CONTROLLED PROPAGATION AND TRANSLOCATION OF RIPARIAN BRUSH RABBITS: ANNUAL REPORT FOR 2002

Report To:

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ABSTRACT

We report on activities related to the controlled propagation and release of captive-born, riparian brush rabbits (*Sylvilagus bachmani riparius*) to the wild during federal Fiscal Year 2002. Three rabbits of each sex were trapped in the wild in the South Delta, San Joaquin County, California, and moved in November 2001 to a 1.24-acre (0.5 ha) pen that contained natural vegetation. The pen is located west of Lodi, San Joaquin County. Subsequent monitoring of the confined population provided information on reproduction, survivorship, growth, and health. The first two offspring were captured on 22-23 February 2002 and were estimated to be 18 and 23 days old. Offspring about 90 days old or greater exhibited evidence of reproduction, including 12 of the young females. We estimated conservatively that there were 22 pregnancies by 15 females producing 64 young that lived long enough to be trapped and marked, probably 14 days or more from birth. Mean number of young per pregnancy surviving to this milestone was 2.9. Mean weight at first capture was 228.5 g and mean estimated age at first capture was 29 days. Mean increase in mass approximately after weaning (14 days old, 110 g) was 8 g/day. Geneotyping using six polymorphic microsatellites (two others were non-variable in this population) demonstrated that all six rabbits contributed to the first cohorts consisting of 15 young. In July, three animals born in the wild and used as brood stock were returned to their original capture locality. One was killed about a month later, probably by a predator. The other two survived into February 2003 when monitoring ceased. Three young released at the capture sites of adult breeders that died in captivity did not survive long enough to breed. Forty-nine captive-born rabbits were released at the San Joaquin River National Wildlife Refuge between July and October 2002. Between 31 July 2002 and 17 February 2003, 18 of the translocated rabbits died—mostly of predation, though two died of radio-collar accidents. Translocated rabbits were confined to a soft-release pen at the Refuge for periods ranging from 2 to 20 days before being liberated. Thirty-three individuals confined for 7 or more days had a 24% mortality rate while those confined for < 7 days had a 50% mortality rate, though the difference was not significant at the 5% probability level. Overall survival of translocated rabbits through 17 February 2003 was 63%. Health exams, necropsy of dead rabbits, and tests for diseases showed that the riparian brush rabbit population in the South Delta, including rabbits in confinement at the controlled propagation facility, was robust and healthy. Rabbits did well in captivity, gaining weight and reproducing sooner and at a rate greater than we have witnessed in the wild. We also censused the wild population of riparian brush rabbits and woodrats (*Neotoma fuscipes riparia*) at Caswell Memorial State Park during January 2002. Traps were operated sequentially for 8 days in each of three sections of the Park. Trapping followed protocols established in earlier years. Sixteen riparian brush rabbits and 31 riparian woodrats were captured in 2002, values greater than any year since 1993, but insufficient to reliably estimate population sizes when partitioned among separate census areas. Capture rates also were low compared to 1993 but higher than all other years since 1993. This report also includes an overview of activities for coordinating emergency responses to natural catastrophes at the sites where rabbits are confined (Pond 6 and the soft-release enclosure on the Refuge) and guidelines for managing vegetation at the controlled propagation facility.

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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.), blackberries (*Rubus* 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 the South Delta area of the San Joaquin River, including Paradise Cut and Tom Paine Slough (Williams and Basey 1986, Williams and Hamilton 2002, 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 streambed 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, mostly only a few meters between developed ground or developed ground and water. 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 and Kilburn 1984, Williams 1993, ESRP unpubl. data).

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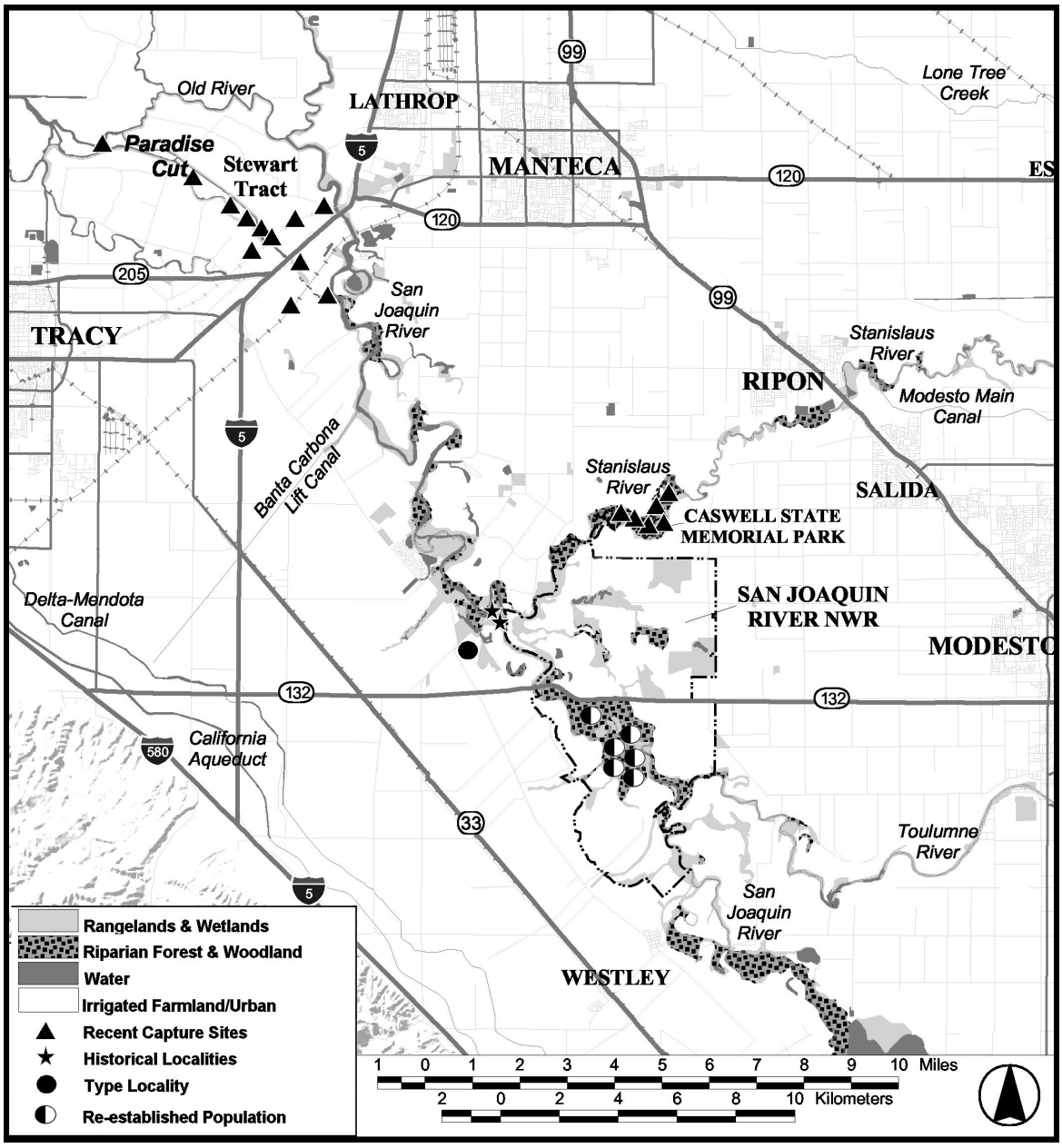

Figure 1. *Distribution map for the riparian brush rabbit based on data current through December 2002.*

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, and predation exacerbated by high numbers of feral cats (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.

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 the 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). The Caswell MSP population is too small and nonproductive to serve as a source of wild-born rabbits for translocation. For these reasons, breeding in confinement to provide a source of animals for reintroductions is called for in the Recovery Plan. To that end, efforts to initiate a controlled propagation program were undertaken in 1999. Subsequently, the U.S. Fish and Wildlife Service, California Department of Fish and Game, and U.S. Bureau of Reclamation decided not to pursue studies of controlled propagation on a surrogate subspecies of brush rabbits, but rather to take advantage of a newly discovered population of riparian brush rabbits in the South Delta.

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, the necessity for offspring to learn about habitat, food, and predator avoidance, and to become acclimated to weather at the translocation site, the Riparian Brush Rabbit Recovery Working Group, decided not to confine and breed rabbits in small cages. Instead, animals were placed in fenced enclosures larger than their typical home ranges (0.33 ha, Dixon et al. 1981) and populated with natural vegetation that provided suitable habitat (Williams and Basey 1986, Williams and Hamilton 2002).

This document reports results of efforts directed to recovering the riparian brush rabbit during the federal fiscal year 2002. It includes descriptions of the location and structure of the controlled propagation facility; activities directed at populating and operating the facility; results of observations on the confined population; activities involving the translocation and release of captive-bred animals to the wild; preliminary results of monitoring the newly established population; and other, related activities.

PART 1:
CONTROLLED PROPAGATION AND TRANSLOCATION OF RIPARIAN BRUSH RABBITS

The principal objectives for holding and breeding riparian brush rabbits in confinement are to conserve a portion of the South Delta population at risk of extinction; produce offspring that will be reintroduced to restored, historical habitat; maintain confined populations until new populations are established in restored habitat; and produce individuals to supplement and invigorate the extant population at Caswell MSP.

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 criteria considered when assessing potential

sites were no flood risk; low probability of vandalism; accessibility in the wet season and directly after or during rainfall; minimum of 3 to 5 acres in size; prime habitat not absolutely necessary but appropriate cover must be present; have climate essentially the same as planned reintroduction sites; be within about 1-hour driving time from Turlock, CA, where staff are based; and hunting prohibited.

The Department of Water Resources' property known as Pond 6, in San Joaquin County, was the only 1 of 50 examined that met all 8 criteria, 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 2). The other half of the property is primarily wetland, natural upland, and open water, with a narrow fringe of riparian plants. 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 can 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.

Construction and Preparation of the Propagation Enclosure

Initially, we planned for the construction of three captive breeding enclosures on the east side of the property during summer 2001. However, only one enclosure was completed and ready for use in October 2001, which limited controlled propagation to one enclosure during the first year, reported here. The remaining enclosures were built in summer 2002.

The first enclosure is 1.24 acres (0.5 ha) in size. It is fenced with hardware cloth, with a 2-ft band of sheet metal flashing at the top. The sides stand 7 feet high). The interior is covered with netting, which is supported by cables strung from the long sides of the enclosures (Figure 3). The netting allows song birds and quail to enter and exit, but not without some difficulty. Raptors, however, are excluded.

Prior to the introduction of brush rabbits, we conducted periodic trapping totaling 22 days to remove animals that could adversely affect the captive breeding population (e.g. snakes, foxes, and weasels, other rabbit species, rodent species). We used both extra long, large, folding Sherman™ traps and double-door, wire-mesh Tomahawk™ traps (Model 203; 61cm long × 15.2cm high and wide). Half were baited with canned cat food and half with a mix consisting of apples, oats, walnuts, and molasses. We also placed nine 1 x 1-m track plates and three automated camera stations and baited them in separate areas with cat food and the mix.

