

**Amendment to Desert Tortoise Translocation Plan for Fort Irwin's  
Land Expansion Program at the U. S. Army National Training Center  
(NTC) & Fort Irwin**

**Prepared for**

**U.S. Army National Training Center, Directorate of Public Works**

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# 1. Clearance Procedures for the Western Expansion Area

## General Information

The Western Expansion Area (WEA) will be searched in its entirety (250 km<sup>2</sup>) using one pass by tortoise survey teams. If >4 adult tortoises are found within one square-km, then that area will be surveyed a second time in its entirety. In an intensive search of a portion of the SEA, we found approximately 70% of the adult tortoises with 2 passes, and >95% with 2 human- plus 2 canine-team passes (Nussear et al. 2008). However, based on apparent densities observed on previous surveys of the Western Expansion Area (WEA), the decision was made to limit surveys in low-density areas and repeat surveys in grid cells (1km<sup>2</sup>) on which >4 tortoises are found. Tortoises remaining in the WEA post-translocation will be subject to safety protocols in place for the rest of the National Training Center.

Removal of tortoises from the WEA must begin by Spring 2010 and be completed by Spring 2011 if military activities are to commence by July 2011 (see Appendix 1 - Timeline). This requires determining the number of tortoises in the WEA, and complete preparation of translocation sites (selection of specific release sites, screening the health of resident tortoises, planning for fencing where required, contracting, etc.). Tortoises will be counted and disease testing of tortoises in the WEA will be completed by fall of 2010. Permits and authorizations for all activities related to tortoise capture and handling will be acquired prior to any surveys from appropriate agencies (i.e., Fish and Wildlife Service, California Department of Fish and Game, and Bureau of Land Management). All work identified below is subject to state and federal permits and may be altered or modified to meet permit conditions or based on new information, as appropriate.

The development of this document was guided by input from a variety of sources beyond that of the authors and contributors. Other sources included guidelines from the IUCN (1998), and the Science Advisory Committee to the US Fish and Wildlife Service, Desert Tortoise Recovery Office, and several anonymous reviewers.

## WEA Clearances

### *Tortoise Encounter Procedures*

Upon locating each tortoise during surveys the following information will be recorded: date and time tortoise is located, sex, location of each animal (determined using a GPS), air temperature at 5cm above the ground, tortoise identity (see below), carapace length (mm), mass (g), general notes on appearance and health/condition. All data will be recorded on standardized data sheets provided in this document (Appendix 2A) and input into the online database at [www.deserttortoise.gov/dtms](http://www.deserttortoise.gov/dtms).

Tortoises found during clearance surveys will be fitted with an external label and notched using the highly modified Honegger System (Appendix 2B), and adult tortoises will have a light-weight radio transmitter attached with a battery life of at least two years. Smaller tortoises are to

be fitted with transmitters with an 11- or 12-month battery life. Transmitters will be attached using methods similar to those described in Boarman et al. (1998). All transmitters will be monitored at least monthly until they are translocated to a release site. Approved handling techniques will be used as required by the State and Federal permits. After processing and data collection, tortoises will be released as soon as possible at the point of capture. Time of release will also be recorded.

All tortoises that are too small to receive an 11 or 12-month transmitter will be removed from the field and transported to a temporary outdoor holding facility. The holding facility will be maintained according to all legal and ethical requirements for treatment of captive animals (e.g., Animal Care and Use Guidelines from an official university ACUC program, ASIH 2004).

### ***Health Screening Of Tortoises Prior to Translocation***

All tortoises (juvenile and adult) will be inspected for clinical signs of upper respiratory tract disease (URTD), signs of a herpesvirus infection (lesions in the mouth), or signs of other debilitating diseases. Minimally, blood samples will be collected for laboratory analysis; collection of additional biomedical samples may be added as approved techniques for monitoring desert tortoise health are developed. For example, although diagnostic tools for the identification of herpesvirus in some tortoise species have been developed, there are currently no diagnostic tools that have been shown to confirm the presence of herpesvirus in desert tortoises. Based on discussions among CMWG members, the development and validation of diagnostic herpesvirus tools seems imminent (University of Florida – Small Animal Clinical Sciences 2009). Should they become available they will be added to the toolkit for diagnosing disease. In the meantime it is likely that field samples will be requested for testing and this project is prepared to accommodate some of that work. Future references to herpesvirus work in this document should all be considered with this in mind.

Only healthy tortoises will be translocated, although classifying individual tortoises as sick or healthy includes uncertainties. For the purposes of this translocation, “healthy” tortoises are defined as those: a) lacking clinical signs of acute infection and; either b) testing negative for *Mycoplasma testudineum*, *M. agassizii*, and herpesvirus antibodies using an ELISA test (similar to Martel et al. 2009, but requiring testing on desert tortoises before approval as an appropriate test for determining the fate of animals in this program), or; c) if testing positive to *M. agassizii*, and herpesvirus antibodies with the ELISA test, showing a natural antibody response with a Western blot (Hunter et al. 2008) and Polymerase Chain Reaction (PCR), respectively. Complete details for conducting health evaluations on desert tortoises are provided in Appendix 3.

### ***Monitoring of Tortoises***

Tortoises will be tracked at least monthly in the WEA until they are picked up and moved to the translocation area. Upon locating each animal the following data will be recorded: tortoise number, date, time, location (acquired with a GPS), general location description, temperature (°C, measured at 5 cm above ground as per permit terms and conditions). Any pertinent information related to any change in the condition or health status of the individual will also be recorded upon locating each animal, if possible. These are the minimal data to be collected, and the needs may be increased with further discussion.

## **Western Translocation Area (WETA)**

The area considered for prospective translocation covers 1,153.6 km<sup>2</sup> to the southwest of the National Training Center at Fort Irwin (NTC) in southern California, USA, which is entirely within the Superior Cronese Critical Habitat Unit (Figure 1). Criteria for prioritizing potential translocation sites included biological and anthropogenic factors affecting desert tortoise populations in the Western Mojave Desert Tortoise Recovery Unit. We identified the translocation area by considering potential release sites relative to land ownership, habitat, proximity to unfenced roads and highways, proximity to urban areas, road density, potential areas with depleted tortoise populations, and utility corridors. The site-selection decision support model is described in Appendix 4. The areas selected for desert tortoise translocation include any map unit (square-mile sections on Figure 2) with a weighted value greater than 0.5, which includes all green-shaded areas and indicates that these would be the most favorable sites for translocation, considering all of the criteria identified. Only lands owned and managed by the Army or BLM shall be used for translocation sites. State lands are not being considered due to administrative burden related to such activities.

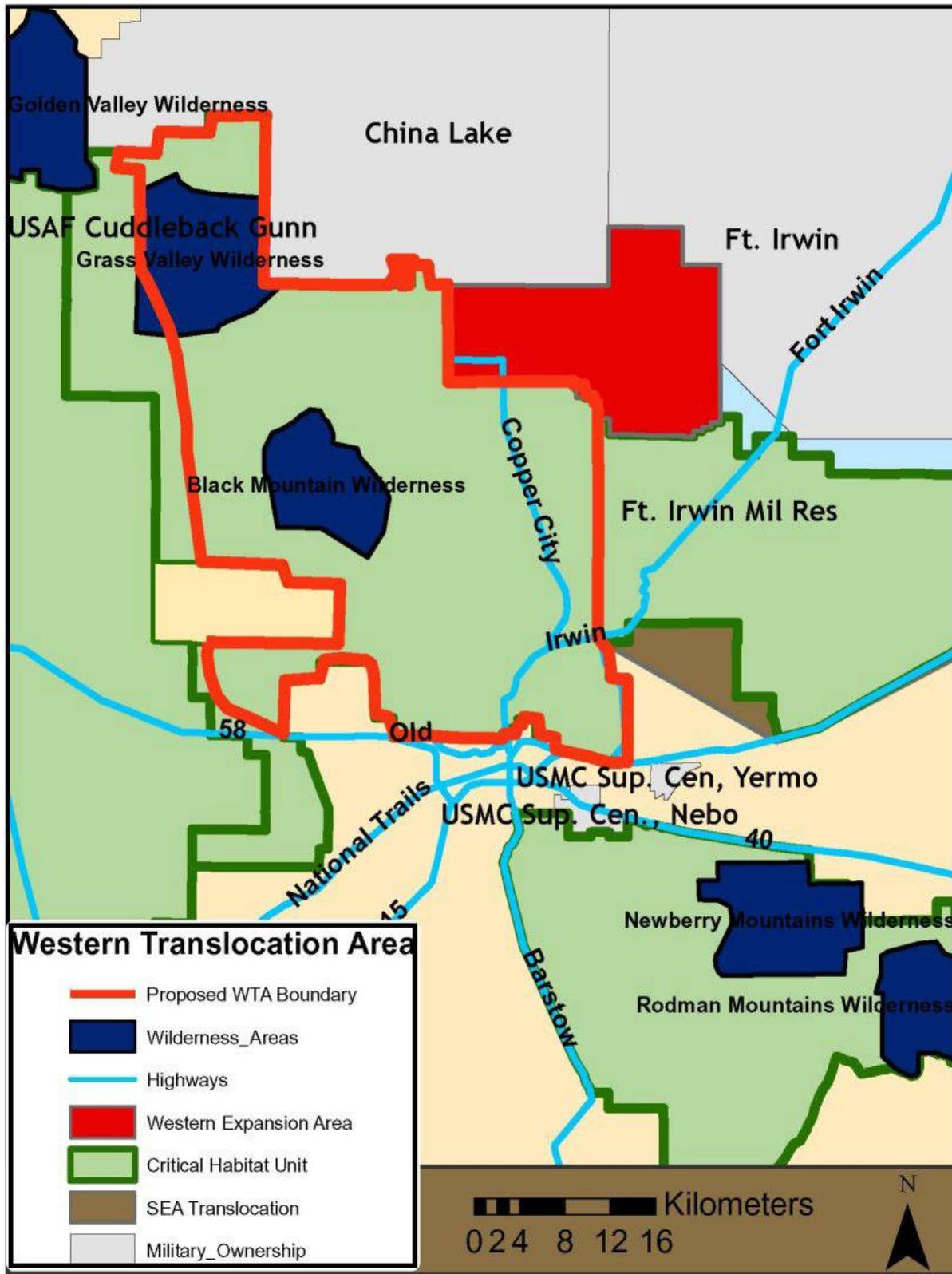


Figure 1. Location of the area considered for translocation of tortoises from the WEA.

One important topic for the translocation of desert tortoise is the disease status of those tortoises being translocated versus the disease status of the resident tortoises in the area where other will be translocated to. This is a topic of current debate in wildlife management and the issue must be balanced with respect to many factors regarding translocation which we consider here. The fact that disease occurs in the WETA may or may not be a problem for translocated tortoises, especially in light of the fact that disease also exists in the WEA. Thus, the animals that are proposed to be translocated are at risk of disease exposure in either location. Anonymous reviewers of this plan (in addition to the original translocation plan) noted that if tortoises reside in a population where disease is present, then it may make no difference if they are translocated into a population with disease. Furthermore, technically, these animals are all part of the same population as there are no known geographic or genetic barriers between the two areas where tortoises occur at this time, thus one would expect tortoises and disease to move through the area over time. One of the benefits of the 5 yr monitoring program for the WETA, and continued monitoring of tortoises in the Southern Translocation Area, is to determine if this is a problem worth worrying about during future management actions.

