CONTROLLED PROPAGATION AND REINTRODUCTION PLAN FOR THE RIPARIAN BRUSH RABBIT (*SYLVILAGUS BACHMANI RIPARIUS*)

by

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EXECUTIVE SUMMARY

We present a plan for controlled propagation and reintroduction of riparian brush rabbits (*Sylvilagus bachmani riparius*), a necessary set of tasks for its recovery, as called for in the *Recovery Plan for Upland Species of the San Joaquin Valley, California*[1]. This controlled propagation and reintroduction plan follows the criteria and recommendations of the U.S. Fish and Wildlife Service's *Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act*[2]. It is organized along the lines recommended in the draft version of that policy and meets the criteria of the final policy. It should be viewed in an adaptive management context in that as events unfold, unexpected changes and new information will require modifications. Modifications will be appended to this document.

Controlled Propagation is necessary for the riparian brush rabbit 1) to provide a source of individuals for reintroduction to restored habitat for establishing new, self-sustaining populations, 2) to augment existing populations if needed, 3) and to ensure the prevention of extinction of the species in the wild. We propose to establish three breeding colonies in separate enclosures of 1.2-1.4 acres each. Predator-resistant enclosures will be erected around existing, but unoccupied natural habitat for brush rabbits. Enclosures will be located on State land surrounded by irrigated agriculture that provides no habitat for brush rabbits. Further, the pens are designed to prevent escape of rabbits. Thus, there is little risk that escaped rabbits could found a colony outside the known historical range of the species. Each enclosure will house three rabbits of each sex. Females in the 3 enclosures could collectively produce as many as 81-180 young per year for translocation, though a more conservative estimate, assuming lower fecundity and 67% survival to translocation, is about 54-72. Breeders will be obtained from the South Delta population, which is under severe threats of extinction because of urban development, flooding, and habitat destruction. After one annual season of breeding, extending from about December to May or June, they will be returned to the wild and new breeders will be selected for the following year's production. They will be released where they were captured, or at Caswell Memorial State Park to augment that population, or at re-establishment sites, as genetics and circumstances indicate. Individual breeders to be housed together will come from different areas within the existing habitat of the South Delta population to ensure that parents have relatively low kinship values. In these ways we will maximize genetic diversity in the reintroduced populations and prevent depletion of genetic diversity in the source population.

Young will be reintroduced first to the San Joaquin River National Wildlife Refuge where restored and protected habitat is available. Each cohort of young will be removed from the enclosures and translocated when they reach a size where they would normally disperse. Translocated rabbits will be held for up to 5 days in temporary enclosures around natural habitat at the reintroduction site before release. Nest boxes and escape

[1] U.S. Fish and Wildlife Service, Portland, OR, 319 pp., 1998.
[2] U.S. Fish and Wildlife Service and U.S. National Marine Fisheries Service, Federal Register 65:56916-56922, 20 Sep. 2000.

structures will be provided at the release site to provide more places to retreat from predators. Reintroduced rabbits will be closely monitored by radio telemetry and periodic livetrapping to assess success. We expect to release young from the controlled propagation program to the Refuge over a 3-5-year period as habitat restoration proceeds. Additional reintroductions are being planned, but specific release sites have not been identified. Releases on public land are preferred, but private land will be considered if appropriate public release sites are limited. Although releases within the historical range are categorically exempt from NEPA, the extensive habitat restoration needed may require NEPA.

This effort differs from traditional captive breeding and reintroduction plans in that no animals will be held permanently in captivity and large numbers of individuals will not be held. Breeding of successive generations in captivity is not planned in order to prevent genetic adaptation through natural selection to conditions in confinement. Brush rabbits could be considered to have a short lifespan as defined in the Policy because relatively few live more than a few months and fewer still survive to breed. They are not known to breed until the year after their birth, though it is probable that some will breed sooner under some circumstances, and very few survive in the wild to breed a second year. Further, to avoid the numerous other problems that arise from raising animals in small pens or in zoos, which include behavioral naïveté in terms of social and environmental interactions, enclosures surround natural habitat for brush rabbits and will be stocked at densities similar to populations in the wild.

The plan includes protocols for data collection and record keeping, monitoring of genetic, demographic, life history, phenotypic and behavioral characteristics, disposition of individuals that may be surplus to the program needs, disease screening, and maintaining health of the rabbits in captivity. The plan has measurable objectives for both propagation and reintroduction, and the program will be conducted in a manner that takes known precautions to prohibit the potential introduction or spread of diseases and parasites. Further, when appropriate, protocols follow the accepted standards for captive propagation.

ABOUT THE ENDANGERED SPECIES RECOVERY PROGRAM

The Endangered Species Recovery Program (ESRP) was established on 1 July 1992 by the U.S. Fish and Wildlife Service and U.S. Bureau of Reclamation to carry out recovery planning and implementation measures for listed and sensitive species in the San Joaquin Valley region. The impetus for its establishment was to assist the agencies with implementation of the Friant Biological Opinion[3] regarding long-term water contract renewals in the Friant Service Area of the Bureau of Reclamation. ESRP is a research and planning group that is administered through the non-

[3] U.S. Fish and Wildlife Service. 1991. Biological opinion for the Friant Division water contract renewals. Fish and Wildlife Enhancement, Sacramento Field Office, Sacramento, CA, 47 pp. + Appendices.

profit auxiliary Foundation of California State University, Stanislaus. Program activities are executed under contracts with the supporting agencies. ESRP also receives grants from and contracts with other governmental and non-governmental entities for projects consistent with its mission. Recovery actions on endangered species are carried out under the authority of scientific take permits issued to the directors or principal investigators of ESRP and by consultation and collaboration with personnel from state and Federal permit-issuing agencies. ESRP is not a governmental agency and has no authority concerning policy or regulation.

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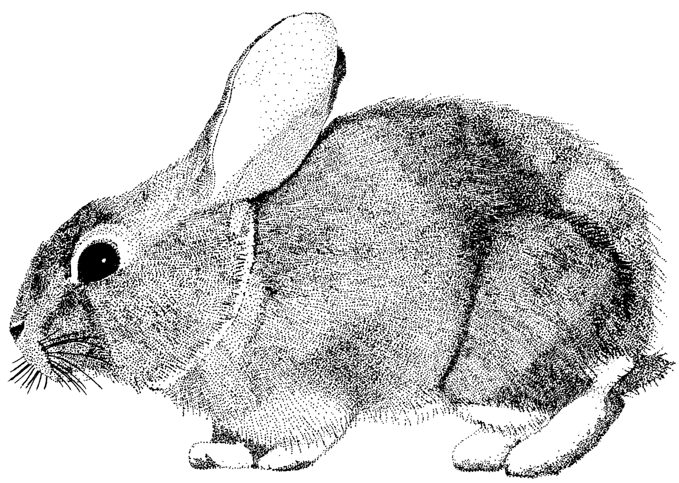

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by

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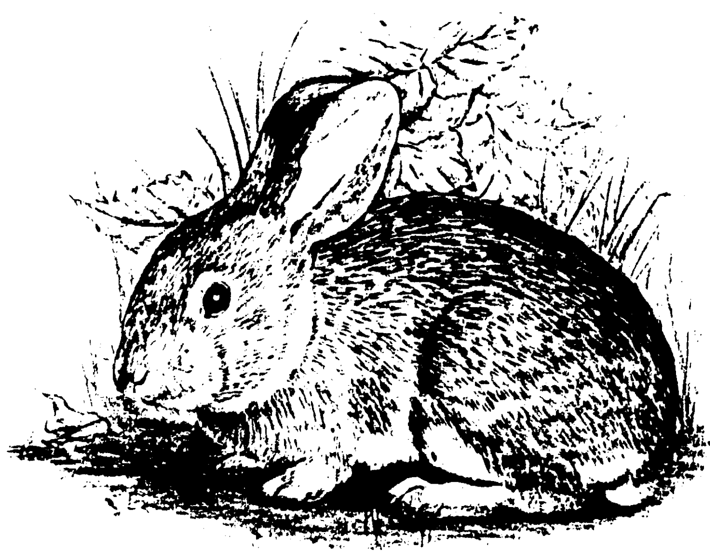

CONTROLLED PROPAGATION AND REINTRODUCTION PLAN FOR THE RIPARIAN BRUSH RABBIT (*SYLVILAGUS BACHMANI RIPARIUS*)

INTRODUCTION

The riparian brush rabbit (*Sylvilagus bachmani riparius*) is California- and federally-listed as an endangered species (U.S. Fish and Wildlife Service 2000). It also was identified as a *Critical Needs Species* under terms and conditions of the *Friant Biological Opinion* (U.S. Fish and Wildlife Service 1991). It occupies riparian communities dominated by thickets of willows (*Salix* spp), wild roses (*Rosa* spp.), and other successional trees and shrubs, and when available seasonally, dense, tall stands of herbaceous plants adjacent to patches of riparian shrubs in the northern San Joaquin Valley (Figure 1). Such communities in the San Joaquin Valley have been reduced to less than 1% of their historical extent, primarily by clearing natural vegetation, irrigated cultivation, impoundment of rivers, and stream channelization.

Today, the only known populations of riparian brush rabbits are confined to Caswell Memorial State Park (MSP) on the Stanislaus River, and along Paradise Cut, an overflow channel of the San Joaquin River about 10 miles northwest of Caswell MSP in the South Delta area of the San Joaquin River (Williams and Basey 1986, ESRP unpubl. data). The Park is 253 acres in size. The South Delta population is located on private land, mostly along Paradise Cut. Paradise Cut's stream bed is private property and the waterway is managed for flood control, not wildlife habitat. The South Delta population exists on an estimated 270 acres, spread linearly over several miles in discontinuous patches (Figure 1). As far as is known, all other historical habitat along the San Joaquin River and its tributaries has been lost or degraded beyond use by irrigated agriculture, livestock grazing, and impoundment and channelization of streams (Williams and Basey 1986, Williams 1993, ESRP unpubl. data).

Both populations of riparian brush rabbits are under significant, proximate threats of extinction. The population in Caswell MSP faces threats from random demographic events in small populations, inbreeding and loss of genetic diversity, wildfire, flooding, disease, predation exacerbated by high numbers of feral cats, and possibly from competition with desert cottontails, *S. audubonii* (Williams and Basey 1986, Williams 1988, 1993, U.S. Fish and Wildlife Service 1998). The South Delta population faces threats from stochastic demographic and genetic events, flooding, disease, predation, competition, and habitat conversion on private land.

Current population numbers are too low to provide sufficient captures to estimate population sizes with capture-recapture population estimator models (Figure 2). In Jan-

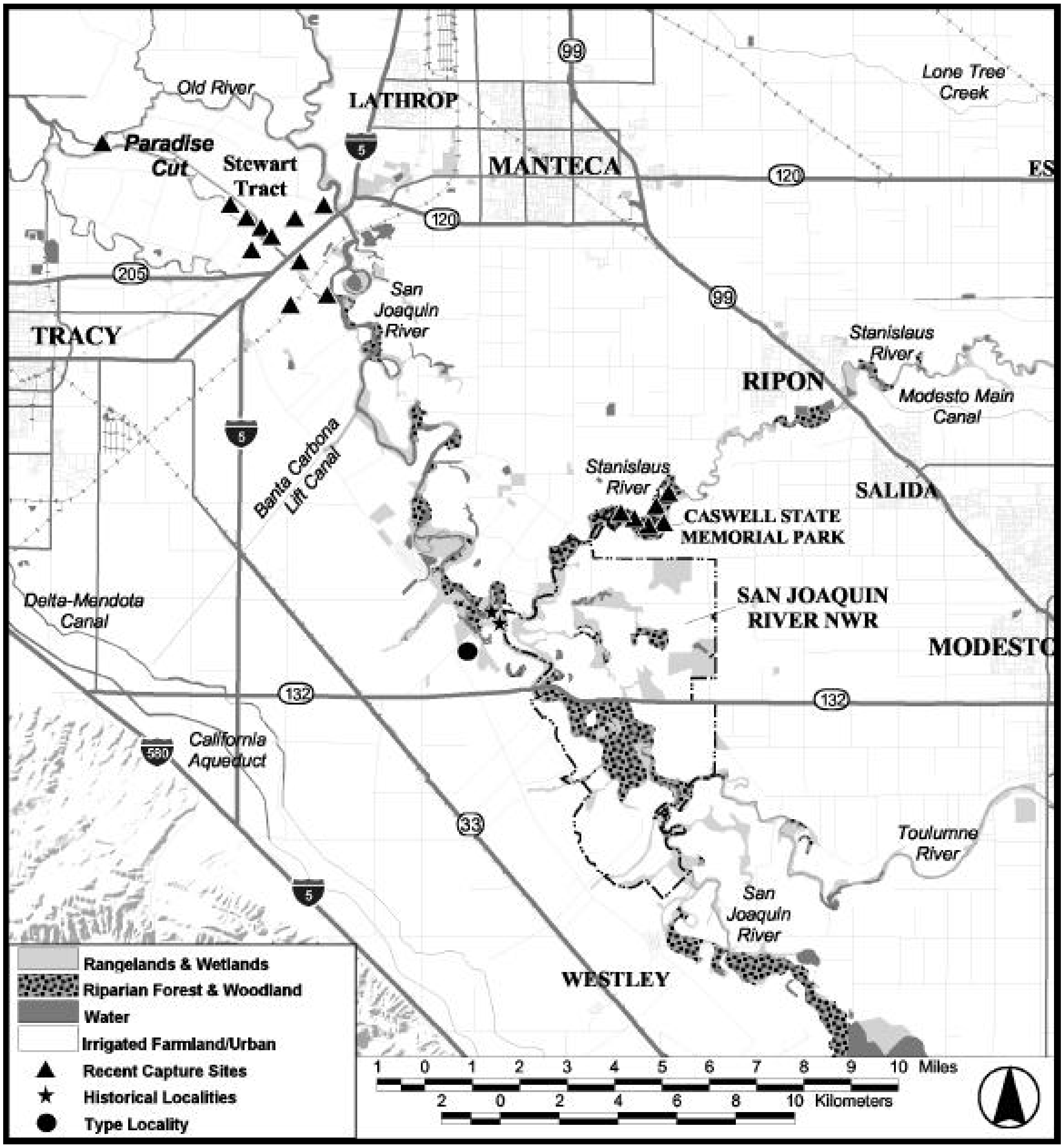

Figure 1. *Historical and recent (current) records for the riparian brush rabbit,* Sylvilagus bachmani riparius*. Not all private land along the San Joaquin River in San Joaquin County has been thoroughly surveyed for brush rabbits because of access restrictions. Shaded relief in the lower left of the map is upland non-native grassland and chaparral.*

ary 2001 in Caswell MSP, 2 riparian brush rabbits were captured, whereas 37 brush rabbits were trapped during the standardized surveys in January 1993 (Williams 1993, ESRP unpubl. data). An additional four rabbits were caught in 1993 in a portion of the Park that apparently is now uninhabited. Furthermore, in 2001 both rabbits were caught in a single portion of the Park that had not been part of previous surveys. None were taken in portions of the park that traditionally had yielded captures in the past. Sign of rabbits

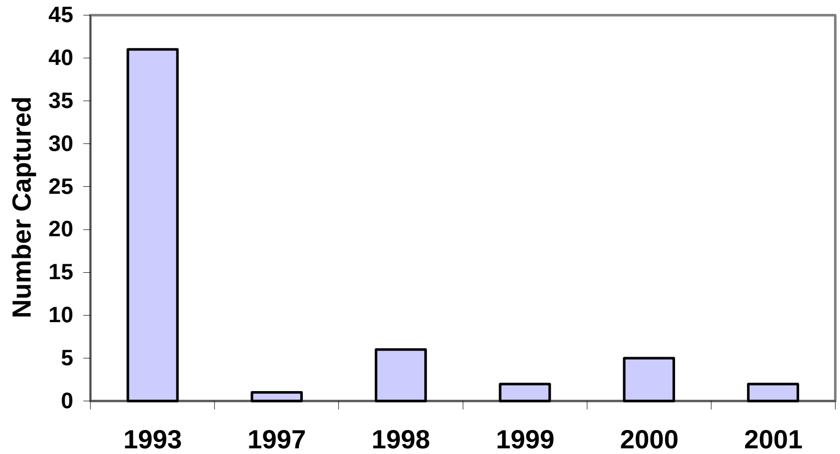

Figure 2. *Numbers of riparian brush rabbits captured in annual population censuses during January at Caswell Memorial State Park (Williams 1993 and ESRP unpubl. data).*

elsewhere were scarce or absent between 1997 and 2001, unlike 1993, when fresh sign of rabbits could be found throughout the Park (Williams 1993, ESRP unpubl. data).

Access restrictions for trapping on private land have prevented extensive trapping to estimate population size at the South Delta site, but based on captures in 1998-1999 and the amount and distribution of habitat, the population probably consisted of 25-100 individuals (18 were captured). The numbers captured may be inflated above carrying capacity of available habitat because extensive habitat on private land was temporarily destroyed by clearing for flood just before we trapped some of the adjacent habitat. We believe that the clearing operation displaced individuals into remaining habitat, which temporarily increased density.

ASSUMPTIONS AND HYPOTHESES

In articulating a set of hypotheses that are both practical and testable within the framework of restoring populations of endangered riparian brush rabbits we first offer the following set of observations and assumptions. Each assumption associated with an observation can be restructured as one or more testable hypotheses; however, it is impractical or unnecessary to scientifically test most of these. These statements are mostly general. Working through them will result in an understanding of the framework for the proposed processes for recovering riparian brush rabbits.

- Habitats for riparian brush rabbits and riparian woodrats (*Neotoma fuscipes riparia;* also an endangered species and known only from Caswell MSP and its immediate vicinity) are found only in Valley Oak (*Quercus lobata)* woodland and riparian communities on the northern San Joaquin Valley floor (Williams and Kilburn 1984). Their abundances are greatest in different seral stages and microhabitats within these communities. Essential habitat elements for riparian brush rabbits include appropriate size and distribution of clumps of shrubs for cover, suitable types and amounts of plant species providing cover and food, and access to non-flooded ground with cover and food. Tree canopy is not an essential element of brush rabbit habitat. Herbaceous vegetation is used extensively when it provides sufficient cover for the rabbits (Williams and Basey 1986, Williams and Hamilton 2002). Essential habitat for riparian woodrats includes Valley oaks, tree canopy cover of moderate to high percentage, shrub understory, and shelter with food and nest sites above flood levels (ESRP unpubl. data). The differing needs for habitat by these two species creates potential conflict in management objectives of their essential habitats and makes habitat manipulation as the primary recovery strategy problematic.

- Impoundment and channelization of streams resulted in alteration of the northern San Joaquin Valley landscape, including the development of cultivated agriculture and human structures on former floodplains where habitat for these species was found. All permanent Valley streams in the northern San Joaquin Valley have one or more up-stream impoundments and are channelized downstream.

- Conversion of riparian and woodland communities to agricultural and urban uses has eliminated more than 95% of the natural communities that riparian brush rabbits and woodrats depend upon for their existence. Remnants of these communities today are found only within the levees of the stream channels. Less than 1% of historical riparian communities is considered potential habitat for brush rabbits today (U.S. Fish and Wildlife Service 1998).

- Permanent cultivation and other developments on the land sides of stream levees prevent access by brush rabbits to non-flooded ground with appropriate food and cover during times of high stream runoff. The levees provide no food or cover for rabbits during high water. Flood control levees raise the level of in-channel flooding, thereby eliminating most or all patches of non-flooded ground within levees. Most stretches of existing riparian communities along Valley streams have no high ground suitable for brush rabbits to refuge from flood, and either are too small or lack essential habitat elements for supporting a permanent population of brush rabbits.

- Some essential elements of habitat for brush rabbits were partly created and maintained by natural flood dynamics and ecological succession.

- Impoundment and channelization of streams changed flood dynamics and altered ecological processes.

- Long-term fire suppression and reduction or elimination of scouring floods because of up-stream impoundments have resulted in a decadent, climax community with a high fuel load and very little ground dominated by secondary seral communities in Caswell MSP (Williams 1988). Resulting changes have degraded habitat and reduced carrying capacity for riparian brush rabbits. Invasive exotic trees, shrubs, and other plants, feral cats, and black rats have further altered the composition and structure of the community, but the ecological effects on the native animal community are unknown. Invasive exotics, however, pose additional management challenges for the Park where preservation of native biota is a major objective.

- In combination with the reduction and degradation of habitat for brush rabbits caused by human-induced changes in the landscape, environmental, demographic, or genetic stochasticity separately or in combination extirpated all but two, isolated populations of brush rabbits (Williams and Basey 1986, Williams and Hamilton 2002). In retrospect and without any information on former distribution and numbers of riparian brush rabbits it is impossible to determine whether environmental, demographic, or genetic factors caused extirpations at particular sites under consideration for restoration. What is important is to identify sites containing ground higher than recent flood levels with habitat where brush rabbits can refuge during floods, and giving those sites high priority for restoring habitat and re-establishing brush rabbits. It also is important to create high ground where potential habitat exists on sufficient acreage to support a permanent population of brush rabbits.

