Translocation Model for the California Ground Squirrel (*Otospermophilus beecheyi*) to Facilitate California Grassland Ecosystem Recovery

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INTRODUCTION

The native grasslands of the Western United States, and California in particular, are among the most endangered ecosystems in the temperate world (Samson and Knopf 1996). In California approximately 90% of species listed in the Inventory of Rare and Endangered Species can be found in grasslands (Barry et al. 2006). Grasslands support both high wildlife abundance and diversity and are one of the signature ecosystems of the west. Due to their suitability for grazing, agriculture and housing developments, grasslands are also among the most favored ecosystems for human use. Grasslands are vulnerable to invasion by exotic plants, particularly if disturbed. In California, many native bunch grass systems have been invaded by annual grasses, mainly Mediterranean in origin. It is not surprising that remaining native grasslands in California support a number of species of conservation concern. One of the most notable species is the charismatic and highly visible western burrowing owl (*Athene cunicularia hypugaea*). Another signature species of this ecosystem, the California ground squirrel (*Otospermophilus beecheyi;* CAGS), is not threatened with extinction, but is an integral component of this ecosystem and may be critical for burrowing owl recovery.

California ground squirrels play a key role in engineering grassland ecosystems, yet this species has received little attention in conservation planning and policy. Fitch (1948) and Linsdale (1946) long ago noted the diversity of animal life associated with ground squirrel burrow systems. More recent quantitative research has substantiated these observations. Sites with ground squirrel colonies have greater diversity of reptiles, amphibians, insects and birds than sites where squirrels are absent (Lenihan 2007). Similarly, black-tailed prairie dogs (*Cynomys ludovicianus*) on the Great Plains are associated with higher bird diversity (Smith and Lomolino 2004) and other plant and animal diversity (Kotliar et al. 1999). It is also possible that ground squirrels are a "keystone" species responsible for maintaining the vegetation community itself. Kangaroo rats (*Dipodomys* spp.) play this role in Arizona where investigators found that their removal resulted in the invasion of annual grasses into the native shrub habitat (Brown and Heske 1990).

Lenihan (2007) also found that the presence of ground squirrels may be a prerequisite for burrowing owls in her study area in northern California: only those sites with ground squirrels had burrowing owls. The decline of fossorial mammals has been implicated as a key factor for the decline of burrowing owls in British Columbia (Howie 1980), the Great Plains of the U.S. (Kotliar et al. 1999, Desmond et al. 2000, Smith and Lomolino 2004), Argentina (Machicote et al. 2004) and here in San Diego County (Lincer and Bloom 2007). Human development of burrowing owl habitat is obviously responsible for the loss of many burrowing owl breeding sites in San Diego, but the loss of burrowing owl populations in undeveloped areas, such as

Camp Pendleton and Warner Springs, cannot be explained by habitat destruction. Elimination of ground squirrels or crushing of burrows may have made these areas unsuitable as burrowing owl breeding sites (Lincer and Bloom 2007).

Despite significant positive impacts on grassland ecosystems and key species of conservation concern, the role of ground squirrels remains a neglected aspect of conservation action in California. This can perhaps be attributed to the commonly held belief that ground squirrels are a “pest” species and are commonplace. However, because they are viewed as a nuisance, “…eradication campaigns have poisoned California ground squirrels by foot, horse, vehicles, and aircraft using a variety of chemical toxicants…, anticoagulants…, and burrow fumigants” (Lenihan 2007). These continued efforts toward eradication keep ground squirrels at 10-20% of their historical carrying capacity (Marsh 1987) in numbers too low to adequately perform their role as ecosystem engineer.

Where ground squirrels are absent and their ecosystem engineering role needed, translocation to re-establish squirrel populations is a potentially useful conservation tool. Prior to this program, translocations of ground squirrels were ineffective. Salmon and March noted “Our experience has been California ground squirrels released into an area will rarely stay”. In one translocation study, 83% of ground squirrels translocated using a hard release without acclimation immediately abandoned the release site (VanVuren et al. 1997).

We developed this CAGS translocation model as part of a long-term adaptive management conservation program designed to develop new approaches to assist in the recovery of western burrowing owls and their grassland ecosystem in San Diego County. Translocations occurred 2011-2013 and included a total of 703 squirrels. Squirrel activity and/or survival was monitored for up to four years post-release allowing us to assess population viability of release colonies.