### ***Disease Testing of Resident Animals in the WETA***

Preliminary results of disease surveys in the Western Expansion Translocation Area (WETA; Berry 2009) indicated that a more thorough and complete survey is required to capture the spatial distribution of disease in the WETA. Data from the disease surveys conducted during the Southern Expansion Area clearances and from the residents in the southern translocation area were analyzed to determine the scale of autocorrelation in the presence of disease. For this analysis, we compared the spatial distribution of animals that tested positive or suspect with those animals that were considered negative using ELISA-based tests for *Mycoplasma agassizii* and *M. testudineum*. The analysis was used to evaluate the likelihood that sick versus healthy tortoises are clustered and if we could identify clusters where disease is prevalent. Areas of disease prevalence could then be avoided while deciding where to place translocated animals. To accomplish this we analyzed the presence/absence of disease using spatial glm (sglm) with binomial error distributions using R (version 2.8.1, R Development Core Team 2009) and the geoRglm package (version 0.8-24, Christensen and Ribeiro 2002). Estimates indicated that the presence or absence of disease as measured by animals that tested positive or suspect was spatially autocorrelated, with an effective range of ~ 5 km (Figure 3). This indicated that in order to sample the WETA with sufficient precision to detect areas that contained clusters of potentially diseased animals we should sample the area at this scale. We selected center points within a regular grid of sections in the WETA that were predicted to be suitable for translocation such that the maximum distance diagonally between sampling locations was 5-km (Figure 4). Where the pattern of suitable sections on the landscape caused larger areas not to be sampled we adjusted the sampling grid accordingly. This resulted in a pattern of 64 sample points within alternating sections within which health sampling of tortoises should be conducted. The goal of the health surveys should be to find animals for health surveys in and around the sample points, and not 100% coverage of the sections themselves.

Each survey will include walking surveys at 7.5 m intervals throughout each selected survey area (2.6 km<sup>2</sup>). A minimum of 10-15 tortoises should be located on each survey area. Each animal encountered will have full health surveys and sufficient blood sample collected for analysis of

known pathogens including: *M. agassizii*, *M. testudinum*, and herpesvirus ELISA, PCR and Western Blot where applicable.

If a sampling location containing diseased resident animals (including suspect laboratory test results) is detected during disease sampling in the WETA, then a 5 km buffer will be placed around the “diseased” animal(s). Translocated tortoises will not be released within this 5 km buffer. Buffer size was determined by an analysis of the spatial distribution of disease found in the SEA (Fig 3). This distance is also one-half the average first-year, straight-line distance moved by translocated tortoises as reported by Field (1999), Nussear (2004), and SEA translocation monitoring (Drake et al. 2009, Berry 2009, Walde et al. 2009). This will minimize

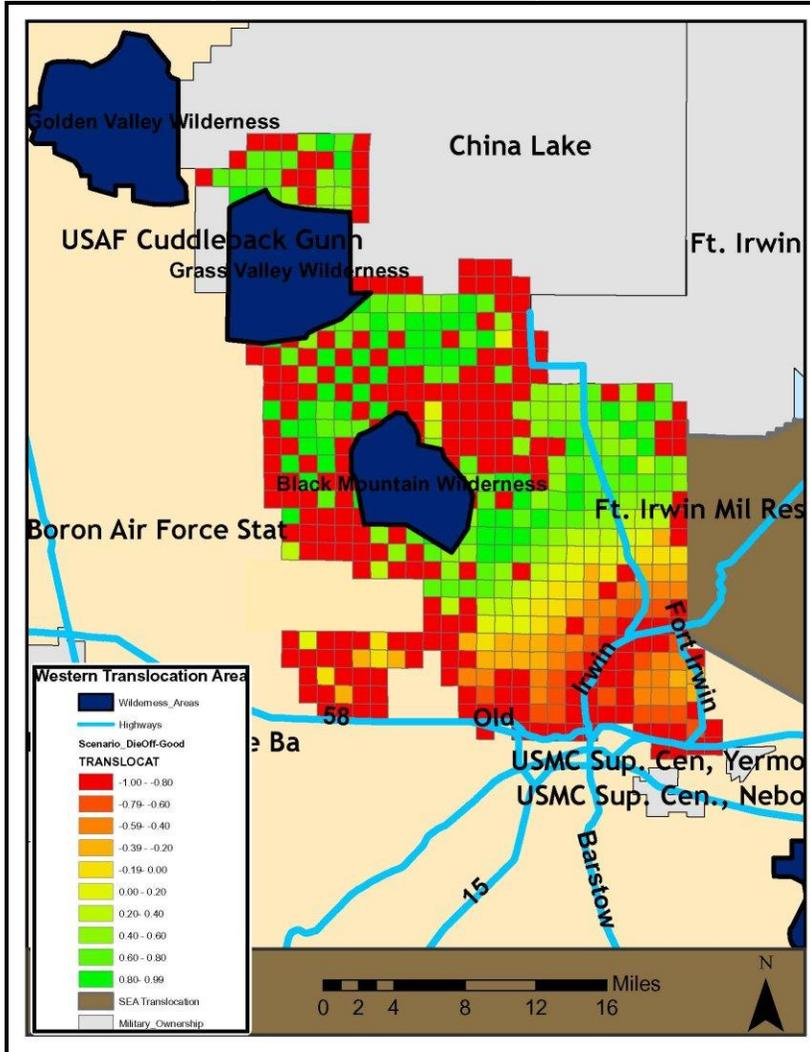
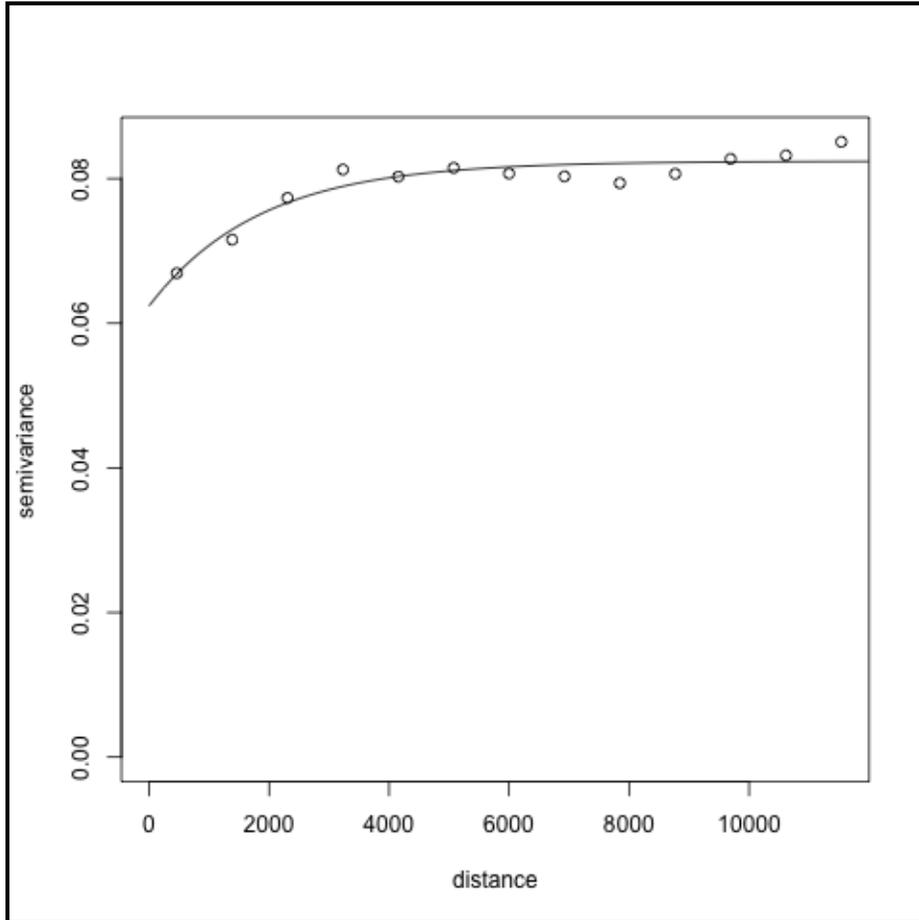


Figure 2. Results from the Translocation Suitability model for the Western Translocation Area. Colors indicate suitability where red is considered unsuitable through green considered highly suitable.

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translocation monitoring (Drake et al. 2009, Berry 2009, Walde et al. 2009). This will minimize contacts between translocated tortoises and potentially ill resident tortoises, thus minimizing the risk of spreading of disease in the WETA. If additional release sites are required for this translocation action, a re-evaluation of disease sampling around affected areas should be conducted.

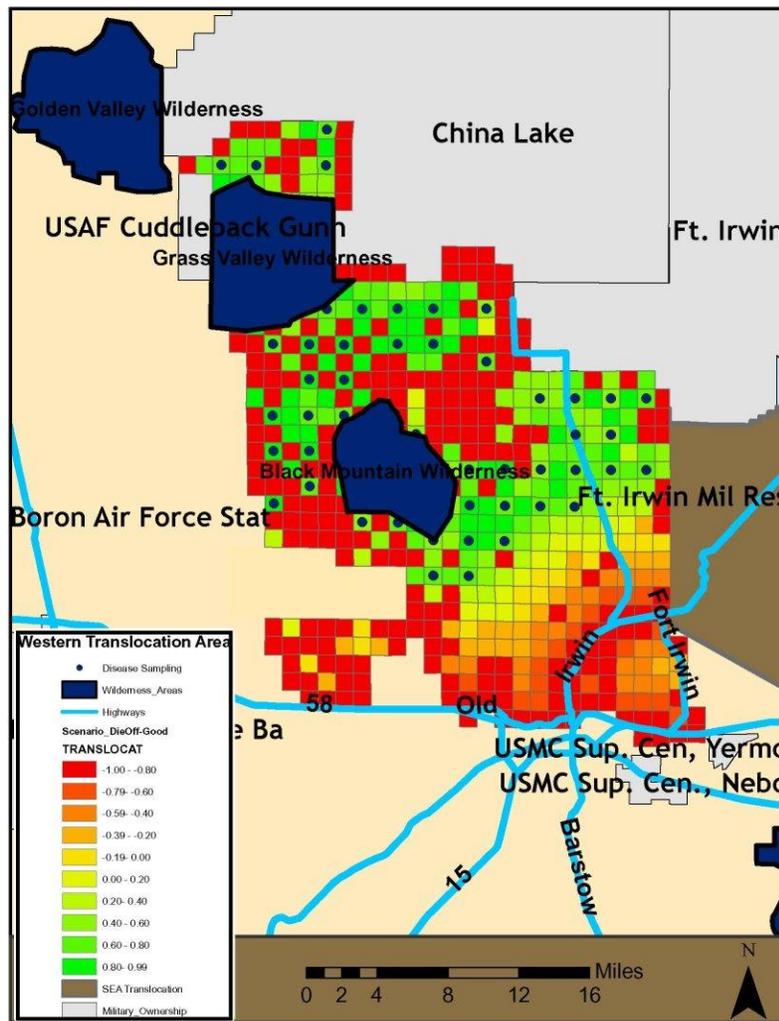


*Figure 3: Semivariogram showing the autocorrelation in the spatial aggregation of positive/suspect and negative tortoises for *Mycoplasma agassizii* and *M. testudineum* as evaluated by ELISA. Here, lower semivariance indicates a higher covariance among animals that are closer together, leveling off at approximately 5,000 meter distance (5km).*

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***Predation and Predator Control***

High levels of predation were observed immediately prior to and subsequent to translocation from the southern expansion area. Analyses of the southern expansion area data indicated that translocated tortoises were not preyed upon differently from resident tortoises or resident control animals that were at large in the area (Esque et al. Unpublished Data). Moreover, additional data from more than 10 sites spanning the Mojave Desert and representing sample populations of desert tortoises that were monitored throughout the Mojave Desert in the same time period as the translocation illustrate that very high predation rates were Mojave Desert-wide in their extent (Esque et al. Unpublished Data). Although on-the-ground predator control was initiated in 2008, it was not possible to identify offending individual predators, and only minimal results on coyote control were obtained (2 coyotes removed). Under these and related circumstances, predator control is unlikely to be successful for protection of desert tortoises in relation to this particular project (Goodrich and Buskirk 1995). The U.S. Fish and Wildlife Desert Tortoise Recovery Office Science Advisory Committee (DTRO-



*Figure 4. Results from the Translocation Suitability model for the Western Translocation Area. Colors indicate suitability, where red is considered unsuitable through green considered highly suitable. Blue circles indicate the center of selected disease sampling areas.*

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SAC) recommended that large-scale predator control is not a valid management action, based on a lack of evidence of its effectiveness, unless conducted under an experimental design.