- The dangerously small population sizes of brush rabbits and woodrats at Caswell MSP precludes any substantial experimental manipulation of existing habitat, or extensive trapping and handling of rabbits there that might result in reduced carrying capacity or mortality.

- The South Delta population is distributed in patches along Paradise Cut, a channel of the San Joaquin River where it enters the Delta, and two railroad right-of-ways near where they cross the channel (Williams and Hamilton 2002). All the land except the Interstate Highway right-of-way, which provides less than an acre of temporary habitat (a few willows and mostly annual weeds), is privately owned and is either managed for cultivated agriculture, transportation, or flood control. Currently there are no opportunities for replicated, experimental manipulation, acquisition, or expansion of habitat for this population on private or public land.

- Remaining natural communities inhabited by riparian brush rabbits are separated by more than 24 river-miles of degraded, channelized, and flood-prone stream channels without all essential habitat components. Though study of integrated flood management is being done in response to the floods of the late 1990’s, it will be some time before wide-scale implementation will allow significant areas along the rivers to restored and reoccupied by brush rabbits. Even then, dispersal between populations may not be possible because water barriers also exist between the extant populations of brush rabbits. Except for Caswell MSP, land supporting riparian vegetation and brush rabbits is privately owned (Williams and Basey 1986, Williams and Hamilton 2002).

- Conflicting management objectives at Caswell MSP, including preservation of the climax Valley Oak forest, differing habitat needs for woodrats and brush rabbits, recreation, and preservation of archaeological and historical resources, prevent large-scale conversion of the community to secondary successional stages more suitable for brush rabbits. However, expansion of the Park by acquisition of contiguous land now in walnut orchards would provide from about 50 to 90 acres that could be restored and managed as high-quality habitat for brush rabbits (Williams 1988). Small-scale restoration projects, such as removing exotic trees and shrubs, and reducing fuel loads would then be accomplished without the high level of risk these activities currently pose.

- Other sites, such as the San Joaquin River National Wildlife Refuge (NWR), within the historical geographic range of riparian brush rabbits, are in public ownership or wildlife habitat easements and suitable areas are being or can be protected and restored as habitat for brush rabbits, but cannot be re-colonized by natural dispersal from existing populations because of non-habitat barriers that isolate them. Translocation is the only feasible way to repopulate most of the land on the Refuge (U.S. Fish and Wildlife Service 1998).

- At the San Joaquin River NWR over 700 acres of contiguous potential habitat for brush rabbits exists today and sufficient ground is now suitably protected from flooding so that all life requirements for brush rabbits are met. The management plan for the refuge (in development) includes not repairing former breaks (from the 1997 flood) in the west-side levees along the river and allowing flood waters to flow through formerly cultivated ground, creating a more natural hydrological cycle, maintaining more ground in secondary seres, and eliminating the need for flood-control levees. The existing levees are being re-vegetated by natural processes and some now provide cover on their banks for rabbits to refuge from high water. Additionally, ground higher than the levee tops has been created at one site to provide an additional site where rabbits could refuge from flood. The restoration plan for the Refuge calls for creation of high ground at additional sites.

- The process of restoring riparian plant communities in the northern San Joaquin Valley is well-established and restoration at the San Joaquin River NWR is well underway—268 acres have been planted to various riparian trees and shrubs and over 800 additional acres are being prepared for restoration. Habitat needs of brush rabbits also are understood and do not need to be determined through experimentation (Williams and Basey 1986, Williams 1988, 1993, Williams and Hamilton 2002). Potential habitat exists along stretches of the Stanislaus and San Joaquin rivers that we believe could support populations with no need for restoration of riparian vegetation—what is needed are accessible sites with habitat above flood level where animals can escape flooding. For some properties targeted for acquisition, reduction or elimination of livestock grazing in the riparian zone should result in rapid increase in habitat suitability for brush rabbits.

The principal hypothesis for this proposed program is that stream impoundment and channelization and the resulting lack of refuge sites above flood levels have resulted in the endangerment of riparian brush rabbits, primarily by habitat degradation and loss. Restoring some of the natural flood dynamics, such as is being done on the west side of the river on the San Joaquin River NWR, or otherwise providing refuges from flooding for brush rabbits and managing habitat by promoting a mosaic of shrub and herbaceous patches will result in successful re-establishment and persistence of populations. In this context, we hypothesize that for some sites such as the San Joaquin River NWR, self-sustaining populations can be established through translocation of animals from existing populations or a controlled propagation facility. We also hypothesize that other populations (e.g., Caswell MSP) can be enhanced and protected so that they become self-sustaining by acquisition of additional land contiguous to existing occupied habitat and restoration to provide refuges sites above flood levels, management to maintain the community in succession, and perhaps through genetic intervention.

The Recovery Plan for these species (U.S. Fish and Wildlife Service 1998) does not envision connecting all existing or future populations as practical options. This is because populations are either separated by water barriers, private lands with land uses that preclude establishment of habitat for these animals, or long stretches (i.e., miles) of channelized streams where flood control considerations are paramount and preclude restoration until an integrated strategy is developed. Prior to European settlement, populations probably were not continuous and when disease, flood, fire, or other events periodically caused local extirpations, the clumped spatial pattern of populations allowed some to escape these events. To lower the risk of mortality-causing epidemics and other environmental stochastic events, some of these small, highly vulnerable populations should be kept isolated. Where genetic or demographic considerations require, animals can be periodically moved between isolated populations.

In light of the foregoing, we believe that an adaptive management approach to restoration of riparian plant communities and re-establishing populations of riparian brush rabbits and woodrats is the most appropriate course, overall, in contrast to a step-wise, controlled experimental approach. We do believe that some processes should take place within a controlled, experimental framework, and are planning for or implementing such an approach when needed. Limited funding and the necessity to act quickly to prevent possible extinction of riparian brush rabbits, however, have precluded controlled experimentation on reproduction and translocation using surrogates of riparian brush rabbits. We plan to confine a few pairs of a non-endangered subspecies of brush rabbit and measure aspects of behavior, reproduction, and development that cannot be carried out on the rabbits being confined within the propagation enclosures. Details of those studies still are being developed and probably will be part of a graduate research project.

Because of the severe and proximate threats of extinction to these populations and the particular threats to their habitat on private land, we believe it is essential to bring some of the South Delta population into captivity. The Caswell MSP population is too small to serve as a source for controlled propagation without jeopardizing remaining individuals because it is extremely unlikely that all individuals could be captured. Furthermore, until the genetic relationships of the two populations are determined, it would not be advisable to mix individuals from the two sites for controlled propagation. Finally, the widely scattered, but clumped distribution of brush rabbits in the South Delta population will make it possible to select individuals for pairing that are unlikely to have high kinship values, allowing schemes to enhance outbreeding to be designed and implemented.

RECOVERY OBJECTIVES

The objectives are to recover the riparian brush rabbit from endangerment. Providing and protecting or maintaining habitat and re-establishing brush rabbits populations are essential elements in achieving recovery. The riparian brush rabbit will be recovered when three or more self-sustaining populations are established outside of Caswell MSP (U.S. Fish and Wildlife Service 1998). Self-sustainability cannot be accurately quantified with precision now. The adaptive process of recovering brush rabbits will involve several iterations of increasingly more precise and, we hope, accurate estimates of how much habitat and how many rabbits are needed as more data on demographics are gathered. The current guidelines are mostly based on generalized wildlife conservation principles and a partly qualitative assessment of the species habitat needs and demographics because of lack of needed data. We believe that the primary areas targeted for restoration and reintroduction of riparian brush rabbits are sufficiently large that self-sufficiency can be achieved. Though there are other criteria for recovery in the Recovery Plan (U.S. Fish and Wildlife Service 1998), recovery mostly will be achieved by these activities related to controlled propagation and reintroduction or translocation:

1. establishing and protecting continuous habitat that includes several refugia above levee elevations, and a self-sustaining population of brush rabbits along the south side of Stanislaus River, extending across the river from Caswell MSP to the San Joaquin River and southward along the San Joaquin River from its confluence with the Stanislaus River through to the south end of the San Joaquin River NWR (Figure 1), a stretch of about 25 river-miles in extent that potentially could support several thousand brush rabbits; The 25 river-miles represent about 12.5 straight-line miles along the rivers' paths.

2. establishing self-sustaining populations on the west side of the San Joaquin River on the San Joaquin River NWR, an area with more than 700 continuous acres of existing potential habitat for these species and over a thousand more currently being restored or being prepared to be restored—an area that could support several thousand rabbits;

3. protecting and expanding the population in Caswell MSP by acquisition of contiguous cultivated ground inside the flood-control levee, restoring habitat and creating flood refugia with appropriate vegetation structure and composition for brush rabbits and woodrats, and enhancing habitat through selective removal of fuel and decadent shrubs; these enhancements could result in double or more of the current carrying capacity of the Park for rabbits, to around 300 or more, and greatly reduce the threat of population extinction by fire or flood;

4. enhancing and protecting the South Delta population on private land through habitat management and redesign and construction of new, broader, flood channels to lower the elevation of floodwaters—portions of existing levees could be left in place and re-vegetated and serve as permanent habitat and refugia during flooding;

5. additionally or if one or more of the preceding goals are not achieved, establishing self-sustaining populations elsewhere on the northern San Joaquin Valley floor on public land, such as the San Luis NWR, to achieve the goal of at least three protected, self-sustaining populations outside Caswell MSP.

PURPOSES FOR CONTROLLED PROPAGATION

The principal objectives for holding and breeding riparian brush rabbits in confinement are to:

1. conserve a portion of the South Delta population at risk of extinction;

2. obtain brood stock to produce offspring that will be reintroduced to restored, but uninhabited, historical habitat on the San Joaquin River NWR and other state and federally-owned properties within their historical range;

3. maintain confined populations until new populations are established in restored habitat—for the purposes of this criterion, establishment means increasing numbers over the number of founders through natural reproduction in the relocated populations until they reach a viable population size, as determined by analysis of the minimums of several population cycles and other demographic factors that we are learning through research and monitoring of the species;

4. and possibly produce individuals to supplement and invigorate the extant population at Caswell MSP—whether or not this takes place will depend upon the results of phylogenetic analyses and disease screening of the two existing populations.

JUSTIFICATION FOR CONTROLLED PROPAGATION

Threats to Existing Populations

***Flooding*.—**Since we began population studies of riparian brush rabbits in the early 1980's, the population in Caswell MSP has fluctuated from a estimated low of 10-25 rabbits to a few hundred (Williams and Basey 1986, Williams 1988, 1993, ESRP unpubl. data). Major floods were observed in the winters of 1982-83, 1985-86, 1996-97 (Figure 3), and 1997-98. After the 1985-86 flood, the population increased from an estimated 10-25 rabbits to its high point in January 1993, when the population was estimated to be 241 rabbits (Williams 1988, 1993). After the flooding in 1997 (Figure 3), the most severe since the 1970's because of its duration, the population has remained very low into spring of 2001. In the 2001 population census, just two brush rabbits were captured. A desert cottontail also was captured in the Park in February 2000, the first since the 1980's (ESRP unpubl. data).

Figure 3. *Photograph of flooding at Caswell Memorial State Park in January 1997. Dense brush thickets, tangles of vines, and tree branches provide sites for brush rabbits to climb above flood waters. High water, however, persisted for about 6 weeks in winter 1997, making this survival mechanism by brush rabbits problematic (photo by D.F. Williams).*

The South Delta population was discovered by ESRP in 1998, so we do not have a history for the dynamics of this population. When we livetrapped a portion of the area, we captured 18 riparian brush rabbits over about a 6-month period of sporadic trapping. Our goals were to confirm the species identity of the rabbits, obtain tissues for genetic analyses, and determine the distribution of brush rabbits in the area. We have not attempted to estimate population size using capture-recapture techniques because of restrictions on trapping on private land.

Existing habitat is found along one overflow channel of the San Joaquin River and an adjacent slough that also floods. Habitat also is located on contiguous high ground along railroad right-of-ways (Figure 4). Brush rabbits probably refuge from flooding along the railroads. The shrubs, vines, and small trees along Tom Paine Slough were cleared in 1998-99, as was most of the vegetation along the levees and bottom of the overflow channel. The remaining habitat along the railroads is under immediate threat because San Joaquin County has ordered the railroad company to clear its right-of-ways to lessen fire hazards. For many years, the Southern Pacific Railroad right-of-way on Stewart Tract has not been cleared, allowing Valley Oak trees, willows, roses, and other woody plants

Figure 4. *Photograph of the Southern Pacific Railroad bed and north-side right-of-way, facing eastward, on Stewart Tract. The larger, darker-colored trees are Valley oaks; willows, wild roses, blackberries, and other woody and weedy plants grow in the borrow pits along the bases of the railroad embankments. Riparian brush rabbits are found intermittently along the railroad where there is sufficient habitat for home ranges (photo by D.F. Williams).*

to grow in dense stands. Clearing the right-of-way would eliminate the only known refugia for rabbits during flooding.

Wildfire.—Caswell MSP is a climax riparian forest with thick, relatively untouched understory, abundant ladder fuels, and little historical vegetation management. The park has a dense understory of shrubs and vines and an overstory of mostly old trees. Much of this vegetation is decadent and thus highly flammable. The ground is covered with deep accumulations of duff and woody litter. Fire suppression and lack of a comprehensive fuels management program for more than 50 years has left the Park with extreme fuel loading. These conditions pose a serious threat of catastrophic wildfire. Ladder fuels of heavy vines contribute to the likelihood of uncontrolled fire spreading into the tree canopy (Figure 5). Adding significantly to the fuel load, many trees were downed in a severe storm and flooding during January 1997 (Close and Williams 1998).

Figure 5. *Photo, taken in January 2001, showing typical vegetation in Caswell MSP. Vines and abundant downed logs and limbs together with many decadent shrubs and vines provide abundant fuel and high risk of wildfire (photo by D.F. Williams).*

Predation.—Though the factors that are keeping the Caswell MSP population very low are unknown, predation may be a major contributor. Coyotes (*Canis latrans)*, gray foxes (*Urocyon cinereoargenteus*), bobcats (*Lynx rufus*), several species of hawks and owls, and feral cats all have been documented as preying on riparian brush rabbits (ESRP unpubl. observ.). Feral cats are especially numerous in the Park. Park Personnel have observed that it is a popular place to abandon unwanted pets. Other potential predators are long-tailed weasels (*Mustela frenata*), striped (*Mephitis mephitis*) and western spotted skunks (*Spilogale gracilis*), feral and domestic dogs, black rats (*Rattus rattus*), and snakes. We believe that the high population of black rats poses an extreme threat to nestling rabbits, as well as other animals (Williams 1993, ESRP unpubl. data). The California Department of Parks and Recreation (CDPR) has initiated a control program for feral cats and the interactions between black rats, woodrats, and songbirds are being investigated (ESRP unpubl. data).

Other Threats.—Both the Caswell MSP and South Delta populations face threats of unknown magnitude from random demographic events, inbreeding, and loss of genetic diversity. These threats are presumed to be high because both populations are probably small. The Caswell MSP population has experienced reductions to numbers of less than 25 individuals at least twice since we first undertook studies there, and probably two other times in the mid 1970's to early 1980's, due to flooding (Williams and Basey 1986, Williams 1988, 1993, Williams et al. 2000). These events must have significantly reduced the historical genetic diversity in the Stanislaus River population. We have no history of the dynamics of the South Delta population, which probably has fewer than 200 individuals in scattered subpopulations, but presume that it too has undergone one or more population reductions in recent decades because of flooding.

Also of unknown significance is the threat of disease epidemics. We have no information on diseases in either population, but presume that diseases are either causal or contributing factors to mortality of riparian brush rabbits. Several diseases are known to occur in populations of brush rabbits and related species of *Sylvilagus*—tularemia, plague, myxomatosis, silverwater, California encephalitis, equine encephalitis, listeriosis, Q-fever, and brucellosis have been recorded in California populations of *Sylvilagus* spp. (Williams 1988; see Appendix A for additional discussion of diseases).

Why Measures to Improve the Species Status in the Wild Are Ineffective

The riparian brush rabbit was listed as endangered by the state of California in May 1994 (Title 14, Division 1, California Administrative Code, Section 670.5, *Animals of California declared to be endangered or threatened*). Hunting of all rabbits was prohibited within the then known and probable range of the riparian brush rabbits. Hunting has never been allowed in Caswell MSP since its establishment almost 50 years ago, so the change in the Fish and Game laws had no effect in this area. Hunting has been allowed where the South Delta population occurs because this population was only recently discovered outside the no-rabbit hunting area. Hunters typically do not distinguish species of *Sylvilagus* and killing of brush rabbits adds to the other threats to the population, especially because the population is so small. We have no data on the number of riparian brush rabbits killed by hunters, but have seen spent shotgun and 22-caliber shells in inhabited areas. We expect that a prohibition on hunting rabbits in the area of the South Delta population will soon be enacted, so hunting is not viewed as a long-term threat.

Recommendations for improving the status of riparian brush rabbits have been made in several plans. A habitat management plan for riparian brush rabbits in Caswell MSP was prepared for the CDPR in 1988 (Williams 1988). The plan recommended habitat restoration, fire and flood control, changes in pest management, and acquisition of adjacent property to construct refuges from flooding that are higher than existing levees. This assumed that the land owner would willingly sell. The riparian brush rabbit was a featured species in the Recovery Plan for Upland Species of the San Joaquin Valley (U.S. Fish and Wildlife Service 1998), in which further recommendations for conservation and recovery of the riparian brush rabbit were made. A fire and flood management plan also was developed by ESRP for the U.S. Bureau of Reclamation (USBR) and CDPR in 1998 (Close and Williams 1998). Collectively, these plans have presented and discussed recommendations for improving the status of the riparian brush rabbit in the wild. Yet because of persistently low population numbers for unknown reasons, the species is more vulnerable to extinction now than when the studies of its conservation status began in 1982 (Williams and Basey 1986). Habitat enhancement and fuel reduction programs at Caswell MSP only were begun in 1999—lack of funding and low priority from state and federal agencies were the major reasons nothing was done sooner.

Habitat enhancement in Caswell MSP probably will not result in a significant increase in carrying capacity for brush rabbits because most of the Park already provides average habitat and because of conflicting management objectives for the Park (Williams 1988, 1993). Instead habitat manipulation is designed to renew vegetation that provides habitat for brush rabbits while reducing the fuel load in the Park and improving the infrastructure for containing and fighting wildfire (Close and Williams 1998). These actions will result in maintaining average to good habitat for brush rabbits into the future and reduce the risk of extinction by wildfire. The fuel reduction program will take several years to accomplish. To try to create good to excellent habitat throughout the Park would result in significantly diminished values for other listed and sensitive species, require removal of most old, overstory trees and decadent shrubs, and conflict with the recreational and other missions of the Park. Removal of all decadent shrubs in an area also would result in loss of habitat for brush rabbits and woodrats until new shrubs were planted or naturally recruited and grew to a size sufficient as habitat—a process expected to take several years during which carrying capacity of brush rabbit habitat would be substantially reduced.

An informal program by CDPR of controlling feral cats has been ongoing for many years, but new cats regularly appear in the Park, at least some being abandoned by the pets' owners. In the past, this program only operated periodically, when the Park had no visitors in the winter months. CDPR began trapping and removing feral cats in winter 1999-2000. It is too soon to determine if this has any effect on the riparian brush rabbit population. Control of feral cats needs to take place year-round, yet currently there is no funding available for such a control program.

Control of native predators has not been tried at Caswell MSP. Gray foxes, bobcats, and red-shouldered hawks (*Buteo lineatus*) probably are the most significant predators other than feral cats. We do not believe that controlling native predators in Caswell MSP is either practical or in keeping with the recovery objectives of the riparian species.

We are working with some of the land owners to maintain habitat for brush rabbits where the South Delta population is found, but the general area is slated for future urban development. San Joaquin County officials want some vegetation eliminated along railroads to control possible wildfires, and flood-control entities periodically eliminate vegetation in stream channels and on levees. If the County's position were to be reversed, there is potential to modestly enhance habitat and increase its carrying capacity along railroads. However, the population would remain very small and highly vulnerable to flooding, fire, effects of urbanization, and stochastic demographic and genetic processes that might lead to extinction within the next 10 years unless protective measures are accomplished.

Recommendations for Captive Breeding in the Recovery Plan

The Recovery Plan for the riparian brush rabbit lists the establishment of three additional self-sustaining, wild populations outside of Caswell MSP and within the historical range of the species as being necessary for recovery (U.S. Fish and Wildlife Service, 1998, p. 169). Because the extant populations at Caswell MSP and South Delta are isolated from other suitable sites that currently are uninhabited, reintroductions of individuals derived from existing populations will be required to achieve this goal (U.S. Fish and Wildlife Service, 1998, task 2.2.5, table 8, p. 202). The Caswell MSP population is too small and nonproductive to serve as a source of wild-born rabbits for translocation now. For these reasons, breeding in confinement to provide a source of animals for reintroductions is called for in Tasks 4.43 and 7.8 in the Recovery Plan (U.S. Fish and Wildlife Service, 1998, table 10, pp. 214, 227).

