

Translocation of Mojave Desert Tortoises from Project Sites: Plan Development Guidance

U.S. FISH AND WILDLIFE SERVICE



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Glossary

Biological samples: Samples collected from desert tortoises (*e.g.*, blood or oral swabs). Refer to the *health assessment* procedures (USFWS 2019) for the samples currently being collected.

Clearance(s)/clearance survey(s): Surveys to locate and remove as many desert tortoises as possible prior to initiation of development at a project site to reduce likelihood of death or injury.

Control tortoises: Desert tortoises at a location separate from the project or *recipient population*, selected for monitoring purposes relative to *translocated* and *resident tortoises*.

Depleted population: Areas where tortoise densities are below a minimally viable level of 3.9 adult tortoises/km². Populations expected to decline to this level within 3 years based on trends estimated in Allison and McLuckie (2018) are also considered depleted. For regions with increasing populations, depleted-population density is set at 3.9 adult tortoises/km². See Table 1.

Development footprint: Boundary of the project within which tortoises will be cleared and *translocated* to another location. The development footprint typically will be fenced with tortoise *exclusion fencing*.

Disposition plan: A specific proposal of the fate of each desert tortoise from the project site (*e.g.*, *translocate* to specific *release area* at *recipient site*, transport to veterinarian for evaluation and treatment, remove from population, etc.). The disposition plan template (Appendix H in USFWS 2019; contact USFWS for most recent version) includes summary health information for all assessments of each tortoise. It must be completed within the season in which translocation is proposed to occur and is one part of the *Translocation Review Package*.

ELISA: Enzyme-linked immunosorbent assay used to detect antibodies in blood plasma to targeted pathogens, in this case antibodies to *Mycoplasma agassizii* and *M. testudineum*.

Exclusion fence/fence/fencing/project fencing/perimeter fence: References to fencing in this document refer to fencing that is impermeable to most tortoises and is used to either confine or exclude them. Specifications can be found in Chapter 8 of the [Desert Tortoise Field Manual](#).

Health assessment: A standardized physical examination, which includes measurements and evaluation for presentation of key clinical signs of disease. *Biological samples* may or may not be collected at the time of a health assessment. See USFWS (2019).

Post-translocation density: Density of the tortoise population at the recipient site immediately following release of tortoises from the project site. Post-translocation density = (estimated # adult *resident tortoises* + # adult tortoises *translocated*) / (area of *recipient site*). Adults are \geq 180 mm midline carapace length.

Post-translocation monitoring: Monitoring of *translocated* and *resident* tortoises in the *recipient population* and one or more associated *control populations* to evaluate the success of the *translocation* (see p. 20).

Pre-project survey(s): Standardized surveys to determine presence/absence and abundance of desert tortoises (<http://www.fws.gov/carlsbad/PalmSprings/DesertTortoise.html>).

Prevalence: The proportion of tortoises within the population that are infected or diseased (Gray *et al.* 2017). For the purposes of this guidance, prevalence includes seroprevalence (*i.e.*, the proportion of tortoises seropositive to antibodies of *Mycoplasma agassizii* or *M. testudineum*, as well as those with clinical signs that disqualify them from being *translocated* (USFWS 2019). Note that presence of antibodies provides an indication of previous pathogen exposure, not current illness.

qPCR: Quantitative polymerase chain reaction technique for detecting DNA of a target organism, in this case *Mycoplasma agassizii*, *M. testudineum*, and Testudinid herpesvirus 2 from oral swabs.

Recipient site/population: The location/population to which desert tortoises removed from a project site will be translocated. This is a larger area than the specific *release site* into which tortoises are physically moved and includes the full, anticipated *release area* (Example 1).

Regional population-augmentation site: Desert tortoise population that the USFWS has determined to be in specific need of augmentation for recovery purposes. Such populations may be used to receive tortoises from development sites within a specified radius.

Release area: The area into which most tortoises are expected to move and settle after release (Le Gouar *et al.* 2012). For tortoises released ≤ 300 m from their points of origin, the release area is a 1.5-km radius around the release points, and for those released > 300 m the radius is 6.5 km.

Release site: The geographic area containing the release locations of *translocated* tortoises (Example 1). Translocated tortoises will move from the release site throughout the *release area*.

Resident tortoises: Desert tortoises living within the *recipient population* prior to the release of tortoises translocated from the project site. Once identified, they continue to be designated as resident tortoises after the *translocation*.

Small number of tortoises: When a regional population-augmentation site is unavailable certain components of the translocation guidance may be relaxed when translocating a small number of tortoises. Determining precisely what constitutes a “small number” will depend on project-specific details, such as the scope and scale of the project, the area across which tortoises will be displaced, and the area within which the tortoises are proposed to be moved. However, relaxing guidance described herein will be based on the principles that translocating few tortoises 1) will negligibly increase the density of a *recipient site*, 2) will minimally disrupt *resident-tortoise* social dynamics and contact rates, and 3) will lack sufficient power to evaluate translocation effectiveness via a detailed monitoring program.

Translocated tortoises: Desert tortoises that have been moved from a *development footprint* to a *recipient site*.

Translocation: The human-mediated movement of living organisms from one area with free release in another (IUCN 2013). Translocation is an overarching term, and synonyms that may have been used previously include “relocation.”

Translocation plan: A document detailing the proposed *translocation* and *post-translocation monitoring*, as per the contents of this guidance. Projects using an established *regional population-augmentation site* need only complete a *translocation review package*, rather than a full translocation plan.