BIOLOGY OF THE CALIFORNIA GROUND SQUIRREL

The California ground squirrel is a semi-fossorial rodent widely distributed throughout its range, which includes most of California, western Oregon, southwestern Washington and northern Baja California (Linzey et al. 2008). California ground squirrels occupy a variety of natural and urbanized habitats from grasslands and chaparral, to agricultural lands and urban parks. As herbivorous generalists, their diet consists of a wide range of foliage and seeds, but will also occasionally include invertebrates. CAGS are a semi-social species with small overlapping home ranges (Owings et al. 1977), ranging in average size from 270 to 375 m^2 for males, and 616 to 902 m^2 for females (Boellstorff and Owings 1995). The burrows of adult males are usually found around the outer perimeters of ground squirrel colonies.

Application of Behavioral Ecology to Improve Translocation Outcomes

Translocation is the intentional capture and transfer of wild animals or populations from one part of their historic range to another. Translocations are used to establish, re-establish or augment a wild population to increase the viability of a species or to supplement game populations. They are also used as a control measure to remove nuisance animals from areas where they are causing damage, and thus alleviate human-animal conflict. Most translocation research has focused on mammals and birds, but translocations have also been conducted with fish, amphibians and invertebrates. Though translocation has become an increasingly popular conservation tool, many translocations fail to produce sustainable populations and involve risks such as disease transmission. This has led to an increased interest in determining the factors that influence the success of translocations and designing methodology to minimize transmission of disease.

Post-translocation mortality is highest in the first days to weeks following release as animals make settlement decisions and modify the release-site habitat to accommodate their needs. Problems associated with this initial establishment phase include: 1) post-release dispersal (i.e. long-distance movement away from the release site), 2) predation, 3) stress response to the novel environment, 4) difficulty finding food, and/or 5) competition for resources (e.g. territories) with either conspecific residents or fellow releasees.

Among the proposed explanations for the high mortality during the establishment phase, post-release dispersal and predation are thought to be important factors. Immediate rejection of a release site indicated by post-release dispersal has been documented in many species. In some cases, translocated animals travel all the way back to their natal habitat (i.e. “homing”). Long-distance movement and, for many species, the required habitat modifications (e.g. digging burrows) leave translocated animals particularly vulnerable to predators immediately after release.

Post-release dispersal may initially be high for a variety of reasons. From an ecological perspective, animals may leave release sites because the habitat at the site is unsuitable or of low quality. From a behavioral ecological perspective translocated individuals may leave because they are site faithful, are not familiar with the physical characteristics of the release site or with the individuals with whom they were released, or because they are at a disadvantage when competing for resources with residents. Biologists studying translocations are beginning to understand these problems and modify translocation methodology to address these issues. For example, to dampen post-release dispersal and decrease stress, the IUCN recommendation is to select release sites with high quality habitat (IUCN 2012). Further, biologists have used “soft” release techniques, where the animals are provided with some form of support during the release (e.g. a period of time in an enclosure on the release site and/or supplemental food provided after release). Compared with hard releases (e.g. direct release without an acclimation period), soft releases are generally thought to enhance the likelihood of translocation success in small mammals, by increasing site fidelity and post-release survival. However, there are some species that exhibit distress behaviors when held in captivity or their habitat requirements are such that holding them in a soft-release cage may have a negative effect on their survival. For these species, hard releases are the best option.

Newly translocated animals may also leave the release site because there are no resident conspecifics, which may indicate that the habitat is unsuitable. For example, black rhinoceros (*Diceros bicornis*) move less during the first 5 days after release if cues that indicate conspecific presence (i.e. dung) are broadcast at the release site (Linklater and Swaisgood 2008).

Alternatively, or in addition, founder group composition may influence translocation success. Group size, age and sex class ratios, and familiarity between founder group members have all been shown to affect survival post-release. While there is a positive relationship between the size of the founder group and survival across taxa, suggested age and sex class ratios may vary by social system and life history strategy. For example, black bear (*Ursus americanus*) are polygynous. Males have large home ranges that overlap with as many as 9 females. Thus, suggested translocation schedules include a release of males prior to release of females to allow males time to establish breeding territories into which females can settle (Miller and Ballard 1982, Huber 2010).

Composing founder groups of intact social groups has been shown to have a significant influence on translocation success (Shier 2006, Shier and Swaisgood 2012). For black-tailed prairie dogs, founder groups composed of intact families were more successful in terms of post-release survival, reproductive success (Shier 2006), and population viability. Maintaining family unity appears to reduce the effects of successful predation on prairie dogs and dampen post-release dispersal. Results from this research suggest that any species dependent upon social interactions for survival and reproduction may benefit substantially from the maintenance of social groups during translocations. Perhaps surprisingly, maintaining social relationships may be just as important for some territorial species as for highly social ones. In solitary and territorial Stephens’ kangaroo rats (*Dipodomys stephensi*), individuals translocated with familiar neighbors survived at higher rates and had 24 times more offspring compared to kangaroo rats translocated without neighbors (Shier and Swaisgood 2012). These results may be explained in part by post-release behavior. Immediately following release, kangaroo rats translocated without known neighbors fought more and spent less time foraging compared to kangaroo rats that were translocated with known neighbors. Like unfamiliar kangaroo rats, territorial black rhinos translocated without attention to familiarity among founders exhibit high levels of intraspecific aggression following release, leading to serious injury and death (Linklater and Swaisgood 2008). Thus, choosing the right number and composition of animals and providing sufficient space at the release site can reduce these problems.

