from foot traffic, additional weed seed from supplemental food sources (e.g., alfalfa), and irrigation water, eventually overtaking native vegetation across 25% of the enclosure habitat. Further, repeated foot traffic led to soil compaction and high rabbit densities and long duration of occupancy likely contributed to degradation of the enclosure habitat within the enclosures by over utilizing the natural forage. Attempts to rehabilitate vegetation within enclosures (e.g., plug plantings, mechanical and chemical weed treatments) proved too difficult to implement with pygmy rabbits present.

Concurrent with noxious weed problems was the extensive outbreak of coccidia within the breeding enclosures. Spread of coccidia occurs through fecal material and soil contact. The frequent concentration of rabbits at supplemental feeding stations likely contributed its spread. Prolonged high-density occupancy of these enclosure sites built up coccidia loads in the soils that ensured continuous exposure. To address this outbreak, we implemented changes in husbandry techniques (reduced the number of feeding stations, raised feeding screen to avoid soil contact, and regular disinfection of sites) as well as administering antiprotozoal agents (Amprolium) in gravity water units during the breeding season. Consultation with veterinarians on the Science Advisory Team estimate persistence of coccidia in the soil could last 2-3 years and the only true management option is to move rabbits to uninfected sites.

The most significant event in the breeding enclosures occurred in 2017, as the Sutherland Canyon Fire destroyed the BH breeding enclosure. This was the largest and most productive breeding enclosure, ultimately losing nearly half the semi-wild breeding population from this event. Following this loss, plans for a replacement enclosure were quickly implemented, but we utilized this opportunity to develop a prototype for new enclosure design. The permanent breeding enclosure infrastructure design was expensive and labor intensive to build and maintain. The new design would be less costly and labor intensive, but also needed to address the biological problems of noxious weeds, site degradation, and coccidia.

We developed the prototype for a mobile breeding enclosure in the late summer of 2017 (Appendix A). The main feature of the new design is its mobility, being constructed of interlocking panels it can be relocated to different sites every couple of years. There is no digging or mowing involved with installation and therefore minimizing soil disturbance and noxious weed infestations. Implementation of this new breeding enclosure design allows managers to locate breeding rabbits on “fresh pasture” on a short rotation cycle, minimizing or breaking the coccidia infection cycle, build-up of noxious weeds, and enclosure habitat degradation. Further modifications included eliminating many husbandry and infrastructure components that were determined to be no longer necessary (e.g., artificial burrows, irrigation, raptor netting, and supplemental feed).

The first mobile breeding enclosure was built in 2017 with kits introduced to the structure in 2018, and successful kit production occurring in 2019. Following this successful trial, we then implemented a full transition away from the permanent enclosures to the new mobile enclosures. Two additional mobile enclosures were established in 2019 and scheduled for kit production in 2020. Remaining pygmy rabbits were removed from the two permanent breeding enclosures at SBF in the fall of 2018. As of 2019, only the Dormaier permanent enclosure site was still occupied. We expect nearly all the kit production in 2020 and beyond will be in the mobile breeding enclosures.

Reintroduction Infrastructure

While the reintroduction infrastructure and techniques have adapted to meet changing circumstances, their overall goal remains the same. We aim to maximize survival of released pygmy rabbits during the post-release period. Reintroduced animals, especially those from manipulated origins, experience increased risk of mortality by predation after release (Seddon et al. 2007). Released animals are unfamiliar with the area, meaning that these animals do not have havens from predators, often do not know the specific predation risks associated with the area and in some cases display behavior and movements that increase predation risk (Banks et al. 2002). Pygmy rabbits are vulnerable to a wide range of abundant generalist predators with naturally high mortality in the first months of life (Price et al. 2010). Juvenile pygmy rabbits naturally disperse from natal territories (Estes-Zumpf and Rachlow 2009), thus requiring an acclimation and release technique that does not interfere with this natural behavior.