### ***Fencing and Other Considerations***

No additional fencing is scheduled to occur in relation to the removal of tortoises from the WEA and into the WETA. The CMWG considered fencing the section of Old Irwin Road that crosses the southeast corner of the WETA. If it were possible to fence that area, fewer desert tortoises would likely be killed attempting to cross the road. However, after extensive discussions between the Army and the County, fencing was considered to be logistically unfeasible due to the propensity of the area to sheet flood and the resultant extensive washouts of fencing. Investing in fencing that area of highway would only provide a false sense of security because the county could not assume the cost of maintaining the fence. The southern and eastern boundary of Naval the Air Weapons Station, China Lake, will be fenced to prevent desert tortoises from entering Fort Irwin from the weapons station.

## **2. Translocation Procedures**

Selection of recipient sites (by CMWG) and all pertinent inter-agency agreements will be finalized prior to translocation of animals. In addition, the Army will coordinate with any ongoing research in the area. The need for fencing of any tortoise containment facilities will be identified so that construction of those fences can be planned, contracted, implemented and completed in time for the sites to receive tortoises from the expansion areas prior to training activities (see Appendix 1, Time Line of Activities).

### **Disposition and Distribution of Desert Tortoises from the WEA**

Those tortoises found to be healthy (Appendix 3) will be moved to predetermined, dispersed release points within the Western Expansion Translocation Area (WETA). Tortoises will be dispersed in a regular pattern throughout the WETA so that tortoise densities will remain as low as possible. Tortoises will not be purposefully re-distributed randomly, whenever possible, they

will be released in cohorts that include individuals that were collected in proximity to one another. There were ~ 205 sections that were identified by the model to have a suitability value of 0.5 or higher, indicating that these would be the most favorable sites for translocation, considering all of the criteria identified (Figure 2). Selected suitable habitat sections will be evaluated and ground-truthed by qualified personnel prior to translocation under the auspices of the U.S. Fish and Wildlife Service and California Department of Fish and Game. Current estimates indicate that as many as 1,000 adult tortoises (MCL>180mm) will need to be translocated from the WEA (Walde et al. 2009). If all of these animals are healthy and all sites are suitable for translocation, we will need to translocate approximately 4 animals per section to distribute them evenly across the suitable landscape. However, should there be any areas excluded by buffering diseased resident tortoises in the WETA, then the total area available for translocation will be decreased and the release density of tortoises in each area will increase accordingly.

### ***Translocation Procedures***

Translocations will only occur in the spring (i.e., March – early May) and fall (i.e., late September to mid-October), to avoid extremely high or low temperatures (Cook et al. 1978, Nussear 2004). Tortoises will not be released in the summer (i.e., June - August), or winter (i.e. late November through February) for any reason. No desert tortoise will be captured, moved, transported, released, or purposefully caused to leave its burrow for whatever reason when the ambient air temperature is above 95 degrees Fahrenheit (35 degrees Celsius). No desert tortoise will be captured if the ambient air temperature is anticipated to exceed 95 degrees Fahrenheit before handling or processing can be completed. Tortoises found in burrows will be “tapped” to encourage them to exit (Medica et al. 1986) or they may require careful excavation. Multiple visits will be necessary if tortoises are inaccessible in caves. Tortoises with radios that were attached during clearances or other activities will be collected from field sites and transported in vehicles or helicopters to the translocation sites by biologists that have been approved by the U.S. Fish and Wildlife Service and California Department of Fish and Game to handle desert tortoises, and released within 24 hours. Juvenile tortoises (those too small for radio attachment) housed elsewhere after clearance, will be translocated at this time as well. During translocation, tortoises will be transported in clean, disinfected protective containers to ensure their safety during translocation. If re-used, these containers will be disinfected using a 10% bleach solution before being used to translocate other tortoises.

Upon release, any tortoise that defecated will be rinsed with clean water. All tortoises will be provided drinking water for 30 minutes and will then be released into an unoccupied tortoise burrow (if available) or in the shade of a shrub. Previously, desert tortoises released into artificially made burrows showed no fidelity to those sites, often leaving them immediately (Field 1999, Nussear 2004, Boorman et al., unpubl. data). Suitability of release depends on the severity of the daily ambient temperature at the time of release (Lohoefer and Lohmeier 1986, Corn 1991, Field 1999, Nussear 2004).

In previous studies on translocation, animals were observed after release under similar conditions to those proposed herein, and virtually all those animals were able to find suitable shade resources generally without showing signs of overheating or thermal duress; only two individuals

showed temporary signs of thermal stress, by frothing, but both of them survived this episode (Field 1999, Nussear 2004). More recently, during the southern area expansion translocation of >640 adult tortoises, two were observed to exhibit behaviors related to overheating and subsequently one of those individuals died (K. Berry per comm.). Thus, it is imperative that these procedures be followed and desert tortoises be monitored for signs of problems even when conditions seem conducive to translocation.

A subset of tortoises (20%) will remain equipped with transmitters upon release and will be monitored with a similar cohort of residents and resident control animals at least biweekly for the first year after the translocation (see subsequent section on post-translocation monitoring in the WETA). Thereafter, tortoises will be monitored at least monthly for a period of 5 years.

### ***Post-translocation Disposition of Tortoises in the WEA***

Tortoises that are not found during the clearances of the expansion areas may be encountered at a later date during military training or other activities. Tortoises found in the WEA after the translocation will be left in place unless they need to be moved from imminent danger if encountered as per current Directorate of Public Works (DPW) procedures on the National Training Center (NTC).

### ***Monitoring Design for Resident Tortoises in the WETA***

Guidance from the CMWG has indicated that post-translocation monitoring of tortoise populations in the WETA is warranted in order to be consistent with the basic tenets of the Translocation Plan: 1) humane treatment of desert tortoises; 2) contribute to the conservation of the species by adding to the knowledge base; and 3) incorporating the most up-to-date and best science practices in all activities. After the first year, tortoises should be monitored at least monthly for a period of 5 years. With this in mind additional hypothesis-driven monitoring in the WETA should focus on basic health and well-being of the tortoises involved while providing new information and testing tools when logistically and fiscally feasible. The monitoring program will include basic assessments of survival of the affected and control populations, fundamental measurements of tortoise movement and behavior, testing of basic and experimental health physiology profiles, and development of new tools for tortoise conservation.

Although monitoring the population with controls is costly, it has also proved to be one of the most important tools for understanding the potential effects of translocation versus other factors that can affect tortoise populations. Although translocation is a large focus of the work with these animals, the southern expansion area translocation illustrated how changes in local land use, other management activities, and direct or indirect results of environmental changes can also affect the tortoises and can have important ramifications for the translocated population. When properly designed, information on the movement and behavior parameters are acquired with little cost if the bi-weekly (first year) and monthly (thereafter) monitoring of tortoise survival and locations is properly organized. Basic health profiles for known tortoise diseases such as *Mycoplasma* spp. and various strains of herpesvirus will be taken annually at a minimum.

Considering all these factors, the best monitoring design incorporates 3 populations of tortoises

for monitoring which is consistent with the design in the Southern Expansion Translocation Area (SETA). This design can accommodate a variety of research questions and has already proven very important during the translocation in the SETA. One year of post-translocation experience has illustrated that this design has successfully handled unforeseen problems that arose due to excessive predation across the desert (Esque et al. *Unpublished Data*). The 3 populations include the translocatees, residents, and resident control animals (Esque et al. 2005). The overall design will require slight modifications because of general differences in the translocation that have already been accepted by the CMWG. For example, the dispersed releases of tortoises could compromise the integrity of control tortoises. To resolve this potentially confounding fact, we plan to study tortoises in Wilderness areas as control animals with perhaps some additional animals spread throughout the study area. In addition, any ELISA-positive tortoise with an innate-immunity banding pattern that is translocated (see below) should be monitored. We expect this design to include all ~1100 translocatees (Walde et al. 2009). We plan to sample and monitor 20% of those tortoises and to study comparable numbers of residents and control animals for a total of somewhere near 660 tortoises in the monitoring program.

### 3. Literature Cited

- ASIH. 2004 Guidelines for use of live amphibians and reptiles in field a laboratory research. Second Edition. Revised by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists. (Committee Chair: Steven J. Beaupre, Members: Elliott R. Jacobson, Harvey B. Lillywhite, and Kelly Zamudio).
- Berry, K.H. 2009. An evaluation of desert tortoises (*Gopherus agassizii*) and their habitats at 20 sample plots in the Western Expansion Area, Fort Irwin Translocation Project, San Bernardino County, California. Report from U.S. Geological Survey to NTC & Fort Irwin. 44p.
- Boarman, W. I., T. Goodlett, and P. Hamilton. 1998. Review of radio transmitter attachment techniques for turtle research and recommendations for improvement. *Herpetological Review* 29:26-33.
- Christensen, O.F. and P.J. Ribeiro Jr. 2002. geoRglm: A package for generalised linear spatial models. *R-NEWS*, Vol 2, No 2, 26–28.
- Cook, J. C., Wever, A. E., and G. R. Stewart. 1978. Survival of captive tortoises released in California. *Proceedings of the Desert Tortoise Council*, Las Vegas, NV.
- Corn, P. S. 1991. Displacement of desert tortoises: Overview of a study at the apex heavy industrial use zone, Clark County, Nevada. Pages 295-303 in *Proceedings of the Desert Tortoise Council*.
- Drake, K.K., T.C. Esque, K.E. Nussear, P.A. Medica, K.M. Nolte, and B.M. Jacobs. 2009. An annual report for the Fort Irwin desert tortoise translocation project (2008). Unpublished Report. 24 pp plus appendices.
- Esque, T. C., K. E. Nussear, and P. A. Medica. 2005. Desert tortoise translocation plan for Fort Irwin's land expansion program at the U. S. Army National Training Center (NTC) at Fort Irwin. Prepared for U. S. Army National Training Center, Directorate of Public Works. <http://www.fortirwinlandexpansion.com/Documents.htm>
- Field, K. J. 1999. Translocation as a conservation tool applied to the desert tortoise: effects of the

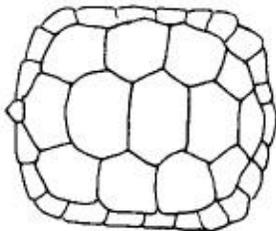
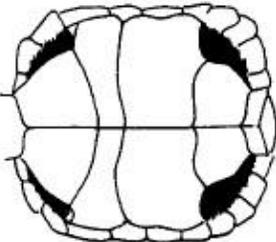
- pre-release availability of water. Masters Thesis. University of Nevada, Reno, NV.
- Goodrich, J.M., and S.W. Buskirk. 1995. Control of abundant native vertebrates for conservation of endangered species. *Conservation Biology* **9**: 1357-1364.
- Hunter K.W. Jr., S.A. Dupré, T. Sharp, F.C. Sandmeier, and C.R. Tracy. 2008. Western blot can distinguish natural and acquired antibodies to *Mycoplasma agassizii* in the desert tortoise (*Gopherus agassizii*). *Journal of Microbiological Methods*. **75**(3): 464-71.
- IUCN. 1998. Guidelines for Re-introductions. IUCN/SSC Re-introduction Specialist Group, IUCN, Gland, Switzerland, and Cambridge, UK.
- Lohofener, R., and Lohmeier, L. 1986. Experiments with gopher tortoise (*Gopherus polyphemus*) relocation in southern Mississippi. *Herpetological Review* **17**:37-40.
- Martel, A., S. Blahak, H. Vissenaekens, and F. Pasmans. 2009. Reintroduction of clinically healthy tortoises: the herpesvirus Trojan horse. *Journal of Wildlife Diseases* **45**(1):218-220.
- Medica, P. A., C. L. Lyons, and F. B. Turner. 1986. "Tapping": A technique for capturing tortoises. *Herpetological Review* **17**:15-16.
- Nussear, K. E. 2004. Mechanistic investigation of the distributional limits of the desert tortoise, *Gopherus agassizii*. Unpublished Ph.D. thesis. University of Nevada, Reno.
- Nussear, K.E., T.C. Esque, J.S. Heaton, M. Cablk, P.A. Medica, and C. Valentin. 2008. Are wildlife detector dogs better at finding tortoises than humans? *Herpetological Conservation* **3**(1): 103-115.
- University of Florida – Small Animal Clinical Sciences. 2009.  
<http://www.vetmed.ufl.edu/college/departments/sacs/research/documents/SUBMISSION%20PROTOCOL%20FOR%20DNA%20PCR.pdf>. Accessed on 1 May 2009.
- Walde, A., W.I. Boarman, and A.P. Woodman. 2009. Desert tortoises estimates on the Western Expansion Area of Fort Irwin. Unpublished Report. 6 February 2009. 13 pp.

