2011 COLUMBIA BASIN PYGMY RABBIT REINTRODUCTION AND GENETIC MANAGEMENT PLAN
Washington Department of Fish and Wildlife

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

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EXECUTIVE SUMMARY

This plan addresses the recovery strategy for the federal and state endangered Columbia Basin pygmy rabbit (*Brachylagus idahoensis*) in shrub-steppe habitat of central Washington. It is a consolidated update of the 2010 genetic management plan and the 2007 reintroduction plan for the pygmy rabbit. Technical background for the plan, covering the history, biology, and ecology of pygmy rabbits, has been reviewed extensively in a 5-Year Status Review (USFWS 2010) and an amendment to the federal Draft Recovery Plan (USFWS 2011) for the Columbia Basin distinct population segment of the pygmy rabbit.

Currently, there are no wild pygmy rabbit populations known to occur in Washington's Columbia Basin. As a result, the recovery strategy relies on the reintroduction of captive-bred pygmy rabbits originating from the joint captive population maintained since 2001 at Northwest Trek, Oregon Zoo, and Washington State University, in conjunction with the release of wild pygmy rabbits captured from other populations within the species' historic distribution. The reintroduction plan was formulated with information gleaned from studies of pygmy rabbits in the wild, results of the 2002-04 pilot-scale reintroductions in southeastern Idaho, results of a trial 2007 release of animals into Washington, and comparable reintroduction efforts for other endangered species.

Beginning in the spring of 2011, pygmy rabbits were reintroduced at Washington Department of Fish and Wildlife's Sagebrush Flat Wildlife Area. Released rabbits included captive Columbia Basin lineage rabbits and wild rabbits from other range states to obtain the numbers and genetic diversity needed for the likely establishment of the population. A combination of small soft release enclosures and large enclosures were used for the releases with different enclosures and holding times tailored to the needs of wild, captive-bred, adult, young, male or female pygmy rabbits. Reintroduced adult individuals were fitted with radio collars and will be tracked to document habitat use, dispersal, mortality factors, reproductive success, seasonal and annual survival rates, and changes in population genetics. After the release of most captive-bred pygmy rabbits in 2011 and 2012, the breeding program will be de-emphasized.

Wild animals from other range states will be used for subsequent translocations in the years after the majority of the captive population has been reintroduced. Numbers, timing, and sites selected will be dependent upon the success of the initial releases, as well as the availability of rabbits from other states. If the reintroduced population at Sagebrush Flat achieves a desirable growth trajectory and population size, and assuming rabbits remain available from other states for continued release efforts, reintroductions will proceed sequentially to previously identified and prioritized recovery areas. Baseline stochastic population models suggest that a single reintroduced subpopulation of pygmy rabbits could grow rapidly so that multiple sub-populations could be created in a managed metapopulation in approximately 10 years.

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INTRODUCTION AND BACKGROUND

Natural History of the Columbia Basin Pygmy Rabbit

Pygmy rabbits (*Brachylagus idahoensis*) 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.

The mean adult weights of pygmy rabbits range from 375 to slightly over 500 grams (0.83 to 1.1 pounds), and lengths from 23.5 to 29.5 cm (9.3 to 11.6 in.; Orr 1940; Janson 1946; Wilde 1978; Gahr 1993; WDFW 1995). Overall color is slate-gray tipped with brown. Their legs, chest, and nape are tawny cinnamon-brown, while the entire edges of their ears are pale buff. Their ears are short, rounded, and thickly furred outside. Their tails are small, uniform in color, and nearly unnoticeable in the wild (Orr 1940; Janson 1946; WDFW 1995). The pygmy rabbit is distinguishable from other leporids by its small size, short ears, gray color, small hind legs, and lack of white on the tail.

Pygmy rabbits are typically found in dense stands of sagebrush (*Artemisia* spp.), and are highly dependent on sagebrush to provide both food and shelter throughout the year (Orr 1940; Green and Flinders 1980; WDFW 1995). The winter diet of pygmy rabbits may be comprised of up to 99 percent sagebrush (Wilde 1978), which is unique among leporids (White et al. 1982). Pygmy rabbits are known to climb among the upper branches of sagebrush plants to forage (Green and Flinders 1980).

The pygmy rabbit is the only rabbit in the United States that digs its own burrows (Nelson 1909; Green and Flinders 1980; WDFW 1995). Pygmy rabbit burrows are typically found in deep, loose soils. However, they occasionally make use of burrows abandoned by other species, such as the yellow-bellied marmot (*Marmota flaviventris*) or badger (*Taxidea taxus*) (Wilde 1978; Green and Flinders 1980; WDFW 1995). Pygmy rabbits, especially juveniles, use their burrows as protection from predators and inclement weather (Bailey 1936; Bradfield 1974). The burrows frequently have multiple entrances, some of which are concealed at the base of larger sagebrush plants (WDFW 1995). Pygmy rabbits evade predators by maneuvering through the dense cover of their preferred habitat, often along established trails, or by escaping into their burrows (Bailey 1936; Severaid 1950; Bradfield 1974). Burrows are relatively simple and shallow, often no more than 2 m (6.6 ft. in length and usually less than 1 m (3.3 ft) deep with no distinct chambers (Bradfield 1974; Green and Flinders 1980; Gahr 1993). The number of active burrows may not be directly related to the number of individuals in a given area (Price and Rachlow, in press). Individual rabbits have unique burrows per rabbit as well as shared burrows (Gahr 1993; WDFW 1995). Pygmy rabbits may be active at any time of the day or night and appear to be most active during mid-morning (Bradfield 1974; Green and Flinders 1980; Gahr 1993).

Remote video observation of captive pygmy rabbits indicates that they are most active at night (L. Shipley, Washington State University (WSU) pers. comm.).

Pygmy rabbits begin breeding at age 1 and breeding may occur from February through July (L. Shipley, WSU, pers. comm. 2003). 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). Information based on the behaviors of captive pygmy rabbits indicates that females may excavate specialized "natal" burrows for their litters in the vicinity of their regular burrows (P. Swenson, Oregon Zoo, pers. comm. 2001; L. Shipley, WSU, pers. comm. 2001). The gestation period of captive pygmy rabbits is approximately 25 days and kits emerge from their natal burrows at roughly two weeks of age (L. Shipley, WSU, pers. comm. 2003).