The first method utilized were soft release pens (Appendix A). These units were small and robust, ensuring maximum protection from predators and raptors. The goal was for kits to remain within them for up to two weeks for site acclimation and to promote burrow development. More than 30 soft release pens were established throughout the core release area on Sagebrush Flat, 200-450 ft apart, and each included an artificial burrow. Supplemental feed, water, and natural forage (cuttings/potted sagebrush, grasses and forbs) were provided. Fences enclosing the pens were breached after two weeks to allow for local dispersal and continued use of the pen area.

Soft release pens were used exclusively during the 2011 release and post-release kit survival was 10% for rabbits using this release method. Site fidelity was minimal as nearly all kits dispersed from the release sites. Kits that survived to the winter dispersed an average of 764m (WDFW *unpub data*). Given the intensive labor, maintenance, site disturbance associated with this release method, and the minimal kit survival, an alternative release method was investigated.

Beginning in 2012, a hard release method was field tested using only artificial burrows (Appendix A). Kits were released using both soft and hard release techniques on SBF to determine which method was more effective and efficient. Initial kit survival and site fidelity did not appear to differ between the two methods. With no negative impact to released kits, the lower labor, maintenance, and installation costs warranted switching to the hard release method. The hard release method, utilizing artificial burrows, was the standard technique used through 2015. Approximately 142 artificial burrow sites were established on SBF and another 225 on BH. This method was very effective in handling the extraordinary number of kits released from 2013-2015.

Initial results using the hard release method were very encouraging, with a minimum 37% of released kits confirmed surviving to the winter in 2012 (DeMay et al. 2015). Dispersal from release sites averaged 900-1000m, with no significant differences between sex or age of the released rabbit (DeMay et al. 2017). Survival rates declined dramatically in the following years, dropping to 11 and 10% respectively in 2013 and 2014 (DeMay et al. 2017). The post-release survival was lowest in 2015, with <1% of released rabbits (SBF and BH) confirmed surviving to the winter. Averaged across all sites and years, the minimum survival rate utilizing hard release artificial burrows was approximately 8.5%. The observed annual variation suggests other ecological factors are contributing to post-release survival. Aside from the variable and declining survivorship of released pygmy rabbits, we began to experience increased maintenance needs with artificial burrows as small mammals continuously filled the piping up with dirt, requiring multiple clean outs per season. The soil disturbance from their installment and maintenance created noxious weed infestations on mound sites. All of these factors contributed to looking at alternative release methods.

We adapted the hard release method in 2016, now utilizing inactive pygmy rabbit burrows occurring throughout the release areas. We anticipated survival and site fidelity would improve by utilizing these natural structures. We selected 101 burrow sites within the SBF release area, placing 1-2 kits in each one. Game camera monitoring confirmed some initial occupancy of burrow sites up to two weeks. Overall, site fidelity was still low and minimum kit survival was only 4%. While this was an improvement over the previous year and by far the least intensive method labor wise, survival was still too low for effective population augmentation.

Improving post-release survival was the priority as we adapted the soft release approach and developed temporary acclimation pens (release pens) for 2017. Kits are most vulnerable to predators when dispersing from natal burrows (Estes-Zumpf and Rachlow 2009). The basis of this approach was to limit initial kit dispersal and predation risk, allowing for settlement or burrow establishment of multiple kits on site. The pens are circular, approximately 1 acre in size (Appendix A). The pen design is not predator or rabbit proof, but is economical and lightweight for easy installation/relocation. There is no digging required for installation, which will reduce the spread of noxious weeds. Once released rabbits begin or fully establish burrows, the pen fencing will be removed, leaving the settled rabbits to fully utilize a suitable home range. Similar methods of short-term containment documented increased survivorship in translocations of European wild rabbits (Rouco et al. 2010). Release pen fencing is relocated to new sites each year.