## **Appendix 1. Time Line of Activities Related to the Translocation of Desert Tortoises.**

- a. Spring 2009**
  - Evaluation of potential recipient areas
  - Interagency agreements, i.e., NEPA, land uses and right-of-ways
  - Apply for State and Federal permits
  - Apply for animal care and use committee approval for animals held in captivity
  - Order equipment (transmitters may take 6 month prep time)
  - Fencing plans in place for conservation research area
  - Test and telemeter residents in the recipient sites and begin monitoring them
    - Note: the timing on disease testing dictates that tortoises tested in one season (e.g. spring or fall) are not eligible for activities involving other tortoises until the subsequent season (fall or spring, respectively).
  - Collect baseline environmental data on WEA and recipient sites
  - Build juvenile tortoise holding pens
  - Continue surveys and radio attachment in WETA
  - Sample residents for disease in WETA
- b. Fall 2009**
  - Begin full scale surveys in the WEA / radio attachment / blood work at WEA
  - Place juvenile tortoises in holding pens within an outdoor facility
  - Annual review with Conservation Mitigation Working Group
  - Blood sampling in WETA
  - Finish translocation all remaining SEA tortoises
- c. Spring 2010**
  - Continue clearance surveys / radio attachment / blood sampling at all WETA recipient sites
  - Begin translocation tortoises from WEA if possible
  - Blood sampling in WEA
- d. Fall 2010**
  - Continue translocating tortoises from WEA
  - Annual review with Conservation Mitigation Working Group
- e. Spring 2011**
  - Complete translocating tortoises from WEA
- f. Summer 2011**
  - Military training begins
- g. Fall 2011-2015**
  - Continue to monitor tortoises
  - Annual review with Conservation Mitigation Working Group

## Appendix 2A. Field data sheet for use in the WEA

The following data sheet is to be used for tortoise encounters on this project.

LIVE TORTOISE TRANSMITTER DATA FORM					
Fieldworker(s) _____		Date (dd/mmm/yy) _____		Tortoise ID# _____	
		Time : Start _____ End _____		WEA <input type="checkbox"/> Sex _____	
Fort Irwin, NTC Expansion		Temp. (2 cm above surface, °C) _____		SEA <input type="checkbox"/> Control <input type="checkbox"/> Resident <input type="checkbox"/>	
County <u>San Bernardino</u> State <u>CA</u>		Photo: Digital <input type="checkbox"/> Film <input type="checkbox"/>		Translocation Site No. _____	
<b>TORTOISE LOCATION</b>		<b>ACTIVITY</b>		<b>TRANSMITTER</b>	
<input type="checkbox"/> At sheltersite <input type="checkbox"/> Not at sheltersite <input type="checkbox"/> in tunnel <input type="checkbox"/> in open <input type="checkbox"/> at mouth <input type="checkbox"/> under cover: <input type="checkbox"/> on mound <input type="checkbox"/> of shrub <input type="checkbox"/> face in <input type="checkbox"/> of rock <input type="checkbox"/> face out <input type="checkbox"/> pallet <input type="checkbox"/> sideways <input type="checkbox"/> unknown <input type="checkbox"/> nearby (dist-m) _____ m		Resting <input type="checkbox"/> Basking <input type="checkbox"/> Walking <input type="checkbox"/> Feeding <input type="checkbox"/> Digging <input type="checkbox"/> Asleep <input type="checkbox"/> Mating <input type="checkbox"/> Combat <input type="checkbox"/> Unknown <input type="checkbox"/>		NEW    OLD Brand _____ Number _____ Freq. _____ Best Freq. _____	
				<b>PURPOSE OF VISIT</b>	
				Transmitter, Retransmitter, etc. _____	
				Transmitter Type	
				Round(Big) <input type="checkbox"/> Single-Bat. <input type="checkbox"/>	
				Round(Med) <input type="checkbox"/> Double-Bat. <input type="checkbox"/>	
				Round(Small) <input type="checkbox"/> Juvenile <input type="checkbox"/>	
<b>VOIDING (All Encounters)</b>		<b>MEASUREMENTS/Shell Wear Class</b>		UTM (NAD 1983)	
Urine Amt. (ml) _____		MCL (mm) _____		(Easting) _____	
Particulates Amt. (ml) _____		Max. Height (mm) _____		(Northing) _____	
Color (Circle) _____		Max. Width (mm, at bridge) _____			
Clear Yellow Brown Color (Circle) _____					
White Grey Pink Color (Circle) _____					
<b>NARES (Circle)</b>		<b>POSTURE/BEHAVIOR</b>		<b>LEGEND FOR DIAGRAM</b>	
YES NO UNK Signs of Disease ONLY Nasal exudate dry/wet <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Exudate color _____ Rt/Left naris occluded <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Amt Occlusion % (Rt/Left) _____ / _____		YES NO UNK Appropriate for time of year <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Appropriate for time of day <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Alert, responsive <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Can withdraw tightly in shell <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Lethargic <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Limbs hanging loose <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>		Cutaneous dyskeratosis <input type="checkbox"/> Trauma <input type="checkbox"/> Note source, severity if active/healed/healing	
<b>FORELEGS</b>		<b>EVIDENCE OF SHELL DISEASE (Draw also)</b>		DRAW TRANSMITTER, NOTCHES, & LABEL LOCATIONS  	
Dried exud. on scales <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>		Cutaneous dyskeratosis <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Surface area Carap/Plast/F-Limbs (%) _____ Fungal areas <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>			
<b>EYES (Circle as needed)</b>		<b>EVIDENCE OF TRAUMA TO:</b> (draw also)			
Rt/Left Eye sunken <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Rt/Left Eye bulging <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Rt/Left Eye clear, bright <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>		Head <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Forelimbs <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Hindlimbs <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Gular <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Carapace <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Marginals <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Plastron <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Type (predator, impact, etc.) _____			
<b>EXTERNAL PARASITES:</b>		<b>SCUTE NUMBER ANOMALIES:</b>			
Ticks <input type="checkbox"/> <input type="checkbox"/> Number _____ Ass. w/ Trans. Equip. <input type="checkbox"/> <input type="checkbox"/> Mites <input type="checkbox"/> <input type="checkbox"/> Number _____		Notched This Encounter <input type="checkbox"/> <input type="checkbox"/> Gel-epoxy "Notch" (juveniles only) <input type="checkbox"/> <input type="checkbox"/>			
Epoxy Type(s) & Color: Transmitter _____ Antenna _____					
Other ID No.'s: _____					
<b>OTHER NOTES:</b> _____					
Version: May 4, 2008					

## Appendix 2B. Notching protocol for newly marked tortoises

### Notching Protocol for Newly Marked Fort Irwin Tortoises

By A. Peter Woodman and William I. Boarman

September 11, 2007

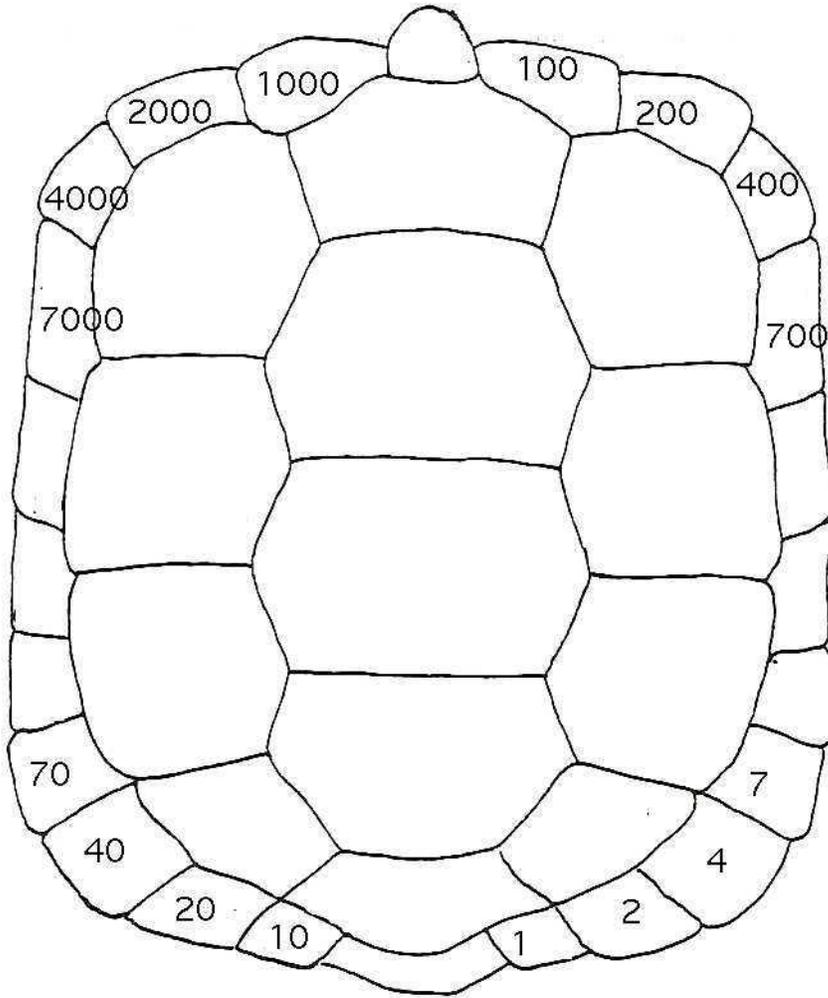
All tortoises will be notched with the Highly-modified Honegger notching system (Fig. 3B-1; see below). The tortoise should be held firmly to the ground and the notches filed forcefully with a downward motion making sure that the animals head and legs are not in the path of the file strokes. All notches will be filed with a sharp, triangular file. Files will be replaced as they get dull or begin to rust (due to bleach used for disinfection). Notches will be filed deeply, but not so deeply as to scar the bone. The flat surface or “V” at the apex of the notch cut with a triangular file are diagnostic and will be more likely to be observable if deep. As much as possible, notches will be placed on the anterior or posterior portions of the scute to minimize impacts to the bone sutures. Locations of notches will be first marked with a felt pen or in a similar manner and double checked to help ensure that notches are made on the correct scutes.

A number of previous surveys have been conducted on the Southern Expansion and Translocation Areas and some tortoises have been notched using the Berry System. The notches used for the previous surveys were shallow nicks. All existing notches on relocated tortoises will be notched more deeply when part of the new tortoise ID number. Previous notches on scutes that do not need to be notched for the current effort will be left, but noted on the data form.