Washington's Columbia Basin Pygmy Rabbit Population

The Washington population was historically confined to the Columbia Basin of central Washington. The pygmy rabbit has been present within the semi-arid Columbia Basin shrub-steppe biome in Washington State for over 100,000 years. This distinct population segment of the pygmy rabbit is believed to have been isolated from the remainder of the species' range for at least 10,000 years, as suggested by the fossil record and population genetic analyses (Lyman 1991; Warheit 2001; Lyman 2004). There are however, few detailed historic accounts of pygmy rabbits in the Columbia Basin and reports are conflicting as to whether the species was common or scarce (Taylor and Shaw 1929; Booth 1947; Dalquest 1948). Pre-1962 museum specimens were collected in four counties: Adams, Grant, Douglas, and Lincoln (WDFW 1995). Little was known about the distribution and status of pygmy rabbits in Washington until Washington Department of Fish and Wildlife (WDFW) conducted surveys between 1987 and 1990 (Dobler and Dixon 1990). Pygmy rabbits were known from six relatively small, isolated populations during the 1990's in Washington. Population sizes were never known; relative numbers of animals were estimated through counts of active burrows. Number of active burrows ranged from 10 – 590 at the six sites. The pygmy rabbit was listed as a threatened species in Washington in 1990 and was reclassified to endangered status in 1993 (WDFW 1993). A state recovery plan for the pygmy rabbit was first written in 1995 (WDFW 1995, 2001, 2003).

Between 1997 and 2001 five of the six populations disappeared (USDI 2003). Populations with the fewest active burrows generally disappeared first. Two populations dramatically declined after fire. By March 2001, rabbits remained only at Sagebrush Flat Wildlife Area, near Ephrata, and that population suffered a sudden large decline during the winter of 2000-2001. Large-scale loss and fragmentation of native shrub-steppe habitats, primarily for agricultural development, likely played a primary role in the long-term decline of the Columbia Basin pygmy rabbit. However, once population numbers dropped below a certain threshold, a combination of other factors such as environmental events (e.g., extreme weather), predation, disease, loss of genetic diversity, and inbreeding likely contributed to the extirpation of all but one subpopulation by 2001. WDFW conducted genetic analyses of pygmy rabbits in 2001. The results indicated that the Columbia Basin population of pygmy rabbits was genetically distinct from other populations and appears to have suffered from a reduction in genetic diversity over the past 50 years (Warheit 2001).

Under emergency provisions of the federal Endangered Species Act in November 2001, U.S. Fish and Wildlife Service (USFWS) listed pygmy rabbits of the Columbia Basin in Washington, with a final rule continuing the endangered listing in March 2003 (USFWS 2003). Sixteen individuals were brought into captivity in 2001 to establish a founding captive population to support future recovery efforts.

Breeding and Genetic Management of Captive Pygmy Rabbits

Captive breeding of Columbia Basin pygmy rabbits began in 2002 at Washington State University and Oregon Zoo. A Science Advisory Group, with members from state and federal wildlife agencies, universities, and zoos was formed to review and direct all aspects of captive breeding and population recovery. Members of the Advisory Group have included individuals from WDFW, USFWS, WSU, Oregon Zoo, Northwest Trek Wildlife Park (NW Trek), University of Idaho and University of Arizona. The theoretical relatedness (*sensu* Queller and Goodnight 1989) was calculated between each combination of males and females and those with the lowest pairwise values were selected to breed, with priority given to the most genetically diverse individuals (i.e., those that possessed rare alleles). Although the captive population existed at multiple localities (a third locality, NW Trek, was later included) it was managed as a single population, with individual rabbits being moved between localities in order to achieve the most optimal pairings. Similar to most captive breeding programs, the Columbia Basin pygmy rabbit breeding program aimed to produce as many purebred animals as possible. Unfortunately, from the first breeding season reproductive output was very poor and Columbia Basin pygmy rabbits produced far fewer young than captive Idaho rabbits in the same facilities.

When the genetic diversity of the Columbia Basin founder population from 2001 was compared to a population of Idaho pygmy rabbits trapped in 2000, the Idaho population was approximately two-times as diverse as the Columbia Basin population, despite the fact that the sample sizes were roughly equal and the geographic extent from which the samples were drawn was the same (Warheit 2001). Therefore this lack of genetic diversity in the founder population suggests that inbreeding depression is linked to the poor reproductive success, as well as skeletal deformities in a few offspring and increased susceptibility to disease (Elias 2004; WDFW 2005a; USFWS 2006).

The two principal disease issues of concern for captive pygmy rabbits have been coccidiosis and mycobacteriosis, both related to the use of soil for maintaining pygmy rabbits in captivity. Disseminated mycobacteriosis due to *Mycobacterium avium* has been the most common cause of death of adult captive pygmy rabbits with fatal cases diagnosed in 28 captive pygmy rabbits between June 2002 and September 2004 in two captive holding facilities. *M. avium* is a bacterium that commonly exists in soil and water, can survive for long periods of time in soil and can be shed in feces. Coccidiosis is caused by a protozoan that invades the intestines and other tissues of animals. A new pathogenic species of coccidian, *Eimeria brachylagus*, has been identified from captive pygmy rabbits (Duszynski et al. 2005). Four captive-born Columbia Basin pygmy rabbit young and three captive-born Idaho pygmy rabbit young died of intestinal coccidiosis during the 2002 breeding season. In 2003, four captive-born Columbia Basin pygmy rabbit young and at least six young Idaho pygmy rabbits died of intestinal coccidiosis. Since the deaths in spring 2002, coccidia levels are now monitored in captive pygmy rabbits. Animals with elevated coccidia levels are treated with antibiotics and the treatment has been effective at decreasing parasite loads. The high incidence of mycobacteriosis and coccidiosis led the Oregon Zoo to collaborate with National Institute of Health to initiate an investigation into the pygmy rabbit cellular immune function. In general, Columbia Basin pygmy rabbits had a significantly poorer immune response than Idaho pygmy rabbits (Harrenstien et al, 2006; K. Mansfield, WDFW, pers. comm.). While it is not possible to say with certainty that this finding is related to the higher degree of inbreeding in Columbia Basin pygmy rabbits, a relationship between diminished genetic diversity and higher susceptibility to mycobacteriosis has been demonstrated for a number of other species (Harrenstien et al. 2006).

In an attempt to address the breeding and disease issues in captivity, experimental intercross matings were performed pairing male or female Columbia Basin pygmy rabbits with individuals originating from Idaho populations. These demonstrated that the two populations were readily capable of interbreeding and producing viable offspring that exhibit higher fitness in captivity than purebred Columbia Basin pygmy rabbits (Sayler unpublished). Reproductive output was markedly different between Idaho, Columbia Basin and intercross rabbits. Pregnancy success was 100% for Idaho females (when paired with Idaho males N = 20) and 51% for Columbia Basin females (when paired with Columbia Basin males N = 35). Pregnancy rates when Idaho or Columbia Basin females were mated to produce intercross offspring were intermediate, approximately 70% (N = 36). Perhaps most importantly, Idaho pygmy rabbits produced 1.75 litters/ breeding female and 3 litters per breeding male, while Columbia Basin pygmy rabbits produced 0.66 litters per breeding female and 0.79 litters per breeding males. No purebred animals produced during the 2005 or 2006 breeding seasons survived to maturity. Demographic models of the purebred Columbia Basin population also independently indicated a population declining to extinction (Warheit 2001), matching the empirical observation of extirpation in the wild.