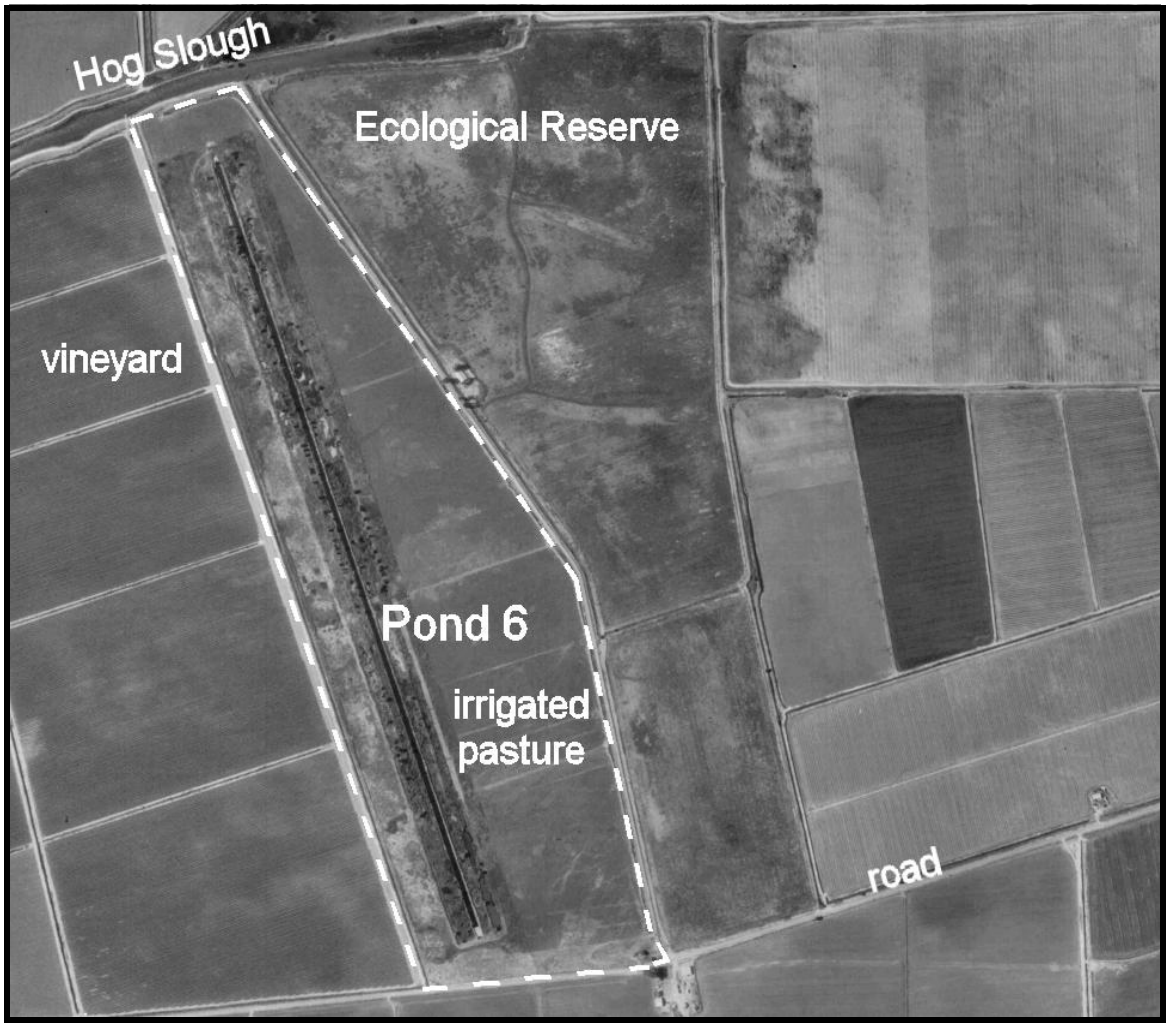

Figure 2. *Satellite image showing the configuration of Pond 6 and surrounding ground. The property is bounded by a dashed line. The pond is the dark-colored, narrow, linear structure along the left side of the property. The pond is bordered on the east (right) by a narrow (about 150 feet) band of natural vegetation including wetland and upland components. The pens were constructed in the upland area closest to the irrigated pasture.*

Additionally, we placed cover boards (3-ft pieces of corrugated, galvanized sheet-metal roofing) on the ground as shelter for snakes and other animals. Traps were checked twice a day and captives were released outside the enclosure. Track plates, cameras, and cover boards were checked daily when traps were set and checked. Animals under cover boards were captured by hand. Tracks were identified and film was replaced and developed.

Over the 22 days of trapping, we removed 7 desert cottontails (*Sylvilagus audubonii*), 73 California voles (*Microtus californicus*), 30 house mice (*Mus musculus*), 1 black rat (*Rattus rattus*), 1 California ground squirrel (*Spermophilus beecheyi*), and 15 birds. Two

Figure 3. *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 7 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).*

small (about 1 foot in length) gopher snakes (*Pituophis melanoleucus*) were captured under cover boards during these trapping efforts. Because we did not continue to capture predatory animals, we believed the enclosure was safe for the introduction of rabbits. However, similar efforts continued throughout the year to assure that the risk of predation on the captive population remained low, and to keep the vole population in check.

We constructed six artificial nests with 8-inch PVC pipe (Figure 4), and placed them into openings carved into the blackberry thickets. We used gasoline-powered hedge trimmers to carve openings for nests and tunnels of pipe. Various lengths (approximately 2-3 ft) of 6 and 8-inch PVC pipe were placed along the edge of the blackberry bushes to serve as the initial runways in and out of the thickets. In addition, various lengths and widths of concrete and terra cotta pipe were placed throughout the enclosure to provide refugia from climatic elements. Piles of concrete pipe and rubble were placed in cleared areas near the fence on the short-sides of the pen to serve as refugia in case the vegetation in the pens caught fire.

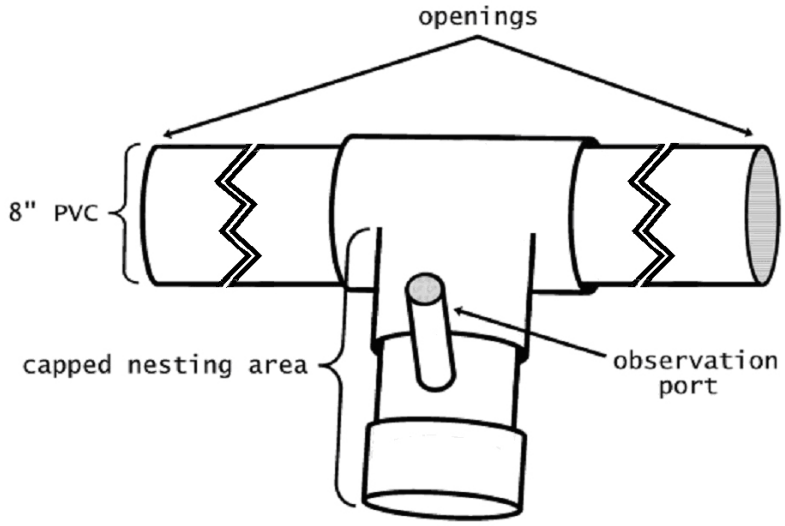

Figure 4. *Top view of an artificial nest structure for captive brush rabbits. Nests were made with 8-inch diameter PVC pipe with a T-fitting; one arm is fitted with a cap, the others were open. Open arms are not shown to scale—each was approximately 3 feet long.*

Introduction of Rabbits to the Propagation Pen

In November and December 2001, traps were set at 10 sites along Paradise Cut and Tom Paine Slough in an effort to capture rabbits for relocation to the controlled propagation enclosure (Figure 5). Our objective was to capture six individuals (three males and three females) from six separate areas to allow for low kinship values and, presumably, maximum genetic heterozogosity, thereby reducing the effects of inbreeding depression.

We searched closely in potential habitat for sign of rabbits (fecal pellets, runways, fur, clipped sedges). Where sign was found at sites separated by 1 or more kilometers, we set Tomahawk™ traps. We set a total of 107 traps. Traps were the double-door design and were placed directly in runways or paths in dense vegetation. Traps were baited with a combination of walnut meats, rolled oats, molasses, and sliced apple. Traps were set in the afternoon or early evening, checked about 2 hours after dark and again in the early morning. Traps were left open around the clock unless weather conditions threatened the health of the rabbits. Captured brush rabbits were permanently marked with metal ear tags and PIT tags, weighed, and measured. A 1-2 mm diameter plug of ear tissue was taken with a biopsy punch from brush rabbits and preserved in 95% ethanol (reagent grade). Animals that were not selected for propagation were released at the site of capture.

Twelve riparian brush rabbits were captured during three days of trapping (Table 1). Traps were open only for about 2-3 hours on day 1 because of an unexpected storm. Days 2 and 3 represent one episode of overnight trapping. Thus, though rabbits were captured on three different dates, the total trapping effort was little more than one "trap night." Nine of these rabbits were new captures, and three were previously caught and

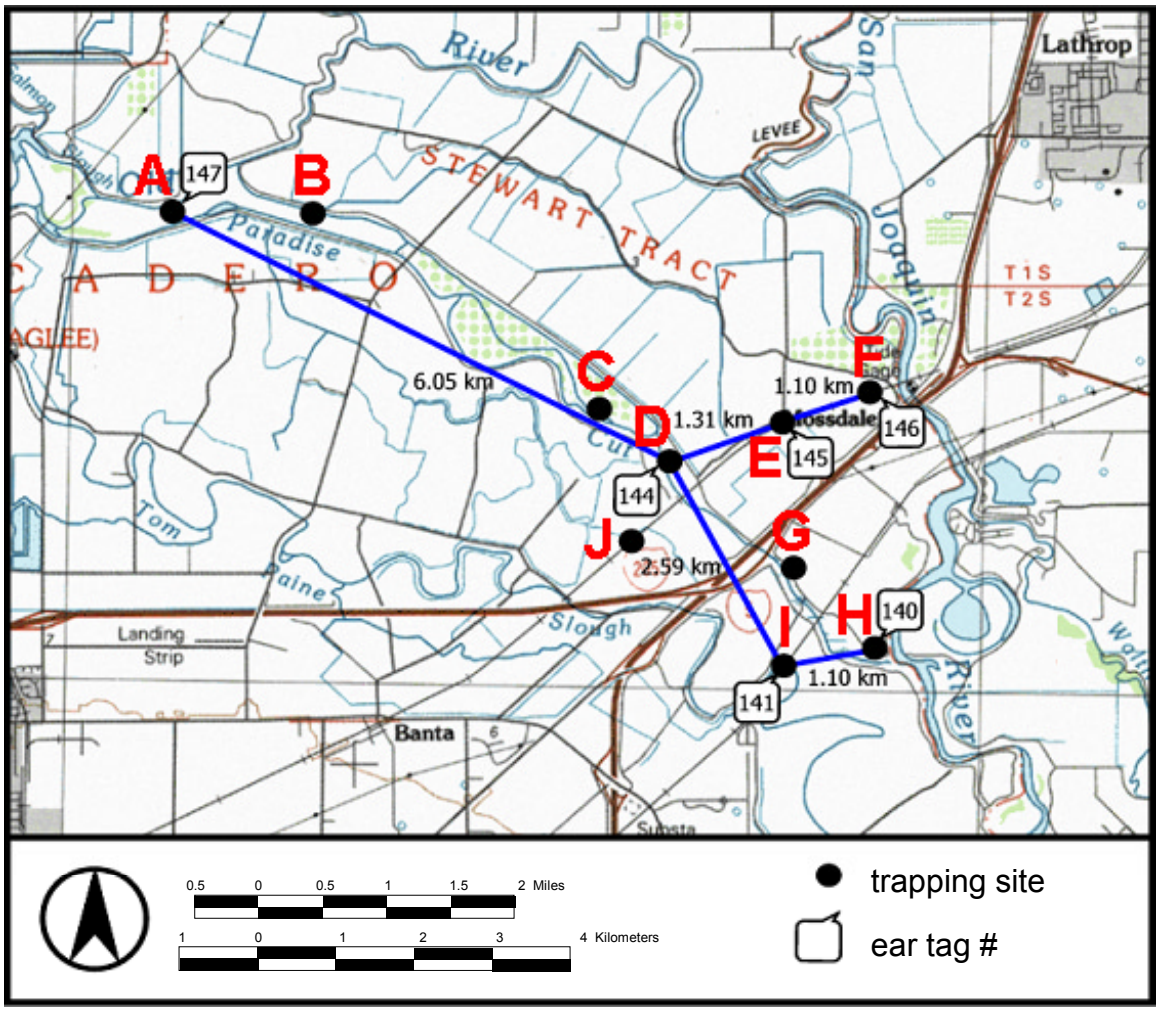

Figure 5. *Map of South Delta depicting the distribution of habitat where we trapped for riparian brush rabbits (sites labeled A-J) for relocation to the propagation enclosure. Locations of rabbit selected for controlled propagation, identified by ear-tag number, also are shown. Values (km) on lines between capture sites indicate straight-line distances between animals selected for controlled propagation. Unconnected sites are where rabbits were either not captured or not used in the propagation program.*

marked during a brush rabbit survey in August 2001 (Williams and Hamilton 2002). The six rabbits selected for the propagation enclosure were captured from distinct clumps of vegetation (Figure 5). These rabbits were processed according to standard protocol, and fitted with Holohil™ radio-collars so they could be monitored for movement and mortality inside the enclosure.