At the time of notching floy tags will be inspected to ensure they are legible. If not, they will be replaced with numbers printed on paper then epoxied onto the shell (fourth right costal) Epoxied and other numbers that are not legible will be replaced. Un-notched tortoises will be notched when they are re-transmitted, but not when they are translocated, since doing so may cause additional stress with unknown effects, potentially confounding interpretation of results.

One standard system for marking turtle shells was described by Rene Honegger (Marking amphibians and reptiles for future identification. International Zoo Yearbook 19:14-22; 1979) of the Zurich Zoological Garden and used widely throughout Europe. It apparently is a modification of a system developed by Froese and Burghart (A dense natural population of the common snapping turtle (*Chelydra s. serpentina*). Herpetologica 31:204-208; 1975). It uses the numbers 1, 2, 4, and 7 and marginals 1-4 and the last four marginals (Figure 1). At Fort Irwin, all tortoises will be marked using the following modification to the Honegger System (Fig. 1). The scute next to the supracaudal will be the number 1 (on right) and 10 (on left), the next one would be 2 (or 20), the third would be 4 and 40, and the fourth 7 and 70. This progression is somewhat more intuitive than the Honneger System and will likely reduce errors in notching and deciphering the code under field conditions. The four right front marginals will represent the hundreds (100, 200, 400, and 700), and the four left front marginals will represent the thousands (1000, 2000, 4000, 7000). In juvenile tortoises, the four bridge scutes (scute numbers 4, 5, 6, and 7, counted from the pygal scute, on right and left) will be avoided whenever possible. Hence, tortoise numbers in the 700, 800, 900, 1700, 1800, 1900, etc., and 7000, 8000, and 9000 series will be avoided whenever possible. To minimize confusion, tortoises will be marked and notched using the number series (FW5000-FW5999) within the WEA and number series (FW7000-

FW7999) within the WETA.



*Figure 2B-1. Highly Modified Honegger System for marking desert tortoises at Fort Irwin, California.*

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## Appendix 3. Health protocols for desert tortoises

### Background

One goal of the Fort Irwin Translocation Project is to translocate healthy tortoises that have high potential to establish themselves at new sites. Tortoises that are debilitated from disease or previous traumas may be unsuitable for translocation. Protocols are already available to evaluate tortoises for general health and disease and to identify tortoises suitable for salvage (Berry and Christopher 2001). The protocol in this appendix is focused on evaluating and testing of tortoises for infectious diseases in the Western Expansion Area (WEA). Tortoises with infectious disease should not be translocated because they present a threat to naïve individuals and populations.

The most commonly known infectious diseases in wild desert tortoises are upper respiratory tract diseases (URTD) caused by *Mycoplasma* spp. and *Pasteurella* (Snipes and Biberstein 1982, Roberts et al. 2008). Some evidence exists for herpesvirus (Christopher et al. 2003, Johnson et al. 2006), but a strain from wild desert tortoises has yet to be isolated, characterized, and sequenced (Francesco Origi, pers. comm.). There are other infectious diseases as well (Homer et al. 1998, Jacobson 1994, 2007).

No single test or clinical sign of disease is useful in determining whether a tortoise has or is capable of transmitting an infectious disease (e.g., Brown et al. 2002, Ritchie 2006). Enzyme-linked immunoassay (ELISA) tests for *Mycoplasma*, for example, may provide an indication of prior infection or of current anti-*Mycoplasma* antibody status, but they do not reveal whether a tortoise was shedding the bacteria at the time the blood sample was taken (Brown et al. 2002, Wendland et al. 2007). A recent study by Hunter et al. (2008) has found evidence of natural antibodies in tortoises to *M. agassizii*, indicating that caution should be applied in interpreting ELISA-positive results because these tortoises may not have been previously exposed but simply carry natural immunity that can only be distinguished from acquired immunity through the use of western blots. *Mycoplasma* species can be cultured by taking oral swabs and nasal lavages, but are generally very difficult to grow. Once cultured, they can be identified by polymerase chain reaction (PCR) tests. Thus a combination of clinical signs, ELISA and PCR tests, western blots, and cultures can be useful in diagnoses. Herpesvirus presents similar problems: many strains exist and others need to be identified (e.g., Origi et al. 2004, Ritchie 2006, Martel et al. 2009). For some herpesviruses, ELISA and serum neutralization tests are used for antibody detection and may be available; diagnostic testing can include PCR tests, biopsies, identification of virus particles with electron microscopy, cell cultures, and several other techniques (e.g., Origi et al. 2004, Ritchie 2006). In summary, even though we prescribe all known assays (i.e. those with at least 1 published and positive validation of their efficacy) in this plan, it is possible that the generality of the ELISA tests and the specificity of the PCR testing in combination with clinical observations may present us with information that individual tortoises are not well, but specific diagnoses of what disease is present are not possible with the tools available now.

Tortoises with an infectious URTD caused by mycoplasmosis or herpesvirus may have a nasal discharge (Jacobson et al. 1991, Brown et al. 1994, Schumacher et al. 1997, Ritchie 2006). When the nasal discharge is present, the tortoises may be more likely to transmit pathogens to other tortoises. For example, in early studies of *M. agassizii* in desert tortoises, the relationship

between clinical signs of URTD and the ELISA test for *M. agassizii* was evaluated by Schumacher et al. (1997). Ninety-three percent of tortoises with mucous nasal discharge tested seropositive, and the presence of nasal discharge was highly predictive for exposure to *M. agassizii*. In transmission experiments, naïve tortoises were infected with *M. agassizii* by using the nasal discharge (Brown et al. 1994).

Tortoises can have subclinical disease or latent infections. They may have no clinical signs and be shedding bacteria or viruses (Schumacher et al. 1997, Ritchie 2006, Martels et al. 2009). For example, the ELISA test for *M. agassizii* also detected potential subclinical infections in 34% of tortoises without clinical signs of disease (but see Hunter et al. 2008). Less is known about the relationship between clinical signs for tortoises with *M. testudineum* or herpesvirus, ELISA and PCR tests, and cultures. Veterinarians recommend that tortoises surviving herpesvirus infections be kept isolated from other tortoises and not translocated because they are still capable of infecting other individuals (Ritchie 2006, Martels et al. 2009).

When tortoises with positive serological tests for either *M. agassizii* or *M. testudineum* or both species were necropsied, they were found to have mild to severe lesions in the nasal cavities (Jacobson et al. 1995, Homer et al. 1998, Jacobson and Berry 2009). Tortoises without clinical signs of URTD may have negative serology for *M. agassizii* but may have lesions in the nasal cavities; these tortoises may have subclinical disease (Jacobson et al. 1995). We do not have similar information for *M. testudineum*. We do not know the frequency or prevalence of tortoises with negative ELISA tests and lesions in the nasal cavities typical of mycoplasmosis in a population.

### **Field Protocols for Health Evaluation**

This health evaluation protocol has been designed to identify tortoises with clinical and subclinical infectious diseases and to remove such tortoises from the translocation program. These actions are essential to safeguard both the recipient population from exposure to potentially infectious diseases, as well as the translocated individuals. The following procedures are illustrated in Figure A3-1, and the numbers that accompany each part of the procedure in the following paragraphs are used to label the procedure (Figure A3-1).

The first step (1, Fig. A3-1) is to identify tortoises with clinical signs of disease, particularly infectious diseases that would render them unsuitable for translocation using the standard health evaluation form (Berry and Christopher 2001, modified appendix). For the purposes of this translocation project, clinical signs of acute infection are defined for URTD as nasal or moderate-to-severe ocular discharge (U.S. Fish and Wildlife Service 2008 [Appendix B], Berry and Christopher 2001). Clinical signs of a previous or dried nasal discharge include eroded nares or partially or completely occluded nares. Clinical signs of dried ocular discharge can be manifested as crusts and dried mucus on the palpebrae, periocular area, fornix, and beak. Signs of dried nasal and ocular discharge must be obvious and should not be confused with dried dirt or mud on the beak and nares from recent rain events. For herpesvirus, typical clinical signs are plaques on the tongue, palate, and other parts of the mouth (Origgi et al. 2004, Ritchie 2006). Emaciated or moribund tortoises should be salvaged for necropsy.

A subcarapacial or brachial blood sample will be taken (Hernandez-Divers et al. 2002) with special attention given to avoiding lymph in the sample and dilution. Notations shall be made on the data sheet about potential presence of lymph in the sample, and where necessary, sampling may need to be repeated. For small and large adult tortoises >180mm CL, up to 2 ml may be collected. Tortoises <100mm may have <5% of total body weight drawn in blood samples (ASIH 2004). The protocol provided by Dr. L. Wendland, based on the following equation with estimates in Table 1, is useful:

$$\text{Maximum blood draw (ml)} = \text{Body Weight of tortoise to be bled (kg)} * 1000 \text{ g/kg} * \text{estimated 6\% blood volume} * 10\%$$

Table 1. Amounts of blood that may be drawn from small tortoises by carapace length at the midline (mm, MCL).

Size of tortoise (mm, MCL)	Amount of blood to be drawn (ml)
< 80*	0.15–0.25, with the upper level more desirable. For the 45 g tortoise, the lower number must be used.
80–100	0.5–0.6
>100–140	0.6–1.0
>140–179	>1.0–2.0

The mouth will be examined by a person trained in identifying the clinical signs of herpesvirus infections and may be swabbed for use in analyses of potential herpesvirus infection research (University of Florida-Small Animal Clinical Sciences 2009). If the tortoise has no acute clinical signs of infectious disease, a radio transmitter shall be attached, and the tortoise shall be released *in situ* (Step 2, Fig. A3-1). Tortoises with acute clinical signs of infectious disease (Step 7, Fig. A3-1) will be removed from the field after the health evaluation is completed, a blood sample is collected (Hernandez-Divers et al. 2002), a swab of the mouth taken, and a nasal lavage is conducted for cultures and a PCR test for *Mycoplasma* spp. (Brown et al. 2002). These tortoises will be taken to previously established quarantine facility at the southeast corner of the Western Expansion Area, where they will be maintained as 1 tortoise per individual isolated compartment (suggested size  $\geq 100 \text{ m}^2$ ) while the laboratory samples are being analyzed.

Juvenile tortoises encountered will be processed in the same manner as adult tortoises, with the same protocol. However, all animals too small to receive an 11- or 12-month transmitter will be removed from the field and transported to a temporary outdoor holding facility. The holding facility will be maintained according to all legal and ethical requirements for treatment of captive animals (e.g., Animal Care and Use Guidelines from ASIH 2004).

### Management of Blood Samples in the Field and in USGS Labs

Blood samples will be immediately placed on ice and centrifuged within 4 hours of sampling. After centrifuging, plasma will be separated from the red blood cells and stored in liquid nitrogen, dry ice, or in a freezer until samples are shipped to a reputable laboratory for testing. The plasma samples sent to the lab should contain a minimum lymph (<10%) to minimize the potential for dilution and a false negative test. Red blood cells that are a by-product of the centrifuging process will be stored for potential future genetic analyses. Nasal lavage sample will

also be chilled immediately and fast frozen on dry ice or in a freezer within 4 hours of collection. A separate protocol shall be developed for swabs of the mouth for testing herpesvirus. Where and how this protocol is to be developed is under consideration (K. Berry – *personal communication*).

## **Laboratory Testing**

Blood samples from both groups of tortoises (acute clinical signs vs. no acute clinical signs) will be submitted to a qualified laboratory for testing. For all tortoises, the tests shall include ELISA tests for *M. agassizii* and *M. testudineum*; Western Blot for *M. agassizii*; and available ELISA, serum neutralization and other appropriate tests for herpesvirus. For tortoises with acute clinical signs, cultures and PCR will be undertaken for *Mycoplasma* spp.