Although the genetic management plan for the captive population included the objective of producing additional purebred Columbia Basin pygmy rabbits, this objective was abandoned due to continuing poor reproductive success and likely reduced fitness of the population. Most captive breeding programs for endangered species are designed to maintain a specific percentage of the wild populations' genetic diversity for a specified time period. Soulé et al (1986) originally proposed that there should be an effort to retain 90% of the wild population's genetic diversity for a period of 200 years, although in practice this goal has been relaxed to a lower percentage for a shorter period of time. Attempts to retain in captivity a certain percentage of the wild population's genetic diversity are grounded in two fundamental assumptions: (1) the wild population is still relatively large (e.g., Ne [effective population] > 500, or N > 1000 individuals) and genetically diverse, and (2) the founding population for the captive program is a random sample of the wild population, and is large enough to retain most of the genetic diversity (measured as allelic diversity or heterozygosity) of the wild populations (see Frankham et al. 2002 for discussion of specific goals). Both the WDFW's Science Advisory Group and the USFWS' Pygmy Rabbit Recovery Team (which has included members from WDFW, WSU, BLM, Oregon Zoo, The Nature Conservancy, Foster Creek Conservation District, Washington Department of Natural Resources and U.S. Natural Resources Conservation Science) concluded that it was necessary to produce intercross animals with a lower percentage of the founders' genes and higher genetic diversity for release. This was expected to result in increased fitness and likelihood of successful reintroduction to the wild because of the genetic rescue effect. Genetic rescue, or genetic restoration (Hedrick 2005), has been achieved for a number of wildlife species (e.g., Florida panther) by introducing more genetically diverse individuals from another population (Tallmon et al. 2004). The primary difference in the situation with the Columbia Basin pygmy rabbit was that genetic restoration had to be effected using the captive population because the wild population was potentially extirpated.

Genetic diversity did increase as a result of intercrossing animals, with reproduction largely improving for captive rabbits progressively over the years (Table 1). Unfortunately, while production of kits increased, the survival of emerged kits decreased, with maternal neglect and disease (i.e. coccidia) the most common causes of mortality. High levels of disease occurrences continued to hamper attempts to increase the size of the captive population and it is believed that cell-mediated immunosuppression persisted as a result of inbreeding depression (Harrenstien 2006) despite attempts to infuse the population with new genes.

No purebred Columbia Basin pygmy rabbits remain today in captivity; and as a result, recovery now depends upon the reintroduction of intercross animals with varying percentages of representation from Washington founders. In 2010, the percentage of Columbia Basin genes in captive individuals ranged from 42 to 87%, with the largest proportion of the population at 75% (Table 2). Intercross breeding has conserved some of the Columbia Basin population's remaining unique genetic characteristics while restoring genetic diversity lost during early bottleneck events (Warheit pers. comm.).

Project Goals and Objectives

The goal of the recovery program for the Columbia Basin pygmy rabbit is to re-create free-ranging populations of sufficient size and number to ensure the long-term existence of this population segment in Washington (WDFW 1995, USFWS 2011). Specifically, the Washington State Recovery Plan (1995) indicates that pygmy rabbits could be considered for delisting from State Endangered status when a minimum population of 1400 adult rabbits is comprised of at least two areas supporting at least 500 adult rabbits and four additional areas that support at least 100 adult rabbits. All of the areas must be in secure habitat with long-term management plans in place which conserve pygmy rabbits and their habitat. For delisting from State Threatened status a minimum population of 2800 adult rabbits must comprise at least four areas of at least 500 adult rabbits and eight additional areas with at least 100 adult rabbits. All of these areas must be in secure habitat with long-term management plans in place.

Now that recovery relies on the reintroduction of pygmy rabbits to Washington, the first step of the project is to establish a founding population of pygmy rabbits to Sagebrush Flat Wildlife Area. The measure of success will come from studies to assess demographic and genetic outcomes as well as population viability models.

Other specific conservation objectives of the recovery program are to:

1. Refine translocation and reintroduction methodologies for pygmy rabbits that could be used for pygmy rabbit populations throughout the western United States and other lagomorph species worldwide;
2. Create a demographic and molecular (i.e., genetic and epigenetic) database for the newly-established Columbia Basin pygmy rabbits that could be useful for future population management and conservation in Washington and elsewhere throughout the species' historic distribution;
3. Collaborate with zoos, universities and federal and state wildlife agencies to accomplish in situ conservation of an endangered population;
4. Train new students in demographic and molecular monitoring and reintroduction methods for an endangered mammal; and to
5. Disseminate information through regional reports and scientific journals for use in this and future lagomorph conservation programs across North America and the world.

LESSONS LEARNED TO INFORM REINTRODUCTION EFFORT

Several prior studies have provided good background for planning reintroductions of Columbia Basin pygmy rabbits in Washington. They include: a pilot reintroduction study completed in Idaho (Westra 2004); a small-scale trial release into Sagebrush Flat in 2007 (Sayler et al., unpublished manuscript); results of ongoing studies of captive pygmy rabbits (Elias et al. 2006); and field investigations of wild pygmy rabbits in Idaho (J. Rachlow, pers. comm.).

Idaho

A pilot reintroduction study was conducted for the pygmy rabbit using captive-reared, non-endangered pygmy rabbits reintroduced in southeastern Idaho (Westra 2004). Thirteen rabbits were reintroduced in August 2002, 7 in September 2002, 7 in July 2003, and 15 in February 2003 using small soft release enclosures.

Some key observations and conclusions from that study were:

• Captive-bred pygmy rabbits transported well to the release site and continued to feed during transport and immediately after placement in the temporary confinement enclosures around the artificial burrow openings. Individuals also appeared to be consuming natural forage by the first visual observation (usually the first day) after full release.

• About 86% (n = 36) of the released pygmy rabbits remained within 1 km of the original release site and extensively utilized artificial burrows and natural burrows. Excluding 6 animals that were censured because of lost radio signals, pygmy rabbits dispersed 0 – 859 m during the first week after release (Sayler, unpublished data).

• More than half of the release population died within the first 18 days, however, this mortality rate may have been reduced considerably if the initial soft-release containment enclosures had adequately protected pygmy rabbits from weasels. Predators killed at least 20 of 42 rabbits (47.6%), with long-tailed weasels (*Mustela frenata*) confirmed to have killed at least 12 individuals (Sayler et al., unpublished manuscript). Also, the artificially high density of burrows and pygmy rabbits on the release site may have created a locally concentrated population that attracted predators more than if the pygmy rabbits had been released over a larger area.