Health screening was delayed because of unavailability of the consulting veterinarians. Thus, the rabbits were transported to the enclosure where they were placed inside individual artificial nests, which were then capped and set into openings in the tangle of blackberry canes (Figure 6). About 3-4 hours later, the nest caps were removed and the rabbits were allowed to move freely throughout the enclosure.

Figure 6. *Photograph of artificial nest in a cave carved in a Himalayan blackberry thicket (photo by D.F. Williams).*

Table 1. *Sites trapped[1], number of traps used, number of riparian brush rabbits captured to relocate to the propagation enclosure in 2001, and locations of selected rabbits.*

Location Trapped and Tag # of Selected Rabbit[1]	Sex of Relocated Rabbit	Days of Trapping at Site	# of Traps	Males	Females	Total
A – 147	female	3	16	0	1	1
B		2	10	0	0	0
C		3	10	0	0	0
D – 144	female	3	15	4	1	5
E – 145	male	3	10	2	0	2
F – 146	male	3	10	1	0	1
G		1	10	0	0	0
H – 140	male	1	16	1	0	1
I – 141	female	1	10	0	2	2
J		3	10	0	0	0

[1] Locations shown in Figure 5.

Approximately two times each week, the rabbits were monitored for location and mortality by radio telemetry. Within the first 2 months of captivity, two of the rabbits had shed their radio-collars.

Health-Screening of Brood-Stock Rabbits

The purpose of incorporating health screening into the riparian brush rabbit reintroduction program was 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 was to ensure the greatest chance for successful reintroduction by maintaining and releasing healthy rabbits.

In February 2002, we trapped within the propagation pen in an effort to replace a lost radio-collar, as well as collect blood and urine samples for health screening. This effort resulted the capture of the six original founders, as well as two young riparian brush rabbits. The young rabbits appeared to be about 3-4 weeks old and weighed 140 and 179 g, about 25 to 33% of average adult, non-reproductive weight.

Health exams were performed on the six adult riparian brush rabbits between February 21 and March 3, 2002. After the exams, all were released back into the controlled propagation pen. All three males had scrotal testes, two of the three females were pregnant, and the third female had an inconclusive progesterone test for pregnancy.

Mal-positioning of radio collars was the most significant health problem in this group of rabbits, with three out of six rabbits exhibiting mal-positioning—abrasions on the neck, under the collar, in two and mortality in one. The rabbit that died had advanced lymphoma.

More accurate assessment of laboratory blood-work would benefit from more complete published reference ranges for *Sylvilagus* sp. The incidental finding of lymphoma in one rabbit, suspected to be caused by a herpesvirus-like pathogen (Hesselton et al 1988), underscored the importance of thorough necropsy examinations of all carcasses, and also indicated that a serosurvey of the source and captive populations of *S. bachmani riparius*, as well as of sympatric *Sylvilagus* sp. at the release site, is warranted. At that point, without more information on the presence of *Herpesvirus sylvilagus* in the population, and without further evidence that this virus was having a clinical impact on individuals, it was not possible to assess the level to which this viral pathogen, if present in the population, represented a risk to the reintroduction program.

Radio Collars

The six founders were fitted first with radio transmitters with wire and Tygon tubing collars manufactured by Holohil (Ontario, Canada), model RI-2D. These collars weighed approximately 7.5-8 grams, with a battery life of 12 months. When we noted the mal-fitting radio-collars on three of the six rabbits during the health screening in February, we reattached collars to improve fit. On March 1, 2002, we discovered that founder rabbit #147 died when her radio collar became wedged in her oral cavity. Subsequently, we

captured and removed collars from the remaining founders while confined in the pen. We decided that the basic design for attachment of the Holohil collar was not suited for brush rabbits, whose necks are nearly the same diameter as their heads, and who can sometimes manipulate their short front legs under snug-fitting collars. The wire crimps that secure the ends of the wire sometimes slipped when force was applied by the rabbits forefeet. This may have happened because of improper crimping, but we did not want to risk further harm to rabbits by trying to perfect this method of attaching transmitters.

After considering factors such as weight, battery life, and the method of collar attachment, we decided to test radio transmitters produced by Advanced Telemetry Systems (ATS; Isanti, MN), model M1750, with neoprene-impregnated cotton duck belting collars. These units weighed approximately 13 grams, with a battery life of 7 months to 1 year. The strap-type collar of fixed size and placement of holes was secured by a nut and bolt.

To test the ATS collar design we moved the three adult males to individual enclosures (66 x 16 ft) at the Small Mammal Research Facility administered by the Department of Wildlife, Fish, and Conservation Biology, University of California, Davis. Each rabbit was radio-collared prior to placement in its enclosure. Brush piles, pipes, and nest boxes were provided as shelter for the rabbits in the enclosures. A strip of grass (commercial sod) measuring approximately 16 x 3-4 ft was placed in the center of each enclosure to enhance the environment and as a supplemental food source.

Animals were provided rabbit pellets, alfalfa hay, and timothy hay ad libitum. This food has successfully maintained male and female pygmy rabbits at the Oregon Zoo (Swanson undated, Shipley 2001) Water was provided for each animal via poultry watering stations. Water and food dishes were disinfected weekly with a chlorine solution. Feces and old browse were removed from pens weekly. Rabbits were checked at least twice daily, and their health and behavior were noted.

On 24 May 2002 rabbit number 146 died. The death did not appear to be related to the radio-collar and a necropsy was performed by Dr. Karen Terio, pathologist with the UCD small animal clinic. Results were inconclusive but we later established that they were not inconsistent with poisoning by nightshade, which was found growing in its pen. After approximately 6 weeks with no problems with radio-collars, the two remaining rabbits were released at their original capture locations at Paradise Cut.

Reproduction and Development of Captive Rabbits

At biweekly intervals between 22 February and October we trapped within the controlled propagation pen to recapture the brood stock and their offspring. Our objectives included assessment of the general health and appearance of all captured individuals, reproductive condition of the breeders, number of offspring present in the enclosure, and growth and development of captive-bred offspring.

The first offspring was captured on 22-23 February 2002, and weighed 140 and 179 g. Given the approximate 30-day gestation period for brush rabbits, conception probably occurred in late December, a few weeks prior to date we projected for the beginning of mating season. A total of 64 offspring (34 female, 28 male, 2 unsexed) were produced. The unsexed individuals were very small and still nursing, and were not recaptured, so presumably died. The overall sex ratio was 1:0.82, female to male. We captured many more females in the early part of the summer and more males in August and September (Figure 7).

All three of the adult females exhibited evidence of producing young; two probably had three or four litters each. Though five litters was considered the upper number that might be produced by females, we had expected only 2 or 3.

Although, we did not expect the offspring to reproduce during their first year, 12 juvenile females exhibited evidence of reproduction such as estrus, lactation, or pregnancy. Of these, two individuals probably had two litters. As a conservative estimate, there were 22 pregnancies by 15 females producing 64 young that lived long enough to be trapped and marked. The mean number of young per pregnancy surviving beyond the first few weeks after birth was 2.9. We found no evidence of estrus or pregnant females after 19 September. We removed from the pen all the males that we could trap and which were of sufficient size to radio-collar, starting on 31 July.. Numbers of newly trapped and marked young peaked in May and August and declined in September and October (Figure 7).

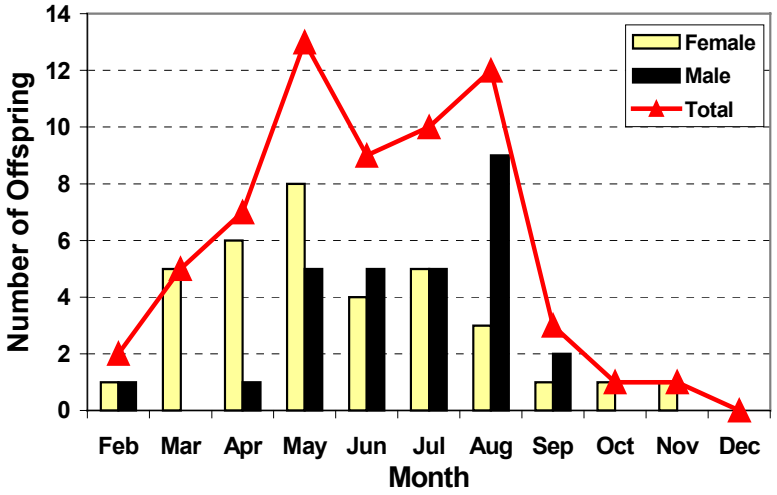

Figure 7. *Number of young rabbits newly trapped and marked in the controlled propagation enclosure each month during 2002.*

The smallest individuals captured weighed 110 g and were probably no more than about 2 weeks old (Davis 1936, Mossman 1955, Chapman and Harman 1972, ESRP unpubl. data). They probably were just weaned or about to be weaned. Mean mass at first capture for 62 young was 228.5 g (s = 86.46 g). Least and greatest mass at first

capture were 110 g and 480 g, respectively. Distribution of weight classes for mass at first capture are shown in Figure 8. A majority of rabbits weighed less than 300 g when first captured—the modal weight class was151-200 g.. Figure 9 depicts the relationship between time and gain in mass for 20 young rabbits weighing < 600 g; weight of animals heavier than 600 g probably was influenced by reproduction and was not included in calculating rate of gain. Mean gain in mass was 8.0 g per day for rabbits weighing 110 g or more initially. Extrapolating from these data and published data on development of

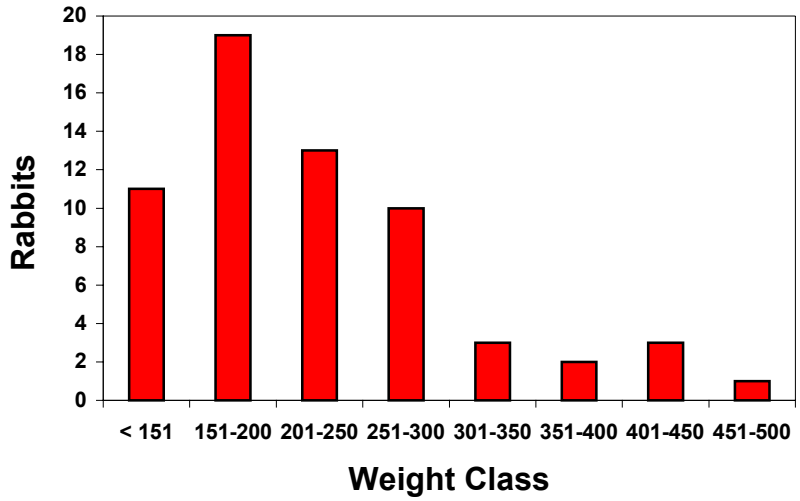

Figure 8. *Distribution of mass (g) at first capture of 62 young in 50-g weight classes for rabbits born in the Controlled Propagation facility at Pond 6 in 2002.*