The first release effort using temporary release pens was 2017 in BH, however we lost all of them to the Sutherland Canyon wildfire. We continued the use of release pens in both BH (2) and BD (3) during release efforts for 2018 and 2019. The fencing was largely successful in deterring predators, as only one breach occurred (badger) in these two years. Kit breaching of the pens was higher than anticipated. Small kits were observed squeezing through the 1 x 1 in wire mesh and larger ones were able to climb over it. This was not necessarily a problem as we confirmed many of these kits dispersing within 300m of the release pen sites. Burrow development was rapid among the kits remaining within release pens. Most sites had burrow digging within the first week of placement in them. One burrow was measured at nearly 1m deep within three weeks of being initiated. Site fidelity was high with 22% of kits developing burrows within release pens in 2018. Pens were fully breached during the winter and game cameras confirmed rabbits remaining on site until breeding season. Initial results are encouraging as total post-release kit survivorship increased to 33% for the 2018 release effort. Overall, this method is efficient and adaptable to use and most importantly it has improved kit survival. We expect this to be the main release method used in the coming years.

Release Protocol

Release protocol elements, like all aspects of this reintroduction effort, continue to evolve and adapt to changing circumstances. Pygmy rabbits raised in captivity will require different approaches than ones caught in the wild or breeding enclosures. Below are general guidelines for releasing and handling pygmy rabbits with respect to their origin.

- Captive-bred rabbits: Our experience has shown that rabbits having been born or lived in captivity most of their life are poorly suited for survival in the wild. These rabbits are best used in semi-wild breeding enclosures. The husbandry and infrastructure help them acclimate to living on their own and they will be able to contribute to the collective breeding effort.
- Wild rabbits captured from other states: Given the high cost associated with travel and capture of these rabbits, they should not be directly released into the wild given typically high mortality with any translocations. They are best utilized for the semi-wild breeding enclosures. When translocating them, they require a veterinarian check before importing into Washington state. To reduce stress, we should minimize their transport time from initial trapping to release within breeding enclosure to less than 48 hrs.
- Wild rabbits captured within Washington: Because they are wild and locally adapted, they can be either placed within semi-wild breeding enclosures or direct augmentation between wild populations. To maximize their survival following translocation, temporary release pens would be the best method for use. Time in transit should be restricted to less than 24 hrs.
- Enclosure born rabbits: Their purpose is primarily intended for both release into the wild and sustaining the semi-wild breeding enclosure population. Release methods have included supplemental food, soft release infrastructure, and hard release methods. While not truly wild or captive, they possess the necessary instincts to survive with little accommodations. The type of release method can be dictated by the number of kits being released as well. Enclosure born kits should be released to the wild as young as possible to minimize habituation. Generally, a kit more than 4 weeks old (approximately 100g) would be independent in the wild (Elias et al. 2006, Price et al. 2010).

All pygmy rabbits, regardless of origin will be handled and released under the following guidelines:

- Minimize handling time and stress during trapping, sampling, and transportation.
- Pygmy rabbit transport crates will minimize thermal, visual, and auditory stress. Sagebrush clippings can be added for forage if animals are held beyond a couple hours.
- All pygmy rabbits will have tissue samples taken for DNA identification and genetic monitoring.
- Any pygmy rabbits showing injury, illness, or physical defect should not be released into the wild.
- If desired for research, released juveniles may be fitted with glue-on transmitters and adults may be fitted with radio collars

- Pygmy rabbits being retained within breeding enclosures should have a microchip (PIT tag) implanted in its nape to allow for field identification.

Post-release Monitoring

The primary objectives for post-release monitoring are to estimate survival and distribution of released pygmy rabbits into the wild. We have used a variety of methods to accomplish these objectives. Each year, data collected during post-release monitoring helps evaluate factors affecting survival and dispersal. Among the variables evaluated are the physical condition of released rabbits (age, weight, parasite load, sex, genetic heritage, breeding enclosure origin), method of release, timing of release, and release sites (DeMay et al. 2017). Where possible, we aim to release pygmy rabbits in conditions associated with high survival for effective population establishment.