Predation is a significant impediment to translocation success (Kleiman 1989, Beck et al. 1994). The effects of predation are especially prevalent during the establishment phase. Most attempts to minimize predation on newly translocated animals have involved installation of predator exclusion fencing at the release site, monitoring of release sites post-release and/or predator removal.

In our program, we have studied the ways in which behavioral ecology can improve translocation success with California ground squirrels. Through these efforts, we have learned how to successfully translocate this species. While additional improvements to the translocation methodology described here are possible, this report provides a synthesis of the best practices developed to date.

TRANSLOCATION METHODOLOGY

Prior to Translocation

Release Site Selection

The first step in selecting a release site for any species is to restrict the search to sites within the species historic range. CAGS are found in a variety of habitat types throughout California (e.g. grasslands, open coastal sage scrub, open forests). Nevertheless, our research provides direction for the selection of ground squirrel release sites.

Habitat suitability

The next step is to find habitat that matches the species habitat preferences. Ideal grassland release sites for CAGS include areas with pristine open native habitat. However, most grasslands in California today are comprised of non-native grass species that create thick and tall vegetation structure that is suboptimal for ground squirrels likely because visual occlusion limits squirrel ability to detect predators (Ordenana et al. 2012) and interact with conspecifics. Therefore, if translocation of ground squirrels into these habitats is required, some form of manipulation will be needed to reduce vegetation.

In addition, the habitat requirements for translocated animals may differ from—and be more restrictive than—the type of habitat the species can survive and thrive in when they are established. As a prey species, CAGS are more vulnerable to predators following release because they do not yet have access to adequate burrows and other escape refuges. Two of the biggest impediments to CAGS translocation success are dispersal and predation. The first days to weeks following release show the highest morality rates while animals are making settlement decisions and establishing burrows as refuges. During this period, habitat that provides shelter and promotes burrow establishment is critical (Shier 2006, Moorhouse et al. 2009). Ground squirrel predators include rattlesnakes, raptors, and coyotes. Some predation is expected to occur, but efforts should be made to limit predation pressure by avoiding sites adjacent to raptor nests, perch sites, and established game trails. Raptors use old fence lines, power poles, trees, site markers, etc. as perches. If these features are present on the release site, they should be removed or the release location sighted at least 200 m away from these features to reduce the effects of predation by raptorial predators post release.

High quality soils are likely most important for early establishment following translocation as newly released CAGS are at a greater risk of predation until new burrow systems are in place. While the animals may be able to expand into less suitable (e.g. compacted) soils once established, the additional time required to dig a burrow into compacted soil may be the difference between life and death. Our results show better establishment of translocated California ground squirrels at release sites in which the soil clay content was below 20%. This association with soil type matches other ground squirrel species. Washington ground squirrels *(Urocitellus washingtoni)* and Townsend's ground squirrels *(U. townsendii)* prefer soils with higher silt content, lower sand and lower clay content (Betts 1990; Laundré and Reynolds 1993; Greene et al. 2009). Likewise, Lohr et al. (2013) found higher densities of burrows for Idaho ground squirrels (*Urocitellus endemicus)* where the soil content was higher in silt and lower in sand. Rocky or heavy clay soils can slow down squirrel digging rates, while highly sandy soils are prone to tunnel and burrow collapse. Soils with high clay content also have poor drainage and may be subject to flooding.

The level of predation is determined by a combination of habitat features conducive to predator hunting strategies and features that allow prey escape tactics. Release sites that contain some form of cover (i.e. boulders, logs, cacti, etc.) may provide necessary shelter to allow squirrels to effectively avoid predators and facilitate early settlement (Figure 1). Cover is particularly important in open grassland sites. While established CAGS can thrive in open grasslands with no cover (pers. obs.), establishing translocated populations in such sites will be more difficult and may require active management by providing additional cover to facilitate establishment. At sites with more raptor perches, additional effort to provide adequate cover may be required.

Sites that contain non-native vegetation but appropriate soils can be managed to create suitable release habitat for CAGS (see Release Site Preparation – Habitat Manipulation section).