Scientific Principles Leading to Decision for Captive Breeding

Populations experiencing severe declines to a few individuals typically lose much of their genetic diversity, a result of random genetic loss and inbreeding among closely related individuals. This genetic bottlenecking and subsequent inbreeding can result in the expression, through homozygosity, of mal-adaptive or lethal genetic traits that reduce fitness of the population. Repeated bottlenecking over an extended period is thought for some species to be part of the process leading to extinction of populations from the reduced fitness of inbred individuals. Reduced fitness can derive from greater numbers of birth defects, slower growth, higher mortality, or lower fecundity (Ralls et al. 1979).

Though our genetic data, developed by Dr. James Youngblom of CSU, Stanislaus, are limited and the analyses are preliminary, substantial heterozygosity still exists in both populations (Table 1, Figure 6). We have identified microsatellite primers developed for European rabbits (*Oryctolagus cuniculus;* Rico et al. 1994, Mougel et al. 1997, van Haer-

Table 1. *Observed (H_o) and expected (H_e) heterozygosity for eight microsatellites in four populations of brush rabbits (ESRP and James Youngblom unpubl. data).*

Locus	*n* (alleles)	Caswell MSP		South Delta		Diablo Range		Sierra Nevada	
		H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
SOL-44	8	0.600	0.775	1.000	0.985	1.000	0.864	0.619	0.654
OCR-4	5	0.071	0.415	0.444	0.490	0.167	0.591	0.455	0.434
SAT-7	8	0.733	0.733	0.444	0.616	0.118	0.378	0.565	0.618
SOL-8	8	0.800	0.851	0.611	0.535	1.000	0.800	0.591	0.639
SOL-30	17	0.538	0.763	0.667	0.614	0.833	0.682	0.869	0.854
0cBGXL	4	0.500	0.452	0.389	0.608	0.750	0.678	0.087	0.166
SAT-16	8	0.500	0.598	0.778	0.776	0.833	0.883	0.565	0.583
0CLS	7	0.214	0.505	0.556	0.738	0.000	0.303	0.000	0.000

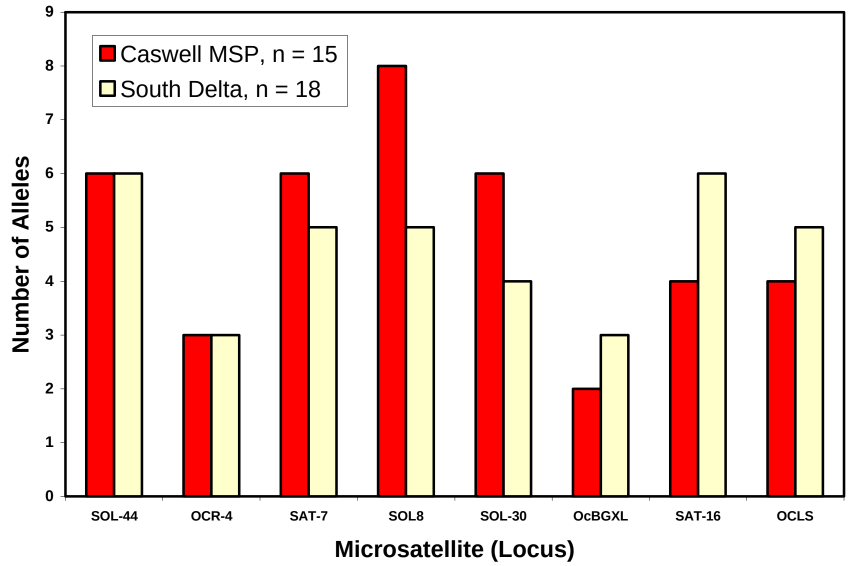

Figure 6. *Number of alleles per locus for eight microsatellite loci in two populations of Riparian brush rabbits (ESRP and James Youngblom, unpubl. data).*

ingen et al. 1996/97, Surridge et al. 1999) that yield consistent results with brush rabbit DNA at eight multi-allelic loci (Sheehan 1999, ESRP unpubl. data). For the eight variable microsatellite loci, there are means of five alleles per locus in both populations. The two populations differ in having from one to three unique alleles at each locus (Figure 7). Using many of the same microsatellites, van Haeringen et al. (1996/97) found an average of four alleles per microsatellite (range 2-11) in 32 European rabbits.

Allele frequencies in brush rabbits reveal considerable differentiation between populations, particularly at loci OCR-4, SAT-7 and OCLS (Table 1). Heterozygote deficits were noted at several loci, particularly for OCR-4 and OCLS in the Caswell MSP and Diablo Range populations.

These data are not what we would expect from repeated genetic bottlenecking. There are several possible reasons for the greater than expected diversity in these populations that cannot be ruled out without further study. Some possible explanations include: low points for populations were not so low as to cause bottlenecking; populations have only recently been reduced in numbers; populations are larger than thought; samples are heterogeneous in that the populations are spatially and genetically subdivided; there are errors made in amplification from microsatellite primers that were developed originally for European rabbits, or the microsatellites mutate at extremely rapid rates. Because of the consistency of amplification products for the selected primers, we can rule out that the apparent diversity is due to mistakes in amplification. Nor is there evidence from studies of other rabbits that these microsatellites have such unusually rapid mutation rates (van Haeringen et al. 1996/97, Surridge et al. 1998, 1999). Because rabbits were found

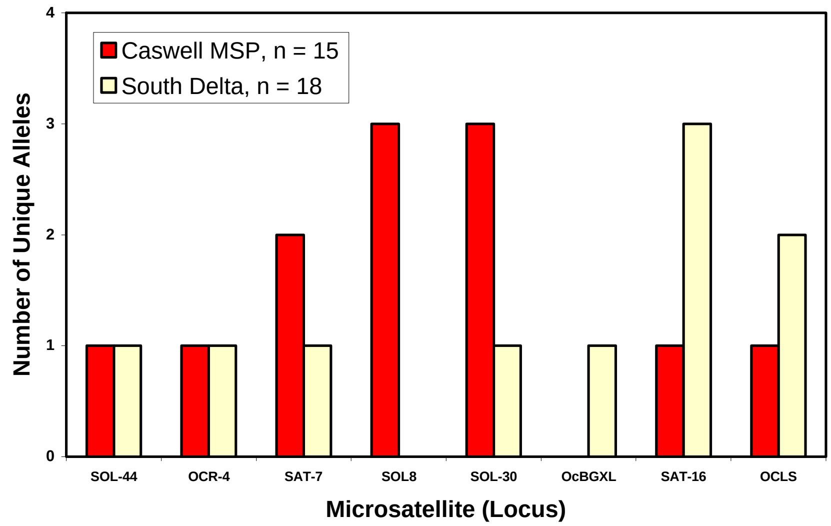

Figure 7. *Number of unique alleles for eight microsatellite loci in two populations of riparian brush rabbits (ESRP and James Youngblom unpubl. data). Unique alleles are ones not shared with the other population sample.*

throughout Caswell MSP in the 1980's and early 1990's (Williams and Basey 1986, Williams 1988, 1993) we do not believe that the Caswell MSP population is spatially subdivided, though we do think the South Delta population is subdivided. The 15 individuals from Caswell MSP were collected over a 5-year period so there is temporal subdivision in the sample, but we fail to see how this could increase apparent diversity to the levels measured.

Another possible explanation is that bottlenecking persisted for only a short time, probably only one generation for brush rabbits. In this context, reductions in population that are severe enough to impact genetic diversity are considered to be "bottlenecks." The number of generations of an animal population that a bottleneck persists is an important determinant of the effect of population reduction on genetic diversity. Data from Buri (1956), appearing in Hartl (1988), clearly show that heterozygosity was depressed in a population undergoing a bottleneck beyond the fourth generation. Random fluctuations in those data obscured whether the effect on the fourth generation was significant. Theoretical calculations indicate that genetic effects on an ideal population of a single generation bottleneck may not be significant if the population is not depressed by any constraints in succeeding generations. In other words, if the population rebounds in less than four generations to a level sufficient to sustain the variety of alleles carried by the population, the loss of genetic diversity can be minimal. However, where the population is slow to recover, there will be a genetic impact even for a single generation bottleneck.

Surridge et al. (1998) also found surprisingly high levels of heterozygosity in some populations of European rabbits that were known to undergo frequent bottlenecking. They believed that the high fecundity and short generation time of the European rabbit played an important role in maintaining genetic variability in those bottlenecked populations. They did not define what constituted a bottleneck. Some researchers consider a population reduction to between 2 and 4 individuals of mixed sex to constitute a bottleneck. Others, including Surridge et al. (1998) used the term more in keeping with Hartl's (1988) definition. Brush rabbits have lower fecundity than European rabbits and longer generation times (Chapman 1974), but their populations also probably can rebound quickly from bottlenecks if habitat is available. The most likely explanations for our data are that the populations have not undergone severe bottlenecking (i.e., down to only 2-4 animals), that reductions in size occurred over only one or two generations (Surridge et al.'s 1998 hypothesis), or that there are more rabbits than estimated from trapping results—these are not mutually exclusive hypotheses.

Regardless of the explanation for the apparent diversity in microsatellite alleles, these data suggest that diversity is sufficient for both populations to serve as sources for a controlled propagation program, assuming that the diversity measured at these presumably selection-neutral loci are somehow indicative of the active genome. We believe, however, that it would be very difficult to obtain enough individuals from the Caswell MSP population for controlled propagation at this time. Further, we doubt that the population is of sufficient size to support removal of individuals.

Because of the magnitude and immediacy of threats, the small sizes of their populations, their isolation, and the lack of population increase in the Caswell MSP population since the flooding of winter 1997, we believe that some individuals from the South Delta population should be captured, confined in large enclosures with natural vegetation where they can breed, and the progeny translocated to restored habitat on public lands within their historical geographic range.

CONTROLLED PROPAGATION FACILITY

To avoid the problems that could arise from confining a rare species in small cages when little is known about its husbandry and mating behaviors, and the necessity for offspring to learn about habitat, food, and predator avoidance, and to become acclimated to weather at the translocation site, animals will not be confined to and bred in small cages. Instead, animals will be placed in fenced enclosures larger than their typical home ranges and populated with natural vegetation that provides suitable habitat. Predators will be excluded from the enclosures, but the animals otherwise will be in a natural setting.

Location and Design

Criteria for Site Selection.—During 1998, ESRP employees surveyed 50 state, federal, and private properties as possible captive breeding locations, both inside and outside of the riparian brush rabbit's historical range (Williams et al. 2000). The following criteria were considered when assessing potential sites:

1. no flood risk; in this context shallow, partial flooding from irrigation for only a day or two was considered acceptable because we believe it is to be well within the species normal habitat conditions;

2. low probability of vandalism;

3. accessibility in the wet season and directly after or during rainfall;

4. minimum of 3 to 5 acres in size;

5. prime habitat not absolutely necessary but appropriate cover must be present;

6. have climate essentially the same as planned reintroduction sites;

7. be within about 1-hour driving time from Turlock, CA, where staff are based;

8. hunting prohibited.

***Location*.—**Based on these criteria, the Department of Water Resources' Pond 6 in San Joaquin County was the only one that met all eight criteria, after the Department of Fish and Game agreed to suspend hunting for the duration of the program, and was the most promising location for a captive breeding program (Williams et al. 2000). Pond 6 is an elongate 180-acre parcel of which approximately half of the acreage is irrigated pasture (Figure 8). The other half of the property is primarily wetland, natural upland, and canal, with an intermittent, reduced riparian corridor. Vegetation at the site includes willows, Fremont cottonwood (*Populus fremontii*), Himalayan blackberry (*Rubus discolor*), Baltic rush (*Juncus balticus*), salt grass (*Distichlis spicata*), poison hemlock (*Conium maculatum*), and stinging nettle (*Urtica dioica*), among others. The parcel's riparian characteristics make this site especially attractive for the project because there is a well established shrub component that the rabbits could use for cover. Additionally, the property is contiguous to part of Woodbridge State Ecological Reserve, primarily managed for wintering sandhill cranes. The environments at Pond 6 and the San Joaquin River NWR are not so different that we would expect a need for acclimation at the translocation site or adaptation to a different regime by the captive animals.

Pond 6 is leased to the California Department of Fish an Game (CDFG) to be managed for wildlife, hunting, and fishing. CDFG has agreed to temporarily suspend hunting on the site for the duration of the captive breeding project. The Department of Water Resources (DWR) leases the grassland portion of the property for grazing, and cattle are typically present all year. Water from nearby Hog Slough is used to irrigate the pasture. DWR's Division of Flood Management performs general maintenance of the pond, including weed control, tree pruning, and litter removal.

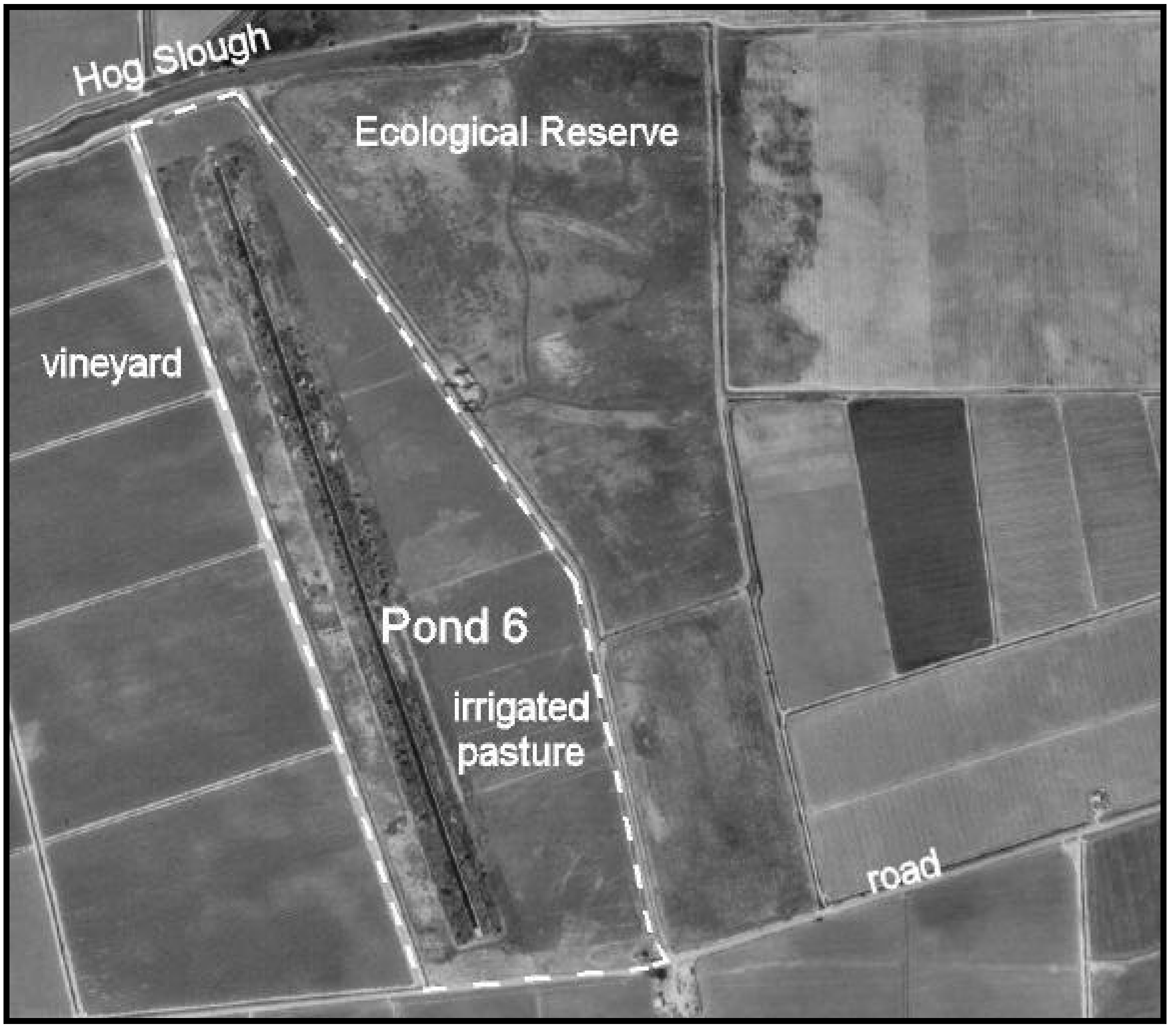

Figure 8. *Satellite image showing the configuration of Pond 6 and surrounding ground. The property is bounded by a dashed line. The pond is the dark-colored, narrow, linear structure along the left edge of the property. It is bordered on the east (right) by a narrow (about 150 feet) band of natural vegetation including wetland and upland components. The pens are to be constructed in the upland area closest to the irrigated pasture.*

Description of Pens for Confining Rabbits

Three captive breeding enclosures are being constructed on the east side of the pond. Each enclosure will be about 1.2 to 1.4 acres in size and encompass large clumps of blackberries. They will be fenced with hardware cloth with a 2-ft band of sheet metal flashing at the top and stand 6 feet high. A horizontal 6-ft band of fencing will be laid on the ground, about 6 inches to 1 foot below the ground surface and the vertical fencing built along the middle of the horizontal band. Earth excavated for the horizontal band will be used as backfill over the band once the fence is erected. The interior of pens will be covered with netting supported by cables strung from the long sides of the enclosures

Figure 9. *Photo of a portion of a pen for controlled propagation of riparian brush rabbits at Pond 6. The pen is approximately 530 feet long, 100 feet wide, and the side fencing is about 6 feet high. The top is covered with netting to prevent raptors from entering. Sides are topped with sheetmetal, shown on the left, but not yet installed on the right. For scale, two vehicles are parked near the center line (photo by L.P. Hamilton).*

and supported by 18-ft poles. The general appearance of an enclosure is depicted in Figure 9.

Each pen will be equipped with several 4-ft lengths of 8-inch diameter PVC pipe laid on the ground under cover of vines and shrubs for rabbits to take shelter from cold and wet weather. Each pen also will be equipped with 6 nest chambers made of 8" PVC pipe as illustrated in Figure 10*a*. Dried grasses and leaves will be provided in the chambers as bedding material. An observation port large enough to accept a video burrow probe will be provided in top of the structure over the chamber. We also will place an equal number of wooden boxes with holes in two sides to serve as nest boxes (Figure 10*b*). For all three types of shelters, the intent is to provide structures to get out of the rain and substitute for hollow logs and hollows under the exposed roots of living trees because neither occur within the pens. Whether or not these will serve as places for the female to bear and nurse young is unknown. We believe that there also are natural shelter sites within

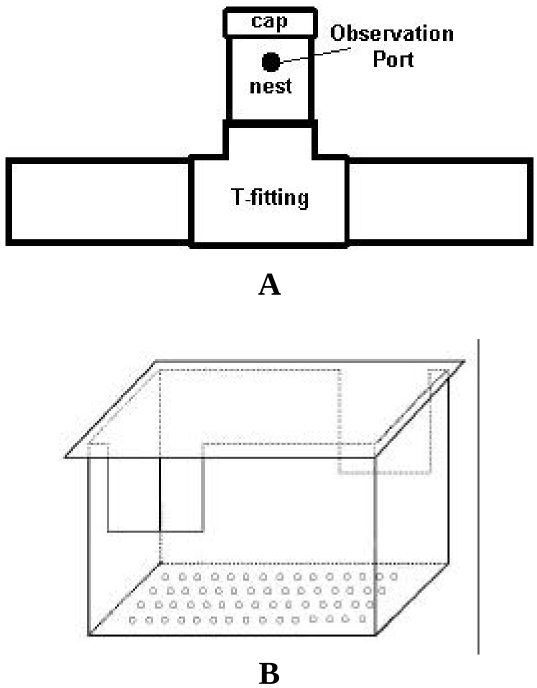

Figure 10. *Drawings of shelters for brush rabbits.* ***A***. *Top view of a nest for brush rabbits. Nests are made of 8-inch diameter PVC pipe with a T-fitting; one arm is fitted with a cap, the others are open. Side pipes are each 4 feet long and are not drawn to scale.* ***B***. *Schematic drawing of a wooden nest box with removable top cover, cutouts in sides, and drain holes in the bottom.*

the pens, but the blackberry thickets are too dense to permit detailed inspection of their interiors.

During construction, the nearly continuous clumps of blackberries will be broken by blading crisscrossing paths through the thickets in order to complete construction of the pens and to make access for monitoring possible (Figure 9). Maintaining paths through the thickets also will create narrow edges favored by brush rabbits (Chapman 1974).