• Despite the small number of released female pygmy rabbits surviving to the first breeding season (n = 4), there was evidence of successful reproduction and pygmy rabbits continued to be observed on the release site for a year after the study was terminated (Sayler et al., unpublished manuscript). Empirical observations suggest that even small numbers of surviving females may produce offspring in their first breeding season that also may survive for a year or more.

Overall, the pilot project in southeastern Idaho demonstrated the potential to restore populations of pygmy rabbits in Washington. Captive-bred pygmy rabbits transported well, adults tolerated radio collars well, and adapted quickly to eating natural forage after release. The majority of released rabbits remained within 1 km of the release site and utilized both artificial and natural burrows.

Washington

In 2007, a small-scale experimental release of Columbia Basin pygmy rabbits was conducted at the WDFW Sagebrush Flat Wildlife Area in eastern Washington. Twenty captive-raised, intercrossed pygmy rabbits (10 males and 10 females) were released at two spatially separate artificial burrow clusters with 10 rabbits each and no soft release containment enclosures. Median survival was only 6.5 days for males (max. 228 days) and 19 days for females (max. 153 days); overall survival to 65 days was very low (11.8%, SE = 0.08). Mortality from predators was the dominant proximal cause of death for most reintroduced pygmy rabbits. Animals were released during the breeding season and all females were bred prior to release. Males did not settle at release sites and dispersed widely.

Predation was the proximate cause of mortality of released pygmy rabbits. Factors contributing to mortality could have included animals being unfamiliar with the site, insufficient existing burrow infrastructure, or naivety of captive raised animals. Most predation mortality that could be determined was attributed to coyotes (*Canis latrans*) and raptors. Short-term survival of pygmy rabbits could likely be improved by modifying the soft-release techniques used in the releases in Idaho in 2002 (Sayler et al., unpublished manuscript).

Population Modeling

Using preliminary data from reintroduced animals in Idaho (Westra 2004), population modeling (Zeoli 2008) demonstrated that annual supplementation for 2 - 3 additional years beyond the initial release is likely necessary to achieve an increasing local population. Baseline population models indicate that a single reintroduction of 20 to 60 pygmy rabbits will result in estimated 10-year populations of 159 to 454 rabbits. When initial reintroduced pygmy rabbit populations of 20 to 60 rabbits are augmented annually for two subsequent years, projected population sizes range from about 339 to 989 rabbits (Zeoli 2008). Thus, planning for continual supplementation after the initial year of releases is an integral part of a reintroduction plan.

If estimates of carrying capacity derived from Idaho studies are relevant to Washington, then in relatively ideal conditions, population models suggest that several populations might be founded in 10 to 15 years with the following scenario: a) introduce 30 animals, b) supplement for 2-3 additional years, c) start second population with 30 animals, d) supplement for 2-3 years, e) in year 5 or 6, harvest 30 rabbits from population 1 to start population 3, and f) continue this sequential process. The projected result of this reintroduction process is a managed metapopulation of six separate populations, ranging in size from 30 to about 400 animals, with a total metapopulation of about 1,065 pygmy rabbits (Zeoli 2008).

However, these baseline population models lack adequate information from studies of wild pygmy rabbits to accurately parameterize the models, which creates significant uncertainty in the projected growth rates of reintroduced pygmy rabbit populations. Consequently, the ecological research conducted during the initial stages of the reintroduction program in Washington will provide important data to be used in an adaptive fashion to adjust reintroduction procedures and develop better population models. Such refined population models will contribute to developing appropriate quantitative delisting criteria and help achieve recovery objectives as rapidly as possible.

Pygmy Rabbits in the Wild

Multiple studies in the wild since 2007 also provided some important insights into pygmy rabbit biology that helped inform the current reintroduction strategy. Research on juvenile pygmy rabbits confirm that mortality was highest in the first two months after emergence from the natal burrow (Estes-Zumpf & Rachlow 2009) and that predation was the largest source of mortality, as has been seen for adults (Price et al. 2010). The use of glue-on transmitters was found to have serious limitations and required the frequent (12-14 day) recapturing of juveniles to reattach the devices (Estes-Zumpf & Rachlow 2007). Therefore, although close monitoring of juveniles to confirm that they are persisting after release would be valuable, the invasive nature of radio-transmitters for juveniles makes it costly to consistently track already vulnerable juvenile rabbits in the long term. In addition, new tools for molecular monitoring of pygmy rabbit populations have become available in recent years. In addition to the seven molecular genetic markers designed for European rabbits that were previously used for pygmy rabbits, ten new species-specific markers (Estes-Zumpf et al. 2008) have also been used successfully to study dispersal, gene flow and population structure in pygmy rabbits (Estes-Zumpf et al. 2010).

Other Lagomorph Species

The reintroduction of other rabbit species provides examples of release techniques that have proven successful. European rabbits (*Oryctolagus cuniculus*) have been restocked repeatedly and multiple studies have investigated how to increase survival after reintroduction. The use of temporary enclosures has shown to increase post release survival, especially for female rabbits (Letty et al. 2008). Letty et al. hypothesized that high levels of stress from translocation for hard released rabbits led to higher mortality, while soft release temporary enclosures allowed the rabbits to adjust and habituate before release. It is likely that pygmy rabbits also experience higher stress levels due to transport, sampling and radio-collaring. Therefore, it is hoped that transition time in soft release enclosures will return stress hormones back to baseline before release and, ultimately, increase survival.

High predation after reintroductions of European rabbits has also hindered establishment of released populations. Calvete & Estrada (2004) determined that initially protecting the released rabbits from predation by fencing with electric wires, shooting problem predators and ensuring that vegetation cover was sufficient inside enclosures increased significantly the survival of rabbits. Similarly, the strategy used in the Washington reintroductions aims to include enclosures for initial protection, electric fencing, problem animal control (e.g., weasel traps around enclosures, control of problem coyotes), as well as high sagebrush density inside enclosures and in the core release area.

The recovery strategy for riparian brush rabbits (*Sylvilagus bachmani riparius*) in California has been centered on controlled propagation of the population in large predator-resistant enclosures (Williams et al. 2002). Once offspring reach dispersal age, they are removed from the enclosures and translocated to a reintroduction site and held for five days in temporary enclosures before release. This approach has been successful in establishing a population that has continued breeding on the site. Since spring is breeding season for pygmy rabbits, some controlled and natural propagation was used during the first 2011 release and is planned for subsequent breeding seasons, when possible. Large enclosures are also incorporated into the release strategy for Washington, though the size will be smaller than those used for brush rabbits to ensure financial and logistical viability. As was done in California, newly-independent juveniles will be moved to the wild to hold them in soft release enclosures before release.