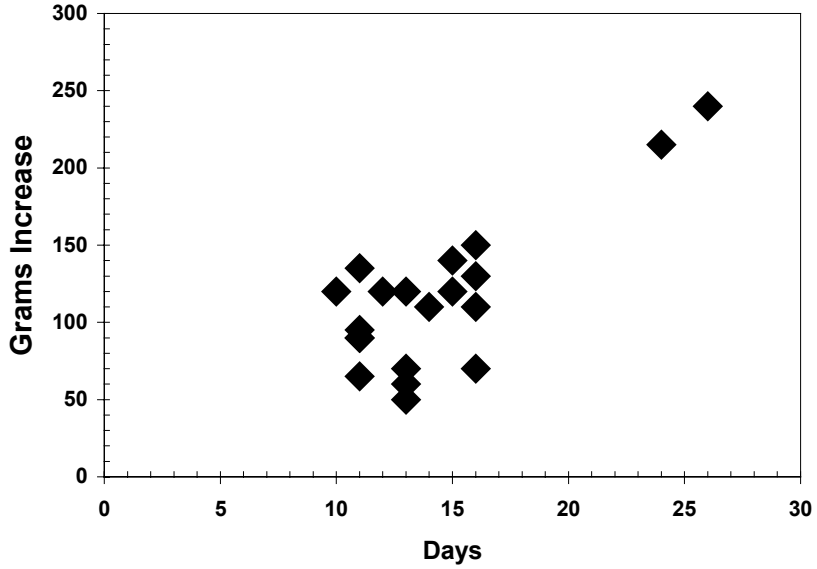

Figure 9. *Scatter plot depicting increase in mass between weighting periods in days for 20 young riparian brush rabbits born at the Controlled Propagation facility.*

brush rabbits and other *Sylvilagus* species (Chapman 1974, Chapman 1975, Chapman and Wilner 1978, Chapman et al. 1980), and assuming 4-day old rabbits weight about 28 g (Davis 1936), we estimated the mean age at first capture as 29 days.

Genealogy of Young Rabbits Produced in Confinement

The genetic variability of the rabbit populations was determined using eight polymorphic microsatellite loci (Table 2). Each reverse primer carried a TET fluorescent tag. Extracted DNA was amplified in a 20 µl PCR (3 µl DNA, 4 µl dNTPs (1.25 mM each), $MgCl_2$ (Table 3), 1 x PCR buffer, Taq DNA polymerase (1 unit), 0.4 µl each primer (50 µM). PCR conditions were optimized for each primer by varying the $MgCl_2$ concentration and annealing temperature (Table 3). In addition, primer Sol-44 required higher concentrations of Taq DNA polymerase (2 units/20 µl reaction). The following thermocycler program was used for each reaction: 5 minute denaturation at 95°C; 8 cycles of 94°C for 30 seconds, primer specific annealing temperature for 30 seconds, and 72°C for 30 seconds; 26 cycles of 89°C for 30 seconds, primer specific annealing temperature for 30 seconds, and 72°C for 30 seconds; 10 minute final extension at 72°C, hold at 4°C.

Following PCR, proper amplification was affirmed by examining the samples after electrophoresis on a 2% agarose mini-gel. Aliquots of amplified samples were sent to the Iowa State University DNA Sequencing and Synthesis Facility for genotyping on an ABI Prism 377 DNA sequencer (high resolution gel electrophoresis system). Gel files were downloaded from Iowa State Univ. and analyzed locally using Genographer 1.2 genotyping software (Benham et al. 1999).

Parental genotypes (Table 4) were determined and compared with the first 15 offspring that were captured and marked (Table 5). Only six microsatellites were used for this analysis because two were non-variable in the brood-stock population. Analyses of other offspring's' genotypes was not completed in the time period for this report and is expected to be more complex because of potential backcrosses and F2 mating.

Table 2. *Base sequences for eight polymorphic microsatellites isolated from riparian brush rabbits.*

Microsatellite	F-Strand	R-Strand	Authors
SOL-8	5'GGATTGGGCCCTTTGCTCACACTTG3'	5'ATCGCAGCCATATCTGAGAGAACTC3'	Rico et al. 1994
SOL-30	5'CCCGAGCCCCAGATATTGTTACCA3'	5'TGCAGCACTTCATAGTCTCAGGTC3'	
OCBGLX	5'TCTAGGAAGAAGCTTTATCCCTC3'	5'GTTTTCTCATCAGAAATCCACC3'	Van Haeringen, et al. 1996/97
OCR-4	5'TTCCTTTCTGTCCTGAGACCATG3'	5'GCAGTTGTGTGGAAATTTGGC3'	
OCLS	5'ACTGCTATATCAAAGGCATGACCC3'	5'TCAGGTATTTGGAAAGTGAATCCC3'	
SOL-44	5'GGCCCTAGTCTGACTCTGATTG3'	5'GGTGGGGCGGCGGGTCTGAAAC3'	Surridge, et al. 1997
SAT-7	5'GTAACCACCCATGCACACTC3'	5'GCACAATACCTGGGATGTAG3'	Mougel, et al. 1997
SAT-16	5'AATCAGCCTCTATGAATTCCC3'	5'AATGCTACATGGTAACCAGGC3'	

Table 3. *Magnesium chloride concentration and annealing temperature (T_A) for each primer*.

Primers	$MgCl_2$(mM)	T_A
SOL-8	3.0	55
SOL-30	2.5	54
OCBGLX	2.5	56
OCLS	3.5	62
SOL-44	4.0	58
SAT-7	3.0	59
SAT-16	3.5	56
OCR-4	4.5	58

The first 15 offspring were collectively produced by all six breeders (Table 5). Paternity was not resolved by microsatellites for three young. Male 140 fathered 6 or 7 of the 15 young while male 146 fathered from 4 to 7 and male 145 from 1 to 3. Female 141 produced 6 young, female 144 produced 8,and 147 produced only 1. Female 147 died of a collar-related accident on 1 March. Weights and dates of first appearance of offspring of females 141 and 144 coincide with two litters each; subsequent evidence of pregnancy or lactation is suggestive of these two females having 4 litters, though genealogical analysis is incomplete.

Table 4. *Genotypes for six microsatellites for six adult riparian brush rabbits comprising the brood stock for the controlled propagation program in 2002.*

	Parental Genotypes					
Rabbit ID/Sex	**OCR-4**	**SAT-7**	**SOL-30**	**SAT-16**	**OCLS**	**SOL-8**
140 male	326, 326	198, 198	159, 153	139, 129	157, 169	120, 120
145 male	326, 326	200, 196	161, 161	141, 133	173, 157	116, 116
146 male	326, 326	196, 196	161, 153	141, 129	173, 157	120, 118
141 female	323, 326	198, 196	153, 151	129, 127	157, 169	120, 116
144 female	326, 326	200, 196	161, 155	139, 139	157, 169	120, 116
147 female	328, 326	200, 198	159, 159	139, 135	169, 165	116, 116

Table 5. *Genotypes of first 15 offspring tested in the controlled propagation pen and their presumed genealogy. Where two males are indicated, either could have been the father.*

Offspring Genotypes							Parents	
ID	OCR-4	SAT-7	SOL-30	SAT-16	OCLS	SOL-8	Father	Mother
5483	326, 326	196, 196	161, 161	141, 139	157, 157	120, 116	146/145	144
54C4	326, 326	198, 198	159, 153	139, 127	169, 157	120, 120	140	141
B1D3	326, 326	200, 196	161, 155	141, 139	173, 157	120, 116	146/145	144
1F63	326, 326	198, 196	159, 153	139, 129	169, 157	120, 116	140	141
434A	326, 326	196, 196	155, 153	141, 139	173, 157	120, 118	146	144
2000	326, 326	198, 196	161, 153	139, 139	157, 157	120, 116	140	144
1F46		198, 196	159, 153	129, 129	169, 157	120, 120	140	141
2006	326, 326	200, 198	159, 159	139, 129	169, 157	120, 116	140	147
2002		200, 198	161, 153	139, 129	169, 157	120, 120	140	144
1F4669	326, 326	198, 196	153, 153	129, 129	157, 157	120, 120	146/140	141
2004	326, 326	198, 196	153, 153	141, 127	173, 169	120, 118	146	141
1F73	326, 326	200, 196	161, 155	141, 139	157, 157	118, 116	146	144
191		198, 198	153, 153	129, 129	157, 157	120, 116	140	141
194	326, 326	200, 196	161, 155	139, 133	157, 157	120, 116	145	144
195	326, 326	196, 196	161, 155	139, 129	173, 157	120, 120	146	144

Repatriation of Brood Stock

An important goal of the program is to have no net effect on the naturally occurring population. After being used as brood stock, the rabbits were returned to their original capture sites in the South Delta. To provide data to use in assessing the impacts to the population of capture, confinement, and repatriation, we monitored repatriated rabbits on a weekly basis using radio telemetry.

In 2002, three brood-stock rabbits were returned to their original capture locations. In addition, three young, born in the propagation enclosure, were translocated to the South Delta to replace founders that died in captivity (Table 6, Figure 11). Rabbits were fitted with ATS radio-transmitters equipped with a "mortality sensor." We monitored to determine if the rabbits were alive or dead and to locate them using the radio signal. We fitted hand-held, 2-Element, "H" style directional antennas (Telonics; Mesa, AZ) to portable receivers (model R1000; Communications Specialists) and located individual rabbits by walking toward their radio signals until we were within a few feet. Dead animals were retrieved and delivered to the University of California, Davis, Wildlife Health Center for necropsy and determination of cause of death.

Repatriated adults, familiar with the site where they were released, survived better than the naïve young born in captivity (2/3 versus 0/3). The radio signal of one young was not heard after the day of release and we have no evidence that this rabbit died. The

Table 6. *Status of rood-stock and captive- bred rabbits repatriated or released to their or their parents' original capture locations in the South Delta. Rabbit 140 was retagged as rabbit 197. Age group signifies brood stock (Adult) and their progeny (Young).*

Ear tag number	Age Group	Sex	Status	Cause of Death	Days from release to death
140 (197)	Adult	Male	Alive	N/A	N/A
141	Adult	Female	Alive	N/A	N/A
146	Adult	Male	Dead	Probable predation	5
218	Young	Male	Missing	Presumed mortality	N/A
221	Young	Female	Dead	No body recovered	47
222	Young	Female	Dead	Probable predation	24

February 2003, when monitoring ended. By that time both had had opportunity to breed successfully after repatriation.

A rabbit (# 146) translocated to Paradise Cut was found dead with only minimal remains submitted to the pathologist. The quality and quantity of remains submitted were insufficient for necropsy or histopathology.

Adults were familiar with their release sites and may have been more experienced with evading predators. Rabbits used for brood stock were relatively long-lived compared to rabbits in the wild (ESRP unpubl. data) suggesting heartiness that may have fitted them better prepared for release back into the South Delta. No conclusions should be drawn from any of these limited observations, however, because there were too few rabbits in each category.

While monitoring the locations of the repatriated founders, we observed that the male (#140/197) moved throughout his patch of habitat frequently, and on at least one occasion traveled across Paradise Dam to the other side of Paradise Cut. This is not a great distance (about 150-200 feet), but his travels would have left him exposed to a greater risk of predation because the top of the rock-fill dam did not provide continuous cover and the plant community immediately on the other side consisted solely of small patches of herbaceous forbs and grasses.