Once pens are constructed we will intensively trap within them to remove any animals accidentally entrapped. Once the enclosures are emptied of unwanted animals, the doors will be sealed. We will use automated, infrared-triggered cameras and baited track plates to help determine if unwanted animals are confined and to evaluate progress in removing them.

Why Only One Facility Is Being Considered

Having all enclosures at a single site is necessary for the reasons already elaborated (see Location and Design, p. 16)—this is the only site that met all criteria (Williams et al.

2000). Three enclosures (pens) are planned at this site. Restraints on the type and size of enclosures include expense of construction, the small number of available rabbits, lack of knowledge about the mating system of the species, lack of suitable sites for confining breeding colonies, and problems that arise when releasing captive-reared animals that are naïve about their environment.

Operation of the Facility

***Populating the Controlled Propagation Pens*.—**Riparian brush rabbits in the South Delta population will be trapped to provide individuals for controlled propagation. Individuals captured will be assessed for captive propagation, have a biopsy sample taken for genetic analysis, and permanently marked with a monel ear tag and a passive integrated transponder (PIT tag). Those individuals selected for captivity will be moved to the controlled propagation facility. Standard pet carriers (cat sized) made of plastic and metal will be used to hold and transport rabbits.

We will follow established guidelines for captive and conservation breeding programs for selecting parents (Lacy 1994) and ensuring that outbreeding is maximized to the extent possible. Factors considered in initially assessing individuals will include sex, relative age, physical appearance, apparent health, and location of capture. Our criteria for selection will include representation of all spatial segments of the population, which will be accomplished by taking individuals from throughout the inhabited lands to which we have permission to work. We will reject obviously sick individuals; and then select a proportionate mix of any different phenotypes noted among animals captured. Animals destined for confinement in the same pen will come from different areas, separated by 0.25 miles or more (Figure 11).

We propose to use this approach because evaluation of the rabbits' genetic markers will take several weeks. We otherwise would have to mark the individuals and return them to the wild until genetic work was completed, then recapture them. Alternately, the rabbits would have to be confined to an appropriate holding facility until evaluations were completed and selections made—an activity that we believe would place the captives under additional stresses and risks. If returned to the wild to await selection, several weeks would intervene between capture and recapture, and it is likely that some individuals would have died or moved. More importantly, the genetic markers we have identified (Williams et al. 2000) are but a small fraction of the genome of the rabbits, and microsatellite DNA is noncoding, therefore is believed to be neutral to natural selection. Though the microsatellites provide tools to assess phylogenetic and familial relationships and relative genetic diversity of the populations, they do not allow us to evaluate the fitness of individuals or determine what mix of markers is best to seek to maintain.

The propagation program we propose is not typical of captive breeding programs in that founders will be held only for a single breeding season and then replaced by new, wild-taken founders. Because we have no information of kinship among the wild popula-

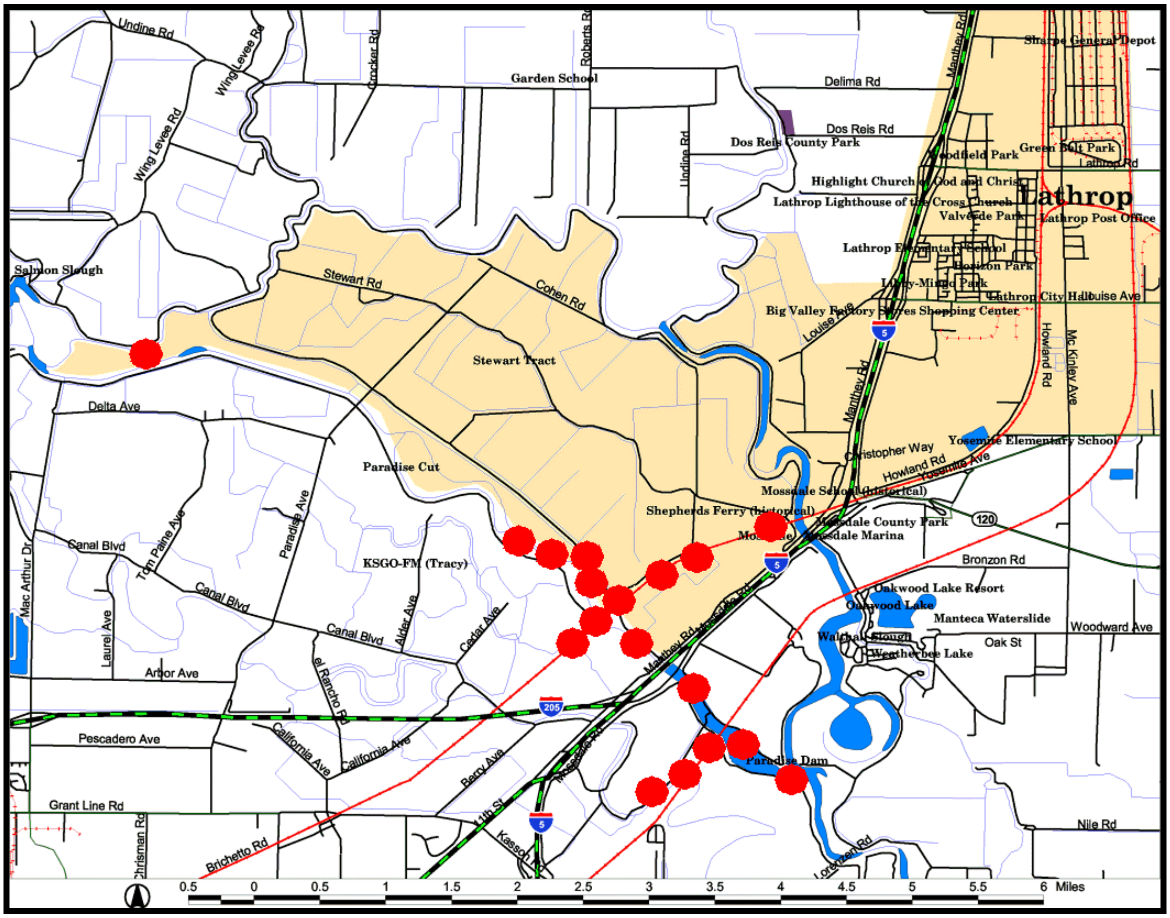

Figure 11.—*Map showing Stewart Tract and known locations for brush rabbits in the South Delta population (dots on map; ESRP unpubl. data). Distribution of brush rabbits is nearly continuous along both railroads and Paradise Cut. Distribution along Paradise Cut may extend the length of Stewart Tract or beyond, and at scattered sites along Tom Paine Slough. The shaded area is part of the City of Lathrop, San Joaquin County; that on Stewart Tract is slated for urban development.*

tions, we will confine animals for breeding that come from widely separated habitat areas. As a guide we will not confine individuals together that were taken from areas less than about 400 m (0.25 mile) apart. This should ensure that kinship values are low given the low vagility of brush rabbits (Chapman 1971). The program we propose will negate the genetic changes occurring during successive generations in captivity that could make captive stock unsuitable for release because we do not plan to hold and breed captive-bred animals. Thus, elaborate pedigree analysis and breeding plans to avoid unintended artificial selection are not required (Lacy 1994).

Monitor Confined and Re-released Individuals.—Animals selected for controlled propagation will be fitted with radio transmitters on neck collars. Collars fitted with transmitters will be less than about 3% of body weight of adult rabbits, or no more than 12-20 g. Young rabbits less than about 400 g in weight will not be collared. Adults typically weigh between 500 and 600 g (Williams 1993). Transmitters will have a mortality-mode sensor. The mortality mode is essential for the timely location of dead animals so that cause of death potentially can be determined. Animals also will have a monel, uniquely numbered ear tag attached and a passive integrated transponder (PIT tag) injected subcutaneously.

Confined rabbits will be monitored by radio telemetry to determine movements and activity periods and to detect mortality. Upon confinement, individuals will be monitored daily for the first week. Depending on results, individuals will then be monitored less frequently, but no less than one session per week, depending on objectives of monitoring. Monitoring to determine activity periods and space use within pens will take place during 3-5 day periods once every 6 weeks. At monthly intervals during the nonbreeding season and biweekly during the breeding season, confined animals will be trapped using Tomahawk™ double-door, wire-mesh live-traps (Model 203; 61 cm long by 15.2 cm high and wide) to assess reproductive condition and to inspect and change defective collars or radio transmitters. Traps are modified from the standard model by construction with smaller mesh wire (0.5 x 1-inch mesh instead of 1 x 1-inch standard for Model 203) and adding a Plexiglas shield to the inner side of the doors to prevent animals from injuring their nose and rostrum on the trap wire by pushing on the door. Animals are removed from traps by coaxing them into cloth bags; animals are held temporarily in the cloth bags. Additional trapping may be required if we fail to recapture some individuals.

***Physically and Genetically Assess Progeny in Confined Populations*.—**We will begin trapping for reproductive checks mid December. Newly captured young animals will be inspected and measured, sexed, marked with ear (if over 300 g) and PIT tags, have a biopsy of ear tissue and a tuft of hair taken, and released at the capture site. Animals will be given health screening exams either immediately, or later, after they have had a chance to accommodate to their new surroundings. The exams will include the drawing of whole blood for disease screening and other analyses. Animals that appear to be healthy may be released to the pens without full screening when necessary to avoid prolonging the stress of capture and handling, as when several animals are captured at the same time. (Health screening is discussed further in Appendix A).

Subsequently, tissue samples will be analyzed genetically to determine parentage. These data, together with sex and physical condition will be used in deciding which rabbits will be released and where they will be released at the translocation site. Animals that are determined to be siblings or parent-offspring will not be released in close proximity although we acknowledge that once free, they may move together by choice or randomly. If only a single temporary holding pen is used to simultaneously release a cohort or mixed cohorts, we will place related animals in different areas of the pen. Obviously sick or deformed animals whose deformities would not permit them to function normally in the wild would not be translocated. Sick animals would be treated and if recovered, would be considered for translocation.

If there are individuals produced that cannot be translocated, they will be released into the South Delta population from which their parents came, or retained in confinement to replace a parent.

Replacement of Parents in Propagation Pens

Parents will be replaced (1) if they do not successfully breed; (2) after they have produced one or more litters during an annual breeding season and there are other individuals available that are unrelated or have under-represented genotypes; (3) if they are discovered to have a serious, transmissible disease; (4) or when they die, assuming in each situation that appropriate replacements are available. Surviving animals removed from the enclosures will be returned to the South Delta population at the point of their original capture. A new colony of breeders will be selected from the wild population each year as long as they are available. Thus, unless new breeders become unavailable, no more than one generation will be produced in confinement, negating the possibility that translocation stock will have become genetically adapted to conditions in captivity. The founder stock for the controlled propagation program will consist of more than the 20 recommended as a minimum, but will not be expanded through captive breeding as recommended (Lacey 1994) because new founders will be trapped and bred instead. Over the 3 to 5-year span of controlled propagation, we expect to use 54 to 90 founders. If fewer than planned are available at some point in the program, individuals in the confined colonies will be selected as breeders for the next year's production, increasing the probability of adaptation to confinement. These would be paired with unrelated animals taken from other habitat areas or the progeny of such animals.

Reproductive Biology of Brush Rabbits

Brush rabbits in coastal California have been found to be pregnant as early as December (Mossman 1955). In a 1-year study, the breeding season extended from February to May or June in the Caswell MSP population (Williams 1988). Onset and duration of reproduction may be associated with the timing and duration of the wet season in California, and probably varies from year to year. Thus, we believe it is necessary to have individuals confined to the controlled propagation pens no sooner than October and no later than the beginning of November to avoid any increased danger of injury that would result from capturing and handling females at an advanced stage of pregnancy, and the danger of removing a mother with dependent young.

Based on numbers of embryos, mean litter sizes reported for brush rabbits varied from 2.7 to 3.4 for 2 years in Oregon (Chapman and Harman 1972) and from 3.5 to 4.0 in 2 areas of California (Orr, 1940, Mossman 1955, respectively). Mean number of young born typically is fewer than the number of embryos for rabbits in general. Annual production of young in Oregon was estimated to be 15.2 per female (Chapman and Harman 1972). From these data, we expect three to five litters annually from the confined rabbits. In natural situations, individuals typically do not live for more than 2 years. There is no evidence of young breeding in the year of their birth (Mossman 1955, Chapman and Harman 1972). However, breeding by young rabbits should be considered as a possibility because some other species of *Sylvilagus* are known to breed when as young as about 90-days old. In reported cases, breeding by young in other cottontail species either was a very small percentage of the population examined or varied by year and geographic setting (Chapman 1975, Chapman et al. 1980).

***Estimated Reproductive Success of the Confined Colonies*.—**We have no data on reproductive success in riparian brush rabbits where success is measured by rearing to weaning or dispersal, nor do we know of data from other subspecies of brush rabbits. Assuming 100% reproductive success to dispersal age, and using 3-4 young per liter (Orr 1940, Mossman 1955), about 27-36 rabbits would be produced in each cohort from the 3 pens combined. A cohort is defined as the products of one litter from each of the females combined. In actuality, all females may not produce the same number of litters in a given year nor will their pregnancies necessarily be synchronized. Using Chapman and Harman's (1972) estimate (3 young, 5 litters), the annual productivity of the captive colony (9 females) would be 135 young. Using a higher value (4 per litter) for California rabbits, on the assumption that litter size is greater at lower latitudes as suggested by these reports, annual productivity in captivity could be as high as 180. However, we presume that fewer litters per year may be produced and that many fewer young may be raised to an age where they could be translocated, but have no way to estimate a value with any acceptable level of confidence.

For planning purposes, we use 0.67 as the survival rate. If only two-thirds of the young lived through birth and adolescence, about 18-24 from each cohort would be expected to be available for reintroduction. As a guideline, we will translocate no less than 18 individuals from the first cohort to the site at the Refuge. If there are fewer than 18 we will wait until individuals from subsequent cohorts are available before translocating them. Subsequent translocations to the same site will depend on the fate of the founders of the new population, but we plan to make at least three reintroductions to the Refuge with animals produced by controlled propagation.

If evidence of breeding by young is found, we will try to translocate rabbits before they reach about 90-days old. Because we do no yet have a growth curve to help establish age from weight or other measurements, we will assume that female rabbits more than about 400 grams in weight (about 70-80% of non-pregnant adult female weight) are capable of reproducing. The actual decision to translocate will depend on the number of young available for translocation, availability of a suitable translocation site, and other considerations. In some instances it may be better to temporarily remove fertile males from the pen than prematurely translocate young.

REINTRODUCTION

Schedule for Reintroduction

The California Department of Fish and Game has granted the use of Pond 6 for a 5-year period for controlled propagation. We are confident that re-establishment of two populations on the San Joaquin River NWR and at least one other, as yet unidentified site, can be accomplished within that time.

Animals are scheduled for reintroduction starting in summer of 2002. This schedule assumes that the three enclosures will be constructed before the beginning of the breeding season in 2001-2002 and that they will be emptied of any animals accidentally enclosed before moving rabbits into the pens.

We will begin trapping individuals of the South Delta population in October 2001 with an objective of assessing the distribution of suitable candidates for captive propagation by November. Individuals will be moved to the enclosures with the objective of having the pens populated by 15 November.

Young might first appear anytime after December, though we expect to see the first progeny of the confined colonies in February or March. Once these progeny have reached about 400 g in body weight, they will become candidates for translocation to the San Joaquin River NWR.

The first cohort produced by the captive colonies will be translocated in summer or early autumn of 2002, depending on productivity of the colonies. Subsequent reintroductions will take place as progeny become available in 2002 through 2005 or 2006, depending on productivity of the confined population and the responses of reintroduced populations.

Location of Reintroduction

Currently, one site on the San Joaquin River NWR (Figure 12) has been protected (Dennis Woolington and Scott Frazier, U.S. Fish and Wildlife Service, pers. comm.) by having a mound of earth higher than the levee on the water side of the levee. This mound has been provided with artificial cover for rabbits and is being vegetated with native shrubs and vines that provide cover and food for brush rabbits. It also has a good cover of herbaceous vegetation. Contiguous to this site along the stream side of the levee are approximately 7.5 acres (3.04 hectares) of continuous brushy habitat for brush rabbits (ESRP unpubl. data). Other clumps of suitable brush are located at various distances from the protected site that probably would be accessible to dispersing rabbits when herbaceous annual plants provided suitable cover. Also, the habitat useable by brush rabbits at the protected site would be greater than 7.5 acres, except during flood, because the vegetation present on the site consists of fairly contiguous patches of tall, dense herba-

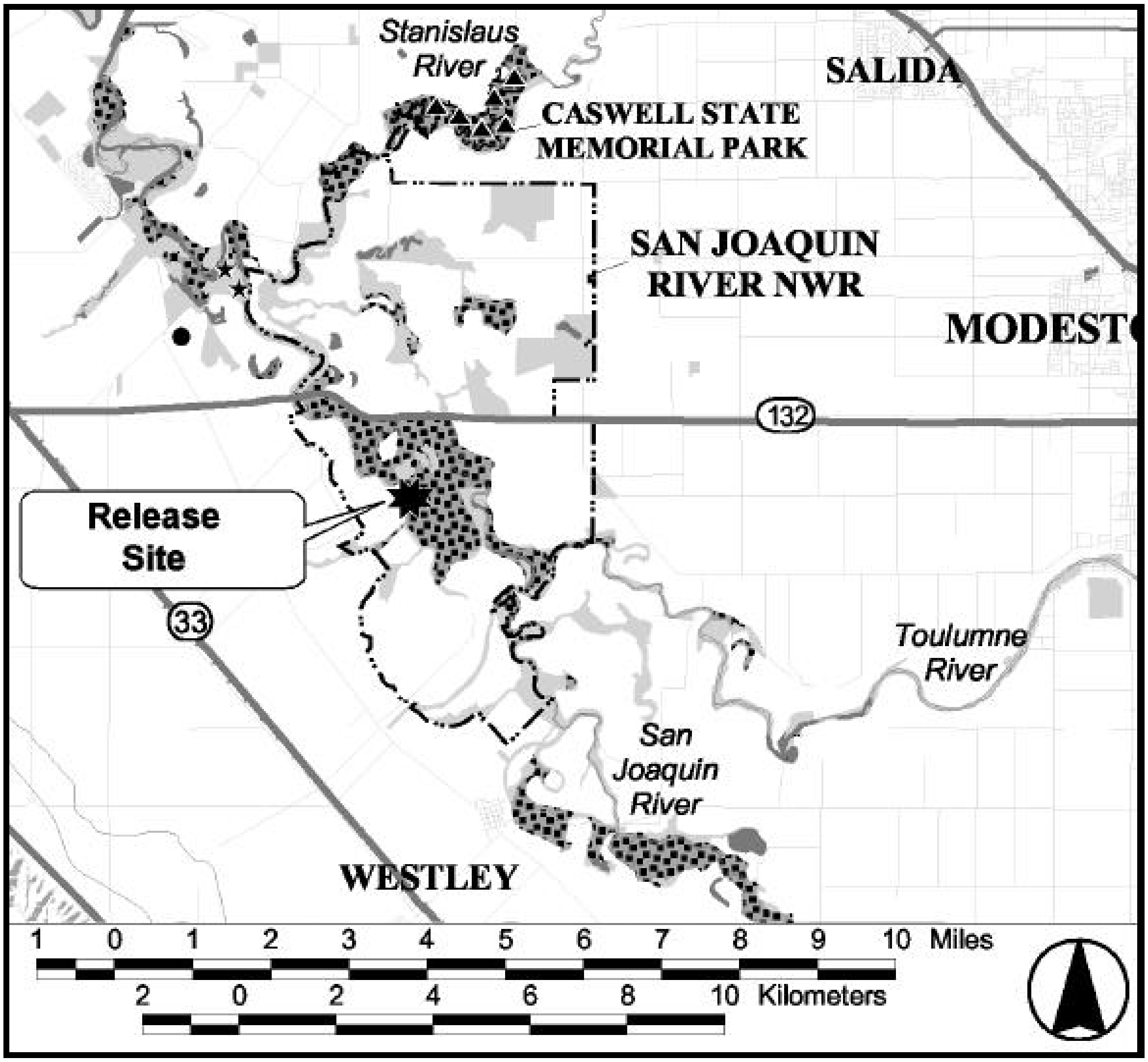

Figure 12. *Approximate location for the first release of riparian brush rabbits on the San Joaquin River National Wildlife Refuge. The Refuge was created primarily for Aleutian Canada geese, and large areas are in permanent pasture and row crops to provide food and wintering habitat for waterfowl. Not all of the land within the approved refuge boundary is publicly owned. See Figure 1 for legend*

ceous weeds and roses, blackberries, and willows and other species of sapling and mature trees. We think that a several hundred acres on and just to the west of Christman Island (all on the Refuge) could become naturally populated from translocations to this 7.5-acre site.