Hamilton et al. (2010) found that brush rabbits were most susceptible to post-release mortality during the first 4 weeks following reintroduction, although longer time in soft-release enclosures resulted in increased monthly survival. They suggest that the duration that rabbits should remain in soft release enclosures will likely vary with species and habitat, and therefore they recommend that an adaptive management approach with ongoing monitoring be used to adjust methods as conditions dictate.

REINTRODUCTION PLAN

Release Sites

Potential reintroduction sites for pygmy rabbits in the Columbia Basin were evaluated by developing maps of potential habitat using geographic information systems (GIS), field surveys for suitable soils and vegetation complexes, and expert opinion of biologists and managers working for state and federal agencies (USFWS 2011). Sagebrush Flat (Douglas County) and Beezley Hills (Grant County) were ranked as the number one and two priority reintroduction sites, respectively because of: 1) previous known occupation by pygmy rabbits, 2) access and management conditions for research and monitoring, 3) habitat condition and restoration activities, and 4) land area available to support a pygmy rabbit population. Sagebrush Flat was selected by the Science Advisory Group as the best initial reintroduction site (Fig. 1). If the reintroduced population at Sagebrush Flat achieves a desirable growth trajectory and population size, and assuming other source populations support continued release efforts, reintroductions could proceed to previously identified and prioritized recovery sites.

Changes to Release Techniques

The goal of the captive breeding program has been to release captive-bred animals at suitable sites within the population's historical distribution in Washington to begin the process of its recovery in the wild (USFWS 2011). Unfortunately, efforts to breed pygmy rabbits in captivity have had limited success over the past ten years. Two major obstacles have hindered reintroduction and recovery efforts of pygmy rabbits in Washington: 1) too few rabbits produced in captivity for large-scale releases, and 2) low genetic diversity that likely led to inbreeding depression.

The current reintroduction strategy has been amended to address these demographic and genetic obstacles. Specifically, to ensure that some rabbits will be able to survive long enough to be supplemented and to increase opportunities to breed in the wild, as many animals as possible will be released initially and populations will continue to be supplemented to encourage persistence on the ground over the course of the three-year program. To accomplish this, releases will contain both captive-bred and translocated wild pygmy rabbits from other range states with large enough numbers to help establish the population. In addition to providing a larger number of individuals, the release of wild individuals from other populations will help to further infuse the reintroduced population with new genes to promote genetic diversity and, hopefully, decrease or eliminate any deleterious effects of inbreeding (e.g. increased disease susceptibility and reduced reproductive performance). Captive-bred and wild rabbits will be placed together in enclosures for more managed breeding on-site and they will also be released on the landscape together to interbreed naturally.

The captive pygmy rabbits will be released between May 2011 and summer 2012. Retaining a small number of captive pygmy rabbits beyond summer 2012 will only occur if it is necessary for continued success of our reintroduction efforts. Numbers and timing of supplementation with additional wild rabbits after the captive population is released will depend upon ongoing assessments of program results and the availability of rabbits from neighboring states.

Several changes were made to the reintroduction techniques to reduce predation and improve survival of pygmy rabbits in the initial post-release period. Because pygmy rabbits are vulnerable to a wide range of abundant generalist and some specialist predators, soft release enclosures, artificial burrows and augured holes will be used to protect rabbits from digging predators (i.e., badgers and coyotes) and raptors. In addition, predator control will be done initially and intermittently throughout the reintroductions in the form of lethal and non-lethal hazing of raptors, and trapping of problem weasels, coyotes and badgers. In addition, soft release enclosures allow an increased ability to monitor the health of the rabbits more closely up until release. Time in enclosures and artificial burrows will hopefully increase fidelity to that site and minimize large-scale and/or long-distance dispersal from the release site.

Reintroduction Infrastructure

Reintroduced animals, especially those that are captive-bred, 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). For pygmy rabbits in the wild, burrows provide their main form of shelter from terrestrial and avian predators. To improve survival of rabbits after releases in Washington, artificial burrows have been constructed and will be maintained in the release area of Sagebrush Flat. In the core release area, approximately 15 artificial burrows are available from the 2007 releases and approximately 60 more have been installed for soft releases. Maps of burrow locations generated just prior to the local extirpation of the pygmy rabbit population at Sagebrush Flat (Siegel 2002) were used as a guide to place artificial burrows on the reintroduction site (Fig. 2).

Selection of specific artificial burrow sites on Sagebrush Flat were made using aerial photographs to identify mounded soils with dense areas of sagebrush. Site preparation for the artificial burrows involved only minimal disturbance of soil from a trench dug about 0.4 m wide by 3 - 4 m long with the soil being replaced over a 3 – 4 m length of 10 cm diameter plastic drainage tubing used to form the burrow. Metal U-shaped stakes were used to secure the two burrow openings to the ground in an effort to deter predators (e.g., coyotes, badgers) from easily digging up burrow systems.

Small, temporary soft release enclosures were erected throughout the core release area, 200-450 ft apart, and each included an artificial burrow and natural growth (i.e. sagebrush, grasses and forbs; Fig. 2 & 3). Along with artificial burrows that the rabbits can use for cover in the long term, these temporary enclosures are anticipated to increase post release survival since this has been shown to be true for other lagomorph species (Letty et al. 2008). The approximately 8 ft diameter soft release enclosures are constructed of 4 ft tall welded wire fencing with a small mesh size (1 in by 0.5 in) to exclude predators such as long-tailed weasels. The bottom of the enclosures are fitted with nylon weed cloth and staked at 1 in intervals into the ground 6 in deep to limit rabbits immediately digging out and to discourage predators trying to crawl under the enclosure (Fig 3). 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. Once the rabbits have been held for a sufficient amount of time, the enclosures will be breached on two sides and the rabbits allowed to come and go.

Large enclosures were also erected on Sagebrush Flat. The large enclosures (approximately 6 and 10 acres each) are semi-permanent structures that could be used throughout the reintroduction efforts. The same small mesh welded wire as the soft release enclosures is used for the large enclosure, but instead the height of the fence consists of two 4 ft widths of fencing. The bottom of the enclosure was buried approximately 12 in to discourage digging predators, while the top is a 'floppy top' design that folds to the outside to prevent weasels from climbing over (Fig. 4). Artificial burrows were placed inside the large enclosure and mounds with concentrations of burrows were covered with netting to provide the rabbits with some protection from avian predators. An electric wire was also placed on the outside of the enclosures to further discourage digging and jumping over the fence.

Preparation of reintroduction sites at Sagebrush Flat included management of old fields to increase shrub cover, construction of large enclosures and soft release enclosures, removal of unneeded fence posts to reduce raptor perches, placement of bird spikes on existing structures, signage to discourage unauthorized public access, weed control, construction of fire breaks, and other management activities designed to improve habitat conditions for pygmy rabbits (USFWS 2011).