Game cameras were useful in monitoring initial release behavior and site fidelity for all release techniques. We have also utilized them to monitor mammalian predator visitation rates to active burrows (Gallie and Zinke 2018) and their temporal activity patterns. Game cameras will likely continue to be a useful means of monitoring released pygmy rabbits due to their low cost to maintain and deploy.

Extensive post-release monitoring in 2011 and 2012 involved radio telemetry tracking. A thorough accounting of methods and results can be found in DeMay et al. (2015). Radio-tracking monitoring provided useful information on directional and long-distance dispersal from the release site and pinpointed some settlement locations. Overall, tracking was difficult as glue on transmitters had limited range and only lasted one to three weeks post-release. In some cases, it was not possible in most cases to determine whether the units fell off the pygmy rabbit or had been pulled off during a predation event. DeMay et al. (2015) additionally compared the efficiency and efficacy of radio tracking versus non-invasive genetic sampling for post-release monitoring.

Non-invasive genetic monitoring requires a DNA profile (established from tissue biopsy) of released rabbits for comparison to DNA profiles of fecal pellets collected at active burrows during winter surveys. Intensive belt transects (50m wide) were conducted on release areas and all active burrow sites mapped and sampled. Snow conditions proved very effective for detecting recent pygmy rabbit activity. Ultimately, non-invasive genetic sampling proved to be the superior method for our effort, as it was able to provide more complete data on survival and dispersal, lower labor and material costs, and involves less handling and stress of the rabbits (DeMay et al. 2015). Both methods struggled to provide certainty on whether released rabbits made long distance dispersals out of the survey area or were lost to predation. This method is labor intensive, often requiring the use of volunteers to cover potentially occupied habitat on and surrounding release sites, typically between 8-15 km^2 annually (DeMay et al. 2017).

Since 2012, winter surveys and non-invasive genetic sampling of fecal pellets at active burrows have been the main tool for post-release monitoring (see DeMay et al. 2015 for details and methods). In addition to monitoring the released rabbits, this method was very effective in documenting breeding in the wild. We determine that a rabbit was wild born if its genetic profile did not match any that were released. The number and proportion of pygmy rabbits born in the wild is perhaps the most important factor in evaluating the success of population establishment (Table 3).

Population Monitoring

As release efforts subside and most of the free ranging population is determined to be wild born, the primary objective will transition to estimating population size, distribution, and changes in genetic composition. Each of these parameters can be challenging to determine when their potential range extends across 100-200 km^2. At small spatial scales, populations ranging across 10-20 km^2 can be monitored by conducting intensive belt transect surveys shortly after snowfall. These surveys document the number and distribution of active burrow sites and when combined with collection of fecal pellets at active burrows for DNA analyses can provide an estimate of minimum population.

Pygmy rabbits often have a patchy distribution across the landscape, reflecting the non-uniform distribution of suitable habitat. One drawback of the intensive belt transect method is that it covers all areas equally and surveyors may spend lots of time covering non-suitable habitat or areas with very low pygmy rabbit density. Belt transects are most effective in uniform habitats and areas with medium to high burrow densities, however they are not effective in surveying patchy and fragmented habitat where burrow densities are low or where pygmy rabbit occupancy is not known. A cluster sampling method was found to be a highly efficient survey method in these settings. Nearest neighbor analysis of burrow sites indicated they are almost always located with 150m of one another (Gallie 2017). When surveying low density areas, surveyors establish a sample plot with a 150m radius surrounding an initial burrow detection. This allows for the detection of neighboring burrows and focusing additional search effort in the most likely occupied areas.

Locating newly established pygmy rabbit sites across the landscape remains challenging, but vitally important for assessing the long-term viability of populations. Landscape-scale ground surveys can be successful by selectively searching areas of suitable habitat within dispersal distance of occupied habitat. Aerial searches (helicopter or drone) have had some success to date (Gallie 2017). However, aerial surveys require clear skies and fresh snow for observing tracks or active burrow sites. Follow up ground visits are required on all aerial detections to verify pygmy rabbit sign and collect fecal samples for genetic monitoring. Additional searches using 150m sample plots should be used when there is a positive detection.