The site chosen, however, is not best placed for populating the entire refuge. Placement was determined by the expediency with which a patch of suitable habitat could be protected by providing a refuge above the elevation of the current levees (Dennis Woolington and Scott Frazier, pers. comm.). The other required criterion is that the site can support one or more of the cohorts produced during the first year of confinement.

Though the mean naïve density for Caswell MSP was estimated at 3 rabbits/ha (Williams 1993), densities in the sections of the Park with the best habitat were 5.5 rabbits/ha, and densities in the best habitat within those sections were about 9 rabbits/ha. The immediate, protected area with continuous habitat on the San Joaquin River NWR has higher habitat values than much of Caswell MSP, based on the greater amount of tall, dense herbaceous vegetation on the Refuge, which borders large patches of willows, shrubs, and vines. In this criterion, the Refuge is more similar to the habitat of the South Delta population, where we had a nearly 330% greater capture rate of rabbits than at Caswell MSP (ESRP unpubl. data). We estimated conservatively that it can support about 6-7 rabbits/ha, or about 20 rabbits in the immediate vicinity of the release site. We think that the area of available contiguous habitat patches can support up to 700-1,000 rabbits depending on how successfully they can disperse.

Release of Rabbits

Prior to translocating rabbits, the release area will be thoroughly censused for predators, particularly hawks, owls, feral cats, bobcats, foxes, and coyotes. Some predators may be trapped and relocated away from the release site, and in choosing the release site, the presence of potential predators will be considered. PVC nest boxes and escape structures (4 ft lengths of 6” PVC pipe) will be scattered throughout the 3-hectare habitat area on the refuge.

After disease screening, rabbits will be trapped, moved from the controlled propagation facility and placed in temporary, pre-release enclosure of habitat about 0.5-1 acre in size, depending on amount and configuration of available high-quality habitat that can be fenced without major disruption to the plant community. Enclosures will consist of 6-ft high poultry wire. The wire will be buried about 6 inches to 1 foot in the ground and supported by T-posts. Rabbits will be confined to this enclosure for 3-5 days, depending on how long it appears to take the rabbits to become adjusted, as shown by behaviors monitored by radio-telemetry and direct observation. The enclosure then will be removed to free the rabbits. We expect that some rabbits might escape before the enclosure is removed. We hypothesize that by confining the rabbits for a few days they will become familiar with places to shelter and retreat and become acquainted with some of the other individuals released at the same time. We expect that this will decrease their tendency to disperse widely when released and give them some additional protection from predators.

***Monitoring Released Rabbits*.**—Released and escaped rabbits will be monitored periodically over the 24 hours each day for the first week after becoming free. Monitoring will assess movements, activities, and possible predation. We will continue to periodically monitor the rabbits to determine location and status (alive or dead). Some animals will be monitored more intensely to determine movements and activities. Monitoring for presence and mortality will continue on a biweekly schedule through the ensuing breeding season in the first half of 2003. Success of the translocations will be evaluated by determining dispersal from the release site, establishment of home ranges, mortality, and reproduction of the translocated individuals. Reproductive success will be quantified to the extent possible by observations and trapping to count and mark unmarked individuals. All unmarked individuals will be considered offspring of the founder population. They will be permanently marked and a biopsy will be taken for genetic analysis. Some will be fitted with radio-transmitters to monitor their movements and mortality.

Locate and Evaluate Additional Potential Release Sites

We expect that more than one cohort of the captive colonies will be translocated to the first site at the Refuge, either because fewer than 20 are produced or because some of the founding cohort will have died, disappeared, or dispersed beyond the area of protected habitat. Yet, additional sites must be quickly protected should productivity and reintroduction exceed expectations, and to meet recovery goals. Recovery goals include managing the population at Caswell MSP to ensure that it will be self-sustaining, plus establishment of at least three additional (four total) self-sustaining wild populations, each with 300 or more adult rabbits during average years (U.S. Fish and Wildlife Service 1998). We do not believe the Caswell MSP population can be described as self-sustaining given recent observations (Williams et al. 2000, Figure 2), and it will require land acquisition to achieve that status, which depends on finding willing sellers among the owners of the parcels contiguous with the Park. We do not know if the South Delta population would be self-sustaining if its current habitat could be maintained, but its habitat is not protected now. Thus, we think it is necessary to reintroduce rabbits to at least three other sites within their historical range. To that end we are working with other agencies and private land owners to locate and protect additional habitat with a goal of having at least one additional site protected each year of 2003-2005. Several potential sites have been located and discussions have started with land owners.

Wildlife habitat easements exist on properties within the stream channel of the Stanislaus River across from and downstream from Caswell, though not all is potential habitat for brush rabbits. Establishing and protecting a population on the south side of Stanislaus River across the river from Caswell MSP to the San Joaquin River, and along San Joaquin River from its confluence with Stanislaus River southward through the San Joaquin River NWR (Figure 12) is a major objective of this program. Rabbits will be established by translocation either on public land or on private land of willing landowners where sites can be constructed and restored above anticipated flood levels. We expect that rabbits will then disperse to suitable habitat along this stretch of more than 25 river-miles.

Disposition of Excess Progeny from Confined breeders

Should more rabbits be produced than can be translocated to the first site at the San Joaquin River NWR, they will be translocated to other, protected sites if there are any available within the historical range. Or, barring that, they will be returned to the inhabited area of the South Delta population if habitat is available and under-occupied. We will determine occupancy level based on the results of trapping during assessment and selection of rabbits for captivity and our other experience with this species. The maximum estimated occupancy level will not be exceeded by reintroduction of rabbits to sites in the South Delta population's habitat. If there are more rabbits produced than we can translocate or return to the wild, we will either temporarily move males and females to separate pens at Pond 6 or close the controlled propagation facility and disperse the surplus to zoos for temporary housing or to academic institutions for research purposes until additional translocation sites can be readied.

COORDINATION TO ENSURE BEST SCIENTIFIC PROCEDURES

Dr. Katherine Ralls of the Smithsonian Institution's National Zoological Park is a collaborator and advisor on this project. She is a member of the California condor and southern sea otter recovery teams and an internationally recognized expert on captive breeding and conservation genetics. She and others in ESRP have been in contact with individuals and officers of the American Association of Zoological Parks and Aquaria (AZA) and the Lagomorph Specialist Group of the International Union For Conservation of Nature (IUCN), seeking advice on this project. One of us (Williams) is a member of the IUCN. We also have been in contact with several researchers who have kept and bred wild species of rabbits in captivity. Another advisor for this effort is Dr. Mark Grobner, Assistant Professor of Biology at California State University, Stanislaus. Dr. Grobner is an expert on the physiology, reproduction, development, and breeding of rabbits.

A Riparian Brush Rabbit Recovery Working Group exists and meets approximately monthly to review all aspects of planning and implementation of controlled propagation, translocation, habitat management, habitat acquisition, and other actions directed at recovering riparian brush rabbits. This informal group consists mostly of biologists representing the California Department of Fish and Game, California Department of Parks and Recreation, California Department of Water Resources, U.S. Bureau of Reclamation, U.S. Fish and Wildlife Service, California State University Stanislaus Endangered Species Recovery Program, University of California Davis Wildlife Health Center, and the Smithsonian Institution. The group is informal in that there is no charter, but its members collectively direct adaptive management responses needed for any particular situation or issue, and seek advice from outside experts when needed.

RISK ASSESSMENT

Genetic and Ecological Effects

***Genetic Effects*.—**Confined populations can provide sufficient numbers of animals needed for successful reintroduction into natural habitats. However, during several generations in captivity, genetic changes could make captive-bred stock unsuitable for release into the wild. A population can be altered by natural selection to conditions in captivity and random genetic drift in small populations can deplete the genetic variation necessary for readaptation to a natural environment when reintroduced (Lacy 1994). The program we outline in this plan is designed to minimize both random and selective changes.

The priorities in this short-term propagation and reintroduction effort are to maximize the genetic diversity of the founders of reintroduced populations to restored habitat while not depleting the diversity in the source population. We plan to use three females and three males in each of the three breeding enclosures. These numbers are based on what we believe the habitat enclosures can support. We have no information on the mating system, but a 1:1 sex ratio for potential breeders would yield the maximum possible unrelated offspring. Even if one male dominates and does most or all the breeding, a 1:1 ratio will provide females with a choice of mates. Depending on what happens among the first groups in confinement, the sex ratio and number of individuals confined in a pen may be altered subsequently.

Starting with 18 founders, and replacing them each year for a 5-year period, will give us a total of 90 founders. Each of the 45 founder females potentially can produce 8-13 young if the mean young per litter for riparian brush rabbits is 4, survival is 67%, and there are 3-5 litters produced per year—360-585 would be available for translocation over the 5-year period. Using lower values for number of young (3) and litters per year (3), and a 67% survival to translocation, the number of young per female is 6 and the total number available for reintroduction over 5 years is 271. The lowest of values meets the first two guidelines for captive breeding programs where individuals are to be reintroduced to the wild: at least 20 founders, with 7-12 progeny per founder (Lacy 1994). The much larger number of founder brush rabbits (90) means that the number of young per founder female (low estimate of 6) more than meets the overall genetic criteria for reintroduction.

It is important to note that this proposed program differs from typical captive breeding programs in numerous ways. These include the plan to return breeders to the source population after a single breeding season and replacing them with new founders with low kinship values to each other and previous founders, reintroducing progeny as they are produced rather than in a single event, and the fact that brush rabbits are a short-lived species, whose reproductive life span in natural settings is typically 3-5 months during a single season. The typical captive breeding program expects founders to live and reproduce over several years.

Depending on the size and diversity of the source population, removal of several individuals for controlled propagation may significantly reduce its genetic diversity. Individuals moved into the confined breeding facility will be returned to the source population after breeding for one season or less. Yet, some mortality of breeders should be expected. Thus, if the source population's habitat is secured, the net effect of this program should be little or no loss of genetic diversity in the South Delta population.

***Ecological Effects*.—**We will monitor conditions in the enclosures and look for evidence of over-impacting the plant community and signs of crowding. Should we find such evidence, we will reduce the number of individuals confined or take other steps to alleviate the condition. We do not expect any significant long-term effects from the temporary enclosures at Pond 6.

Land inhabited by the South Delta population of riparian brush rabbits represents highly degraded strands of riparian communities and the railroad beds and right-of-ways vegetated with some Valley oaks, willows, wild roses, blackberries, and a variety of weedy, annual grasses and forbs. Feral and free-ranging pet cats are part of the community—they and long-tailed weasels and coyotes are probably the most abundant predators. We foresee no adverse long-term ecological effects from removal of rabbits from the South Delta population under the conditions previously described. There will be some slight, temporary reduction in the prey base for predators that might reduce survival of some individual predators, but we don't expect it to be measurable or significant at the population level. Such minor impacts to species requiring federal or state protection are expected for the California-threatened Swainson's hawks (*Buteo swainsoni*) that forage in the area, and brush rabbits probably are a portion of their diet (Hansen and Flake 1995).

Ecological effects from re-establishing riparian brush rabbits in historical habitat is expected to be beneficial. Brush rabbits will provide additional prey for raptors. They also will provide prey for terrestrial predators and ecological services to the riparian plant communities, such as seed dispersal and change in species diversity and abundance (Zedler and Black 1992, Schupp et al. 1997, Dhillion 1999).

Because habitat for the South Delta population borders planned urban development (Figure 11), we simultaneously are working with private landowners there to preserve habitat and a population of rabbits. We believe habitat can be protected from flooding and enhanced in the areas now occupied, and there is good potential for restoring and protecting more habitat during the development process (San Joaquin Co. 2000). There may be other threats from increasing numbers of people and pets as a result of development of Stewart Tract, where most of the existing habitat is located, that will be identified and mitigated. Thus, all considered, the long-term ecological effect would be positive.

Equipment Failures, Human Errors, and other Potential Catastrophes

Every effort has been made to design confined propagation facilities that will exclude predators and confine rabbits. The enclosures will prevent animals from digging out or in, climbing over, or flying into the enclosures. The facilities will be locked, checked frequently, and patrolled periodically. Gates into enclosures will be kept chained and locked. The PVC nest chambers and wooden nest boxes could be rejected as shelters by the rabbits, but we believe that the vegetation is dense enough to provide adequate cover for rabbits year-round at Pond 6. Other than the fencing and netting, there are no other structures or mechanical equipment on the site to fail.

The site chosen is not normally subjected to flooding and has drainage channels to divert sheet flow of water from rains and from flood irrigating the adjacent pasture. However, the area is about at sea level and a slough drains the area to a large pump that could fail. There is little danger of salt water incursion because of the many miles of islands and levees between the site and Suisun Bay, but a pump failure could lead to flooding if it was not quickly put back into operation. This scenario is unlikely because the entire area is developed and great economic losses would ensue if the pumping was not restarted. Portions of the pens are flooded with a few inches of water during irrigation of the adjacent pasture. Each pen has higher ground that does not become inundated where rabbits can seek shelter, and rabbits can climb into vines and on nest boxes to get out of shallow, temporary flooding. We do not believe that this slight flooding is harmful to the rabbits. It is necessary to keep the vegetation in the pens alive and robust.

Should vandals cut or tear down the fencing, or a gate be accidentally left open, the rabbits are unlikely to disperse from the site of the enclosures because they normally confine their movements to areas of continuous cover. If animals were to escape from the pens because of accident or vandalism, they likely would take up residence in nearby patches of shrubs on the same property. Having three separate enclosures reduces the risk of such an event resulting in loss of all confined individuals.

The site (Figure 8) is bordered by a vineyard on the west, with a broad expanse of bare ground between it and the edge of state property; on the north is a slough with bare banks on the side where rabbits will be; on the east is a well-grazed (i.e., grasses less than 2 inches tall, year around), irrigated pasture; and a paved road and row crops border on the south. Additionally, there are open ditches filled with water on all sides of the property, although there is one or more ways to cross these waterways. Should animals escape the confines of the State property, there still is little risk that they could establish a population outside the historical range of the species because there is no habitat for brush rabbits immediately surrounding the state property. It also is possible that the site is within the historical range of the riparian brush rabbit.

There is potential for wildfires. The area has a recent history of arson and accident-caused fires. We are developing a fire-response plan with local and state agencies involved in fire protection. It will include emergency contacts and protocol for removing rabbits should wildfire threaten a pen. Additionally, an area 50-feet wide will be cleared of all combustible vegetation north and south of the pens. Low-growing irrigated pasture grass borders the pens on their east sides—it will not support a wildfire. A wetland and 50-foot wide pond are located on the west side of the pens, the wetland is within 5-10 feet of the fencing. During the dry season it might support fire. We will clear vegetation between the pens and the wetland (a strip of about 5-10 feet) but cannot remove the trees or wetland plants. Fire extinguishers are kept on site in case of accidental fire due to weed cutting machines. These precautions should minimize the possibility of fire in the pens.

Disease

Little is known of the effects of disease in brush rabbit populations, though several diseases have been reported for the species or for other species of *Sylvilagus* (Williams

1988; Appendix A). Tularemia, a bacterial disease spread by blood-drinking insects has been implicated in population regulation of at least one population of the eastern cottontail, *S. floridanus* (Woolf et al. 1993), and is known to be endemic in brush rabbit populations. Disease is a natural component of the environment of brush rabbits and many diseases probably are present in the natural populations. Because so little is known about epidemiology of most of the diseases of brush rabbits, we see no acceptable alternative to bringing healthy-appearing rabbits into confinement. Mixing animals from differed subpopulations increases the risk of disease but those that survive such exposure provide the breeding stock for future generations. Animals that appear sick will be released at the point of capture. Animals moved to enclosures will have a blood sample taken to screen for pathogens or antibodies to pathogens and given a health exam. We do not plan to attemp to restrict propagation only to pathogen-free rabbits, which would be nearly impossible in the enclosed natural areas where they will be confined.

Animals in confinement will be screened for certain diseases (see Appendix A) and monitored for apparent health whenever they are captured for other purposes. Any evidence of disease in the confined individuals will be investigated by veterinarians qualified to recognize diseases likely to infect wild rabbits and to assess the risks to the rabbits as a result of disease. That there is a risk that a disease could adversely affect the confined populations despite these precautions is obvious, but there are no data for evaluating the probability. Having the confined individuals in three separate enclosures will reduce that risk. To reduce the possibility to transporting pathogens on feet or equipment, people entering the pens will have to step through a 10% bleach solution and all equipment use in a pen will either be confined to use in that pen or be sterilized with bleach solution before use (see Appendix B for policy on activities at the Controlled Propagation Facility).

Before translocation, both the animals to be translocated and existing populations of lagomorphs at the translocation sites will be screened for certain pathogens. We will rely on advice from experts on those pathogens in rabbits to ascertain which individuals are to be translocated and what precautions to take to reduce the risk of disease-induced mortality.

Potential for Increased Level of Inbreeding within Populations

Taking only a portion of the population into captivity risks reducing the genetic diversity in both the source and confined populations. We hope to minimize inbreeding risks in the captive population by confining individuals together that were captured from distant points. Because of the clumped nature of their existing habitat and the low vagility of brush rabbits (Chapman 1971), animals from distant points (e.g., 0.25-5 miles apart) probably are not as closely related as those captured close together—in other words, they should have the lowest mean kinship values. By selecting parents from different sites for use in propagation, using different parents for production of young in different years, and making reintroductions from more than one year's progeny, we hope to establish considerable genetic diversity in the reintroduced populations. We also will be assessing the genetic constitution and relatedness of both confined and free-ranging individuals. By genotyping parents and progeny of confined breeders we can determine parentage and assess relatedness of individual progeny and their contributions to founder populations at reintroduction sites. By moving the reproducing individuals through the enclosures and returning them to their home, the source population should not be decreased in diversity. In these ways, we hope to minimize the potential for increased inbreeding in source, confined, and founder populations and maximize outbreeding.

Caswell MSP consists of 253 acres, approximately 70-90% of which is potential habitat for brush rabbits (Williams 1988, 1993). Christman Island on the San Joaquin River NWR is approximately 700 acres in size, with perhaps half or more currently being habitat and the rest being restored by natural succession (some was cleared for farming and livestock grazing diminished shrub cover). A crude estimate of the extent of occupied habitat in the South Delta population is approximately 270 acres. Because there is more potential habitat at the reintroduction site on the San Joaquin River NWR than in either of the extant populations, if the reintroduced population expands to near carrying capacity, inbreeding should not be a significant risk.

We base this assumption on the following. Naïve density of riparian brush rabbits in January 1993 at Caswell MSP was estimated at 3.0 ± 1.13 rabbits per hectare with a 95% confidence interval for the total population of between 215 and 768 rabbits for the entire Park, depending on different assumptions about amount of habitat. Williams (1993) believed that this density was near the maximum carrying capacity at Caswell MSP, but well below what the best habitat could support. If only half the land on Christman Island, San Joaquin River NWR, is useable habitat for brush rabbits, it should support from about 297-1,062 rabbits at a carrying capacity similar to Caswell MSP. As additional restoration of the riparian community on the Refuge proceeds, the amount of contiguous patches of habitat for brush rabbits west of the San Joaquin River should increase to over 1,000 acres and support about 850-3,036 rabbits. When there are sufficient sites for rabbits to refuge above high water, the Refuge should support a self-sustaining population if habitat is maintained through management or natural processes. Inbreeding in the reintroduced population should not be a problem.

Potential Erosion of Genetic Differences between Populations

Our preliminary data suggest that the two populations of riparian brush rabbits share more alleles at eight microsatellite loci than either do with populations of other subspecies from Corral Hollow in the Diablo Range to the west of the Valley, or the Sonora area in the Sierra Nevada to the east (Table 2). So far, we have found a total of 65 alleles for the 8 microsatellite loci in brush rabbits from 4 population samples. The Caswell MSP population shares 27 of its 39 alleles with the South Delta population, 6 of which are uniquely shared. That is, the six unique alleles are not found in the Sierra Nevada or Diablo Range samples. The Caswell MSP population shares 14 alleles with the Sierra Nevada population and 20 with the Diablo Range population, but does not share any alleles with the Sierra Nevada or Diablo Range population that are not also found in the sample of the South Delta population. The South Delta population shares 14 alleles with the Sierra Nevada population and 24 with the Diablo Range population—none of the alleles are uniquely shared with the Sierra Nevada population, but 3 alleles are uniquely shared between the South Delta and Diablo Range populations (Table 2).