The female (#141) stayed in the same small area of rail-road right-of-way through Tom Paine Slough throughout monitoring. This trend is similar to one witnessed inside the propagation enclosure, in which adult females were recaptured in close proximity, and adult males were recaptured throughout the pen.

In the future, we hope to capture a control group of rabbits from the same locations where brood-stock rabbits were captured when it is time to repatriate the brood stock. Rabbits in brood-stock and control groups will be fitted with radio-transmitters, and will be monitored simultaneously to determine if rabbits used in the controlled propagation program survive after return to the wild at the same rate as animals not removed from the wild. Insight into effects associated with familiarity with the release area and living

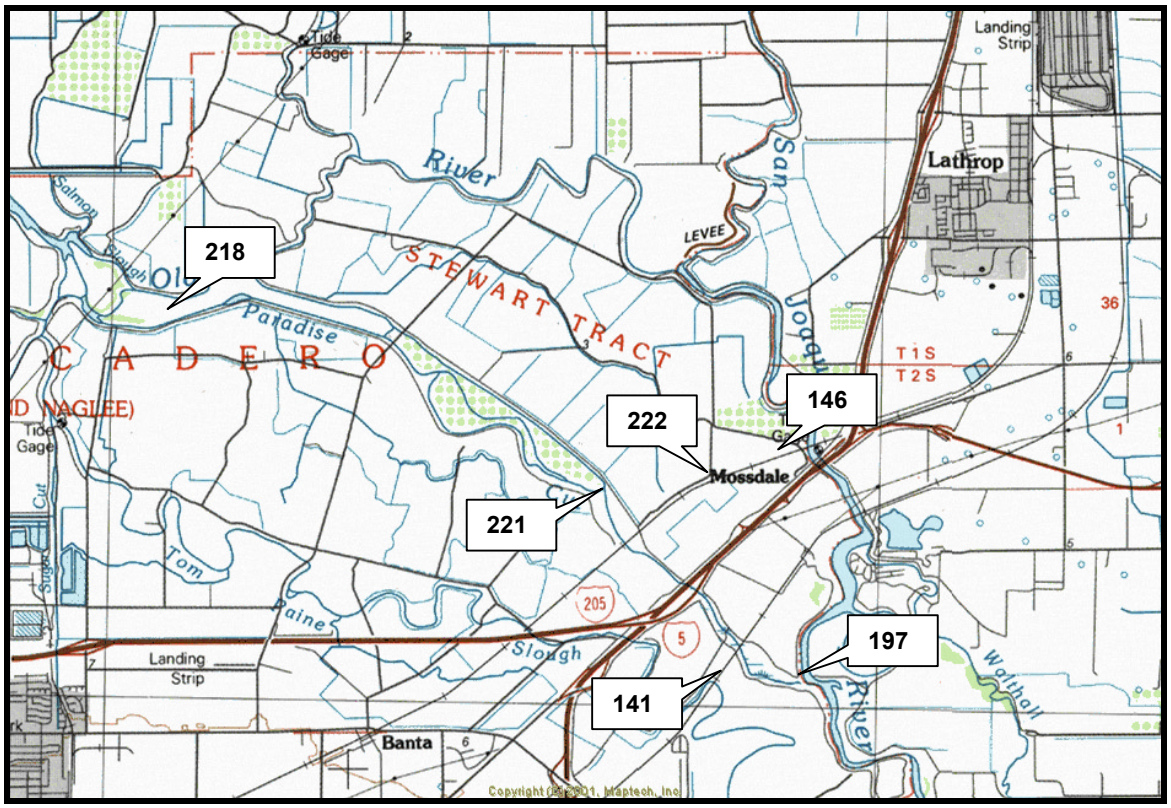

Figure 10. *Map depicting the South Delta locations of repatriated riparian brush rabbits in 2002 (1 cm on the map = 1 km on the ground).*

exposed to predations on subsequent survival will be gained by comparing survival of young born in captivity with that of repatriated adults.

Translocation and Release of Riparian Brush Rabbits

The first translocation took place at the San Joaquin River National Wildlife Refuge (NWR; West Unit, field number H10; T4S, R7E, Section 3, NW ¼ of SW ¼). This site features a dirt mound that was created by the U. S. Bureau of Reclamation to provide rabbits with a refuge above flood level (D. Woolington, S. Frazier, USFWS, pers. comm.). Contiguous to the mound, along the stream-side of the levee, were approximately 7.5 acres (3.04 hectares) of patches of tall, dense herbaceous annuals, rose, blackberry, mugwort, and willow (Griggs 2000) that were distributed more or less continuously. Other clumps of suitable habitat, covering between about 100-200 acres, were located at various distances from the mound and accessible to dispersing rabbits. Cristman Island, an area between 600-700 acres in size, also probably was available to dispersing young when sloughs connected to Hospital Creek had little or no agricultural drain-water or storm runoff.

Prior to moving young to the Refuge, a soft-release enclosure was built to provide them with moderate protection from predation while becoming acclimated to their new

Figure 11. *Soft-release (pre-release) enclosure for riparian brush rabbits at the San Joaquin National Wildlife Refuge (photo by D.F. Willliams).*

surroundings. The enclosure consisted of 1-inch poultry netting attached to steel T-posts and stood 5 feet above ground level and buried 1 foot below ground level. The enclosure encompassed approximately one acre of suitable habitat consisting of willows, blackberries, mugwort, and coyote brush (*Baccharis* spp.; Figure 11).

On nine occasions between July 17-30 2002 we set 100 Tomahawk™ traps throughout the soft release enclosure. Fifty traps were baited with a combination of walnut meats, rolled oats, molasses, and sliced apple. The remaining 50 traps were baited with chicken liver. Fresh bait was prepared each day of trapping. Traps were set in the evening, checked approximately 1 hour after sunrise, and were closed during the daytime hours. Desert cottontails and potential predators were removed from the soft release enclosure. Six aluminum plates measuring 1-m² were placed throughout the enclosure to facilitate the location and removal of snakes. Six automatic-camera and track plate stations were monitored concurrently with the trapping sessions. Camera stations consisted of Trail Master 1500™ active infrared trail monitors with Olympus Infinity Mini DLX™ cameras. Tracking stations consisted of 1-m² aluminum plates covered with a fine layer of flour, baited with either chicken liver or canned cat food. Photographs of tracks were taken for reference. Stations were placed in suitable locations within a mile radius of the translocation site. A spotlight survey was also conducted each evening concurrent with the trapping sessions. Spotlighting was conducted from the levee road, and encompassed the areas a mile to the north and to the south of the soft release enclosure. This route was driven twice to maximize coverage and to increase the possibility of sightings.

We captured 21 desert cottontails and 1 California vole. However, the number of cottontails captured does not represent the total number of individuals. On the sixth day of the session, we began marking the medial side of the ear with a indelible marker. After marking four of eight captures were of previously marked individuals. The cottontails were finding a place where the fence was not properly buried and were re-entering the pen. No snakes were discovered under the cover boards. Four of the six track plates were visited by animals leaving identifiable tracks, and only three of these four plates saw regular activity. Regular visitors included house mice, deer mice (*Peromyscus maniculatus*), striped skunks (*Mephitis mephitis*), and opossums (*Didelphis virginiana*). Less frequent visitors included desert cottontails and lizards. On one occasion, a house cat (*Felis domestica*) was photographed at a station approximately 200 feet north of the release pen. While spotlighting, we saw one striped skunk, one raccoon (*Procyon lotor*), three unidentified mammals, and multiple sightings of great horned owls (*Bubo virginianus*). The owl sightings were likely repeat sightings of two pairs, one residing 0.9 miles to the north of the translocation site, and one residing 0.8 to the south of the site. Throughout the area, coyote (*Canis latrans*) scats were very common, and on one occasion, two coyotes were spotted approximately 0.5 miles south of the translocation sight. Other possible predators included the numerous hawks, mostly red-tailed hawks (*Buteo jamaicensis*), that were present throughout the area.

No predators of rabbits were detected within the soft-release enclosure or its immediate surroundings. Yet owls, hawks, and coyotes were seen in the general area and we assumed that they would present risks of predation on released rabbits. We also assumed that species not detected in the pen, including long-tailed weasels and snakes, presented low risks of predation while rabbits were confined to the soft release pen. By monitoring the confined rabbits daily we hoped to detect any predation in the pen before more than one or two rabbits had been killed and take actions to prevent further losses.

We hypothesized that by confining the rabbits for a few days they would become more familiar with places to shelter and retreat, and would become better acquainted with the other individuals released during the same time period. We expected that this would provide them additional protection from predators.

Health screens performed on desert cottontails and black-tailed hares (*Lepus californicus*) were recommended prior to the release of riparian brush rabbits at SJNWR. On 3 June 2002, twelve adult *S. auduboni* and one juvenile *L. californicus* were collected by gunshot. Heart blood was collected from all carcasses not shot through the thoracic cavity, and the serum was banked. Carcasses were submitted to Dr. Karen Terio for gross necropsies and histopathology. In addition to pathologic examination for disease, these carcasses were screened histologically and with PCR assays for evidence of *Herpesvirus* infection.

Necropsies of the cottontails (eight males and four females) and the jackrabbit (male) revealed the following significant gross findings:

1. mild enlargement of the lymph nodes in the abdominal cavities of five of the cottontails;

2. fleas on all of the cottontails but not on the jackrabbit;

3. pregnancy in three of four female cottontails;

4. minimal multifocal hepatitis in three of the cottontails;

5. multiple parasitic cysts in the abdominal cavity of five of the cottontails

The parasites that caused the cysts were larval cestodes, most likely *Cysticercus* (*Taenia*) *pisiformes*. The characteristics of the multifocal hepatitis were similar to that previously described in wild rabbits with larval cestodiasis. The pathologist also found trichostrongyles and coccidia (most likely *Eimeria* sp.) within the small intestines. A few of the animals had some multifocal inflammatory lesions that may be *Encephalitozoon cuniculi* (use of special stains is pending, but the organisms are typically hard to find). There was evidence in two cottontails of clear-cut lymph node hyperplasia, and the spleen looked reactive in most individuals. All of the parasites mentioned above have been reported before in wild cottontails, and do not represent the kind of health threat to riparian brush rabbits that warrants special veterinary intervention with rabbits being translocated to San Joaquin River NWR.

Tissues from one of the desert cottontails collected has preliminarily tested positive for a herpesvirus. On initial investigation, it does not appear to be *Herpesvirus sylvilagus*, although this is not definite. Further assays to more specifically characterize this virus are currently underway (at no cost to project). Although it is not likely that this herpesvirus presents a significant health threat to the riparian brush rabbits at SJNWR, without knowing what kind of herpesvirus infected the cottontail, this cannot be said with certainty. While we wait for further testing, every effort should be made to recover fresh carcasses, so that in the slight chance this virus is infecting riparian brush rabbits and causing disease, we can detect it.

It was recommended that health screens, to include examinations, oral swabs for herpes detection, and blood work, approximately 10-14 days prior to release should be performed on all rabbits being translocated to SJNWR. Rabbits that exhibited evidence of sub-optimal health (e.g. poor body condition, inflammatory hemograms, palpable or visible lesions that could negatively impact health) were not released until causes of abnormalities were determined or the animal returned to good body condition.

Fifty-five physical examinations and blood collections were performed on 50 rabbits at the controlled propagation pen as part of the pre-translocation health screening effort. In two cases, abnormal findings on physical exam or results of lab work precluded safe translocation of the rabbit on the day of capture. In one of these cases, a rabbit had an elevated white blood cell count, and in the other, the rabbit had a decreased white blood cell count. In both, these hematologic abnormalities had resolved by the next vet check,

and both rabbits were translocated at that time. The only physical examination finding during these pre-release health screen exams that has prevented same-day translocation has been palpable evidence of pregnancy and/or lactation.