## **Disposition of Tortoises After Laboratory Results Are Available**

For the group of tortoises with no acute clinical signs: if all lab tests are negative (Step 3, Fig. A3-1), the tortoise will be translocated (Step 4, Fig. A3-1). If any test is positive (Step 5, Fig. A3-1), then the tortoise will be moved to the quarantine facilities (Step 6, Fig. A3-1), retested and re-evaluated at 6-week intervals until the health status is clarified. Upon re-test, ELISA-positive individuals showing innate-immunity banding patterns with the western blot will be translocated and included in the monitoring program. If a tortoise has a suspect test while remaining in the WEA, it will also be retested and re-evaluated at 6-week intervals until a definitive test result is confirmed. It will not be moved to quarantine unless additional tests are positive or it shows acute clinical signs of infectious disease.

For the group of tortoises with acute clinical signs: if all lab tests are negative (Step 9, Fig. A3-1), the tortoise will be re-tested and re-evaluated after a 6-week interval to double-check test results (Step 8, Fig. A3-1). If any test is positive (Step 5, Fig. A3-1), then the tortoise will remain in the quarantine facilities (Step 6, Fig. A3-1) and a decision made for further disposition (Steps 10-12, Fig. A3-1). If the tortoise has suspect test(s), it will also be retested and re-evaluated after a 6-week interval and will be maintained in quarantine (Step 6, Fig. A3-1). If all disease tests are negative (Step 9, Fig. A3-1) and the tortoise still has acute clinical signs of disease, it will be designated for necropsy (research) to determine the source of disease. Such animals may have an infectious disease, but the protocol for disease testing may be insufficient to identify the pathogen. Those tortoises with no acute clinical signs (after the initial observation) and negative tests, may be returned to the WEA (Step 10, Fig. A3-1) after the translocation has been completed. The potential release locations for these animals will take into consideration their original home-range, low intensity military training zones, other appropriate habitat, as well as proximity to roads and property boundaries.

Tortoises may be maintained in quarantine for up to 6 months after the WETA is cleared in its entirety (Step 6, Fig. A3-1), at which time a decision must be made to include them in a research program (Step 11, Fig. A3-1), incorporate them into headstart or breeding programs (Step 12, Fig. A3-1), or returned to the WEA (Step 10, Fig. A3-1). Only tortoises that are moribund or that show acute clinical signs of disease but all diagnostic tests are negative will be euthanized (Step 13, Fig. A3-1). Tortoises returned to the WEA may be important for future research. Tortoises

found in the WEA after translocation has been completed and during future Army training activities will be removed from immediate danger and remain in the WEA.

### **Risks Associated with Translocation**

In contrast to the SEA phase of the translocation, in which attempts to minimize the risk of disease transmission were made by excluding plots of concentrated seropositive individuals from the research-release plots, the WEA phase of the translocation is not employing a plot-based research or monitoring program. Here, risk of disease transmission is minimized by buffering seropositive or clinically ill tortoises so that translocated individuals are less likely to come into contact with them. However, there are limitations to this approach because tests are not available for all previously identified or suspected diseases. We recognize that health data from a single field evaluation and a single blood sample for a tortoise are for the date of collection only. The tortoise may have been exposed to mycoplasmosis or herpesvirus prior to the field evaluation, be in the process of developing antibodies, and may later break with disease. How soon after exposure will a tortoise have positive serology for *M. agassizii* or *M. testudineum*? For *M. agassizii*, Brown et al. (1994) reported a significant rise in the antibody titer as early as one month after postchallenge and also after 3 months. We don't have an answer to this question for *M. testudineum* and will not have an answer until the test is validated with experimental infections. We have even more limited information for herpesvirus infections.

If the tortoise is not isolated from other tortoises between the time it is first evaluated in the field and a determination is made that it is *Mycoplasma*-free, it may have contact with an infected tortoise and subsequently become infected. Thus, there is a risk of translocating a tortoise that appears to be healthy (negative for all tests and clinical signs) but has recently become infected. The risk probably increases depending on the proximity of the healthy, tested tortoise to a *Mycoplasma*-infected or herpesvirus-infected tortoise. Results of *Mycoplasma* testing in the SEA and WEA between 2005 and 2008 indicate that frequency of tortoises with positive ELISA tests is <5%. Removing individuals showing acute clinical signs of disease at the first opportunity minimizes (but does not eliminate) risks of those individuals infecting susceptible tortoises in the WEA while diagnostic tests are being conducted in the lab.

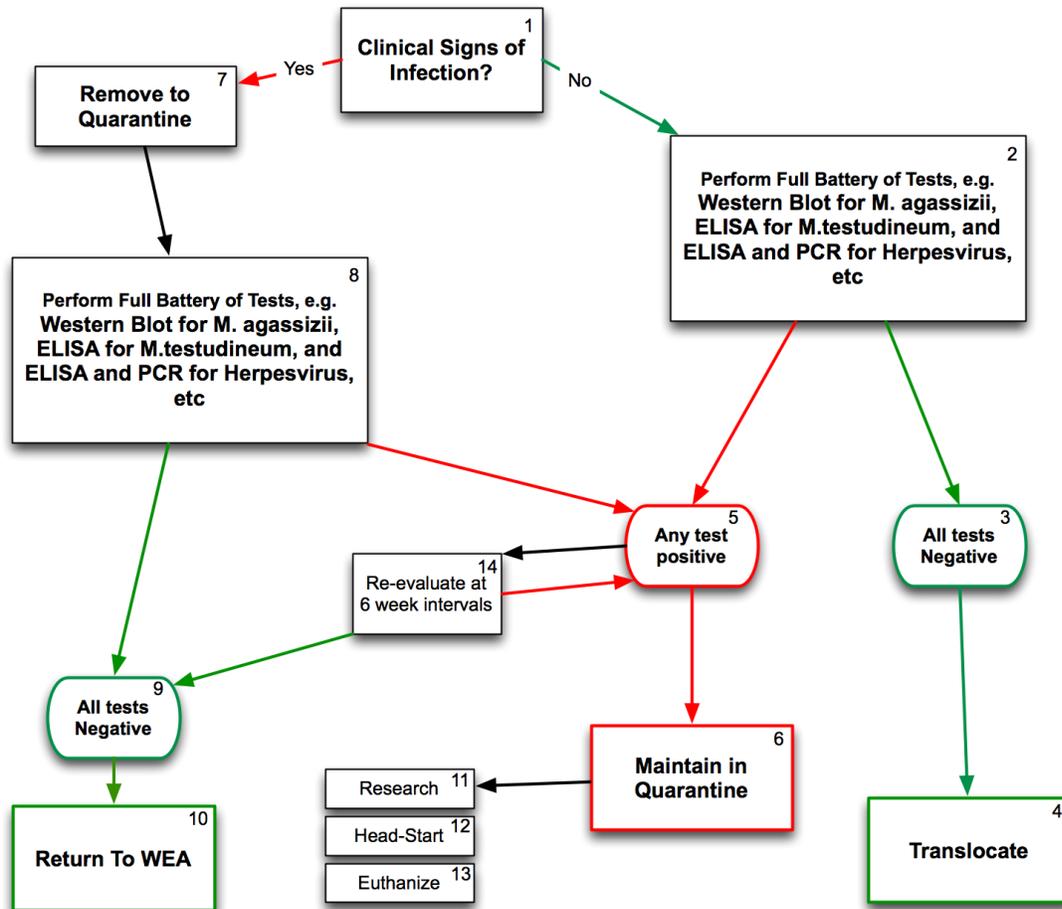


Figure A3-1. Decision tree for health assessment of desert tortoises at Fort Irwin, California.

NOTE: Step 9. If the tests are negative but the tortoise still has a nasal discharge, it should be necropsied (put into Step 11) to determine what disease it has. It may be a tortoise with a new herpesvirus or *Pasteurella*.

## Literature Cited

- ASIH. 2004 Guidelines for use of live amphibians and reptiles in field a laboratory research. Second Edition. Revised by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists. (Committee Chair: Steven J. Beaupre, Members: Elliott R. Jacobson, Harvey B. Lillywhite, and Kelly Zamudio).
- Berry, K.H. and M. M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. *Journal of Wildlife Diseases* **37**: 427-450.
- Brown, M.B., I.M. Schumacher, P.A. Klein, K. Harris, T. Correll, and E.R. Jacobson. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infection and Immunity* **62**: 4580-4586.

- Brown, D.R., I.M. Schumacher, G.S. McLaughlin, L.D. Wendland, M.B. Brown, P.A. Klein, and E.R. Jacobson. 2002. Application of diagnostic tests for mycoplasmal infections of desert and gopher tortoises, with management recommendations. *Chelonian Conservation and Biology* **4**: 497-507.
- Christopher M.M., K.H. Berry, B.T. Henen, and K.A. Nagy. 2003. Clinical disease and laboratory abnormalities in free-ranging desert tortoises in California (1990-1995). *Journal of Wildlife Diseases* **39**: 35-56.
- Hernandez-Divers, S. M., S. J. Hernandez-Divers, and J. Wyneken. 2002. Angiographic, anatomic and clinical technique descriptions of a subcarapacial venipuncture site for chelonians. *Journal of Herpetological Medicine and Surgery* **12**: 32-37.
- Hunter, K.W., Jr., S.A. Dupré, T. Sharp, F.C. Sandmeier, and C.R. Tracy. 2008. Western blot can distinguish natural and acquired antibodies to *Mycoplasma agassizii* in the desert tortoise (*Gopherus agassizii*). *Journal of Microbiological Methods*. **75**: 464-71.
- Homer, B. L., K. H. Berry, M.B. Brown, E. Greiner, and E.R. Jacobson. 1998. Pathology of diseases in wild desert tortoises from California. *J. Wildlife Diseases* **34**: 508-523.
- Jacobson, E.R. 1994. Causes of mortality and diseases in tortoises: A review. *Journal of Zoo and Wildlife Medicine* **25**: 2-17.
- Jacobson, E.R. 2007. *Infectious Diseases and Pathology of Reptiles: A Color Atlas and Text*. CRC Press, Boca Raton, FL.
- Jacobson, E.R., and K.H. Berry 2009. Necropsies of twelve desert tortoises (*Gopherus agassizii*) from California. Annual Report for 2008 to U.S. Geological Survey, Order No. 96WRCN0020.
- Jacobson, E.R., M.B. Brown, I.M. Schumacher, B.R. Collins, R.K. Harris and P.A. Klein. 1995. Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. *Chelonian Conservation and Biology* **1**: 279-284.
- Jacobson, E.R., Gaskin, J. M., Brown, M. B., Harris, R.K., Gardiner, C. H., LaPointe, J. L., Adams, H. P., and Reggiardo, C. 1991. Chronic upper respiratory tract disease of free-ranging desert tortoises (*Xerobates agassizii*). *Journal of Wildlife Diseases* **27**: 296-316.
- Johnson, A.J., D.J. Morafka, and E.R. Jacobson. 2006. Seroprevalence of *Mycoplasma agassizii* and tortoise herpesvirus in captive desert tortoises (*Gopherus agassizii*) from the Greater Barstow Area, Mojave Desert, California. *J. Arid Environments* **67**: 192-201.
- Martel, A., S. Blahak, H. Vissenaekens, and F. Pasmans. 2009. Reintroduction of clinically healthy tortoises: The herpesvirus Trojan horse. *Journal of Wildlife Diseases* **45**: 218–220.
- Origgi, F.C., C.H. Romero, D.C. Bloom, P.A. Klein, J.M. Gaskin, S.J. Tucker, and E.R. Jacobson. 2004. Experimental transmission of a herpesvirus in Greek tortoises (*Testudo graeca*). *Vet. Pathol.* **41**: 50-61.
- Ritchie, B. 2006. Virology. Chapter 24, pp. 391-417 in D.R. Mader (ed.), *Reptile Medicine and Surgery*, 2nd ed. Saunders Elsevier, St. Louis, Missouri. 1242 pp.
- Roberts, J., K. Berry, E. Jacobson, M. Brown, J. Stevens, and F. Origgi. 2008. Causes of illness and death in nine desert tortoises from California: 2005-2007. Progress Report to U.S. Geological Survey. 6 pp.
- Schumacher, I.M., D.B. Hardenbrook, M.B. Brown, E.R. Jacobson, and P.A. Klein. 1997. Relationship between clinical signs of upper respiratory tract disease and antibodies to *Mycoplasma agassizii* in desert tortoises from Nevada. *Journal of Wildlife Diseases* **33**:261-266.
- Snipes, K.P. and E.L. Biberstein. 1982. *Pasteurella testudinis* spp. Nov.: A

- parasite of desert tortoises. Intern. J. Syst. Bacteriol. 32:201-210.
- Snipes K, and E.L. Biberstein 1982. *Pasteurella testudinis* sp. nov.: a parasite of desert tortoises (*Gopherus agassizi*). Int J Syst Bacterial 32:201-210.
- University of Florida – Small Animal Clinical Sciences. 2009.  
<http://www.vetmed.ufl.edu/college/departments/sacs/research/documents/SUBMISSION%20PROTOCOL%20FOR%20DNA%20PCR.pdf>. Accessed on 1 May 2009.
- U.S. Fish and Wildlife Service. 2008. Appendix B, Berry and Christopher 2001 1242 pp. I.
- Wendland, L., L.A. Zacher, P.A. Klein, D.R. Brown, D. Demcovitz, R. Littell, and M.B. Brown. 2007. Improved Enzyme-Linked Immunosorbent Assay to reveal *Mycoplasma agassizii* exposure: A valuable tool in the management of environmentally sensitive tortoise populations. Clinical and Vaccine Immunology 14:1190-1195.