Table 2. *Number of alleles (self-comparisons are in shaded cells), shared alleles (lower triangle of table), and uniquely shared alleles (upper triangle with bold-faced numerals) within and between population samples of brush rabbits* (Sylvilagus bachmani) *for eight microsatellite loci. Interspecific comparisons also are made with the desert cottontail,* S. audubonii *(ESRP and James Youngblom unpubl. data).*

Sample	Caswell MSP	South Delta	Diablo Range	Sierra Nevada
Caswell MSP	39	**6**	**0**	**0**
South Delta	27	37	**3**	**0**
Diablo Range	20	24	31	**3**
Sierra Nevada	14	14	18	32
S. audubonii	13	12	12	13

Effective number of migrants (Slatkin 1985) over all 4 populations of brush rabbits was estimated at 0.498. This value is quite low in comparison to populations of European rabbits in Britain, which was estimated at 1.12 (Surridge et al. 1998). Genetic differentiation and low levels of gene flow also are indicated by unbiased F_{st} estimates between populations (Table 3). F_{st} (Wright 1951) measures the amount of genetic variation in the whole population that is attributable to genetic differentiation among subpopulations. In our analyses (Table 3) we only compared population pairs. The F_{st} values suggest considerable genetic differentiation among populations, particularly between the Sierra Nevada population and the other populations of brush rabbits. Comparisons in Table 3 also include a sample of desert cottontails. Note that F_{st} values between the Sierra Nevada and other brush rabbit populations are nearly as great as between those of brush rabbits and desert cottontails. Desert cottontails and brush rabbits are considered to belong to different evolutionary clades within the genus *Sylvilagus*, therefore, genetic differences between the Sierra Nevada and the other brush rabbit populations may signify different species.

We interpret all the genetic data presented in this plan as demonstrating that the Caswell MSP and South Delta populations, *S. b. riparius*, share more genetic similarities than either does with the other populations, and that the Sierra Nevada population, *S. b. mariposae*, is distinct from both the riparian subspecies and the population from Corral Hollow, which we presume represents *S. b. macrorhinus.* Yet, we currently have too little information to fully evaluate the amount or significance of genetic differences between the populations of riparian brush rabbits. For this and other reasons already elaborated, we plan to confine and translocate only individuals from the South Delta population to establish new populations until genetic information can be fully analyzed and discussed among experts.

Table 3. *Weir and Cockerham's (1984) unbiased estimate of F_{st} between pairs of populations of brush rabbits (ESRP and James Youngblom unpubl. data). F_{st} is a measure of genetic differentiation of population pairs in this analysis. Greater values indicate greater differentiation.*

	Caswell MSP	South Delta	Diablo Range	Sierra Nevada
Caswell MSP	0			
South Delta	0.1956	0		
Diablo Range	0.2046	0.1505	0	
Sierra Nevada	0.3186	0.3411	0.2327	0
Desert cottontail	0.3495	0.3120	0.3184	0.4198

There has been a long-running debate about whether it is better to try to preserve the uniqueness of each small subpopulation of a species or to promote outbreeding in conservation programs (Brown 1994). Ralls et al. (2001) point out that the risks of inbreeding for small populations far exceed the risks of outbreeding. It is probable that the Caswell MSP population would benefit from outbreeding with individuals drawn from the South Delta population, and that translocated populations would benefit from having genetic contributions from both populations. The Caswell MSP population has remained so small for the past 5 years that genetic intervention may be required to preserve it. During that time we have captured a total of 14 animals at Caswell MSP. One other, baby rabbit was found dead during other work in the Park. Our belief is that this population is close to extinction. Thus, although the immediate plan is to preserve the genetic differences by not mixing animals from the Caswell MSP and South Delta populations, it may eventually be necessary or desirable to move some animals that originated in the South Delta population to Caswell MSP in order to recover the species. This would only be done after completing genetic analyses of the relationships of these and other populations, disease screening, and extensive consultations and approval of the responsible state and federal agencies and scientific advisors. For these reasons we believe that it is necessary to reserve the option to conduct outbreeding as part of the controlled propagation program or to translocate some captive bred rabbits from the South Delta population to Caswell MSP.

Exposure to Novel Selection Regimes in Controlled Environments

The confined propagation program is designed to minimize the problems of novel selection operating on individuals raised in captivity. There should be little if any natural selection for conditions of confinement because successive generations will not be raised in captivity. Because animals will not be confined to individual cages in a controlled environment, none of the potential problems such as imprinting on non-natural foods, unbalanced nutrition, stress from being caged, diseases resulting from unsanitary conditions, aggravated fighting with incompatible conspecifics, and artificial heating and cooling will not be factors. Also problems that arise from rearing animals in cages and then releasing them to the wild will not arise. These problems could include lack of experience in coping with natural weather, locating and recognizing food plants, recognizing their species and individual conspecifics, experiencing social interactions, and avoiding predators. By fencing natural habitat, allowing individuals to interact and breed freely within the enclosures at densities similar to natural habitat, and moving animals to reintroduction sites as they mature, they already will be acclimated to weather, habitat, food, and conspecifics. Because the enclosures are in a natural setting, rabbits may gain some experience with predators outside the enclosures.

Whether or not they will be able to avoid predation as well as wild-reared individuals is unknown, and that risk will remain. Conditioning to potential predators will be tried at the pens to learn if conditioned animals survive better after translocation. Under consideration are experiments with muzzled pet dogs and silhouettes of raptors that can be moved on wires strung within the pens to simulate a swooping falcon, hawk, or owl.

DATA MANAGEMENT

Information available is insufficient to develop a detailed genetics management plan similar to that of many species reared in zoos. Nor is that type of plan needed for the short-term breeding program described in this plan. Yet, we will maintain complete records that would allow development of such plan in the future, should it be needed. In the previous sections we have outlined the known genetic information on riparian brush rabbits and discussed how we intend to ensure that neither the source nor confined populations are depleted of genetic diversity. Phenotypic variation in the source population will be noted and we will attempt to preserve that variation in the confined and reintroduced populations. We also will use genetic data from microsatellites to measure genetic diversity and genetic contributions by confined breeders.

Record keeping will be done with a relational database management program. Information recorded for each rabbit will include unique identifier numbers (ear and PIT tag numbers), sex, relative age at first capture, date of capture, locality of capture (using GPS technology), weight, body measurements, any notable phenotypic variation, reproductive status, and genetic composition. Animals used for controlled propagation will have the same data recorded and in addition, date of introduction to a pen, pen number, identity of pen mates, behavioral observations, reproductive observations, number and identity of offspring, estimated date of birth of young, results of disease screening, dates of treatment for ectoparasites, date and cause of death, if known, and other information. Because of the nature of the habitat in the enclosures (dominated by dense clumps of Himalayan blackberries), it may not be practical to locate and identify nests of females, and parentage probably will have to be determined by genetic analyses. Nevertheless, we will periodically check nest boxes and attempt to locate the nests of females through radio-telemetry in order to identify mothers and number of young produced.

Each time an individual is captured during confinement or after reintroduction the pertinent data will be recorded. From the database records, we will be able to track the origin, history, and lineage for every individual considered for translocation.

ALTERNATIVES REQUIRING LESS INTERVENTION

Direct Translocation

Capture and translocation of individuals from the South Delta population directly to restored habitat would require less intervention, but also probably would give only one chance to establish a new population and preserve the diversity in one of the two extant populations. If it failed, there might not be a source of individuals for a second attempt, or if there was, the source population probably would be much reduced in genetic variability. Removal of individuals from small populations without genetic replacement can reduce genetic diversity and, potentially, population fitness. Yet, direct translocation may be used to supplement existing or newly established populations if for some reason, habitat in existing populations must be destroyed for flood control or permitted development.

Habitat Restoration

We do not believe that habitat restoration and enhancement alone can recover the species, nor will enhancement in existing populations lead to repopulation of areas of suitable habitat through natural dispersal sufficient for recovery. This is because there is no suitable habitat along major stretches of the channelized rivers between inhabited sites and sites with potential habitat, and because other societal considerations, especially flood control, make restoring continuous riverine vegetation impractical at this time.

Do Nothing

The do nothing alternative would require no intervention but is likely to lead to extinction of both populations within a few years for the various reasons already elaborated.

ALTERNATIVE REQUIRING MORE INTERVENTION

Rearing Rabbits in Individual Enclosures

The main advantages of rearing animals in small, individual enclosures are that reproduction can be precisely controlled and parentage and number of offspring, dates of birth, and so on can be directly determined. We do not dispute the utility of having this amount of control and the benefits of having these data. Yet, there are substantial disadvantages to this approach. Typically, animals are confined to a fraction of an average home range, and do no live in natural vegetation. They also have limited or human-controlled social interactions with conspecifics. Lack of space can cause life-threatening conflicts when rabbits are put together for reproduction or social interactions. Rabbits born in such confined circumstances are likely to become imprinted on a highly non-natural environment and lack social and other behavioral skills needed in the wild (Konstant et al. 1982, Caldecott and Kavanagh 1988). They also may not be exposed to some diseases endemic in their natural environments and be more susceptible when later released to the wild, for example by being less able to avoid predation while ill. Artificial food or non-natural sources of food generally must be provided that may cause nutritional problems and naïveté about foods in the wild. This approach also requires much more human intervention, which risks undesirable behavioral modifications and transmission of diseases between humans and rabbits. These are often factors in the lack of success in releasing animals into the wild that were reared in captivity.

Rearing rabbits in individual enclosures also is much more costly. Endangered subspecies have low priority for recovery dollars based on U.S. Fish and Wildlife Service policy and the competition for extremely limited funds. The course we propose in this plan is strongly influenced by the amount of funding that is likely to be available to achieve recovery. Yet, the reasons elaborated above were paramount in the decision about where and how rabbits would be bred in this program.

COOPERATORS AND ACKNOWLEDGMENTS

Cooperators whose staff members are part of the Riparian Brush Rabbit Recovery Advisory Group are: the U.S. Fish and Wildlife Service; U.S. Bureau of Reclamation; California Department of Fish and Game; California Department of Parks and Recreation; California Department of Water Resources; California State University, Stanislaus, Endangered Species Recovery Program; University of California, Davis, Wildlife Health Center; and Smithsonian Institution. Funding for work with riparian brush rabbits, including range surveys, trapping censuses, genetic investigations, and controlled propagation has been provided under contracts to ESRP with the California Department of Fish and Game, U.S. Fish and Wildlife Service, and U.S. Bureau of Reclamation. The California Department of Parks and Recreation and Department of Water Resources also have provided significant resources for the recovery of the riparian brush rabbits and permission to work on their properties. Without the cooperation and advocacy of Califia, LLC, in gaining access to private properties to select rabbits for breeding, the controlled propagation program would have had little chance of success. Work on riparian brush rabbits is authorized by permits and memoranda of understanding with the California Department of Fish and Game and the U.S. Fish and Wildlife Service. Dr. Mark Grobner supplied domestic rabbits on which we tested a flea and tick pesticide. We thank the individuals and their agencies and companies for their advice and support in developing this plan and for work with riparian brush rabbits. We also thank the several peer reviewers and ESRP staff who took the time to consider these efforts and to offer their advice and constructive criticisms. The plan is much stronger because of their efforts. In this regard we especially acknowledge the contributions of Lawrence Host of the U.S. Fish and Wildlife Service and Matthew Lloyd of ESRP.

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APPENDIX A

HEALTH PLAN FOR THE ENDANGERED RIPARIAN BRUSH RABBIT (*SYLVILAGUS BACHMANI RIPARIUS*) REINTRODUCTION PROGRAM

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INTRODUCTION

The purpose of incorporating health screening into the riparian brush rabbit reintroduction program is to evaluate the health and well-being of individual rabbits and the captive rabbit population as a whole, minimize risk of disease outbreaks and transmission, and rapidly and accurately determine causes of morbidity and mortality so that informed management decisions may be made in a timely fashion. The overall goal is to ensure the greatest chance for successful reintroduction by maintaining and releasing healthy rabbits. The following suggestions specifically focus on the captivity period, i.e. from the time that rabbits are captured from the source population, transferred to the holding facility, and released to new habitat.

First, recommendations for conducting health checks on captive rabbits (adults and young) are described. Second, an overview of diseases of rabbits is provided, with diseases of most concern listed separately from diseases of less concern. Third, detail is provided on the applicability and interpretation of laboratory diagnostic tests.

RECOMMENDATIONS FOR HEALTH SCREENING

General Principles

Captive riparian brush rabbits should be handled and treated humanely to minimize stress, maximize health, and thereby give the reintroduction program the greatest chance of success. Humane care applies to the capture, handling, housing, feeding, and treatment of all rabbits. Capture should be non-injurious and of as short a duration as possible. Handling should be minimized; sedatives and/or anesthetics should be used for any procedure that is long and/or has the potential to cause discomfort or pain to the rabbit. Housing should be as naturalistic as possible, providing rabbits with adequate shelter and protection from inclement weather and predators. Supplemental food items should be fresh and free of chemical or biological (e.g. fungal) contamination. Injury or illness should be treated when and if it is deemed appropriate and crucial to the reintroduction program (taking into account population size and potential of the disease to significantly impact population size), recognizing that disease is a normal component of the rabbits' biology and ecology. Rabbits should be medically treated when and if the injury or illness is human-induced.

Every effort should be made to avoid disease transmission among captive rabbits, between captive rabbits and wild conspecifics, and between the rabbits and their human handlers. Preventing disease transmission is achieved by:

- holding rabbits in captivity for at least 30 days prior to release (i.e. a "quarantine" period which allows for crudescence[5] of infectious disease and/or identification of morbidity which warrants further investigation);

[5] crudescence—appearance or eruptions making the conditions detectible

- observing captive rabbits frequently to identify illness as early as possible, so that measures can be taken to minimize transmission of an infectious disease to other rabbits;

- using gloves and protective clothing at all times when handling rabbits;

- using foot baths when entering and exiting rabbit enclosures (10% bleach, changed daily, and maintained in a place that prevents exposure of wildlife);

- eliminating opportunity for contact between captive and wild rabbits by constructing escape-proof, double-walled (i.e. with buffer zone) enclosures[6];

- capturing and reintroducing only healthy rabbits; only rabbits free of disease should be released into the wild to minimize the risk of introducing a new pathogen to a susceptible population.

Live Rabbit Health Checks

The following are recommendations for assessing the health of rabbits as they enter the captive breeding facility, and again not more than 10 days prior to release:

1. complete physical examination by a veterinarian;

2. blood collection (ear artery) for a complete blood count, serum chemistry, and for serology where appropriate (see below);

3. fecal sample for parasite examination (direct smear, fecal floatation for nematodes, fecal sedimentation for trematodes);

4. collection and identification of ectoparasites, including external ear canal swab for mites;

5. nasal swab for aerobic culture (for *Pasteurella multocida* and *Bordatella bronchisepticum*);

6. optional—serology test for *Encephalitozoon cuniculi*, rabbit syphilis.

[6] Pen design and construction of one pen was completed and the construction of the two other pens was contracted prior to consultation with Dr. Gilardi. We agree that a double fence would eliminate all possibility of contact between desert cottontails outside the pens and brush rabbits within the pens. The brush rabbits used in this program lived with desert cottontails before being brought into captivity and the ground within the pens was used by desert cottontails. The decision was made to not attempt to isolate the confined population from normal environmental influences except predation. The pens cannot isolate the confined rabbits from the mosquito and tick-born diseases nor the diseases that might be present in the soil. We believe that the hardware-cloth fencing of the first pen is of a small-enough mesh that it is highly unlikely that there could be any direct contact between rabbits inside and outside the pen—nose to nose contact is about all that would be possible. We do not believe that a second fence is necessary.

Examinations should be conducted on rabbits that are chemically sedated, so as to minimize stress to the rabbit, and allow for safest possible handling. Rabbits should be sedated with a combination of acepromazine (0.75 mg/kg) and butorphanol (0.25 mg/kg). All rabbits should be allowed to FULLY recover from sedation prior to release back into the holding facility: full recovery should be assessed by the attending veterinarian.

Contingencies

Positive tests.—If any of the above evaluations and tests reveal information concerning potential disease, the veterinarian shall consult with the reintroduction team to formulate a plan, which may include:

1. ***treatment***, either in situ or at UC Davis School of Veterinary Medicine, if and only if the disease and its treatment does present a risk to other rabbits, and the rabbit can be expected to make a full recovery;

2. ***euthanasia***, if the risk of transmission of the disease to other rabbits is too great, if the treatment process would be considered inhumane or infeasible, or if a definitive diagnosis is necessary and can only be obtained through a full necropsy; or

3. ***no action***, if the disease is considered mild, the rabbit is expected to make a full recovery, and the disease is considered a natural component of the rabbit's biology and ecology (e.g. it is expected that fecal examination will reveal parasites, but unless they are identified as pathogens, and/or the rabbit is showing clinical signs associated with infection, treatment is probably not warranted).

4. ***re-start of quarantine*** for all rabbits that had direct contact with the sick rabbits.

Mortality.—If a rabbit dies while in captivity, or after release, and the carcass is recoverable, the carcass should be necropsied by a veterinary pathologist at UC Davis (Dr. Linda Munson, Chief of Service, Diagnostic Pathology, Veterinary Medical Teaching Hospital; or Dr. Karen Terio, same), and a full histopathologial evaluation should be performed, including special tests as recommended by the pathologist. A full report will be prepared. The carcass should be clearly identifiable (via ear tag), double-bagged in heavy plastic, and the bag labeled with Genus species, common name, identification number of rabbit, date of death (if known), date of carcass collection, and weight of carcass. The bagged carcass should be placed in a refrigerator, and delivered as soon as possible to UC Davis during normal business hours.

Medical treatment.—If a rabbit requires medical treatment that is best provided in a clinical setting, it should be transferred to the UC Davis Veterinary Medical Teaching Hospital, where it can receive a thorough diagnostic evaluation (e.g. radiographs, ultrasound, surgery), and best possible therapy (e.g. fluids, medications, round-the-clock observation).

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OVERVIEW OF DISEASES OF RABBITS

Diseases of Rabbits of Most Concern for the Reintroduction Program for Riparian Brush Rabbits

The following are diseases of special concern for this program, either because they have the potential to cause significant morbidity and mortality in *S. bachmanni*, and/or because they have the potential of causing significant morbidity and mortality to other rabbit species or small mammals, and could therefore hinder the reintroduction effort. As well, diseases of zoonotic[7] concern are listed here.

Tularemia.—ZOONOTIC. Caused by a bacterium called *Francisella tularensis*, which has one of the broadest host ranges of all bacteria, but causes disease principally in rabbits and rodents. Of rabbits, *Sylvilagus* spp. and *Lepus* spp. are the most important hosts. Important rodent hosts in North America are primarily beavers (*Castor canadensis*). Although other mammals are relatively insignificant in the epidemiology of tularemia, an outbreak has been reported in wild gray foxes. The bacteria are transmitted by blood-feeding arthropods (mosquitoes, fleas, tabanid flies, and ticks), contact with infective blood or tissues, inhalation of aerosolized particles, or ingestion of contaminated food or water. Tularemia may act as a regulatory mechanism in rabbit populations, although infection need not be fatal. The course of clinical disease is usually acute: the rabbit develops severe lethargy and rapidly fatal septicemia, with death in 2-10 days. Post-mortem diagnosis is based on gross and histologic lesions and culture of the organism. Available antemortem diagnostics include blood culture (or serum, lung washes, etc) at a biosafety level 2 or 3 facility only, on selective media (glucose cystine blood agar or other media containing sufficient cystine or cysteine). Serologic tests have been developed for diagnosis in people and have limited applicability to wildlife, especially in sensitive species that usually die before they mount a detectable immune response. Serology is better used for more resistant species, in order to detect tularemia activity in a locality. The standard serologic test is the tube-agglutination test. There is also an ELISA[8]. IN MOST ECOSYSTEMS, TULAREMIA CANNOT BE CONTROLLED. IT SHOULD BE RECOGNIZED AS A NATURAL COMPONENT OF MANY ECOSYSTEMS.

Myxomatosis.—Genus *Leporipoxvirus*. Endemic in wild *Sylvilagus bachmanni* in the western US—brush rabbits are natural hosts for this virus. It causes local, benign skin tumors (fibromas) in *Sylvilagus* spp., which spontaneously regress over a period of months. Transmission is via blood-feeding arthropod vectors. Infection of other species of *Sylvilagus* and *Brachylagus idahoensis* (pygmy rabbit) with myxoma virus has not been reported in the wild. Black-tailed hares (*Lepus californicus*) are resistant to infection. The organism is only mildly pathogenic and self-limiting for *Sylvilagus*, but lethal in *Oryctolagus cuniculus*, especially in domestic rabbits. In *S. bachmanni*, antibodies have been detected but are short-lived. CONSIDERING THE ENDEMISM OF THIS

[7] zoonotic—a disease that can be transmitted from other animals to humans
[8] ELISA—Enzyme-Linked Immunosorbent Assay

DISEASE IN THIS REGION, IT IS SAFE TO ASSUME THAT EVERY *S. BACHMANNI* IS INFECTED OR HAS THE POTENTIAL TO BECOME INFECTED.