Long term population monitoring will need to be adaptive as the number, size, and distribution of the pygmy rabbit populations increase. Additional research is needed on the use of active burrow counts as an index for monitoring changes in abundance of pygmy rabbits (Price and Rachlow 2011). As distributions expand and the number of populations increase, we will need to develop or utilize monitoring strategies that require less time and effort.

Adaptation is needed with non-invasive genetic sampling as well. Not all samples provide useful results as fecal DNA degrades from multiple factors including exposure time, temperature, and environmental moisture (DeMay et al. 2013). When fecal pellet sample quality is high, we obtain the individual identity of the pygmy rabbit, its genetic composition, and its parentage. DNA analyses failure rates show high annual variation, ranging from 20% to 75%, averaging 45%. Drawing conclusions on population size and demographics from a data set where only 55% samples provide the needed components can be challenging.

Reintroduction Research Objectives

The proximate factors that contributed to the extirpation of local populations of the CB pygmy rabbit will never be fully understood. Consequently, the reintroduction program offers the opportunity to simultaneously restore local populations while gaining a better understanding of population dynamics and ecological factors critical to long-term population viability.

Many aspects of the population ecology of pygmy rabbits documented in other western states might be vastly different on the Columbia Basin landscape. The objective for nearly all monitoring and research will inform and guide adaptive management for successful reintroduction and population establishment. The following primary research objectives are central to the reintroduction and monitoring:

1. Quantify and correlate factors that affect reproductive success and genetic health of semi-wild breeding methods.
2. Quantify and correlate factors that affect post-release survival and dispersal of released pygmy rabbits.
3. Evaluate dynamics and ecological impact of predation, disease, and parasites.
4. Assess ecological relationships (fine and coarse scales) between pygmy rabbits and the shrub-steppe habitat to develop better quantitative models of habitat use and selection.
5. Assess long term trends in the CB signature, local fitness of genetic adaptation, and the potential need for future genetic management.
6. Develop methods to estimate pygmy rabbit population size, burrow count indices, and suitable survey techniques.
7. Develop empirically based population viability models to guide population establishment, augmentation, and harvest methods.

FUTURE PLANS AND LONG-TERM RECOVERY

The purpose of the following section is to detail immediate reintroduction plans for 2020, the near future (next three years), and potential options for three to five years from now. All of these proposed plans are the culmination of recent adaptive management implementations. Ecological conditions or circumstance can change rapidly and can result in very different actions and timelines than what was originally planned. All significant adaptations have and will continue be discussed with the Science Advisory Team.

Semi-wild Breeding Population and Translocations

Semi-wild breeding populations will likely be needed until multiple wild populations are established. Our immediate priority will be to increase the size of the enclosure breeding population so that it can support release efforts for 2020 and become self-sustaining again. Attempts to translocate wild pygmy rabbits from the SBF population were unsuccessful, leaving out of state populations as the only source option. In 2020, the Department is planning to translocate a total of 30-40 adults from one or more source states beyond the Columbia Basin. These 30-40 breeding adults would be released in the three existing mobile breeding enclosure (joining the resident pygmy rabbits currently within them). Based on productivity rates observed from the last translocations in 2011-2013, a breeding population of this size could yield 80-160 kits in 2020.

If these enclosure breeding adults experience average survival and productivity, we anticipate additional out of state translocations would not be necessary in the next three years. Average productivity should sustain release efforts and maintain an enclosure population over this time frame. Harvesting additional pygmy rabbits from the SBF population could be an option to enhance the number of breeders or influence the genetic composition, assuming population trends of SBF continue to increase. However, if survival and productivity levels are poor and the wild SBF population is unable to support harvest, then we would need to initiate additional out of state translocations to maintain release efforts.