Release Protocol

The release protocol elements are:

1. Captive-bred, intercrossed, young-of-the year kits born at the breeding facilities are transferred to the release area and put into small soft release enclosures to promote residency. The benefit of having these kits released right away is that these rabbits with Columbia Basin genes would not have been exposed to long-term captivity. Since there is no parental care after kits emerge from the natal burrow, it is possible that these progeny already have the instincts that they need to be successful in the wild. They are held for 10-14 days in soft release enclosures that contain growing sage, grasses and forbs, and the enclosures will be supplemented with extra forage. Pygmy rabbits held under these pen conditions have higher growth rates and body mass than individuals kept in smaller enclosures or cages without such vegetation and space (Sayler,

unpublished data), and are expected to enter the release phase in better physical condition.

2. Wild pygmy rabbits are transported from other range states within a day of being captured. They are put into enclosures to discourage homing instincts or dispersal and held in the same way as described above for young captive-bred rabbits or possibly kept for a season of breeding before release (see #3, below).
3. Captive-bred and wild males and females are transported to the release site and placed in the large enclosures for longer term holding. Rabbits can remain in these six and 10- acre enclosures for multiple breeding seasons if needed. Some rabbits may be kept in the large enclosures to promote breeding in the wild. The large enclosure may also serve as a sort of 'half-way house' for captive animals to adapt to the environment. In the large enclosures the rabbits are able to forage and shelter as they would in the wild, and the females have an opportunity to raise multiple litters over the season. Once kits are independent, they are immediately moved to soft release enclosures, similar to the kits born in captive facilities.
4. All pygmy rabbits receive a microchip radio transponder inserted into the nape of the neck prior to release.
5. All wild and captive pygmy rabbits have tissue samples taken and analyzed to produce molecular genotypes for the founder individuals of the reintroduced population.
6. Radio collars equivalent to those being used on pygmy rabbits in field studies in Idaho (J. Rachlow, pers. comm.) are used for all released adults.
7. Released juveniles may be fitted with transmitters (glue-on or another non-collar method) for a limited about of time, but trapping for re-fitting is kept to a minimum.
8. Pygmy rabbits identified for release are inspected for general overall health and approved for release by attending project veterinarians at WDFW, WSU, NW Trek, or Oregon Zoo.
9. Pygmy rabbits are transported to release sites in individual carriers and provided fresh food to allow ad libitum feeding in transport.

Table 3 also outlines the implementation schedule of the reintroduction effort beginning in 2011.

Reintroduction Research Objectives

The factors that originally contributed to the extirpation of local populations of the Columbia Basin pygmy rabbit are largely unknown, because the last known population was extirpated suddenly and before ecological studies could be conducted. Consequently, the reintroduction program offers the opportunity to simultaneously restore local populations while gaining better understanding of population dynamics and ecological factors critical to the long-term survival of the Columbia Basin population as a whole.

Many basic aspects of the population biology of pygmy rabbits are either poorly known or have not been duplicated among independent field studies. Improving the estimates of survival and key reproductive parameters, including their variability, is important to improve the results of population modeling. The following primary research objectives are central to the reintroduction and monitoring program:

1. Describe the behavior, dispersal, and movement patterns of reintroduced wild and captive-bred pygmy rabbits and their progeny.
2. Quantify, describe and compare reproductive success, mortality agents, and ecological factors potentially related to survival rates of wild and captive-bred pygmy rabbits following their release.
3. Evaluate the importance of soft-release techniques (e.g., soft release enclosures and provision of artificial burrows) to determine their influence on dispersal and survival rates of released pygmy rabbits.
4. Assess ecological relationships between pygmy rabbits and the shrub-steppe vegetative community to develop better quantitative models of habitat use and selection.
5. Collect tissue and fecal samples to monitor spatial and temporal trends in the population genetics of progeny of reintroduced wild and captive-bred pygmy rabbits. This information will be used to determine if a genetic signature of the Columbia Basin population remains on the landscape and to evaluate the potential need for future genetic management (e.g., genetic consequences of augmentation, supportive breeding, enhancing gene flow). If possible, fitness and survival consequences related to the genetic make-up of the reintroduced population will be measured over time.
6. From the above data, develop better empirically-driven population viability models and comprehensive systems dynamics models (Ford 1999) to project the timeline and management conditions necessary for evaluating and achieving recovery objectives to enable federal and state delisting of the Columbia Basin pygmy rabbit population (WDFW 1995, 2001, 2003; USFWS 2007, 2011).

Post-Release Monitoring

Information derived from radio-telemetry monitoring of released rabbits will be used to help evaluate the size, distribution, and survival of the reintroduced population in the initial year(s) of the releases. Since the radio-collars have mortality sensors, visual observations are not necessary to ascertain that the animal is still alive. Each released adult rabbit is tracked to determine its approximate location and verify that it is still alive using remote listening stations whenever possible to limit impacts and disturbance. Visual observations are made opportunistically during the first 3 months after release, which is likely to be a period of high mortality. Based upon previous field studies (Westra 2004), tracking is conducted by investigators on foot, using hand-held directional antennas. Previous experience has demonstrated that pygmy rabbits may be approached carefully on foot by circling around the suspected location, which often times may be subsequently confirmed by direct visual sightings or by locating a rabbit in a burrow. Rather than through triangulation, which is difficult in the road-less shrub-steppe habitat of Sagebrush Flat, radio locations are marked directly by taking approximate global positioning system readings of the spot in which the rabbit was originally located, even if it subsequently moves away from the investigator.

When reintroduced pygmy rabbits produce their first progeny in the wild, indirect methods such as non-invasive genetic analyses will be used to estimate population makeup, dispersion and size. Because all the founders of the reintroduced population are genotyped, analyzing fecal pellets can not only establish the species of rabbit, but will also allow individual identification and determine parentage of kits. Once young emerge from natal burrows, pellet samples are collected and recorded on a regular basis.

An annual assessment of the success of the program will be made within approximately one year after release and following the breeding season. Determination of success will be made based on the survival of the captive-bred and wild founders and the reproductive success of pygmy rabbits in the wild.

In addition to radio monitoring and genetic analyses, other techniques may be used to census pygmy rabbit populations, including:
1. Monitoring artificial burrows for occupancy.
2. Monitoring occupancy of new natural burrows or historic burrows.
3. Tracking during fresh snow events in winter to identify the number of active burrows.
4. Mark-recapture estimates as animals are trapped and captured for radio replacement and ongoing studies.
5. Remote cameras installed in key areas to record reproduction and residency.

Using a combination of these techniques in the first several years of the reintroduction program will allow the variability and reliability of different population estimates to be evaluated empirically.