***Pastuerellosis*.—**Caused by a bacterium called *Pasteurella multocida*, a normal constituent of the upper respiratory tract and pharynx of rabbits, which can cause upper respiratory infections, otitis, bacteremia, localized or generalized abscesses and lymphadenitis, and genital infections. Infected rabbits develop antibodies, but usually remain infected. It has been documented to cause outbreaks in wild and farmed European brown hares *Lepus europaeus* in Europe, and is a common cause of mortality in hares elsewhere in Europe. Pastuerellosis has not been documented as a significant cause of morbidity and mortality in wild lagomorphs in the U.S., but is a significant health problem in domestic rabbits, and should be considered a potential clinical disease in any rabbit subject to immuno-suppression or stress. Treatment: antibiotics, but can be difficult. Diagnostics: ELISA for screening; nasal swabs for culture (No. 4 calcium alginate swab 1-4 cm into nares), incubate on blood agar. Swabs for culture can be confusing, since cultures may well be positive in non-clinical animals (because the organism is a commensal), and cultures may be negative in animals with systemic pastuerellosis (the organism can be difficult to culture). It takes 2-3 weeks for titer[9] to rise to detectable levels.

***Bordetella bronchisepticum*.—**A bacterium that causes upper respiratory tract infections. It can predispose a rabbit to Pastuerellosis, or complicate Pastuerella infections. Treatment: antibiotics.

***Rabbit viral hemorrhagic disease*.—**A calicivirus, REPORTED FOR THE FIRST TIME IN THE U.S. IN DECEMBER 2001 in a captive colony of rabbits in New York state. First emerged in farmed rabbits in China in 1984, then reported in farmed *Oryctolagus cuniculus* in Europe starting in the mid-1980s, and was in the United Kingdom by 1992. It very rapidly spread to wild European rabbits. It was first reported in the Americas in 1988 in domestic rabbits in Mexico after the importation of contaminated frozen rabbit carcasses. The disease subsequently was eradicated in Mexico through a test and slaughter campaign. While undergoing testing as a potential biological control agent of introduced rabbits in Australia, the virus "escaped" the island testing site to mainland Australia, where it spread rapidly and decimated *O. cuniculus* populations with a 65-95% mortality rate. The virus causes acute, fulminant and often fatal disease in adults, but essentially spares rabbits < 8 weeks old. European Brown Hare Syndrome, caused by a closely related calicivirus, is not known outside Europe, its distribution apparently corresponding with the distribution of the European brown hare, *Lepus europaeus*. Serological tests are available (ELISA); diagnostics usually via histopathology, PCR, etc.

***Baylisascaris roundworms*.—**E.g. *Baylisascaris procyonis, B. columnaris*. Rabbits can serve as aberrant hosts of the raccoon (*B. procyonis*) and skunk (*B. columnaris*) roundworms. Infection causes neurologic disease. Definitive diagnosis requires necropsy and examination of brain and spinal cord. While *Baylisascaris* spp. are zoonotic, it is highly unlikely that the parasites would be acquired from an aberrantly infected rabbit, as they are not shedding infective oocysts in feces.

9 titer—antibody presence or level in blood

Rabies.—While any mammalian species can be infected by the rabies virus, only a few can serve as reservoirs of infection. In California, the primary mammalian reservoir of rabies are skunks and bats. A rabbit can be infected via contact with a rabid animal, but would most likely not transmit the virus to conspecifics because it would not likely survive attack by the rabid predator, or succumb to the virus without developing a high enough viremia to effectively transmit the virus to other rabbits. From a public health perspective, rabies should always be on the list of diseases to rule out as cause of death in a wildlife carcass, especially if death is acute or the animal was exhibiting neurologic signs prior to death.

Trematodiasis.—Specifically due to the liver fluke, *Fasciola hepatica*. It can infect rabbits that graze in wet pasture or along the banks of streams in endemic areas, where they accidentally ingest the snail intermediate host. Adult flukes live in the gallbladder and bile ducts, and can cause severe weight loss, poor coat quality, lethargy, and death. Diagnosis: eggs seen on fecal sedimentation, or adults seen at necropsy. Treatment: single dose of praziquantel orally.

Aflatoxicosis.—Rabbits are exquisitely sensitive to the aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*, which can grow on improperly stored feeds, and are also ubiquitous in the environment. Rabbits are especially sensitive to the B1 aflatoxin fraction; levels greater than 100 ppm in the diet of rabbits has been shown to cause morbidity and mortality. Supplemental feed should be screened for the presence of aflatoxins prior to providing to captive rabbits.

Diseases of Rabbits of Less Concern for the Riparian Brush Rabbit Reintroduction Program

The following are diseases of less concern for this program, either because they are infrequent, are not likely to cause significant morbidity and mortality in *S. bachmanni*, or because they have been reported in captive rabbits only. These are diseases to be vigilant for during physical examinations and routine laboratory work (e.g. complete blood counts and serum chemistries, fecal examinations) and frequent direct observation, but more specific and thorough diagnostic testing probably is not warranted unless the disease is suspected based on observations.

Brucellosis.—Caused by the bacteria, *Brucella* spp. An important consideration with regard to terrestrial brucellosis in wildlife is to distinguish between a spillover of infection from domestic animals and a sustainable infection in wild species. In 1965, a study showed *Sylvilagus nuttallii* and *S. audobonii* in Utah to be susceptible to *Brucella* sp. *Brucella suis* biovar 2 has been shown to affect European hares, *Lepus europaeus*. *Brucella abortus* is thought to have been eradicated from domestic livestock in the U.S.

Plague.—Primarily a zoonotic disease of rodents, plague is caused by bacterium *Yersinia pestis*. Plague can be spread to rabbits, humans, another animals by infected fleas. California ground squirrels (*Spermophilus beecheyi*) are a major reservoir species for plague. Plague has three forms: bubonic plague, infection of the lymph glands; septicemia plague, infection of the blood; and pneumonic plague, infection of the lungs. Pneumonic plague is the most contagious form because it can spread from person to person in airborne droplets. Periodic outbreaks of plague kill large numbers of rodents (called a "die-off"). The risk of infection to humans and other animals in the area increases when the rodent hosts die and infected fleas look for other sources of blood. Wild rodents become infected but usually do not show clinical signs under normal conditions. The signs in animals are fever, pneumonia, and swollen lymph nodes. Treatment: antibiotics such as streptomycin, chloramphenicol, or tetracycline.

Leptospirosis*.—Leptospira grippotyphosa* has been isolated from wild cottontails in Florida and southwestern Georgia. Rabbits probably are not important as reservoirs for leptospirosis.

***Rabbit syphilis*.—**Caused by *Treponema paraluis-cuniculi*, a spirochaete (bacterium). Usually causes self-limiting lesions of the external genitalia. Asymptomatic carriers are possible. To the best of my knowledge, it is reported in captive rabbits only. Diagnosis: clinical signs, skin biopsy for silver staining, skin scrapes for darkfield microscopy. Serology: rapid plasma reagin test. Treatment: antibiotics.

Tyzzer's Disease*.—Clostridium piliforme* primarily causes a disease of muskrats, but the disease can occur in other mammals, including *Sylvilagus* spp.. Transmission is by ingestion of spores from an environment contaminated by feces of infected animals. Causes a dysentary-like illness in rabbits.

***Listeriosis*.—**Caused by the bacterium, *Listeria moncytogenes*, and also know as Circling Disease or Listerellosis. The infection usually involves the brain. The disease is found in ruminants, pigs, dogs, cats, some wild animals including brush rabbits, and humans. Animals infected with Listeria can show clinical signs of abortions or nervous system disorders. This bacterium can live almost anywhere—in soil, manure piles, and grass. Healthy animals are not usually affected by *Listeria moncytogenes*.

***Q Fever*.—**Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*, a species that is distributed globally. In 1999, Q fever became a notifiable disease in the U.S. Cattle, sheep, and goats are the primary reservoirs of *C. burnetii*. Infection has been noted in a wide variety of other animals, including other breeds of livestock, rabbits, and domesticated pets. *Coxiella burnetii* does not usually cause clinical disease in these animals, although abortion in goats and sheep has been linked to *C. burnetii* infection. Organisms are excreted in milk, urine, and feces of infected animals. Most importantly, during birthing the organisms are shed in high numbers within the amniotic fluids and the placenta. The organisms are resistant to heat, drying, and many common disinfectants. These features enable the bacteria to survive for long periods in the environment. Infection of humans usually occurs by inhalation of these organisms from air that contains airborne barnyard dust contaminated by dried placental material, birth fluids, and excreta of infected herd animals. Other modes of transmission to humans, includes tick bites and human to human intimate contact. Nothing is known of the disease in brush rabbits.

Encephalitozoonosis.—Caused by *Encephalitozoon cuniculi*, a microsporidian protozoa with a predilection for brain and kidney. Spores are excreted in urine. It causes neurological disease (head tilt, hind limb paresis/paralysis, incontinence), but can be subclinical and chronic. Serology: IFA[10] and ELISA; positive titer indicates previous exposure, but does not distinguish between early infection, active infection, chronic asymptomatic infection, or previous exposure and recovery. No treatment. Of unknown significance in wild rabbits.

Sarcocystosis.—Rabbits can serve as intermediate hosts of the protozoan parasite *Sarcocystis* spp., including *S. leporum*. The parasite encysts in skeletal muscle. Cats and raccoons are the definitive hosts. Usually of minimal to no clinical significance in rabbits, noticed at necropsy.

Intestinal Coccidiosis.—*Eimeria* spp. Coccidia are the most common parasites of the rabbit gastrointestinal tract, at least in captivity. Many rabbits are subclinically infected; the presence of only a few coccidial oocysts does not rule out coccidiosis nor does it confirm the diagnosis. Clinical signs vary widely, but are most often seen in young rabbits: weight loss, mild intermittent to severe diarrhea, dehydration.

Hepatic Coccidiosis.—*Eimeria stiedae*, the only coccidium species that is known to occur outside the intestinal tract in rabbits.

Shope's fibroma.—A poxvirus of the Genus *Leporipoxvirus*. Reported in cottontails, *Sylvilagus floridanus*, in eastern and Midwestern North America. Considered an eastern American variant of myxoma virus. In older studies (i.e. from mid 1950s), *S. bachmanni* was shown to be resistant to infection. It causes localized skin lesions (fibromas) that usually are mild and self-limiting.

Rabbit coronavirus.—Discovered in 1980 to cause diarrhea in laboratory rabbits. The virus has been isolated from clinically normal adult rabbits.

Rabbit rotavirus.—Causes diarrhea in captive rabbits, primarily in commercial rabbitries but also in pet rabbits.

Herpesvirus sylvilagus.—This lymphotropic gamma herpesvirus, confined to cottontail rabbits (*Sylvilagus* spp.), is characterized by chronic infection and intermittent low-grade viremia. Transmission is presumably by direct contact, and is not thought to occur transplacentally. This virus has been reported to induce lymphoproliferative disease (lymphoid hyperplasia, lymphoma) in adults.

[10] IFA—Immunofluorescence Antibody Assay

Silverwater.—This bunyavirus-like virus is unassigned within Bunyaviridae. It is known to be transported by ticks. Information on its effects in rabbits was not found. It causes a sleeping-sickness-like disease in humans.

California encephalitis.—Since the original virus, caused by *Bunyavirus* sp., was isolated, other viruses have been isolated that are closely related to California encephalitis. This group of related viruses is now classified as the California serogroup, one of 16 serogroups within the genus *Bunyavirus,* family Bunyaviridae. Information on effects and incidence of infection in brush rabbits is unknown. Little human disease was associated with these viruses until 1960, but now California serogroup virus infections are the most commonly reported cause of arboviral encephalitis in the United States. This virus is transmitted by mosquitoes.

Western equine encephalitis.—The alphavirus, western equine encephalitis (*Alphavirus* sp.), family Togaviridae, is mosquito borne. It first was isolated in California in 1930 from the brain of a horse with encephalitis, and remains an important cause of encephalitis in horses and humans in North America, mainly in western parts of the U.S. and Canada. The enzootic[11] cycle involves passerine birds, in which the infection is not apparent, and culicine mosquitoes, principally *Culex tarsalis,* a species that is associated with irrigated agriculture and stream drainages. Other mosquito species of importance in transmission include *Aedes melanimon* in California. The virus also has been isolated from a variety of mammal species, including brush rabbits, but no information on the disease in brush rabbits was found.

Pinworms.—*Passalurus ambiguus* is the common pinworm of rabbits. It is usually nonpathogenic

Trichostrongyles.—Usually of the species *Obeliscoides cuniculi*. These worms are found in the stomach. Heavy infestations may cause morbidity

Larval tapeworms.—E.g. *Taenia pisiformis*. Rabbits serve as the intermediate host for this tapeworm, for which canids are definitive hosts. They may cause mild to moderate pathology while the larvae are migrating out of the gastrointestinal tract, through the liver, and into the abdominal cavity, where they reside and await ingestion by the definitive host. Diagnosis usually occurs at necropsy.

Tapeworms.—Several species are found in rabbits (e.g. *Mosgovoyia pectinata americana, M. perplexa, Monoecocestus americana, Ctenotaenia ctenoides*). Tapeworms are of little to no clinical significance unless infestations are severe, in which case they may obstruct the gastrointestinal tract.

Flukes.—E.g. *Hasstilesia tricolor*. These flukes usually are an incidental infection (an exception is *Fasiola hepatica,* which is a cause of trematodiasis)

[11] enzootic—constantly present in a wildlife populations, similar to endemic in humans, such as the common cold

Ear mites.—*Psoroptes cuniculi* causes inflammation and crusting of the external ear canal, leading to excessive ear scratching and head shaking in the rabbit, and can disseminate to other parts of body in debilitated rabbits. Diagnosis: clinical signs, swab and microscopy. Treatment: ivermectin

Fur mites.—Specifically, *Cheyletiella parasitovorax* usually causes only mild disease. Fur mites are treatable with ivermectin.

Gastric trichobezoars.—Hairballs are usually only a problem in captive rabbits. Accumulations of hair can partially or completely obstruct the upper gastrointestinal tract. Severely affected rabbits will be anorectic and exhibit weight loss and lethargy. Diagnosis is by physical examination, radiographs +/- contrast agents, and/or endoscopy. Treatment options include medical therapy (hydration, enzymatic digesters, lubrication of GI tract to facilitate passage), or surgery to remove the trichobezoar.

NORMAL VALUES AND DEFINITIONS FOR PHYSIOLOGIC PARAMETERS

The serum chemistry and hematology reference values used in this report represent published normal values for adult European rabbits (*Oryctolagus* cuniculus). Reference intervals were taken from Hillyer and Quesenberry (1997).

SERUM CHEMISTRIES

Abbreviation Ref range	Full name Units of measure What it measures
CPK Ref range	Creatine Phosphokinase (also called CK). iu/L (international units per liter). Increase usually specific for muscle damage. Capture myopathy consistently increases this enzyme, often dramatically.
LDH 34 – 129	Lactate Dehydrogenase, enzyme present in many cell types. iu/L international units per liter. Nonspecific cell damage. Often increased with capture myopathy. Sometimes increased with injury, disease, or cancer in the abdomen.
ALB 2.4 – 4.6	Albumin. g/dl (grams per deciliter). A prominent component of total protein, synthesized in the liver. Decreases with liver, intestinal, or chronic diseases, parasitism.

TP 5.4 – 8.3	Total Protein in Whole Blood. g/dl (grams per deciliter). Decreases with blood loss, parasitism; increases with dehydration. Albumin + Globulin = Total Protein.
BUN 13 – 29	Blood Urea Nitrogen. mg/dl (milligrams per deciliter). Indicator of kidney function, but less specific than creatinine. Increased with kidney failure, dehydration, urinary blockage. Sometimes decreased with severe liver failure.
ALK PHOS 4 – 16	Alkaline Phosphatase An enzyme present in liver, bone, and reproductive cells. iu/l (international units per liter). Increased leakage of this enzyme from liver cells into the blood may indicate liver cell damage, but not specific to the liver—can show high levels in normal rapidly-growing lambs, or bone disease. Decreased levels usually not significant.
AST 14 – 113	Serum Aspartate Aminotransferase iu/L (international units per liter). An enzyme produced by liver, muscle, heart cells. Increase may indicate liver or skeletal/heart muscle damage (ex: often increased with capture myopathy). Decrease not significant.
ALT	Serum Alanine Aminotransferase iu/L (international units per liter). 48 – 80 An enzyme produced by liver cells. Increase may indicate liver damage. Decrease not significant.
CALCIUM 5.6 – 12.5	Ca^{++} . mg/dl (milligrams per deciliter). Important for muscle (including heart) contraction, bone health. Closely linked with phosphorus level. May be abnormally high or low with bone injury, hormone imbalance, toxins, poisonous plants, etc.
PHOS 4.0 – 6.9	Phosphorus, $(PO_4)^{3-}$. mg/dl (milligrams per deciliter).

Affected by kidney function, bone growth, hormonal imbalance (parathyroid hormone). Linked metabolically with calcium.

CL
92 – 112

Chloride, CL^-.
meq/l (milliequivalents per liter).
Linked directly with sodium.

K
3.6 – 6.9

Potassium, K^+.
meq/l (milliequivalents per liter).
Affected by dehydration, hemolysis, end-stage kidney failure.

Na
131 – 155

Sodium, Na^+.
meq/l (milliequivalents per liter).
Affected by dehydration, kidney/adrenal gland function.

T. BILI
0.0 – 0.7

Total Bilirubin. DIRBILI + INBILI = TOTBILI.
mg/dl (milligrams per deciliter).
Pigment produced in liver and byproduct of red blood cell destruction. May be elevated during a severe hemolytic crisis (rupture of red blood cells).

CREAT
0.5 – 2.5

Creatinine.
mg/dl (milligrams per deciliter).
Specific indicator of kidney function. Increased with kidney failure.

GLUCOSE
75 - 155

Blood Sugar.
mg/dl (milligrams per deciliter).
Increased with stress (and diabetes in some species of animals). Decreased with sepsis (severe infection). Rapidly declines even in normal blood samples with time after collection.

GLOB
1.5 – 2.8

Globulin.
g/dl (grams per deciliter).
Component of total protein. Indicator of immune response and antibodies.

HEMATOLOGY

WBC
5.2 – 12.5

White Blood Count.
Cells X 10^3/ìl.

	Immune functions; phagocytosis (destruction of disease organisms and abnormal cells); WBC often increases during bacterial infection, stress; decreases during viral or severe infection and sepsis (blood infection).
RBC 5.1 – 7.9	Red Blood count. Cells X 10^6/ìl. Increases during dehydration; decreases during blood loss, chronic disease, hemolysis (rupture of red blood cells).
HGB 10.0 – 17.4	Hemoglobin. gm/dl = grams per deciliter. Respiratory pigment (carries O^2) within red blood cells. Decreases with iron deficiency; increases with athletic animal, or high altitude.
HCT 33 – 50	Hematocrit (also called PCV = Packed Cell Volume). % volume of red blood cells in whole blood. Decreases with blood loss, chronic disease, hemolysis (breakup of red blood cells). Increases with dehydration, athletic animal, or high altitude.
MCV 57.8 – 66.5	Mean Corpuscular (= Cell) Volume. μm^3. Species-specific red blood cell volume size; sheep (domestic and bighorn) have relatively small red blood cells. May be affected by dehydration. Calculated by (HCT/RBC).
MCH 17.1 – 23.5	Mean Corpuscular Hemoglobin. pgm (Picograms). The weight of hemoglobin in an average red blood cell. Decreases with anemia, iron-deficiency, after lambing. Increases with high altitude, athletic animal. Calculated by (HGB/RBC).
MCHC 29 – 37	Mean Corpuscular Hemoglobin Concentration. % of the red blood cell that is hemoglobin. Similar changes as MCH. Calculated by (HGB/PCV).
NEUT 20 – 75	Neutrophils. Cells/µl = ABNEUT. % of white blood count = NEUT. A first line of defense against infection. The major component of pus. Often increases with bacterial infection or stress.

LYMPH 30 – 85	Lymphocytes. Cells/µl = ABLYMPH. % of WBC = LYMPH. Immune cells, some of which produce antibodies, others produce killer substances to fight invaders (bacteria, viruses, cancer cells, etc.).
MONO 1 – 4	Monocytes. Cells/µl = ABMONO. % of WBC = MONO. Phagocytic cell (destruction of abnormal cells and invaders).
EOSIN 1 – 4	Eosinophils. Cells/µl = ABEOSIN. % of WBC = EOSIN. Indicator of response to allergen, hypersensitivity, parasites.
BASO 1 – 7	Basophils. Cells/µl = ABBASO. % of WBC = BASO. Uncommon cell; may respond to parasites, hypersensitivity.
PLAT 250 – 650	Platelets. x 10^3/µl. Indicator of ability to form a blood clot; may respond to bleeding events, sepsis, bone marrow damage.

APPENDIX B

POLICY DEALING WITH RIPARIAN BRUSH RABBITS

The following Endangered Species Recovery Program (ESRP) policy relates to the trapping and controlled propagation and translocation of riparian brush rabbits.

1. As a matter of principle, as few entries as possible to the Controlled Propagation Pen to meet our objectives should be made only for research, monitoring, and maintenance, and with as few people as possible.

   No one should enter the pens when not on official ESRP business. Only people directly authorized by the ESRP Directors should be allowed in the pens. A list of authorized people will be maintained and distributed to ESRP employees, and representatives of agencies and other entities working on the cooperative recovery effort.