Distance to Conspecific Residents

Conspecific interactions among releasees and residents may influence the establishment of new colonies. Within the release sites, low densities of resident squirrels may allow for conspecific attraction and dampen dispersal. On the other hand, if the release site is already occupied by a high density of resident ground squirrels, the releasees will have to compete with residents for resources which is likely to negatively affect release success. We conducted releases both into unoccupied habitat and occupied habitat via supplemental releases in which a second group of squirrels was released into the same site. Our results suggest that supplemental releases may have been more successful. However, because supplementation was confounded by other changes in release methods (i.e. release timing, addition of cover, etc.), we are unable to definitively determine if supplements were more successful than initial releases. Thus, in the absence of additional information, we recommend selecting unoccupied release sites or sites containing low numbers of squirrels with ample unoccupied habitat for translocated animals to settle. This recommendation follows the IUCN reintroduction guidelines (IUCN 2012).

Another consideration related to conspecific interactions that may influence release success is the presence of resident squirrel populations near the release sites (within 1000 m). Resident squirrels located off-site but within auditory or visual range can attract newly released squirrels away from the release site, reducing the density of released squirrels remaining on the site. Thus, we recommend selecting a site that is greater than 1000 m from an existing squirrel colony if possible.

Anthropogenic influences

Finally, it is important to consider anthropogenic influences when selecting a release site. Most obviously, humans may impact ground squirrels during holding in acclimation cages and after release if they expand into areas where there is a potential for persecution. If releases need to be conducted into a site in which human traffic is expected, signage and/or public education may be required to ensure that squirrels are safe while held in cages on site. Best practices dictate translocations be planned into areas where the target species can persist without impacts in the foreseeable future (IUCN 2012). Thus, because ground squirrels are often considered pest species by private landowners, care must be taken to locate release sites into areas in which long term protection is assured (e.g. away from private land not held in conservation). In addition, paved roads are a known source of direct mortality through vehicle strikes for squirrels (California ground squirrels, pers. obs.; red squirrels, Shutteworth et al. 2015), thus, another important constraint on release site placement is to select a site away from roads.

Release Site Preparation

Site Preparation

To limit dispersal and allow ground squirrels to acclimate to the new site, we recommend a soft release protocol. We prepared our sites for “soft release” by installing one acclimation cage per adult or group of two to six juveniles. Acclimation cages were set 10 m apart and consisted of an underground chamber connected to an above-ground retention cage made of hardware cloth (91.4 cm x 91.4 cm x 30.4 cm; mesh size of 1.27 cm [1/2 inch]) via 1.8 – 2.4 m-long sections of 10.16 cm (4 inch) perforated irrigation tubing. This design allows movement of ground squirrels between the underground chamber and the above-ground retention cage, but precludes escape during the acclimation period (Long et al. 2006; Figure 2). We constructed the underground portion of the acclimation cage out of hardware cloth, but switched to concrete form tubes to allow artificial burrows to become naturalized (Figure 3). The underground chamber was 30.4 cm diameter by 30.4 cm height (we used 12-inch concrete form tubes cut to the appropriate height), and was installed 91.44 cm underground using a backhoe. The dimensions of the trench were 121.92 cm long by 61 cm wide and 121.92 cm deep. We settled the chamber at the bottom of the trench and positioned the tubing in a curved configuration to simulate a squirrel burrow tunnel. The entrance end of the tubing protruded above the opposite corner of the trench. We placed cardboard over the top of the chamber to prevent soil from filling it once dirt was replaced. We installed a layer of chicken wire 10.2 – 15.2 cm below the soil surface then compacted the top layer of soil over the chicken wire to discourage excavation by coyotes. Once the belowground portion of the acclimation cage and the artificial burrow were installed, we set the above ground portion in place. It is also important to cover the entrance of the artificial burrow to prevent animals from taking residence in the acclimation cages prior to squirrel release. To do this, we covered external tube entrances with a fine mesh screen (aluminum window screen). We left the acclimation cage in place for several weeks to allow the materials to weather and to allow human smells to dissipate.