Upon passing individual health exams, rabbits were removed from the propagation pen and placed in the soft-release enclosure. The first group of captive-bred riparian brush rabbits were placed in the enclosure on the San Joaquin River NWR on July 31 2002. Nine subsequent translocations were made between August 14, 2002 and October 19, 2002. Forty-nine individuals were released at the Refuge in 2002.

Rabbits were confined to the soft-release enclosure for 2-20-day intervals, and were monitored by radio-telemetry and direct observation to ensure that they remained alive during the acclimation period. The enclosure was then opened in up to seven separate sections, to allow the rabbits to leave at their will. No animals died while confined in the pre-release pen.

Monitoring after release from the pen had multiple objectives, including measuring dispersal distance, survivorship, causes of mortality, habitat characteristics of established home ranges, and patterns of dispersion of rabbits over the colonized area. The first two cohorts of translocated rabbits were monitored daily for 5-day periods upon opening of the enclosure. Rabbits in subsequent translocations were monitored a minimum of twice per week every week. Radio-collared rabbits were initially hand tracked using 2-Element "H" style directional antennas and portable receivers. Signals were followed to determine which patch/clump of brush each individual was using. The animal's position was then estimated to within a few meters and recorded in UTM coordinates with a global positioning system (GPS; Garmin). The time, weather conditions, signal quality, and habitat patch in which the rabbit was located were recorded.

As additional individuals were released it became impractical to locate each by hand tracking. The rabbits dispersed more widely than expected and we found that we occasionally disturbed them when hand tracking. Consequently, we began to monitor rabbit survival and movements via radio telemetry from fixed stations where we set up precision direction finding arrays.

To acquire location information, bearings were taken simultaneously by each researcher on radio-collared rabbits. Synchronous collection of bearings was achieved by communication via hand-held radios. Each researcher carried an active radio-collar or beacon. For each location fix, a total of four bearings were collected, one from each researcher to the rabbit and one from each researcher to the other. An ArcView® (Environmental Systems Research Institute, Redlands, CA) program extension was used to calculate rabbit locations. The time, weather conditions, and signal quality was recorded by each researcher for every location fix. Readings on collared individuals were separated by at least 1 h to prevent autocorrelation of the data.

Tracking occurred during one of four monitoring stages (0400-1000 h, 1000-1600 h, 1600-2200 h, and 2200-0400 h). We intend to use these stages to attempt to determine

periods of peak activity. Once periods of peak activity are identified, monitoring may be reduced or stopped during periods of low activity. Currently, tracking has occurred during a different period (alternating among the four) each monitoring day.

To evaluate researcher error, location fixes were taken by triangulation on carcasses prior to their collection. Once the carcass was found a GPS reading was taken at the site to allow the calculated position to be compared with the known location (Bond 2001). In addition, readings generally were taken on at least one test collar during telemetry sessions.

Dead rabbits were collected following the procedures outlined by Gilardi (2001) and available remains were collected and transferred to Dr. Karen Terio, Diagnostic Pathology, Veterinary Medical Teaching Hospital; UC, Davis.

As of 17 February 2003, 31 of the 49 (63%) radios on translocated rabbits were transmitting live signals. Fourteen (of 23) of the surviving individuals were female and 17(of 26) were male. Female survivorship (61%) was not significantly different (P = 0.77) than that of males (65%).

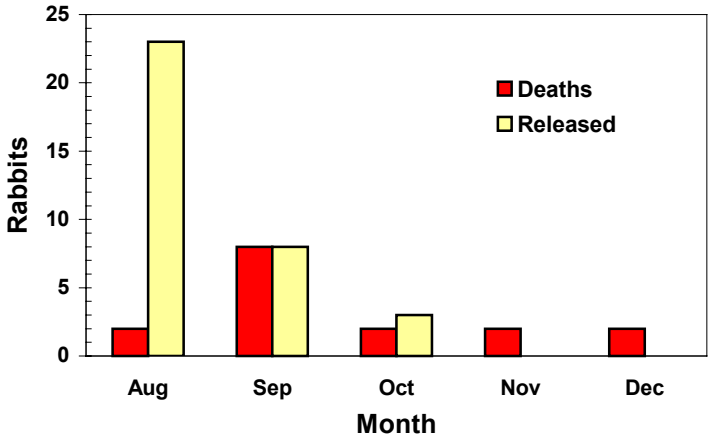

Figure 12. *Numbers of captive-born riparian brush rabbits released on the San Joaquin River National Wildlife Refuge and deaths by month from August 2002 through May 2003. *Six rabbits released on 31 July are combined with 17 released in September.*

Most rabbits died shortly after release in August and September (Figure 12), when most of the rabbits also were liberated. We located remains of 12 of 18 dead rabbits. Four of these were entire carcasses and five consisted of only intestinal remains. Only fur, feet, or bones were present for the other three individuals. Two intact carcasses were rabbits that had slipped the radio collar strap over their lower jaw to become lodged in the oral cavity. The majority of rabbits probably died of predation; however post-mortem scavenging also may have occurred, obscuring actual cause of death. Fifty percent (8 of 16) non-radio-collar-related mortalities occurred within 10 days of release from the soft release enclosure. Excluding the radio-collar related mortalities, 16 of 47 translocated rabbits died by 17 February 2003 (34%).

Based on necropsy results, at SJNWR, predation was the cause of 11 mortalities—5 were confirmed and 6 were assumed because only the radio collars were found. Other mortalities included two with pending histopathology reports, two that were too decomposed for pathology, and two due to a foreign body in the oral cavity of the rabbit (radio collar).

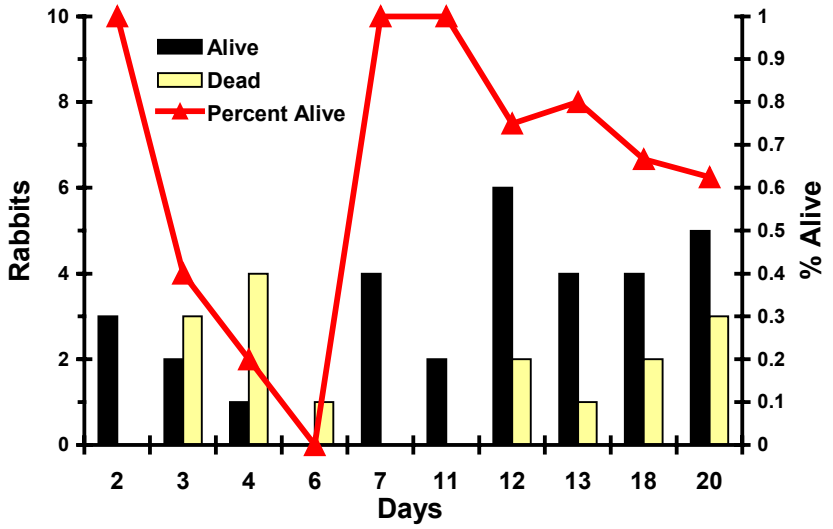

Figure 13. *Status of rabbits (numbers alive and dead) after release and through 17 February 2003, and percent of individuals surviving within each period.*

Rabbits were held in the soft release enclosures for 10 time intervals, ranging from a minimum of 2 days to a maximum of 20 days (Figure 13). The number of individuals within each interval varied from 1 to 8. Excluding the two collar-related deaths, 33 individuals confined in the enclosure for 7 days or more had 24% mortality (8 of 33) through 17 February 2003. The 14 individuals that spent 6 days or less in the soft release pen had 50% mortality (7 of 14), but both rabbits confined for only two days survived through the reporting period. The proportions in the two groups did not differ significantly at the 5% probability level ($P = 0.0867$). Six of eight individuals (75%) in the first translocation cohort, held in the soft-release enclosure for 12 days, survived for 189 days as of 17 February 2003.

Maturity and reproductive status at the time of translocation may have been factors influencing the survival of relocated rabbits. Using weight as an indicator of maturity and reproductive condition (in females), mortalities occurred in all female size classes except 500-700 g and 4 of 5 females in the 800-900-g range died (Figure 14). All 6 males that weighed > 600 g survived (Figure 15), but 9 of 21 males in the 400-600-g range died. The heaviest females probably were pregnant when released and this somehow may have increased risks of death. Rabbits of both sexes less than about 500 g had not attained adult size, which also may be associated with increased risks of death. We noticed this trend after the first few releases and adjusted the protocol to collar and

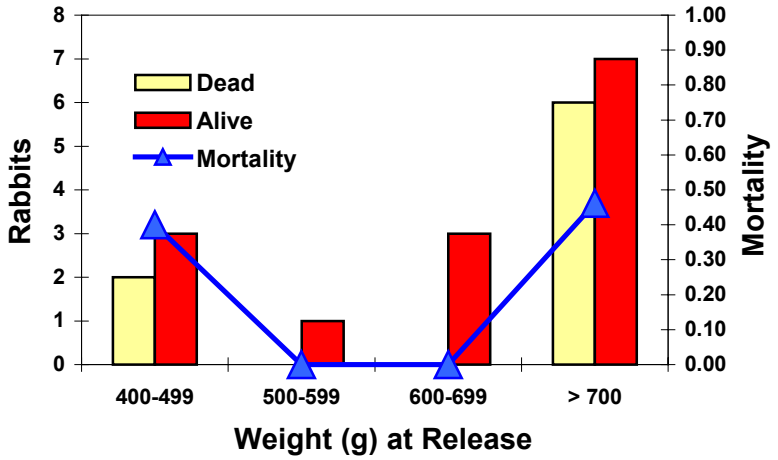

Figure 14. *Mortality within weight classes for female rabbits released at the San Joaquin River National Wildlife Refuge between 31 July 2002 and 17 February 2003.*

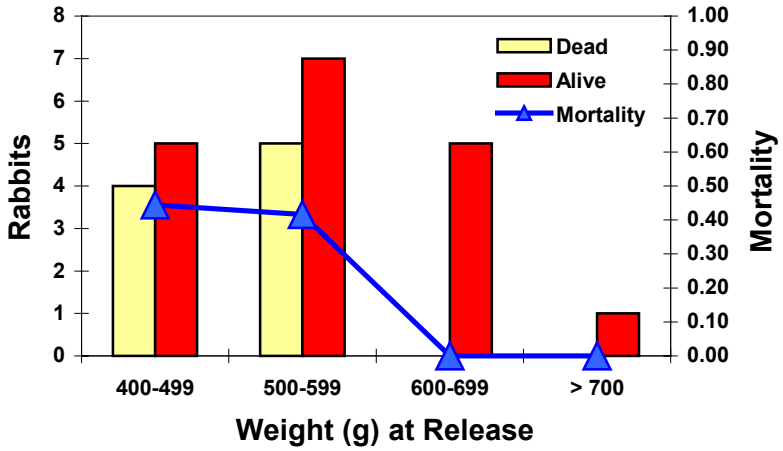

Figure 15. *Mortality within weight classes for male rabbits released at the San Joaquin River National Wildlife Refuge between 31 July 2002 and 17 February 2003.*

translocate rabbits only after attaining a weight of 500 g or more instead of 400 g or more. Both rabbits that died from collar-related accidents also weighed less than 500 g.

On the whole, the riparian brush rabbit population is robust and healthy: animals appear to do well in captivity (gain weight, reproduce), and approximately 60% of rabbits reintroduced to the refuge were alive as of February 2003. There have been no epizootics of infectious disease to date. The primary causes of morbidity and mortality have been predation and radio collar trauma. Findings on physical examinations conducted by the veterinarian have been for the most part unremarkable, and complete blood counts and serum chemistries have usually been within normal reference range for *Sylvilagus* species

(although domestic *Oryctolagus* normal values have been used where values for *Sylvilagus* are unavailable). To date, necropsies of dead rabbits have yielded the most detailed information on disease status of individual rabbits, including a case of lymphoma (etiology undetermined) as well as evidence of a herpesvirus infection (virus species to be determined). Necropsy will likely continue to be the most informative diagnostic method in the event of an infectious disease outbreak.