Longer Term Recovery

The main recovery goal is to establish a viable, managed metapopulation of pygmy rabbits in Washington. The Washington State Recovery Plan for the pygmy rabbit (WDFW 1995) states that the species will be considered for delisting from State Endangered status when the following criteria have been met:
1. The state supports a minimum 5-year average of at least 1400 adult pygmy rabbits in six populations; two populations with at least 500 adults each and four populations with at least 100 adult rabbits each.
2. Habitat security for the six populations has been established.

In addition, the pygmy rabbit will be considered for delisting from State Threatened status when the following criteria have been met:
1. The state supports a minimum 5-year average of at least 2800 adult pygmy rabbits in at least 12 populations; four populations with at least 500 adults each and eight populations with at least 100 adult rabbits each.
2. Habitat security for the 12 populations has been established.

The success of the current recovery efforts in Washington will depend on establishing a pygmy rabbit population on Sagebrush Flat Wildlife Area as a source of founder individuals. To assist in creating this population, the adult rabbits remaining in captivity will be released into large enclosures on Sagebrush Flat Wildlife Area in 2012. These animals will be used for breeding in the enclosures for as long a possible to provide an additional source of kits for reintroduction to the wild.

Since additional supplementations are necessary to achieve population growth (Zeoli 2008), wild rabbits will continue to be translocated from other states for two to three years following the 2011 translocations. While preliminary population models with limited data indicate that up to six new populations might be established in 10 to 15 years (Zeoli 2008), population estimates collected in the field as well as population models with more refined inputs over time will determine the number of supplementations needed to achieve population viability, when new populations can be founded, and when supplementation can be slowed or stopped. Similarly, the federal Recovery Plan emphasizes that additional information on appropriate sizes, number, distribution, and configuration of free-ranging subpopulations necessary to delist the Columbia Basin pygmy rabbit need to be established as the reintroduction effort progresses (USFWS 2007, 2011). The timing of reintroduction of additional populations in new locations will also depend on finished preparations of new areas (e.g. safe harbor agreements, construction of reintroduction infrastructure, habitat improvement) as well as the availability of wild rabbits from other states to ensure sufficient numbers to continue recovery.

CONCLUSIONS

The Columbia Basin pygmy rabbit was historically found in at least four counties in central Washington. Their numbers likely declined as a majority of shrub-steppe was converted to cropland. Furthermore, the fragmentation of shrub-steppe habitat and the resulting isolation of populations led to further decline in previous decades to the point of extinction.

After initial reintroductions, recovery of pygmy rabbits in this state will require that larger populations become established through connecting existing populations and reestablishing additional populations. Maintaining genetic connectivity may require periodic translocations between subpopulations if habitat connections cannot be re-established. Restoring sufficient resources and habitat for recovery will require a sustained effort involving many partners, and will not be possible without cooperation with many agencies and landowners. Partnerships with individuals and organizations with goals for habitat restoration and wildlife conservation will be crucial in the long-term recovery process.

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Table 1. Reproductive performance of all captive pygmy rabbits, 2003 - 2010

	2003	2004	2005	2006	2007	2008	2009	2010
Kits/female	4.52	3.90	5.81	5.17	6.16	6.13	7.61	7.4
Pregnancies/ Female	1.38	1.29	1.76	1.81	2.06	1.75	1.03	1.89
Pairings/female	3.62	2.81	4.05	3.28	3.90	3.58	4.19	2.84
Kits/male	6.79	5.47	8.13	7.44	7.35	7.00	8.43	7.26
Sirings/male	2.07	1.80	2.47	2.60	2.46	2.00	2.5	1.89
Pairings/male	5.43	3.93	5.67	4.72	4.65	4.10	4.53	2.76
Survival of emerged kits*	66.7%	56.9%	61.1%	60.2%	51.3%	49.5%	38.6%	40.6%

*Survival to weaning

Table 2. Number of Columbia Basin pygmy rabbits in captive breeding program by proportion of Columbia Basin genes as of December 2010

Proportion of Columbia Basin genes	Northwest Trek	WSU	Oregon Zoo	Total
100%	0	0	0	0
87.50%	0	1	0	1
84.38%	0	1	1	2
81.25%	0	8	3	11
80.87%	0	1	0	1
79.69%	1	10	0	10
78.91%	0	1	2	3
78.58%	0	1	0	1
78.13%	0	4	2	6
77.34%	0	4	0	4
76.95%	0	2	0	2
76.56%	0	0	0	0
75.78%	0	3	0	3
75.00%	1	5	11	17
74.24%	0	3	0	3
74.22%	1	2	2	5
73.44%	0	1	0	1
73.05%	0	2	0	2
71.88%	1	0	2	3
71.09%	1	0	0	1
70.32%	0	0	4	4
69.93%	1	0	0	1
69.53%	0	0	0	0
68.75%	1	0	0	1
67.19%	0	0	0	0
65.63%	1	0	0	1
64.84%	0	1	0	1
62.50%	1	0	0	1
60.94%	0	2	0	2
58.59%	0	4	0	4
58.20%	2	0	0	2
57.81%	0	1	0	1
42.198%	1	3	1	5

Table 3. Timing of reintroduction effort beginning in 2011.

IMPLEMENTATION SCHEDULE	
Spring-Summer 2011	• Captive adult and juvenile rabbits moved to enclosures on-site for release
Fall 2011	• Wild rabbits translocated from OR and NV and captive adult rabbits brought to large enclosures for over-wintering and breeding in 2012
Winter 2011-2012	• Surveys for released rabbits conducted on Sagebrush Flat, surveys on other possible habitat for presence
Spring-Summer 2012	• Wild rabbits translocated from NV and UT to Sagebrush Flat for release to wild or large enclosures • Juvenile rabbits from captive facility and large enclosures moved to soft-release enclosures on-site and released • Remaining captive adults are likely released to large enclosures
Fall 2012	• Wild rabbits translocated from other range states to Sagebrush Flat for release or over-wintering in large enclosures
Winter 2012-2013	• Surveys for released rabbits conducted on Sagebrush Flat, surveys on other possible habitat for presence
Spring-Summer 2013	• Wild rabbits translocated from other range states to Sagebrush Flat for release or held in large enclosures • Juvenile rabbits from large enclosures moved to soft-release enclosures and released
Fall 2013	• Wild rabbits translocated from other range states to Sagebrush Flat for release or over-wintering in large enclosures
Winter 2013-2014	• Surveys for released rabbits conducted on Sagebrush Flat, surveys on other possible habitat for presence