## **Appendix 4. Translocation site selection decision support model**

### **Methods**

Because analysis procedures and the technological framework were identical much of the following text was taken directly from Heaton et al. (2008). The study area is new and in some cases model criteria, data, and model parameterization were changed. These differences are noted below.

### **Study Area**

The area for prospective translocation covered 1,153.6 km<sup>2</sup> to the southwest of the National Training Center at Fort Irwin (NTC) in southern California, USA, including portions of one desert tortoise Critical Habitat Unit — Superior-Cronese. The study area was subdivided into 2.59 km<sup>2</sup> cells that served as units of analysis. The area of each cell was equivalent to one U.S. Public Land Survey System section, typically referred to in statutory units of 1 mi<sup>2</sup>. This unit size was chosen at the request of the decision makers for the purpose of identifying Public Land Survey System sections that could be purchased to fulfill the land acquisition mitigation measure. We scaled all data sets to this cell size.

### **Technological Framework**

The criteria, relationships between criteria, and criteria weights used to evaluate the translocation potential of a site were documented in NetWeaver (Saunders et al. 2005). Using fuzzy logic (Zadeh 1968), we parameterized these criteria, assigning them truth values which ranged from -1 to 1, where 1 was considered completely suitable, and -1 completely unsuitable. The fuzzy logic framework accommodates uncertainty commonly lost in ecological modeling under traditional mathematical models (Openshaw 1996; Reynolds 2001). For example, species distributional limits may be gradual rather than abrupt, or knowledge of these precise limits may be incomplete (Meesters et al. 1998). For this model, each section was assigned a truth value related to the degree to which that section was predicted to be suitable for translocation given the combined suitability of all the criteria at that location.

We pre-processed all data for developing criteria using ESRI ArcGIS 9.2 and the third party products ETGeoWizard and Hawth's Tools. Spatial models for each criterion and all criteria combined were run within the Ecosystem Management Decision Support (EMDS; Reynolds 2001) ArcGIS extension. Ecosystem Management Decision Support provides a framework for open and spatially explicit decision support modeling in ecological investigations at multiple geographic scales (Reynolds et al. 1996, 2003; Reynolds and Hessburg 2005).

### **Model Criteria**

#### *Criteria Selection*

The criteria selected for prioritizing potential translocation sites included biological and anthropogenic factors affecting desert tortoise populations in the Western Mojave Desert Recovery Unit. Seven criteria were selected for assessing translocation suitability. The following base scenario was developed as follows.

### *Ownership*

Because extensive tracts of federal lands suitable for translocation existed within the study area, sections that contained privately held lands or state lands were considered unsuitable. Only complete U.S. Bureau of Land Management sections and complete sections recently purchased by the NTC as mitigation were considered suitable. Thus this criterion was binary, either suitable (1.0) or unsuitable (-1.0).

### *Habitat*

Since the previous translocation effort surrounding the expansion of the NTC (Esque et al. 2005; Heaton et al. 2008) a desert tortoise habitat model has been developed (Nussear et al. *In Review*). This model was used for ranking habitat suitability within each section. The 1 km<sup>2</sup> cell size habitat model was converted to the 2.59 km<sup>2</sup> analysis cell size using area weighted average. As the model values are not linearly related (i.e. 1.00 is not twice as good as 0.50) we developed a non-linear curve (Figure A4-1).

### *Proximity to Major Unfenced Roads and Highways*

Tortoises are known to disperse up to 15 km after translocation (Berry 1986; Nussear 2004), and evidence of tortoise presence is reduced up to 4 km from major roads (Von Seckendorff Hoff and Marlow 2002; Boarman and Sazaki 2006). Since major roads can be a source of mortality, act as barriers, or at least filter tortoise movement (Gibbs and Shriver 2002; Von Seckendorff Hoff and Marlow 2002), areas <15 km from major roads and highways were considered unsuitable and areas >15 km suitable (Figure A4-2).

### *Proximity to Urban Areas*

Urban areas are considered poor habitat; thus, translocation suitability increases with distance from such areas. This criterion was parameterized identical to proximity to major unfenced roads and highways based upon the same knowledge regarding tortoise movement most translocation (Figure A4-2).

### *Road Density*

Within the Mojave Desert, paved and dirt roads have been implicated in the spread of non-native plant species, increased risk of fire, compaction and increased erosion of soils (Brooks 1999; Brooks and Pyke 2001; Brooks and Lair 2009; Lei 2009). Moreover, roads are known to negatively impact small mammal, lizard, and tortoise populations and habitat (Busack and Bury 1974; Brattstrom and Bondello 1983; Bury and Luckenbach 2002; Von Seckendorff Hoff and Marlow 2002; Boarman and Sazaki 2006), destroy native biological soil crust important for soil stability (Belnap and Eldridge 2001; Belnap 2002), and facilitate human access (Trombulak and Frissell 2000). Unfortunately, access is accompanied by illegal activities such as releasing captive tortoises, collecting, shooting, harassing, etc. The deleterious effects of the increase in roads on tortoise populations have not been explicitly quantified; however, more roads presumably pose a greater level of threat to tortoises. Road density was calculated as the total km of paved and unpaved roads per section; most roads were unpaved. Areas with more roads were considered less suitable than those with fewer roads (Figure A4-2). The data for this criterion were identical to that utilized in Heaton et al. (2008) however the parameterization was updated to match the statistical range of the data within the new study area.

### *Depleted Regions*

The ratio of live to carcass encounter rate was calculated for each analysis cell; cells in which carcass encounter rate exceeded live encounter rate were identified as die-off regions. Observation data were obtained from U.S. Fish and Wildlife Service monitoring data (2001-2005, 2007-2008).

### *Die-Off Good*

Parameterization was categorical; areas with more carcasses were assigned a truth value of +1; areas with equal numbers of live and carcass observations were assigned a truth value of 0.0; areas with more live observations were assigned a truth value of -1.0; and areas with no sample transects were assigned a truth value of undetermined.

### *Utility Corridors*

Translocating tortoises to areas already developed as or slated for utility corridor development would be counterproductive to recovery goals, posing significant future management challenges. Areas within utility corridors were considered unsuitable (-1.0), areas adjacent to these corridors were considered somewhat more suitable, but still relatively unsuitable (-0.5), and areas outside and not adjacent to utility corridors were considered suitable (1.0).

### *Additional Factors Considered*

Although additional biological and anthropogenic factors potentially affecting tortoise populations were considered, they were not modeled separately from the habitat model in this exercise for the following reasons: (1) little or no potential influence in the study area (e.g., latitude and elevation), (2) no suitable spatial data for modeling existed, and efforts required to secure them were time or cost prohibitive (e.g., raven distribution, nutritional composition and distribution of forage grazing and soil friability), or (3) the spatial resolution of the data were insufficient for detecting meaningful variability (e.g., precipitation). Several criteria modeled in Heaton et al. (2008) were not considered here. Proximity to the NTC was used as a surrogate for genetic information in the original translocation plan, but genetics were taken into account for the WEA translocation when the study area was selected (K. Berry, *pers comm*). There were no Off-Highway Vehicle areas in or any Projected Urban Growth areas within 15 km of the current translocation area.

There has been some interest and general discussion about the condition of vegetation in the translocation areas and whether or not the condition of the vegetation at any particular point in time is a good indicator of the value of the habitat (CMWG meeting minutes). Conditions describing the value of habitat related strictly to the abundance of vegetation on a landscape scale have not been addressed quantitatively in the literature, to date. It is fair to say that areas with extremely sparse perennial shrubs (e.g. <8 % cover) over large expanses provide very low or highly variable annual primary production on average. In contrast, areas where the vegetation of perennial shrubs is at least 15% cover (e.g., *Larrea tridentata* and *Ambrosia dumosa* association) are likely to have sufficient long-term average production to support desert tortoises (T. Esque – personal observation). However, any snap-shot of the condition of perennial shrubs or annual vegetation at such a site may be a poor indicator of the potential for that site due to inter-annual variation in precipitation. Tortoise populations regularly experience years of very low precipitation which affects their hydration status (Nagy and Medica 1986) as well as the condition of local plant populations, but individual years or even 2 years in succession are

usually not sufficient to create population-level problems for desert tortoises.

### *Relative Weighting of Criteria*

Criteria were arranged in a logical structure and ranked by level of importance for translocation. The criteria were assigned to one of two tiers with each criterion equally weighted. The first tier criteria (ownership, habitat, proximity to urban areas, and proximity to major roads and highways), were regarded as the most influential, such that if any one of the parameters were unsuitable that section was considered unsuitable for translocation. The second tier criteria were road density, die-off ranking, and utility corridors. Model scores for the second tier criteria were averaged such that no single criterion rendered a section unsuitable for translocation. However, their combined effect could influence the model. All first and second tier criteria were combined to create a translocation suitability value for each section.