Habitat Manipulations

Non-native European grasses and/or forbs are found throughout reserves in southern California and if the release site is dominated by non-native plant species, these must be managed prior to setting up the release site to receive translocated ground squirrels. The herbaceous cover of these introduced grasses and forbs often creates an impenetrable thicket to small ground-dwelling vertebrates. To control these grasses and restore native ecosystems, managers and researchers have used controlled burns, grazing by ungulates, herbicide application, soil disturbance and/or mowing (Kenagy 1985, Kelt et al. 2005). The costs and benefits of these techniques for reserve management vary by situation and site. Some studies have shown that grazing is beneficial for decreasing dense cover (Germano et al. 2001), while others indicate that impacts of grazing are complex (Fehmi et al. 2005, Cox and Allen 2008) and grazing favors certain plant traits and thus may differentially affect native and non-native species (Kimball and Schiffman 2003, Middleton et al. 2006) while reducing the nutrient content of the grasses long-term. Kimball and Schiffman (2003) found that grazing negatively affected native species growth while alien species were unaffected and suggest that because European species have been exposed to grazing for centuries, these invaders may have adaptations that better enable them to recover from grazing. Our research shows that newly released squirrels were five times more likely to settle on release habitat that was mowed to reduce above-ground non-native grasses and indicates that translocations conducted into unmanipulated tall vegetation will fail.

Methods to Dampen Predation

As a prey species, the California ground squirrel is extremely vulnerable immediately following release. Thus, methods to reduce predation pressure during post-release establishment are important. Standard practices include predator removal and/or fencing to keep predators off of release sites. However, neither of these methods is consistently effective and if predators get onto release sites, many releasees will be killed. For example, in translocations with the black-tailed prairie dog, releases that incurred badger predation in the first days to weeks following release failed (Shier, unpublished data; Long et al. 2006).

Two of the primary predators of ground squirrels that are especially problematic on release sites are coyotes (*Canis latrans*) and red-tail hawks (*Buteo jamaicensis*). Both of these predators are medium-sized, that arrive on release sites immediately. Evidence from our translocations indicates that coyotes dig out acclimation cages and prey on ground squirrels during acclimation and visit release sites for several months following release.

Thus, to reduce depredation on newly released squirrels, release sites should have some form of cover. In the absence of existing cover, various types of cover can be added to the site and may improve release success. We constructed loose piles of brush with diameter of at least 1.2m and about 0.9m tall to provide refuge from predators and protection during settlement and burrow establishment. While we were unable to conduct a controlled experiment that examined the effect of cover on CAGS translocation success, our results indicate that newly released squirrels used the cover that we provided. We recommend using cover for future squirrel translocation efforts and are currently assessing the efficacy of using cover to encourage passive movement of squirrels. Our woodpiles were diminished over time; thus, larger brush piles or use of boulders would ensure the vertical structure lasts more than a few years.

Founder Group Size and Composition

In general, assuming enough available high quality habitat with little to no resident conspecifics, the larger the release group is, the higher the probability of survival. Our target density was 30-50 individuals per 0.79 ha plot, roughly one squirrel per 250 m^2. For rodents, research has shown that release groups of greater than 100 individuals fair better than those with fewer animals (e.g. prairie dogs; Robinette et al. 1995).

We attempted to move a ratio of one adult male to three adult females. Because females are the limiting sex, it follows that a male-biased release would not be as successful in terms of reproductive success as a female-biased release. We have not investigated this question directly. Translocation often requires moving whatever individuals are present at a source site, therefore when we include juveniles and young of the year our average sex ratios were roughly 55% female to 45% male.

As mentioned above, research on the effect of social behavior and/or familiarity on release success shows that intact social groupings can substantially improve release success in both social and solitary species (Shier 2006, Shier and Swaisgood 2012). Therefore, we released ground squirrels in familiar groups by identifying group associations during mark-recapture studies at source sites before translocations. While mark-recapture may not be feasible, capturing and moving individuals from the same and adjacent burrow entrances (i.e., presumed groups) might improve survivorship and reproductive success with minimal additional effort and cost.

Release Timing

The ideal time of year to translocate ground squirrels is in the early fall. We arrived at this decision based on several criteria. First, one wants to avoid the height of the reproductive period (February-June), if possible. During this period, females are not in prime condition as reproduction is highly energetically expensive and they may have dependent young in the burrow. Second, it is important to avoid periods of extreme temperatures and periods of precipitation. Because ground squirrels are held in acclimation cages for 7-10 days, they are unable to thermoregulate in a natural burrow system, thus, any extreme temperatures or rain may kill animals in holding. In the San Diego grasslands, daytime temperatures rise in the late spring and throughout the summer and can easily exceed 37.7°C (100°F). CAGS are known to burrow 0.6 – 1.8m below ground (Reynolds and Wakkinen 1987) and can have an extensive network of tunnels with multiple chambers (VanVuren and Ordenana 2012). This structure and depth buffer the squirrel burrows from extreme temperatures. In Otay Mesa during the month of June in 2014 and 2015, we found that the average temperature inside squirrel burrows was 26.6°C (79.8±3.5°F) with a mean difference from outside temperatures of 12.5±8.4°F. Since acclimation cages may not have the same buffering ability as a natural burrow against extreme outside temperatures, we recommend not placing squirrels in acclimation if temperatures exceed 32°C (90°F). Precipitation can be problematic if animals are being held in acclimation cages as water can travel down artificial burrows and fill the nest chamber. Thus, it is important to ensure that animals are not being held during a period of medium to heavy rainfall. Comparison of release outcomes of June versus August translocations show that late season release supported higher survival, potentially because juvenile squirrels were independent and better able to fend for themselves and/or the because late summer is when juvenile squirrels typically disperse. If a fall release is not an option, late spring/summer can be used. However, pregnant and lactating females should be avoided.