With respect to harvest from SBF or other future wild Washington population. Several factors should be considered to ensure harvest does not negatively impact the viability of these in-state source populations. In general, our established populations should not have pygmy rabbits removed if population monitoring indicates declining trends in active burrow counts or genetically estimated number of adults. The minimum viable population size is unknown, but it would seem plausible that populations under 100 adults would be too small to harvest from.

Additional out of state translocations should have careful consideration. This action achieves one objective at the cost of another. Continuing reintroductions and establishing multiple wild populations is the top priority, which has required maintaining genetically healthy captive and semi-wild breeding populations. Over the past 20 years of recovery, achieving these objectives has required the introduction of Great Basin pygmy rabbits which has reduced the level of CB genetic ancestry. Where possible, we strive to retain and conserve as much Columbia Basin ancestry as possible, being central to its source of local adaptation.

If genetic or demographic rescue with out of state translocations is needed to maintain reintroduction efforts, priority to specific regions for translocations needs to be considered. As the predominant ancestry of the current Washington population comes from the Great Basin group, priority should be given to this region. The Great Basin group is the largest, comprising California, Nevada, Oregon, Idaho, and Montana. The F_{ST} values (levels of divergence) of the Columbia Basin are lowest in comparison to this region versus the other two regions (Table 1). If a second region must be considered for translocation purposes, it would be the northern Utah/Wyoming. This ancestry is represented in the current Washington population at a much lower proportion (~14%) and is showing a declining trend each year. The southern Utah group should be avoided, if possible, when selecting sources for translocation. The southern Utah ancestry is only represented in trace amounts in the current population (<1%) and is under-represented compared to what was added during translocations, suggesting individuals with this ancestry may have lower reproductive success in Washington. Additionally, the greatest levels of divergence occur between the Columbia Basin population and southern Utah populations, which increases the probability of outbreeding depression.

Release Efforts

Annual survival of released pygmy rabbits, kit production in breeding enclosures, and productivity of wild pygmy rabbit's have been and will continue to be highly variable. Each of these variables will heavily affect the number of rabbits needed for reintroduction and establishment. Our priority will be maintaining release efforts in both BH and BD for the next three years, continuing to utilize temporary release pens. As mentioned before, a viable population size is unknown, but the minimum population size (number of wild adults) goal should be 100. There could be scenarios where release efforts could shift away from BH and BD to SBF. Should the estimated SBF population decline to <100 adults, then the size and trend of all three populations would be evaluated annually to determine which one is in the greatest need of augmentation to prevent local extirpation.

Release Site Strategy

Within 3-5 years, it may be necessary to identify additional recovery areas for reintroductions and establishment of additional pygmy rabbit popaultions. When core populations in SBF, BH, and BD are >100 wild adults or at a determined viable population size, it will be appropriate to initiate reintroductions into new areas to expand their distribution on the landscape. We propose the strategy of reestablishing a metapopulation distribution within the landscape of our current recognized Recovery Areas in Douglas and Grant Counties. Attempting to recreate the natural distribution this species evolved with, we will focus release efforts into suitable habitat patches within the Recovery Areas that remain unoccupied or where dispersal barriers exist (e.g. large patches of agriculture, cliffs). These subpopulations are expected to have some level of habitat connectivity and be within dispersal distance of existing occupied areas. The greater number of habitat patches occupied by pygmy rabbits will be the best defense against catastrophic loss from wildfire or disease and provide resiliency on the landscape to handle natural populations fluctuations without local extirpation.

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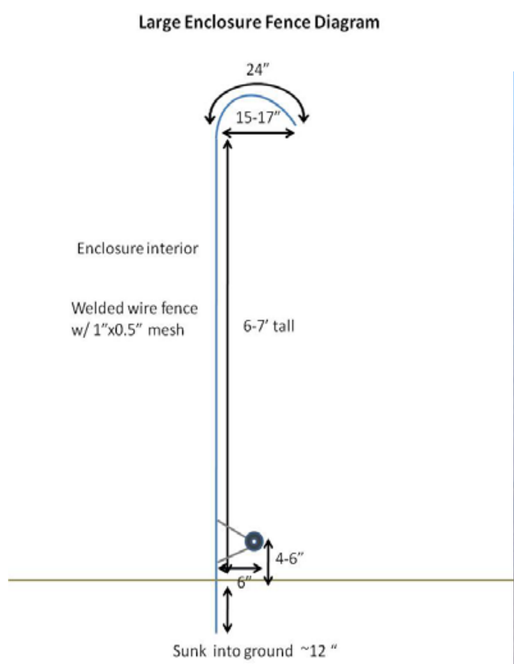
Appendix A. Infrastructure associated with the breeding and release of pygmy rabbits in central Washington, 2011-2019.