Veterinary Options for the 2002-2003 Season

Option #1.—Continue current operation: every rabbit coming in as brood stock, as well as all progeny being translocated out of the propagation pens, receives a full physical examination and blood sampling under anesthesia in the field by the project DVM. All dead rabbits or parts thereof are submitted for pathologic examination to the fullest extent possible given condition of carcass.

Pros: takes advantage of the Project DVM's and the Project Pathologist's experience in assessing animal health, thereby maximally ensuring that project activities are not adversely affecting rabbits, and that only healthy rabbits are being used as founders and/or are being translocated; maximal safety of anesthesia administration achieved; maximal quantity and quality of data acquired from recovered carcasses.

Cons: costly (gross estimate of at least $185 per rabbit for veterinary time, blood-work, mileage and supplies), and would require at least one or two more additional veterinarians to assist the Project DVM in the field, as well as one or two more pathologists available to assist with necropsies and histopathology.

Option #2.—Project DVM trains one or two project biologists to conduct the physical examinations and blood collection in the field. Results of examinations are shared same-day with the DVM, and blood samples are shipped directly to a veterinary diagnostic lab (IDEXX, in West Sacramento), with lab test results sent to the Project DVM for assessment. Any and all concerns regarding the health status of an individual rabbit are brought to the immediate attention of the DVM for further evaluation (either a phone consultation or transporting the rabbit to UCD for a vet exam). In addition, only fresh rabbit carcasses will receive full gross and histopathologic examinations, with remains of insufficient quantity or quality receiving gross necropsy exams only, with tissues archived for further examination in the event of an epizootic.

Pros: maintains involvement of current DVM with most familiarity with project; ensures that rabbits exhibiting ill health either through an abnormal sign on physical examination or a lab result is examined by a DVM; reduces expense by at least $100/rabbit (estimate) in that time and mileage for DVM not incurred for any rabbit not requiring additional veterinary evaluation; reduces pathology costs.

Cons: biologists may not detect evidence of poor health on physical exam; anesthesia not administered by DVM is riskier; up-front costs (vet time to train biologists,

purchase of a portable anesthesia machine); blood samples will have to be dropped off for courier pick-up by noon to guarantee same-day analysis.

Due to the increased scale of controlled propagation the decision was made to train a project biologist to perform health screens with the project veterinarian interpreting all laboratory results. The biologist(s) will receive hands on training and a detailed protocol from the project veterinarian for administering anesthesia, blood collection and physical examinations.

PART 2:
RIPARIAN BRUSH RABBIT AND WOODRAT CENSUS AT CASWELL MEMORIAL STATE PARK—2002

Beginning in January 1993, ESRP began periodic monitoring of the riparian brush rabbit population at Caswell MSP by trapping and marking individuals (Williams 1993). The Park, covering about 253 acres of riparian forest. is located in the San Joaquin Valley of California, along the Stanislaus River, approximately 6 miles west of the city of Ripon. Monitoring objectives included obtaining population numbers from capture-recapture of marked individuals for year to year comparisons and obtaining tissue or hair samples for genetic studies. Because most captures occur only where traps are placed in runways or other natural paths, such as along logs, dispersing traps in a uniform grid or other configurations, conducive to estimating effective area covered by traps and therefore density, does not yield many captures (Basey 1990). Instead we searched closely in standard, defined areas of the Park for sign of rabbits (fecal pellets, runways, fur, clipped sedges) and woodrats (fecal pellets, stick houses). We set Tomahawk™ traps directly in runways, natural paths, and other sites with sign of rabbits or woodrats, and around potential woodrat houses. Traps were baited with a combination of walnut meats, rolled oats, molasses, and sliced apple. Traps were set in the afternoon or early evening, checked about 2 hours after dark and again in the early morning. Traps were left open around the clock unless it rained. We did not trap during heavy rains. Captured brush rabbits, woodrats, and black rats were permanently marked with metal ear tags (all three species) and PIT tags (rabbits and woodrats), weighed, and measured (rabbits only). A 1-2 mm diameter plug of ear tissue was taken with a biopsy punch from brush rabbits and woodrats and preserved in 95% ethanol (reagent grade, not denatured). Animals were released at the site of capture.

These procedures followed the protocol established by Williams (1993), except that we did not trap the middle section of Caswell MSP where rabbits were previously found to be scarce and little or no sign was found in more recent censuses and surveys. Instead, the Crows Loop area (Figure 16) which was added in 1998, was trapped. Instead of the 7 days of trapping in each section, which was standard, we added an eighth day of trapping at each of the three sections in an attempt of increase numbers of captures. Number of days trapped also varied from year to year because of rain storms some years, and because of extra efforts made to capture rabbits in others. Number of traps varied based on amount of sign where traps were placed.

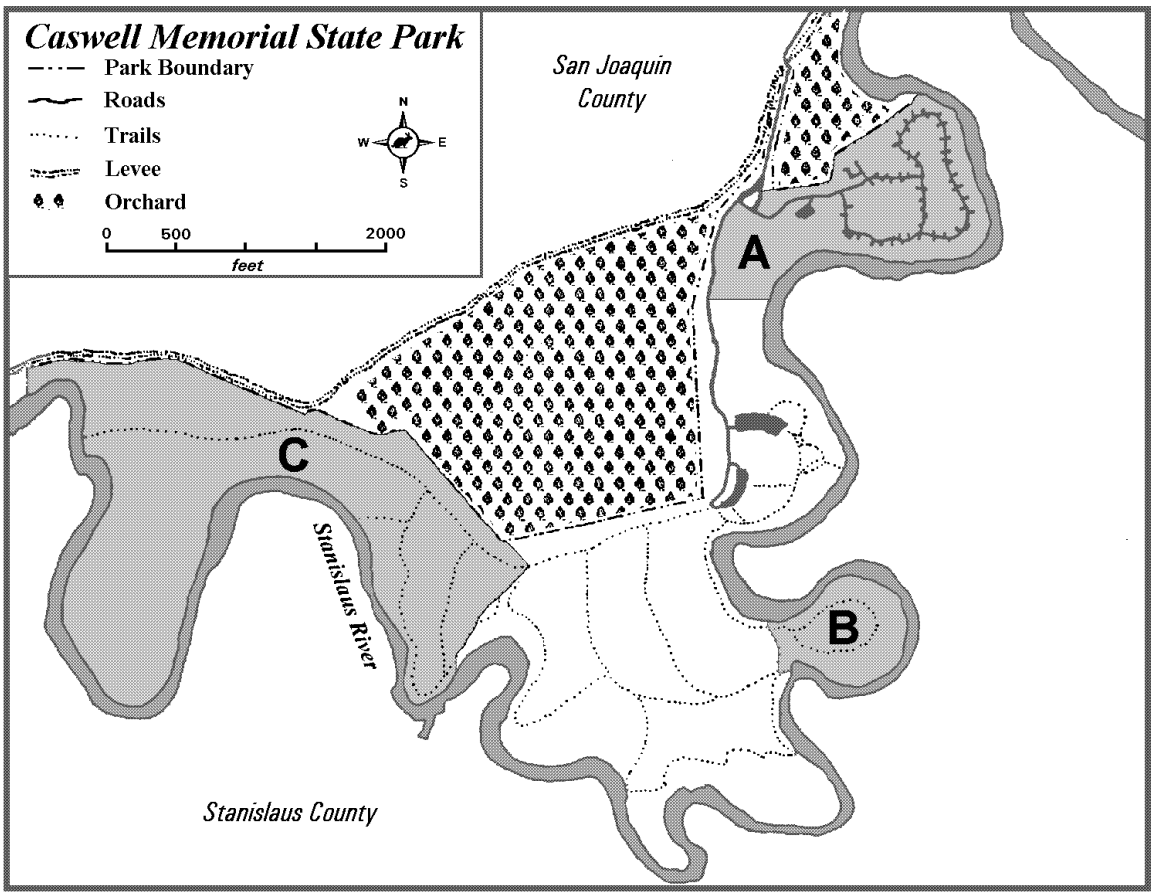

Figure 16. *Map of Caswell MSP depicting the three regularly surveyed areas (lighter shading): A – Campground; B – Crows Loop; C – Fenceline Trail. The levee on the southern side of the Stanislaus River is not shown.*

The census at Caswell MSP, in January 2002, yielded captures of 16 riparian brush rabbits. All 16 rabbits were captured in the Fenceline Trail and Crow's Loop census areas (Figure 16). Sign of rabbits elsewhere were scarce or absent. Brush rabbit and woodrat populations were found to be high in 1993, but very low in 1997 through 2002 (Table 7). This is especially apparent comparing capture rates (Figure 17). Capture rates in 2002 were higher than capture rates any year since 1993. Unfortunately, current population numbers are still too low to allow for a meaningful statistical estimate of population size. A desert cottontail also was captured in the Park in February 2000, the first since the mid 1980's. No additional desert cottontails were captured during the 2001 and 2002 censuses.

Only during January 1993 were captures and recaptures of brush rabbits and woodrats great enough to estimate population sizes using closed population models. The population of riparian brush rabbits was estimated to contain 241 rabbits with a 95% confidence interval of 170-608 rabbits. The population of riparian woodrats was

estimated to be 437 with a 95% confidence interval of 170-608 woodrats. The populations of both species probably were at or near the carrying capacity of the Park (Williams 1993). In 2002, there was an increase in observed rabbit sign and capture rates for both species, suggesting that the populations might be recovering from lows in their fluctuating population cycles.

Table 7. *Numbers of individual riparian brush rabbits, woodrats, and desert cottontails captured (capture rate[1] in parenthesis) at Caswell MSP each year starting in 1993. Traps equals the average number of traps used each night.*

Year	Days Trapped	Traps	*S. b. riparius*	*N. f. riparia*	*S. audubonii*
1993	21	105	41 (1.86%)	55 (2.49%)	0
1997	28	99	0	6 (0.22%)	0
1998	29	78	6 (0.27%)	11 (0.49%	0
1999	16	58	2 (0.22%)	8 (0.86%)	0
2000	14	124	5 (0.29%)	12 (0.69%)	1 (0.06%)
2001	21	123	2 (0.07%)	15 (0.50%)	0
2002	24	124	16 (0.54%)	31 (1.04%)	0

[1] captures divided by trap days—trap days is the number of traps multiplied by the number of 24-h days of trapping

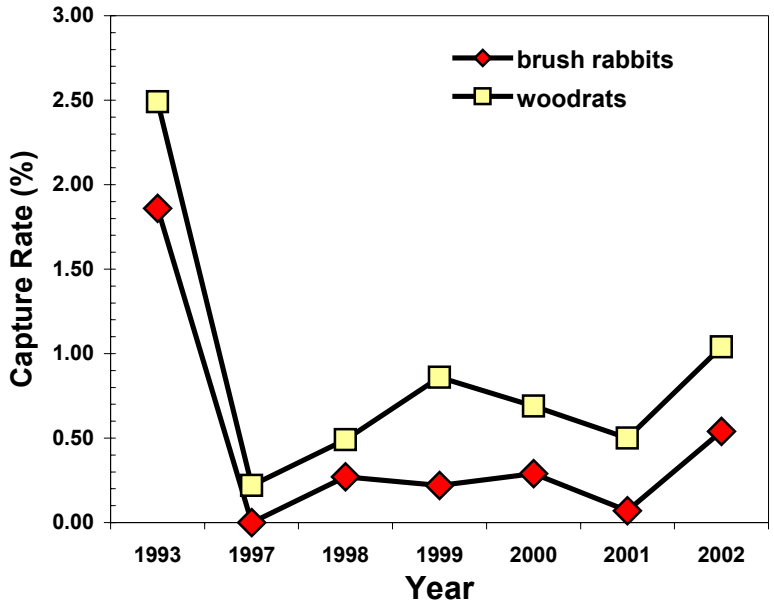

Figure 17. *Capture rates of riparian brush rabbits and woodrats for annual censuses during January between 1993 and 2002.*

PART 3:
EMERGENCY RESPONSE PROTOCOL FOR CONTROLLED PROPAGATION FACILITY

Introduction

An emergency pre-plan and response protocol for the captive breeding pen at Pond 6 was not included in the original Controlled Propagation and Reintroduction Plan (Williams et al. 2002). The need for such planning efforts came about during the early phase of the controlled propagation program when numerous suspicious fires and other vandalism occurred at the propagation site. Subsequent meetings by the Riparian Brush Rabbit Recovery Advisory Group led to funding from the U.S. Bureau of Reclamation and U.S. Fish and Wildlife Service for the purchase of emergency supplies and emergency pre-planning coordination. The goal of emergency planning for the captive breeding program was to ensure the safety of the captive animals and the safety of biologists should an emergency, such as fire or flood, occur in or near any of the enclosures.