Translocation Methods

Capture and Holding Animals for Release

We recommend that the release group be composed of a familiar social group, a recommendation that requires extra effort to capture, mark, release, and recapture squirrels once social relationships are determined. We assessed home range and burrow ownership by trapping, sexing, aging, marking, releasing, and then observing interactions of ground squirrels in source population(s). We captured squirrels in single door Tomahawk traps baited with black oil sunflower seeds. We pre-baited traps for a minimum of two days prior to trapping to acclimate squirrels to entering the traps. At the source sites, we placed traps 10 to 20 m apart in rough grid patterns overlaying areas with existing burrow systems. During a trapping session, we checked all traps within two hours of opening. The number of two-hour sessions scheduled between 7 AM and 1 PM varied based on temperatures staying below 29.4°C (85°F). We placed captured squirrels in conical handling bags (Koprowski 2002), sexed, weighed, marked or identified, and released them. Upon release we recorded the burrow used by the squirrel.

Prior to translocation, all animals within the source colony were identified. We recorded age, sex, weight and reproductive condition for each squirrel. Individuals were marked with standard small animal ear tags (Monel #1005-1) and unique dye markings for individual identification. Another less time intensive way to capture familiar social groups without conducting mark, observation, recapture is to trap heavily in a small core area keeping track of where animals are captured and the relationship of those captures on the landscape. While this method will not ensure that all animals from social groups are moved together, it is likely that most of the animals trapped will be familiar with one another.

During translocation, we temporarily held animals in a quiet temperature-controlled facility (60-80°F) until we captured the necessary number of animals and weather conditions were suitable for release (i.e. no precipitation and overnight temperatures were above 4.4°C (40°F) most nights). Upon capture, animals were checked for physical condition to minimize risk of disease transmission. Animals should be in good body condition (e.g. have no ocular or nasal discharge, no significant hair loss or skin lesions, solid feces, not exhibit any signs of disease/illness such as lethargy, coughing, sneezing, or muscle tremors/seizures, no open wounds or exposed bone), and all ectoparasites should be eliminated by dusting with commercial flea power if necessary (Sikes et al. 2011).

Ear tags may be lost over time, therefore we permanently marked squirrels while in the holding facility with 8.4 mm radio-frequency identification (RFID) tags (Biomark Inc.). The tags are injected subdermally along the dorsum with a sterile Trovan lancet R1.3 x 35mm. Prior to implantation, the site was thoroughly cleaned with isopropyl alcohol. After implantation, we cover the needle insertion point with 2 to 3 drops of Vetbond™ tissue adhesive.

We constructed two sizes of holding cages from hardware cloth, large (0.9 x 0.9 x 0.3 m; mesh size of 1.27 cm [1/2 inch]) for juvenile groups or adult female groups, and small (0.9 x 0.6x 0.3 m) for solitary adults. Young and adult California ground squirrels cannot be housed together during the holding period as conspecific aggression occurred. However, individuals were placed in holding cages together if they were known to use the same burrow systems at source sites. Each cage had hide tubes consisting of four-inch perforated triplewall corrugated HDPE pipe, a

90° PVC elbow and a PVC cap with holes drilled to allow airflow (Figure 4). We provided a tube for each adult or one tube per 3-4 juveniles. Trays beneath the cages were filled with wood shavings, and a one-inch layer was also provided inside the cage for bedding material. We provisioned squirrels daily with commercial rodent pellet, sweet potato (two 1-inch slices per individual per day) and apple (¼ per individual per day). Day-old sweet potato and apple were removed to prevent ingestion of mold and to monitor appetite. Each cage also had a drip water bottle attached. Peanut butter was smeared on the spout to train squirrels to lick. We tracked the water level daily by marking the water bottles with dry erase pen. Large changes in water level indicated leakage.

Translocating squirrels directly to acclimation cages may be an option if sufficient numbers can be captured, moved and released at the same time. Issues arise when animals are released and others are being held in acclimation as the newly released animals may interact with those in acclimation cages. In prairie dogs, animals released on a site prior to other groups pilfered supplemental food from inside acclimation cages and in some cases were able to compromise the acclimation cage and allowed the caged prairie dogs to escape (Shier, pers. obs.).