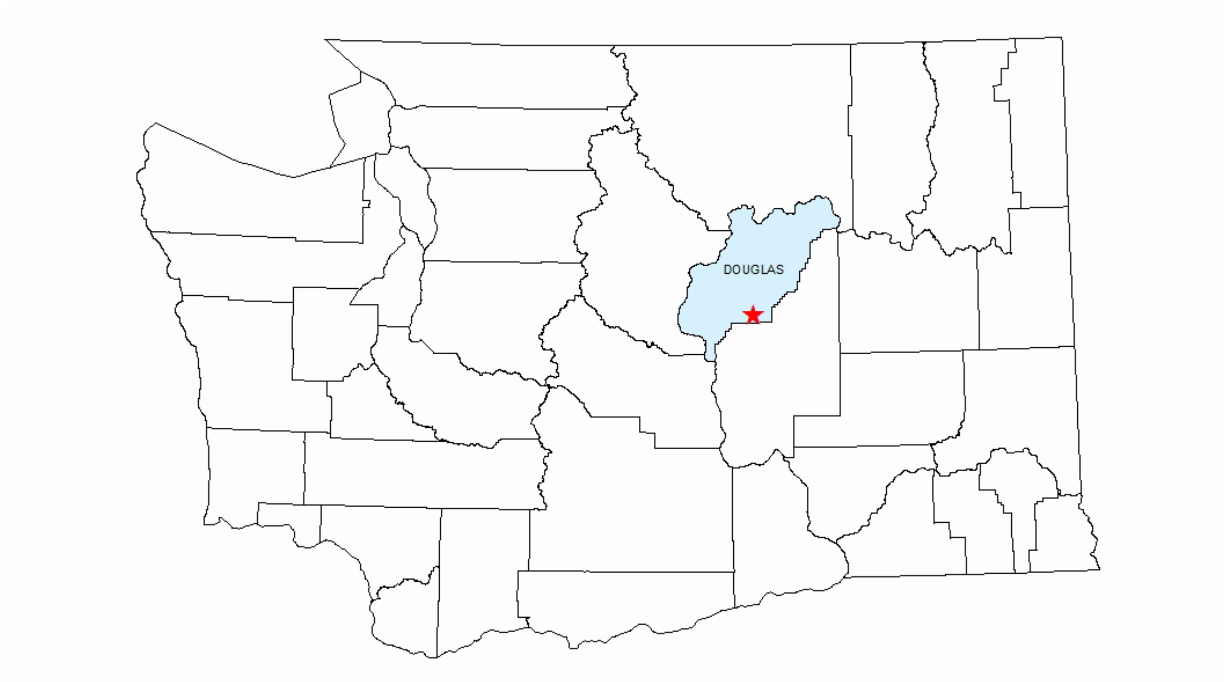

Fig 1. Location of Sagebrush Flat Wildlife Area within Washington State.

Fig 2. Map of aerial view of Sagebrush Flat Wildlife Area with the boundary indicated (green line).

Fig 3. Soft release enclosure with wire mesh and aluminum flashing.

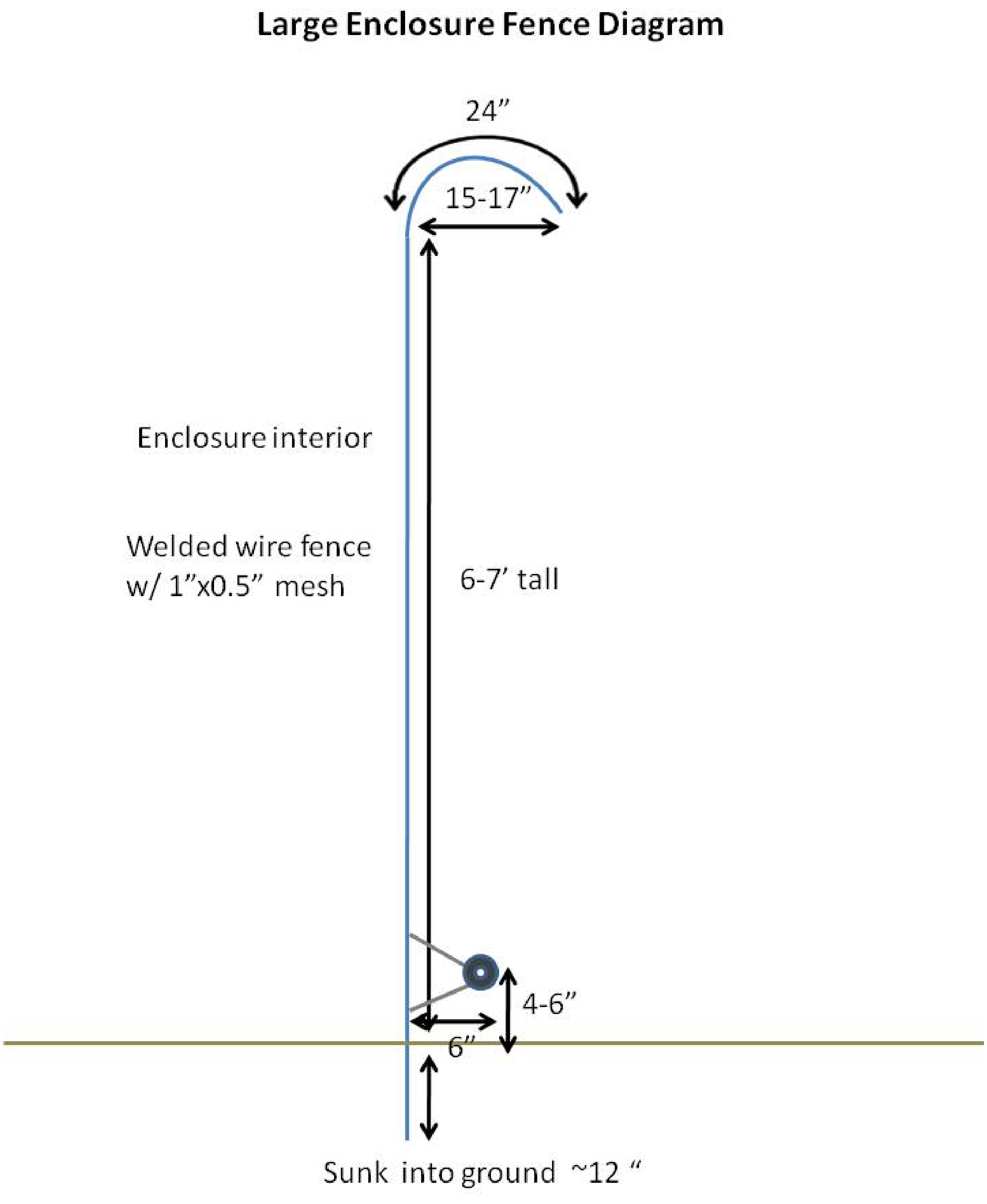

Fig 4. Diagram of large enclosure design with floppy top and electric wire on the bottom.