Permanent breeding enclosures. The large enclosures (between 6 and 10 acres each) are semi-permanent structures that were used throughout the reintroduction effort. Each enclosure was constructed of welded wire mesh fencing of two 4 ft widths (hog ringed together) and vertically anchored to wooden beam or T-post support structures. To deter predators from digging under the fence, the base of the fence was buried approximately 12 in below the soil surface. To prevent weasels from climbing over the fence and into the enclosure, the top of the fence was not structurally supported allowing the fence to droop to the outside of the enclosure. An electric wire was placed on the outside of the enclosures to further discourage predators from digging under fence. Further, to deter raptors from perching on the fence, bird spikes were placed on fence structures.



Construction of semi-permanent breeding enclosures.

Mobile breeding enclosures. These enclosures are smaller in size (2-3 acres) than the semi-permanent breeding enclosures and constructed from interlocking panels. The walls of the enclosures are constructed from weld wire cattle panels (4ft x 16ft) with heavy duty orchard netting (7ft tall, 1 inch mesh) attached to the cattle panels by hog rings. The rigid netting requires no additional support. Metal aviary netting (3ft tall, ½ in x ½ in mesh) is attached along the base of the fence structure is to prevent kits from escaping. The bottom 1.5 ft of the orchard netting is folded to the outside of the enclosure and held in place with 8 in ground staples to deter predators from digging beneath the fence structure. The bottom 6-8 inches of the aviary netting is folded to the inside of the enclosure and ground stapled in-place to deter pygmy rabbits from digging beneath the fenced structure and escaping. Angle iron posts support the panel walls and are secured with metal wire. Construction of the fenced enclosures requires no excavation of soil and panels can be carried to and from enclosure site.


Construction of small, mobile breeding enclosures.

Soft release pens. The approximately 8 ft diameter soft release pens are constructed of 4 ft tall welded wire fencing with a small mesh size (1 in by 0.5 in) to exclude predators (Figure 6). The bottom of the cages are fitted with nylon weed cloth and staked at 1 in intervals into the ground (6 in deep) to limit rabbits from immediately digging out and to discourage predators trying to crawl under the enclosure. The top portion of the welded wire (7-14 in) is covered on the inside and outside with metal flashing to create a slippery surface that prevents pygmy rabbits from climbing out and weasels from climbing in. Soft release enclosures are netted to prevent avian predation from above. After an acclimation period, enclosures are breached on two sides to allow rabbits to move freely. Artificial burrows are provided for cover and shelter.


Construction of soft release pens.

Hard release artificial burrow. Site preparation for provisioning of artificial burrows required excavation of soil to form a trench approximately 0.4 m wide by 3-4 ft long followed by replacement of soil over a 3–4 ft length of 10 cm diameter plastic drainage tubing used to form the artificial burrow. A strip of piping about 1-2 in wide was removed from the long axis of the tubing to create an opening in the bottom to facilitate expansion and facilitate burrow development.

Construction of a hard release site and placement of artificial burrows.

Temporary release (acclimation) pens. The pens are circular, approximately 1 acre in size and constructed from 5 ft. tall metal hex netting (chicken wire, 1-inch mesh size), supported with 4 ft. tall fiberglass electric fence posts. Digging deterrent for kits utilized the bottom 1 ft of the netting folded inward and anchored to the ground with 6-inch ground staples.

Construction of temporary release pens