   There are different categories of authorization. Some ESRP employees are authorized to enter pens as needed. Some employees of regulatory and funding agencies are authorized to enter pens when accompanied by an authorized ESRP employee. Temporary and CSUS student employees should not be allowed on their own within the pens without the permission of an authorized ESRP employee. A list of those so authorized to permit part-time and student employees to work at Pond 6 will be distributed by the Directors. The Directors should be promptly informed when changes in authorization are made by others. Whenever a student or temporary employee goes there at different periods for different purposes, that student must have approval from the responsible person. Student and temporary employees must be made aware and reminded of this policy frequently. They must not take non-authorized people with them when working at Pond 6 or elsewhere for ESRP.

2. Handling of animals must be kept to an absolute minimum to try to prevent habituation to humans and disease transmission. Handling should only be done by those authorized on our State and Federal permits, or under an authorized person's direct supervision, as provided in the permits.

3. Anyone handling rabbits is required to first wash their hands and to wear a mask (dust/vapor grade).

4. There is a sign-in/sign-out book in a waterproof housing just inside the door of the pen. **Everybody** who visits the pen, even if they are part of a group, should sign in and out. The sign-in/sign-out sheets need to be retrieved at least weekly. These sheets are treated the same as data sheets: they are copied as soon as possible and copies are distributed to Turlock and Fresno ESRP offices. The originals should be

filed in a separate binder kept with the other data files on riparian brush rabbits . If something happens at the enclosure, it may become a law enforcement matter, and CDFG/USFWS personnel will want access to this information.

5. The doors to the pens must be kept closed and latched at all times except as needed to enter/exit. Doors must be latched whenever you are in the pen and temporarily are outside the pen, and locked before you leave or are out of the immediate vicinity. Immediate vicinity means out of sight of the door for more than a few minutes. Use your judgment but err on the side of caution on what a few minutes means. In other words, don't take chances. Remember, the area is open to visitors and anglers, and also animals can move quickly.

6. No pets are allowed in the pens unless they are part of a research procedure approved by the Principal Investigators and the permit-issuing agencies.

7. Do not give out the combination for the locks (all of them at Pond 6) to anyone who is not authorized to enter the pen or to anyone outside ESRP without a Director's explicit approval. This includes CDFG wardens, unit biologists, and others. An ESRP employee will be designated to change the combination on the pen gate every month.

8. Everyone entering the pen must step through a 10% bleach solution placed at the door. This applies to EVERY time they enter. Bleach must be mixed fresh each day and must be disposed of in an approved, safe manner.

9. Traps used at Pond 6 must not be used for any other trapping. They must be rinsed in 10% bleach solution before moving into the pen, each time they are removed and then replaced or moved from pen to pen. If sufficient traps are available, they should be locked open and left in place in the pens. Vegetation and debris should be kept clear around the traps so that they function properly.

10. Bait for trapping should be made fresh daily and old bait discarded. Old bait stuck to traps should be washed off between trapping sessions.

11. No rabbits are to be removed from the pens for any purpose without permission from a Director or Principal Investigator; others can be designated to act in emergencies where a rabbit has a life-threatening injury or illness.

12. Cages used to confine and transport rabbits must be cleaned and disinfected with 10% bleach solution, rinsed in clean water, and dried before being re-used. Each rabbit must be placed in a separate, cleaned cage unless they are litter mates or mother-young groups. Cages can be disinfected ahead of time and stored in a clean, sealed, plastic bag. Bedding used in transport cages must be new or disinfected prior to using and discarded or disinfected after each use. Disinfected cages should be kept on hand at the Pond for emergency use.

13. Trimming, cutting, pruning, and other maintenance equipment must not be used anywhere else but at Pond 6. This includes shovels and rakes. If they have been used elsewhere, they should be rinsed in the bleach solution before reusing.

14. Do not advertise the location of the pen and do not tell unauthorized people that they can look at the outsides of the pens. Do not tell them how to get to the pens. Of course, if someone accompanies you to the site for security or other legitimate reasons, they obviously can be invited to look from the outside, but as few people as possible should know about the location.

15. If approached by the press or media representatives about the controlled propagation and translocation of brush rabbits or other ESRP activities, refer them to the Directors of ESRP. Do not provide comments or information to the press without authorization from the Directors. This is standard ESRP policy but it need to be repeated here. It also bears repeating that in your capacity as ESRP employees in your dealings with others do not make comments about governmental policy or regulation. When pressed for opinion or information that involves policy or regulation make it clear that we are researchers under contract and that we have no regulatory authority and have no comment.

APPENDIX C

REVIEWER COMMENTS AND SUGGESTIONS FOR THE RIPARIAN BRUSH RABBIT CONTROLLED PROPAGATION PLAN

Reviewers' comments were considered in revising the text of the draft plan. Substantive comments are summarized in the following section along with our remarks (in italics) where explanation is needed. When the draft was circulated for peer review it did not include the Health Plan. Several of the reviewers' comments concerned health issues that are addressed with the inclusion of the Health Plan.

I. Proposal Omissions, Areas in Need of Clarification, and Strengths

A. Omissions

1. Develop a hypothesis that deals with the identification and rectification of the causes of declines and endangerment of riparian brush rabbits.

   See new sections on Assumptions and Hypotheses, p. 3, and Recovery Objectives, p. 8.

2. Proposal strong on monitoring, but weak on design.

   We agree that the draft was weak on design. We consider all iterations of this plan as part of a living document within an adaptive framework. We drafted it partly on a programmatic level. There is more detail in the revision, but we do not believe that a structured, step-by-step approach is practical because of many unforeseen circumstances that probably will arise. Further, until preliminary steps are taken it often is not clear what processes will need more refinement or testing. Many actions will be planned and reviewed in detail as this process evolves.

3. Rigorous experimentation applied stepwise to produce scientifically defensible results.

   This is an admirable goal for any recovery program, but it is difficult to translate such a statement into actions in many situations. Step-wise experimentation is not the only way to produce scientifically defensible results. We fully intend to produce scientifically defensible results however they are achieved. Our goal is to re-establish and recover riparian brush rabbits within con-

strained circumstances. Those circumstances include limited funding, limited habitat for experimentation, limited time to act to prevent extinction, and limited ability to experiment with endangered species or their surrogates. We believe the course we have outlined in this plan for propagation and reintroduction of riparian brush rabbits can be accomplished within those constraints and be scientifically defensible. Some controlled experiments and other step-by-step actions and analyses are being conducted and others will be performed as the needs arise.

4. Refine long-term success criteria for riparian brush rabbits.

   Success criteria were given in the recovery plan for this species, and are clear at the strategic level. They are stated in fairly general terms in this document because of lack of data needed for realistic modeling of population trends under different scenarios. Our monitoring of the wild and captive populations, plans for monitoring releases, and parallel studies on surrogates of riparian brush rabbits are intended to provide the kinds of observations that will allow us to better define the demographic aspects of the re-established populations. We envision several iterations of increasingly accurate modeling as data become available through this research and monitoring. It also is important to recognize that modeling, even under the best of circumstances, typically is fraught with untested and unrealistic assumptions. See page 8, Recovery Objectives, for more information on the criteria in use.

B. Clarification of Riparian Brush Rabbits Release

1. When and how will riparian brush rabbits be released?

   This was covered in the draft plan in the section on Reintroduction, starting on p. 28 (of the revised version).

2. How many acres is the area of release?

   In the area targeted for the first release, there are approximately 700 acres of land with natural riparian and Valley Oak forest vegetation on the western side of the main channel of the San Joaquin River, and over a thousand acres of formerly farmed land under restoration. In the immediate release site and flood refugium, there are about 7.5 acres of high-quality habitat dominated by shrubs and vines. That site is connected to other such patches of high-quality habitat by lower-quality habitat and seasonal habitat (herbaceous patches), making a few hundred acres easily accessible to brush rabbits, depending on how readily and how far they disperse.

3. How many acres are needed for a self-sustaining population of riparian brush rabbits?
   We do not know. Self-sustainability depends upon many unpredictable factors, both natural environmental and human related. The time-frame of estimated sustainability and number of discrete populations are as important in sustainability as the number of individuals or acres of habitat. Using estimates based on conservation biology principles as a first approximation, we have defined what we believe is the minimum needed to achieve a self-sustaining population level: four or more distinct populations (including the expanded Caswell MSP population), each living on about 300 to several thousand acres of connected habitat of varying quality. Each population would consist of average numbers of adults from about 300 to several thousand. Occasional intervention such as translocation may be needed in the smaller populations, such as Caswell MSP, if only the minimal population size is achieved.

C. Strengths

1. Minimization of potential problems is the strength of the plan.

2. Justification to bring animals into captivity is well developed.

II. Health and behavioral concerns

A. Health/Medical

1. Diseases and ectoparasites

a. Sylvatic plague.

   Plague was recognized as a disease of concern for brush rabbits on page 13 and is discussed in Appendix A, p. 54.

b. Bot and flesh maggots due to collars and nesting boxes.

   We have seen bot fly infestations on only one riparian brush rabbit out of a few hundred in the wild that have been handled and none on desert cottontails. These parasites do not seem to be a problem for rabbits in the area where riparian brush rabbits live. Yet we will be vigilant to the possibility that confined and radiocollared rabbits are more susceptible to blot fly parasitism and look for ways to prevent infestation.

c. Administer ectoparasite pesticide on one or two rabbits before all.

This is good advice and standard practice for us when using something new on an animal. During the time this plan was in review, we tested one such pesticide (Frontline Topspot™) on domesticated European rabbits (Dutch Dwarf breed) with fatal results. Currently we have no plans to use pesticides on rabbits. However, we will continue to seek a safe and efficacious, long-acting flea and tick pesticide because we believe it will lower the transmission rates and risks of several diseases that are known to be or might be transmitted by fleas or ticks.

d. Captive populations at increased risk of disease.

Anytime individuals not previously in contact are brought together increases the risk of disease among them. Captive populations may or may not have further increase in risk of disease depending on the circumstances of captivity. We agree, however, that in this proposed program, without predation operating and with no ability of confined rabbits to disperse, that the pens will attain densities and absolute numbers higher than are likely to occur naturally, and diseased individuals are likely to be in closer contact with their conspecifics for longer periods—these are factors that are known to increase risks of disease. The precautions we have outlined in the operation of the controlled propagation pens and the health plan for individuals should help reduce the risks. Diseases are endemic in wild brush rabbits, but at unknown levels. Disease screening will be used to evaluate the levels in both wild and captive populations. We do not believe that raising rabbits individually in a disease-free environment (or as close as one can achieve without extraordinary efforts) is less of a risk to the ultimate ability of the rabbits to survive in the wild; if anything it presents the greater risks (see p. 41, Rearing Rabbits in Individual Enclosures).

2. Nutrition: provisions for water and food sources

From our observations and experience, brush rabbits live well with only green vegetation as a source of free water. There is green vegetation in the pens at Pond 6 year round. We also believe that the vegetation in each pen includes an abundance of high-quality food plants. We do not plan to provide supplemental food or water unless the animals show indications of under nourishment or malnutrition or the vegetation shows evidence of more than light grazing and browsing.

For animals temporarily confined in smaller pens, adequate sources of food and water will be provided ad libitum*. This will include water, green growing vegetation (e.g., turf grass), freshly gathered grasses and forbs such as alfalfa and clover or other legumes, and dried foods containing whole grains and* *other foods known to be important for nutrition of rabbits in general. All food and water will be free of pesticides and contamination by bacteria and fungi.*

3. Handling and testing

   A. Taking blood samples may be difficult and stressful.

      We agree. The health plan for this program outlines (p. 49) how and with what animals will be sedated for taking blood and other difficult and stressful procedures. Parmenter et al. (1998) found no difference in re-capture rates or mortality from marking, anesthetizing, and taking blood samples from Sylvilagus floridanus, *but found higher mortality and lower trappability for* S. audubonii. *Thus, it is difficult to predict if there will be measurable harm to riparian brush rabbits from such procedures.*

   B. Define blood-testing methodology (e.g. what will be tested for?, who will do the testing?, will the testing be fiscally possible?, how will the results be analyzed?).

      See Appendix A, p.49. Diseases must be recognized as a natural part of the riparian brush rabbit's environment. The principles we will follow are to identify diseases that are present in source and target populations of rabbits (including desert cottontails and black-tailed hares), and not translocate diseased stock to an area where lagomorph populations do not host that disease. Naturally, testing for every potential disease for which these rabbits are or might be at risk is not fiscally possible given the limited funding available. If and when riparian brush rabbits are found to be diseased or dead, we will attempt to determine the causative agents and do more extensive, targeted disease screening. Symptoms and necropsy results will help narrow the number of tests. We also will sacrifice wild caught desert cottontails and black-tailed hares from areas where we plan to translocate riparian brush rabbits for extensive necropsies and disease screening. In these ways we will build a list of diseases for which to screen all or parts of populations. Screening will be limited to diseases that are known to be life-threatening or potentially so, and that exist in the source population (for translocation) but not in the lagomorph populations at the translocation site.

   C. Minimize handling to reduce chance of physical harm.

      We agree and have tried to strike a balance between the need for information that only can be gained by handling and the risk of harm. We consider the amount of handling proposed (i.e., trapping check every 2 weeks during the breeding season, less frequently at other times; an initial health check when brought into captivity and another before translocation) to be *minimal. We will gain only a general picture of reproductive activity of individuals, and some individuals may not be trapped in some sessions and therefore we will have no record of their reproductive state or growth and development at potentially critical times.*

D. Develop protocols for anesthesia.

See p. 49, live Rabbit Health Checks.

E. Need to describe trapping, holding, restraining, and transporting methods.

Transport methods are described on p. 23, and trapping and holding are described on p. 25. For routine examination and tagging, rabbits are restrained by hand. For most activities they are retained in the cloth bags with their eyes covered but their ears and part of their body exposed. Covering the eyes greatly reduces the amount of their struggling and the restraint required.

4. Health-related omissions

a. Proposal does not address provisions for rapid wild and captive population collapse.

Without knowing what causative factors are operating, it is difficult and probably impractical to address such a collapse. As part of the recovery activities for this species, but not included or discussed in this plan, we have developed or are developing emergency response plans to deal with wildfire and flood. Protocols for communicating emergencies and the rescue of rabbits, rescue supplies, and places for temporarily housing rabbits are in place. Assuming, however, that the rapid population collapse is caused by some pathogen, we see little that can be done except to make plans for isolating sick individuals unless the pathogen is first identified. There may be no time for isolating and identifying a pathogen during a rapid population decline. A few sick individuals can be accommodated at the U.C. Davis Veterinary Medical Teaching Hospital, and we have the use of and have refurbished three outdoor pens at U.C. Davis that each can contain a few more rabbits.

Health screening will begin to identify diseases in riparian brush rabbits and help in anticipating and preparing for potential problems (see discussion on disease screening). We know that tularemia has been linked to population declines in cottontails but this disease is endemic in S. bachmani *and we doubt that it can be effectively controlled. Further, it often is not lethal in cottontails. It is not economically feasible or logistically possible to immunize wild rabbits from such a potential disaster or keep a* *large stock of vaccines or antibodies for a possible epidemic without more information. Having animals at more than one isolated location, practicing good hygiene, pursuing additional knowledge about the causes of such collapses, and taking additional steps to avoid or lessen the scope of epidemics are the ways we currently plan to deal with this issue.*

b. Parallel captive breeding facilities would provide important information (e.g. life span in captivity, knowledge of mating behaviors) while increasing resources (e.g. suitable sites for confining breeding colonies, genetic reservoir for subspecies).

We agree and some of the information that can be gained is badly needed. However, funding and scarcity of riparian brush rabbits are major obstacles to the implementation of such a program. Some aspects of mating "behavior" can be learned from genetic analyses. If the facility is designed and operated using riparian brush rabbits, the offspring would be subject to all the potential problems that arise from breeding in individual enclosures and might not be suitable for release in the wild. Instead, we plan to maintain a few individuals of a related subspecies of brush rabbits in small pens to obtain some information that can be used to refine modeling of demographics of riparian brush rabbits, and are seeking funding for this endeavor. We also might use some individual riparian brush rabbits that are not suitable for translocation or release back into their source population in the future, if such rabbits and additional funding become available.

c. Define "alternatives (projects) that require more intervention" in its own section.

We added a section to summarize this alternative on p. 41, Rearing Rabbits in Individual Enclosures.

B. Behavioral

1. Mating/sex ratio of 1:1 does not consider male dominance issues.

We believe it does. The pens are of sufficient size to contain average home ranges of about six individuals (Chapman 1971). Having three males in a pen makes it possible for the three females to choose with whom they mate and gives the males opportunity to interact and form a dominance hierarchy. More importantly, it provides a potentially larger gene pool if one male cannot dominate all breeding.

2. Ferrets and polecats have exhibited heritable changes due to cultural and developmental factors caused by missing or inappropriate stimuli while in captivity.

   This is a risk anytime animals are bred in captivity. We have attempted to minimize or eliminate such changes by 1) not breeding successive generations of brush rabbits in captivity; and 2) confining them in large pens supporting natural habitat where they are exposed to but protected from predators. Clearly some stimuli are missing, though raptors perch in the trees overhanging the pens and fly overhead, and coyotes and gray foxes have been seen around the pens. We plan to develop some predator-like stimuli that can be used in the pens but not harm the rabbits. We cannot anticipate what other stimuli may be missing but we do not think that the conditions in which the rabbits will be raised differ greatly from those of wild-reared rabbits.

III. Structure and Equipment Concerns

A. Propagation Pens

1. Pen design

   a. Perimeter fence would decrease possibility of rabbit escape.

      We agree but the funding is not available now to add a second fence. Firebreaks around pens will discourage rabbits from leaving the cover in the pens under normal circumstances.

   b. Right angles in pens may lead to rabbits banging their heads (both in captive breeding and temporary pens).

      This is a good point. We will place internal "curves" of hardware cloth in the corners of the pens.

   c. Kudos on proposing large enclosures.

2. Pen placement

   a. Construction of pens at or near reintroduction site would be best.

      Yes, but there were no suitable places for pens near the reintroduction site on the San Joaquin River National Wildlife Refuge. See p. 18, on Location and Design.

   b. Are there efforts to control vandalism?

Yes, without going into all details, surveillance equipment is being investigated and other efforts are being made to control vandalism. The best course would be to have someone living on site year round, but providing power, water, and sewage disposal to this rural site and hiring someone to live there present several issues, including costs and permits, that have not yet been resolved.

B. Other equipment

1. Radio collars and ear tags

a. Radio collars can be dangerous (e.g. choking, snagging).

We have experienced problems with radio-collaring brush rabbits and have tried different collar designs in an attempt to find the least dangerous. We also have considered implanting radio transmitters subcutaneously and interperitoneally. We believe the risks from surgery are much greater than the risks from neck collars. Having radio collars on the confined rabbits is not absolutely necessary, but the information we would not have, such as that gained by being able to quickly identify and retrieve dead animals, is vital to the program. We also believe translocated animals must be fitted with radio transmitters to learn their fate and to measure success of translocation.

b. Consider using AVID transponders, they worked well for similar projects.

See p. 23. We have been using Destron™ passive integrated transponders (PIT tags) on brush rabbits since 1993, and on other animals since 1989 (Williams et al. 1997). AVID™ transponders are another manufacturers' brand of passive integrated transponders. We prefer Destron equipment because of the ability to program scanners to indicate previously captured animals (in same trapping session) and to store numbers in different electronic files.

c. Select ear tags carefully because some ear tags catch and tear out.

We have been using U.S. Brand and Tag Company's fingerling-style monel ear tags on brush rabbits for over 20 years. How a tag is attached is also an important factor in whether or not it is torn from the ear or is lost because of infection and tissue necrosis. Although we have not had an incident of a fingerling-style tag being torn from a brush rabbit's ear, it seems inevitable that it will happen. That is why we tag all individuals with both ear and PIT tags. PIT tags also sometimes fail (Williams et al. 1997).

2. Nesting boxes and bedding

   a. Use of non-natural bedding contradicts efforts to not imprint rabbits to non-natural settings.

      This is a good point and we will use dry grasses and leaves as bedding instead of wood shavings.

   b. Will artificial nests be on top of the ground or partially buried (rabbits often dig burrows)?

      Nest boxes will be placed on top of the ground. A very high water table precludes burying or partially burying nests unless they are placed on elevated mounds of soil. We have found no evidence that riparian brush rabbits burrow or use other animals' burrows, unlike some other brush rabbit subspecies (Chapman 1974) and some other cottontail species (Chapman 1975, Chapman and Wilner 1978, Chapman et al. 1980). Never-the-less, mounding earth over pipes or nest structures will provide thermal cover and will be done where conditions permit.

3. Computer software: consider using ISIS software for data management.

   We have considered it and it does not appear to fit our current needs as well as a general relational database management system program.