### **Literature Cited**

- Belnap, J. 2002. Impacts of off-road vehicles on nitrogen cycles in biological soil crusts: resistance in different U.S. deserts. *Journal of Arid Environments*. **52**:155-165.
- Belnap J., and D. J. Eldridge. 2001. Disturbance and Recovery of Biological Soil Crusts. Pages 363-384 in J. Belnap and O. L. Lange, editors. *Biological Soil Crusts: Structure, Function, and Management*. Springer-Verlag, New York.
- Berry, K. H. 1986. Desert tortoise (*Gopherus agassizii*) relocation: implications of social behavior and movements. *Herpetologica* **42**:113–125.
- Boarman, W. I., and M. Sazaki. 2006. A highway's road-effect zone for desert tortoises (*Gopherus agassizii*). *Journal of Arid Environments* **65**:94-101.
- Brattstrom, B. H., and M. C. Bondello. 1983. Effects of off-road vehicle noise on desert vertebrates. Pages 167-206. In R. H. Webb and H. G. Wilshire, editors. *Environmental Effects of Off-road Vehicles: Impacts and Management in Arid Lands*. Springer-Verlag, New York.
- Brooks, M. L. 1999. Alien annual grasses and fire in the Mojave Desert. *Madrono* **46**:13-19.
- Brooks, M. L. and B. M. Lair. 2009. Ecological effects of vehicular routes in a desert ecosystem. In Webb, R. H., L. F. Fenstermaker, J. S. Heaton, D. L. Hughson, E. V. McDonald, D. M. Miller (Ed.), *The Mojave Desert Ecosystem Processes and Sustainability*. University of Nevada Press, Reno, Nevada.
- Brooks, M. L., and D. Pyke. 2001. Invasive plants and fire in the deserts of North America. Pages 1-14 in K. Galley and T. Wilson, editors. *Proceedings of the Invasive Species Fire Workshop: The Role of Fire in the Control and Spread of Invasive Species Fire Conference 2000: The First National Congress on Fire, Ecology, Prevention and Management*. Miscellaneous Publications No. 11 Tall Timbers Research Station, Tallahassee, Florida.
- Bury, R. B., and R. A. Luckenbach. 2002. Comparison of desert tortoise (*Gopherus agassizii*) populations in an unused and off-road vehicle area in the Mojave Desert. *Chelonian Conservation and Biology* **4**:457-463.
- Busack, S. D., and R. B. Bury. 1974. Some effects of off-road vehicles and sheep grazing on lizard populations in the Mojave Desert. *Biological Conservation* **6**:179-183.
- Esque, T. C., K. E. Nussear, and P. A. Media. 2005. Desert tortoise translocation plan for Fort Irwin's land expansion program at the U. S. Army National Training Center (NTC) at

- Fort Irwin. Prepared for U. S. Army National Training Center, Directorate of Public Works. <http://www.fortirwinlandexpansion.com/Documents.htm>.
- Gibbs, J. P., and G. Shriver. 2002. Estimating the effects of road mortality on turtle populations. *Conservation Biology* **16**:1647-1652.
- Heaton, J. S., K. E. Nussear, T. C. Esque, R. Inman, F. M. Davenport, T. E. Leuteritz, P. A. Medica, N. W. Strout, P. A. Burgess, L. Benvenuti. 2008. Decision support for selecting translocation areas for desert tortoises. *Biodiversity and Conservation*. **17**:575-590.
- Lei, S. 2009. Rates of soil compaction by multiple land use practices in southern Nevada. In Webb, R. H., L. F. Fenstermaker, J. S. Heaton, D. L. Hughson, E. V. McDonald, D. M. Miller (Ed.), *The Mojave Desert Ecosystem Processes and Sustainability*. University of Nevada Press, Reno, Nevada.
- Meesters, E. H., R. P. M. Bak, S. Westmacott, M. Ridgley, and S. Dollar. 1998. A fuzzy logic model to predict coral reef development under nutrient and sediment stress. *Conservation Biology* **12**:957-965.
- Nagy, K. A., and P. A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. *Herpetologica* **42**:73-92.
- Nussear, K. E. 2004. Mechanistic investigation of the distributional limits of the desert tortoise, *Gopherus agassizii*. Unpublished Ph.D. thesis. University of Nevada, Reno.
- K.E. Nussear, K.E., Esque, T.C., R.D., Inman, C.S.A Wallace, R.H.Webb, K.A. Thomas, L. Gass, Blainey, J., D.M. Miller, 2009, Modeling Habitat of Desert Tortoise (*Gopherus agassizii*) in the Mojave and Colorado Deserts, California, Nevada, Utah, and Arizona: U.S. Geological Survey Open-file Report XXXX - 2009.
- Openshaw, S. 1996. Fuzzy logic as a new scientific paradigm for doing geography *Environment and Planning A* **28**:761-768.
- R Development Core Team (2008). R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Reynolds, K. M. 2001. Fuzzy logic knowledge bases in integrated landscape assessment: examples and possibilities. Gen. Tech. Rep. PNW-GTR-521. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 24 p.
- Reynolds, K.M., and P. F. Hessburg. 2005. Decision support for integrated landscape evaluation and restoration planning. *Forest Ecology and Management* **207**:263-278.
- Reynolds, K., C. Cunningham, L. Bednar, M. Saunders, M. Foster, R. Olson, D. Schmoltd, D. Latham, B. Miller, and J. Steffenson. 1996. A knowledge-based information management system for watershed analysis in the Pacific Northwest U.S. *AI Applications* **10**:9-22.
- Reynolds, K. M., K. N. Johnson, and S. N. Gordon. 2003. The science/policy interface in logic-based evaluation of forest ecosystem sustainability. *Forest Policy and Economics* **5**:433-446.
- Saunders, M. C., T. J. Sullivan, B. L. Nash, K. A. Tonnessen, and B. J. Miller. 2005. A knowledge-based approach for classifying lake water chemistry. *Knowledge-Based Systems* **18**:47-54.
- Trombulak, S. C., and A. Frissell. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation Biology* **14**:18-30.
- Von Seckendorff Hoff, K., and R. W. Marlow. 2002. Impacts of vehicle road traffic on desert tortoise populations with consideration of conservation of tortoise habitat in southern Nevada. *Chelonian Conservation and Biology*. **4**:449-456.

Zadeh, L. 1968. Probability measures of fuzzy events. Journal of Mathematical Analysis and Applications 23:421-427.

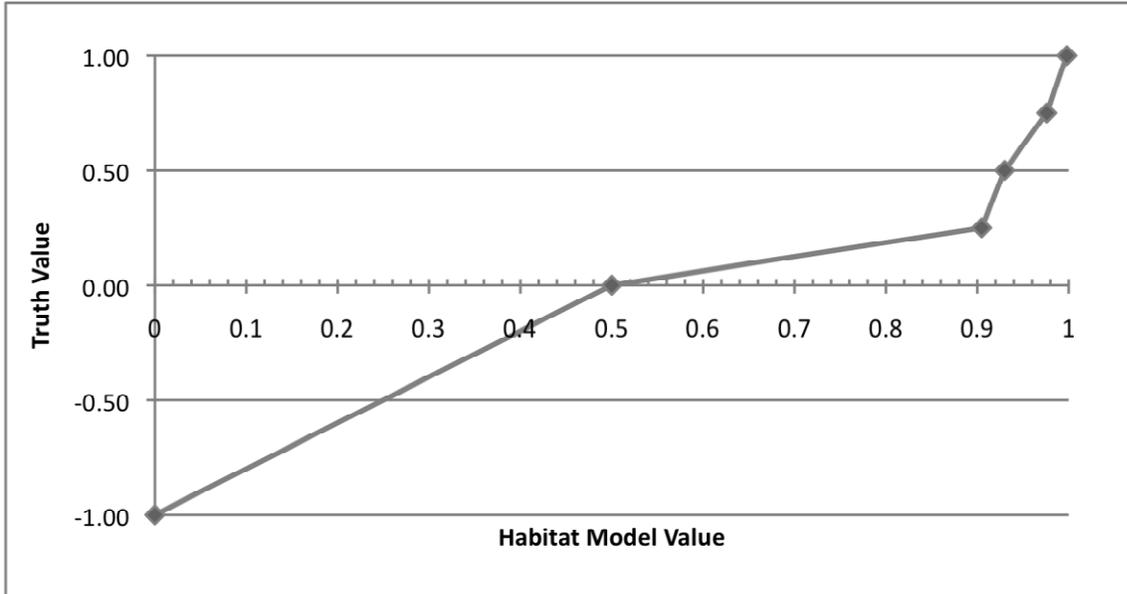


Figure A4-1. Habitat criterion truth value rankings. The highest habitat model value is 0.998.

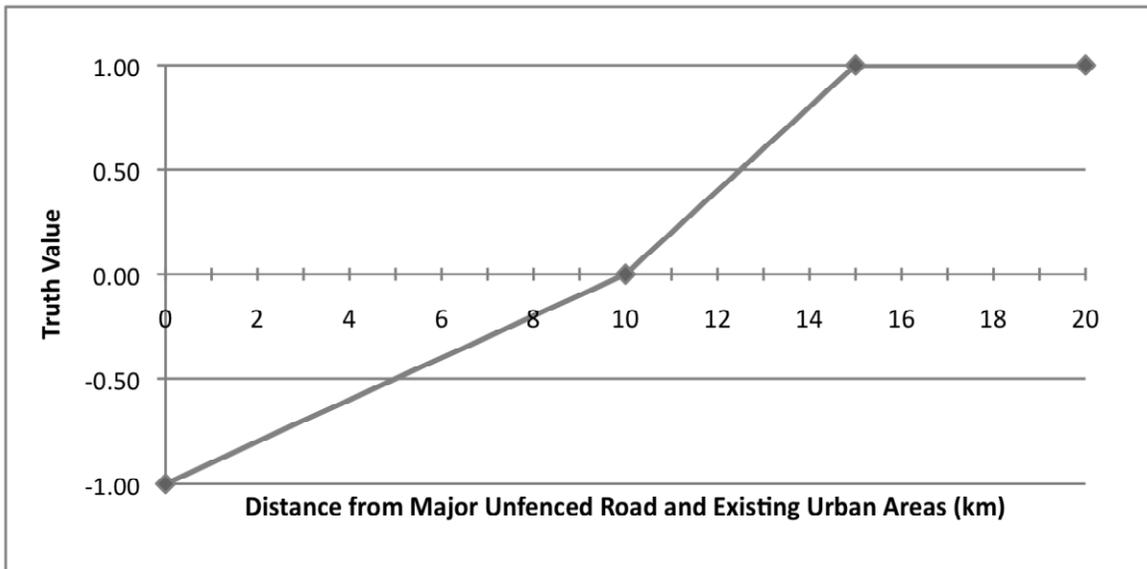
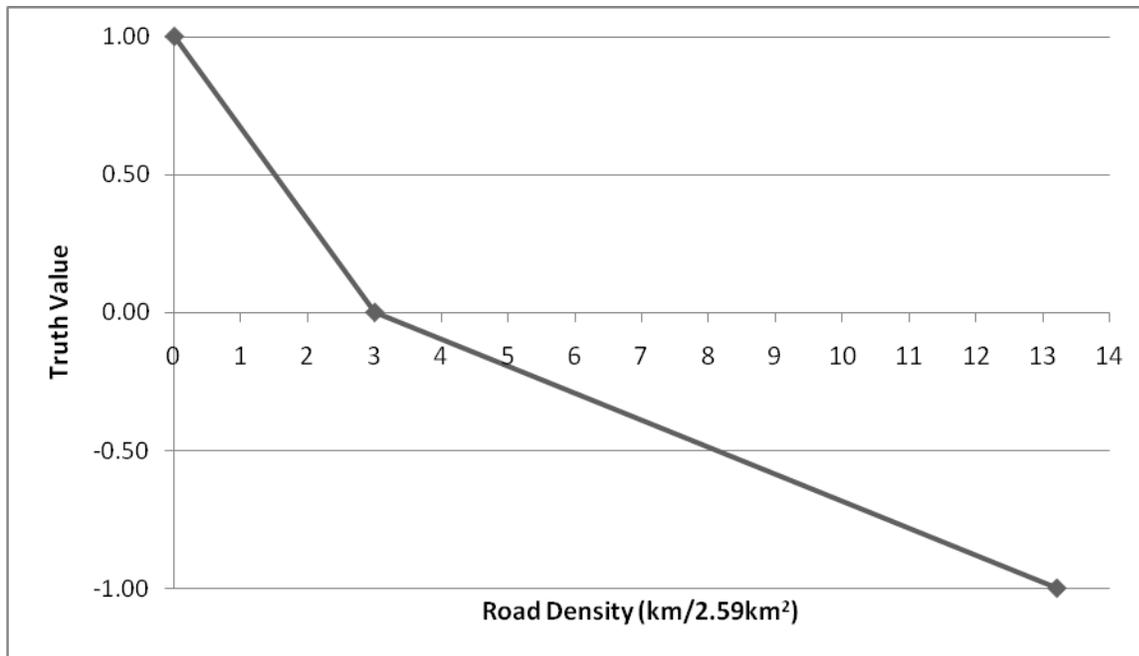


Figure A4-2. Distance from major unfenced road and existing urban areas truth value rankings.



*Figure A4-3. Road density truth value rankings.*

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