Tracking animal care

We maintained a care sheet to track daily information while animals were in holding (Appendix A). In addition, we recommend maintaining a log book to qualitatively record details such as the circumstances of squirrel injuries or deaths.

IACUC and Zoonosis

All squirrel handling should occur under the authorization of an approved animal care protocol plan, which will require planning and protocol development for activities such as: disposition of animals that are not suitable for translocation, humane euthanasia, carcass disposal plan, administration of anesthesia, emergency veterinary care in the case of a trap injury or illness in the holding area, and response to accidental animal bite incidents.

The most significant disease affecting ground squirrels is bubonic plague (caused by *Yersinia pestis*) which is transmitted by fleas. Fleas must therefore be eliminated prior to moving squirrels from one location to another. We recommend dusting all squirrels with evidence of fleas prior to bringing them into a holding facility. This disease is also zoonotic (transferable to humans). Biosecurity protocols should be developed to establish plans for:

- Cleaning and disinfection of animal holding area and equipment
- Personal protective equipment requirements (Leather gloves and non-latex exam gloves)
- Hand washing facilities/hand sanitizer
- Awareness of zoonotic diseases, e.g. plague

Release

Release to New Site

Once all of the animals needed for the translocation are captured, they are transported to the release site and kept in the acclimation cages for approximately one week. We placed adult males in separate cages around the outside perimeter of the plots. Adult females were placed either alone or in pairs. For mixed-age cages, the mean number of squirrels/cage was 3.45. While in acclimation, ground squirrels were fed daily with the same diet as in holding which allows monitoring of their food intake and activity, based on whether there was movement of soil within the acclimation cage. At the end of the acclimation period, the above-ground portion of the cage was removed. Supplemental feeding should continue for one to three months after release while conditions are dry, or until vegetation begins to grow after rain. We found that released squirrels continued to forage on sweet potato and quartered apples but often ignored the rodent pellet. We attempted to minimize predation by chasing potential predators off the release site at least three times per week for the first month following release.

Post-release Monitoring

Assessments of short-term survival provide a snap shot in time but without multiple data points, release population trends cannot be assessed. We recommend a post-release monitoring period of five years to determine release success and population viability of the release population. There are several options of determining the success of the translocation, each with pros and cons. Some are more labor-intensive, but also more informative for adaptive management (e.g., provide insights as to why translocations succeed or fail).

Translocation success is usually determined by a combination of: settlement at release site, survival, and reproduction. Reproduction is essential for long-term population establishment, and squirrels born at the release site will be less likely to disperse from the site compared to founders.

We conducted 4 different types of assessments to determine release success: behavioral observations, trapping, radio-telemetry, and burrow surveys.

Behavioral Observations

We conducted behavioral observations on focal individuals for four weeks immediately following release. We also followed radio-collared individuals for at least three months post-release. These data provide information on how individuals interact with conspecifics, heterospecifics and the environment at the release site and whether there are any immediate threats that may cause failure of the translocation (e.g. immediate dispersal from the release site, conspecific fighting, depredation, etc.). Sightings of marked squirrels also complement trapping for mark-recapture population monitoring, and it is not uncommon to sight squirrels that have not been re-trapped.

Trapping protocol

Live-trapping is the standard method for monitoring small mammal populations. It relies on the use of marks (permanent and temporary can be used) so that individuals can be identified reliably (see marking methods above). We have found that the small number of squirrels at release sites and low capture rate (perhaps as low as 30% of the population) makes it impossible to use formal mark-recapture analysis due to lack of statistical rigor. Low capture rates also provide rationale for complimentary approaches such as behavioral observations. Thus trapping data reflect the minimum number of translocated squirrels (and their offspring and any immigrants) remaining at the release site, rather than a population estimate.

Trapping can be done at any interval following release, depending on management needs for determining translocation outcome. In a low-effort low-information translocation, squirrels may be trapped at 2 years and 5 years post-release. This would establish whether or not the translocation was ultimately successful, but would not allow managers to make management actions that improve the likelihood of successfully establishing squirrels. More frequent trapping would allow managers to determine if predation, drought, dispersal, non-native vegetation regrowth or other factors jeopardized the translocation outcome, and allow them to take action, such as supplemental translocations, habitat modification, supplemental food, or predator control.