Preplanning for emergencies at the controlled propagation facility and the soft-release pens at the San Joaquin River NWR required communication and coordination between dispatch centers for Stanislaus and San Joaquin Counties and the Endangered Species Recovery Program (ESRP). Several meetings were held between ESRP and both dispatch centers and with the local fire departments that would be responding to the locations. This coordination process introduced the local fire fighters and emergency personnel to the existence of the facilities and encouraged them to begin their own pre-planning efforts for emergency response.

The emergency plan provides information about the project site at Pond 6, including physical location and pre-fire planning objectives. The plan includes protocol for the use of a USBR-supplied pager that is alerted by San Joaquin County Dispatch. The pager notifies ESRP biologists and others of an emergency at the propagation facility. It describes how to respond during an actual emergency and what to expect from the Incident Command System. The plan details the acquisition of information during an emergency, and includes information about emergency supplies stored at various locations.

The plan also includes vegetation management guidelines that will help reduce the likelihood of fire spreading into the enclosures (see Appendix). Detailed maps, equipment lists, and animal-intake information sheets are provided in the appendices of the plan. Copies of the plan are being circulated among appropriate agencies for signatures.

Incidents at Pond 6 and San Joaquin River National Wildlife Refuge

Suspicious wildland and vehicle fires on Woodbridge Road in San Joaquin County are not an unusual occurrence (D. DeAnda, CDFG, pers. comm.). During the 2002 fire

season there were seven wildland fires at the Pond 6 Wildlife Area. In May during Memorial Day weekend alone, there were three separate fires of suspicious origin. One of these came to within 200 ft of the enclosure. This indicated an alarming increase in suspicious fire activity in the Pond 6 area (F. Lopez, Thronton Fire Department, pers. comm.).

During the fire season, the pager was alerted approximately 25 times, many of which were for fires not immediately adjacent to the Pond 6 facility. On one occasion there was a fire at Pond 6 and the pager was not activated.

The initial stages of formulating this agreement with the Dispatch Center took several phone calls, letters, and meetings over several months. During this time the dispatch center was learning about the program and instead of making a mistake and not alerting the ESRP, they erred on the side of caution and dialed the pager anytime there was a fire near Woodbridge Road and Interstate 5. These accidental pages ceased near the end of the 2002 fire season.

There were no fires or other emergencies that threatened the soft-release pen at the San Joaquin River NWR. The pre-planning for emergency notification of ESRP was less developed than it was for the Pond 6 location and we are currently working to solidify an emergency notification process at SJRNWR that is similar to our arrangement at Pond 6.

An initial meeting was held in June 2002 with the Stanislaus County Regional 911, the West Stanislaus County Fire Protection District, and the County Fire Warden's Office. At that meeting participants decided to create a new "Fire Demand Zone" that would ensure adequate protection of the pre-release pens and prompt notification of the ESRP pager should a fire occur at the Refuge. An important feature of this emergency plan was to allow swift notification of an emergency to the ESRP biologists so that appropriate emergency action could be taken.

Subsequent conversations between the Refuge staff, Stanislaus County Fire Department, and ESRP resulted in some confusion—as of February 2003, it was unclear if the Stanislaus County Regional 911 Dispatch Center will contact the ESRP pager in the event of an emergency at the Refuge. According to a fax transmittal by Deputy Refuge manager Robert Parris, any USFWS staff contacted by their own dispatch, Sierra National Forest Dispatch, is to notify the ESRP emergency pager. This process is likely to take longer than it would if a call came directly from Stanislaus Regional 911 because Sierra Forest Dispatch would likely get their notification from the County Dispatch Center.

According K. Laughton, Stanislaus County Regional 911 Dispatch Center (pers comm.) emergency notification would take much longer for ESRP to learn of an event if the message was transmitted through the Sierra National Forest Dispatch. The San Luis NWR Complex Fire Dispatch Plan gives directions to San Joaquin River NWR staff to call 911 should a fire be seen on the refuge, so it is apparent that the regional 911 dispatch center would be the first to be notified and more quickly page ESRP.

Importantly, ESRP will have to maintain phone contact with the Stanislaus Regional 911 Dispatch Center, the West Stanislaus Fire District, and Refuge staff to ensure the soft-release pen is opened if fire threatens and rabbits are confined.

Needs Assessment

Part I of the plan covers many aspects of emergency planning for the controlled propagation program and was as complete as possible with the allocated funding. Unfortunately, some situations may arise for which no solutions currently exist. The following are unresolved aspects of this planning effort:

a. There is no long-term emergency holding facility in the event animals must be moved from the controlled propagation facility. Although Micke Grove Zoo has agreed to house rabbits in the 24 cages placed there by ESRP and USBR, three to four times that as many cages and more space could be needed.

b. Should wild rabbits be captured during an emergency such as fire, what should be done with them? For example, a fire at Caswell Memorial State Park or Paradise Cut has the potential to destroy the majority of useable habitat for riparian brush rabbits.

In order to minimize length of captivity in any temporary holding facility, several decisions about the final disposition of captive rabbits must be made very quickly. There are three choices for the disposition of captive rabbits:

a. release back into the area from which they were taken;

b. place in a pen at the controlled propagation facility;

c. release to a different location with natural habitat.

In order to release the rabbits back to their point of origin, the determination must be made that suitable habitat remains after the emergency. The following questions need to be answered:

a. how much habitat is enough; this clearly would depend on the number of rabbits;

b. at what point is the habitat too degraded for use by the rabbits;

c. how long will it take for the plant community to recover to a point where it is habitat?

In the case of flooding, the captured rabbits could be held and then released back to their original capture site (or pen in the Controlled Propagation facility) once the water recedes. In the event of a wildfire, the site of capture may no longer be suitable for the

rabbits. We believe that guidelines can be developed to assist in deciding what to do with rabbits rescued from a wildfire, but that the questions can only be answered based on the conditions at and after such an emergency.

The second alternative to the disposition of rabbits captured during an emergency at an inhabited site or soft-release enclosure is to move them into a controlled propagation pen at Pond 6. This decision can only be made if there is space in the pen to accommodate them, and if adding them to a pen would not create unwanted genetic consequences, such as excessive inbreeding, or risk infecting rabbits already in the pen with a disease that is not found in the confined population.

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APPENDIX

Vegetation Management Guidelines for the Controlled Propagation Facility

OBJECTIVES

The objectives are to reduce the possibility of wildfire spreading to pens, give a clear space around pens to inspect integrity of the wire mesh, and prevent climbing vines from encroaching onto the structure sides, cables, supports, and roof. Vegetation control will:

1. Reduce the likelihood of black rats gaining entrance into a pen by climbing onto vegetation.

2. Reduce the likelihood of wildfire spreading into a pen via contiguous vegetation from the outside.

3. Maintain vegetation-free, 3-foot wide footpaths on the outside of the enclosures that will enable inspection for pen integrity.

4. Maintain vegetation-free, 5-foot wide footpaths on the inside of the enclosures that will enable inspection for pen integrity; the inside and outside footpaths will provide a vegetation free fire-break of 8 ft around the pens.

5. Maintain up to 5-foot wide footpaths inside the enclosure, down the middle and at several intervals across the short axis to facilitate researchers' movements.

METHODS

Vegetation will be controlled with a gas-powered trimmer, ATV-pulled mower and tiller, occasional spot application of herbicides, and various hand tools.

1. Every day a log of accomplished tasks will be recorded.

2. Any evidence of predators within the pens or any RBR death that is found should be left in place. Then project leaders should be immediately contacted for procedure on how the situation should be handled.

Outside Enclosure.—Perimeter vegetation will be removed on a regular basis, as needed to meet the following objectives:

1. Three-ft wide footpath around the pen will be maintained in bare state by cutting or tilling vegetation, or selective use of herbicides. Cutting or tilling should be done bimonthly or more often, as needed.

2. The outside and inside perimeter and the netting of the pens should be closely inspected for breaches at the beginning of every week. If breaches are found they should be investigated, reported and repaired immediately.

3. East side: all vegetation to be removed between pen and fence-line of grazed pasture; vegetation must be cut from around poles and wires. This trimming should be done bimonthly or more often if needed to keep the area bare.

4. West side: vegetation will be cut to ground level to least 15 feet from the enclosure. This trimming should be done bimonthly or more often if needed to keep the vegetation at ground level.

5. North and south of the enclosures (50 feet south of pen 1 and 50 feet north of pen 3, all space between pens 1 and 2 and 2 and 3) a 50-ft wide band will have vegetation mowed and the trimmings removed to facilitate fire control. All vegetation between the pens and around the storage containers must be kept at ground level (a height of 2 inches or less). This trimming should be done bimonthly or more often as needed.

Inside Enclosure.—Vegetation will be removed as needed to maintain the following:

1. A 5-foot perimeter around the inside of the fence will be kept to bare ground to access for breaches. This perimeter with be tilled bimonthly, or more frequently as needed.

2. Five-foot perimeter paths through the pens. On these footpaths, vegetation will be maintained to about 3 inches tall or less. To accomplish this, the vegetation will need to be cut back bimonthly, or more frequently as needed.

3. Existing discreet clumps of vegetation will be maintained by keeping vegetation between them cut down; this vegetation should not exceed 3 inches tall.

4. All vines will be prevented from growing on the guy-wires, large support poles, roof netting, and enclosure sides. To accomplish this the vegetation will need to be cut back bimonthly, or more frequently if needed. Vegetation should be kept 4 feet below all roof netting at all times.

5. Nest chamber openings and permanent trap site locations will be preserved by maintaining narrow paths. Non-mechanized hand tools will be used in the immediate area of chambers and trap stations. Trimming to keep the trap stations free of debris will be done by hand clippers bimonthly so that traps and equipment are not damaged and to facilitate locating and operating traps. Vegetation above

and beside the traps should be left, creating a tunnel to encourage rabbits to use the artificial runways and to provide shade and protection from wind and rain while confined in traps.

S*afety precautions*.—The following safety precautions and hygiene will be practiced:

1. All tools will be cleaned in a 10% chlorine solution before using in the pens.

2. Fifteen-yard^2 sections will be pre-scouted before beginning any vegetation removal. This will involve walking slowly in one direction and moving vegetation aside by hand or stick until the bare ground is observed. This will ensure the area is free of rabbits or other animals.

3. Slash will be removed from the enclosure immediately following cutting. This will prevent accumulation of debris that may subsequently be used for nesting sites, and prevent buildup of dry, flammable material that may contribute to the spread of a fire.