If possible, we recommend conducting five days of trapping at one, six and twelve months post-release and annually thereafter. This trapping allows for the documentation of both survival and reproductive success. Traps were placed along three intersecting transects overlaying the plots. We conduct trapping surveys once in the morning and once in the evening, ensuring that traps are not open for more than two hours. Also, if temperature exceeds 85°F, traps are checked and closed. Timing of trapping is also important, as there are pronounced seasonal trends in above-ground activity by squirrels. Winter hibernation and summer aestivation should be avoided; the timing of these periods can vary with inter-annual variation in climate and resources.

Radio-telemetry

By far the most costly and labor-intensive method of post-release monitoring, this method is largely reserved for more research-oriented translocations, or those where managers need to closely follow the outcomes for individual squirrels in order to make management adjustments to improve translocation outcomes. It might be selected, for example, following a failed translocation attempt in order to determine why it failed and establish improved translocation methods for future translocations. Because large numbers of squirrels are typically translocated, a more manageable subset of squirrels is often selected for radiotracking. Radio-telemetry is the best method for determining post-release movements and the only way to reliably distinguish between mortality and dispersal. Trapping and other methods will invariably yield many “unknown” outcomes, whereas with radiotracked, squirrels mortality can be reliably detected. Radio-telemetry is also the best method for determining cause of death, which can be extremely useful for informing translocation and release site management strategies. For radio-tracking squirrels during our project, we used Holohil 4-gram PD-2C VHF transmitters attached via collars and ensured that the total package weight did not exceed 5% of the animal’s body weight (Sikes et al. 2011). If detailed data on post-release movements are of interest, 1 fix per day over the course of 1-2 months post-release would be sufficient to capture settlement decisions.

Burrow surveys

A simple management tool that allows translocation outcome to be evaluated is burrow-count surveys. Although burrow numbers do not correlate perfectly with squirrel population size, they are a good indicator of establishment of a squirrel population and, more importantly, they allow the manager to document the outcome of the translocation in terms of the most important ecosystem service the squirrels provide: burrow creation. Unlike other methods discussed above, this method provides little insight into why translocated squirrels succeed or fail to establish and does not distinguish between low survival on site versus high dispersal away from the site, two very different problems for a manager to address.

To monitor burrow creation, observers walk a grid pattern through the release site and record California ground squirrel activity. Burrows with an opening of at least 7 cm at the point of maximum diameter are considered probable California ground squirrel burrows (Lenihan 2007). Burrow locations are marked with GPS, and the size and shape of both the burrow entrance and the burrow apron (the disturbed area around the burrow cleared of all vegetation) are recorded. The condition of the burrow entrance (i.e. clear, cobwebbed, collapsed) is recorded, as well as other field notes about burrow condition and use.

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Figure 1. a) California ground squirrel utilizing agave as cover while vigilant and b) CAGS burrow adjacent to cover.

a)

b)

Figure 2. a) Above and below-ground components of the acclimation cage.

b) Below-ground chamber of acclimation burrow made of concrete form tubing.

Figure 4. Refuge tubing for squirrels in holding cages. Note: the green grate seen in the photo was used only during transport of squirrels from holding to acclimation cages.

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Appendix A. Example Care Sheet

California Ground Squirrel Care Sheet Explanation

Room ________ CAGS Care Record ____ Page ____

Date	Cage #	Source Site	Food √	Appetite	Water	Line? √	Notes	Health	RB? √	Init

DATE: Date of feeding/care

CAGE #: ID of cage

SOURCE SITE: Source location of squirrels

FOOD (check): Check off when fed

APPETITE: Good, Fair, Poor (way to track if animals are eating, poor appetite may indicate that they don't like the food/a particular food they are being given or might indicate a health problem)

WATER: Y or N to track if they are using the water bottle or if the bottle is leaking; we use peanut butter on the bottle spout to encourage them to use it, so we might also write "p" in the column to indicate peanut butter was put on the spout

LINE? (check): We use wet erase markers to draw a line at the water line on the water bottle (for tracking water use)—we do this in holding and in acclimation

NOTES: We mostly use this column to keep track of how much food was put in each cage (e.g. a=apple slice, y=yam slice, c=carrot, RB=rodent block (pellet), so we might say 8a, 1y, 2c, RB to indicate 8 apple slices, 1 yam slice, 2 carrots, and a scoop of rodent block); we also use this column to make note of specific food NOT eaten (e.g. carrots not eaten)

HEALTH: This column is to make short notes on health status or concerns that may or may not be further explained in the Red Book. We also use this column to keep track of how many squirrels we actually see at the time of feeding (usually they are hiding in their tubes).

RB? (check): We check this column if we made a note in the log Book .

INIT: Initials of person(s) that fed/cleaned that particular cage. This is important if there is a problem later or if a note doesn't make sense so you can ask the person who initialed about it.