Translocation-review package: Documents submitted to the USFWS for review and final approval of a *translocation*. It includes a *disposition plan* for the project-site tortoises and health summary of *resident* and *control* tortoises; photographs of individual tortoises as specified on the *health assessment* data sheet; health assessment data sheets for *resident*, *control*, and project-site tortoises; maps of the *recipient site*, including digital GIS layers, illustrating health sampling results of the *resident* tortoises, and showing proposed release points of project-site tortoises; maps of the *development footprint* illustrating distribution and health status of project-site tortoises; and any other project-specific information that supports or clarifies translocation decisions.

Introduction

This guidance is based on three overarching principles:

- 1) Once translocation of desert tortoises from a project site has been deemed necessary and appropriate, the first consideration is *how can those tortoises best contribute to recovery of the species*.

Convenience in moving the tortoises out of the way of development is secondary. The guidance prioritizes **regional augmentation sites** to boost **depleted populations** that are important to recovery.

- 2) *Rigorous monitoring* will document the contribution of **translocated tortoises** to recovery as well as the effectiveness of translocation in minimizing impacts to the affected tortoises.

Long-term monitoring of projects with a **small number of tortoises** for which a **regional augmentation site** is not available lacks statistical power to rigorously evaluate translocation effectiveness. Such projects will be evaluated on a case-by-case basis to determine the appropriate level of monitoring.

- 3) Translocation protocols will *minimize risks to both translocated tortoises and the recipient population*, especially relative to disease transmission.

No wildlife populations are completely free of disease. Therefore, the purpose of disease risk minimization is not to maintain a disease-free state within a population but rather to maintain resilient and self-sustaining populations capable of adequately responding to disease occurrences (Rideout 2015). Diagnostic tests of **biological samples** are used to evaluate populations, not individuals (see **Health assessments: Health-assessment protocols**), so projects requiring translocation of a **small number of tortoises**—when a **regional augmentation site** is not available—may require less invasive **health assessments** that do not include collection of **biological samples**.

This document provides guidance for the development of project-specific **translocation plans** for activities that will impact Mojave desert tortoises (*Gopherus agassizii*; hereafter, “desert tortoise” or simply “tortoise”) when avoidance of these impacts is not feasible. Prior to drafting a **translocation plan**, however, project proponents should discuss with the U.S. Fish and Wildlife Service (USFWS) the most appropriate course of action with regard to disposition of affected desert tortoises. Options include translocation to an augmentation site, moving or reducing **development footprints** within occupied desert tortoise habitat, and designing projects that would allow experimental evaluation of continued occupancy of the site by tortoises, even if at a reduced level.

Although recent research on translocation of desert tortoises has shown potential as a conservation tool at more than a dozen sites, results are generally available only for durations ≤ 5 years, and long-term success has not been documented (Brand *et al.* 2016, Dickson *et al.* 2019, Drake *et al.* 2012, Esque *et al.* 2010, Farnsworth *et al.* 2015, Field *et al.* 2007, Harju *et al.* 2019, Hinderle *et al.* 2015, Nafus *et al.* 2017, Nussear *et al.* 2012). As a result, we do not fully understand the long-term impacts of translocation, including for example, altered disease dynamics (Aiello *et al.* 2014) or changes to effective population size (*i.e.*, recruitment into the adult population as a result of successful reproduction; see Mulder *et al.* 2017). Likewise, impacts on population fragmentation and gene flow as a result of large-scale development within occupied desert tortoise habitat, for which translocation is increasingly used as an impact-minimization tool, are unclear. Nonetheless, new information continues to emerge from recent

translocation projects and related research (*e.g.*, Germano *et al.* 2012, Hall *et al.* 2016, Walde *et al.* 2011). New translocation projects should be designed so that effectiveness monitoring addresses questions related to the success or impacts of the translocation. The information gathered from such efforts can be applied to further reduce incidental take resulting from projects and to improve the success of translocation as a conservation tool (see Germano *et al.* 2015).

If translocation can be justified as the most appropriate course of action, this document should be used as a reference that, when combined with project-specific input from the USFWS and other permitting agencies, will facilitate the completion of a **translocation plan**. This guidance does not constitute a **translocation plan** “template.” Rather, it describes considerations that must be taken into account and steps that must be taken when developing a **translocation plan** and preparing to move tortoises across the landscape. Deviations from the guidance may be appropriate and acceptable if addressed by a project-specific effectiveness-monitoring program within the **translocation plan**.

This guidance is complementary to existing protocols for the desert tortoise that should be referenced when planning and implementing surveys, **translocation plans**, and other activities involving this species. To ensure that you are referring to the most current guidance and protocols, contact your local USFWS field office or download the **Pre-project Survey Protocol** (USFWS 2018a or more recent), Desert Tortoise Field Manual (includes **Clearance Survey Guidelines**, Handling Guidelines, and Exclusion Fence Specifications; USFWS 2009 or more recent), and Responsibilities and Qualifications for Authorized Biologists at <http://www.fws.gov/carlsbad/PalmSprings/DesertTortoise.html>. Health-assessment protocols (USFWS 2016 or more recent) referenced in this document may also be downloaded from http://www.fws.gov/nevada/desert_tortoise/dtro/dtro_trans.html. *In addition to this guidance, project proponents need to confer with the State wildlife agency relevant to the project area to ensure compliance with State-specific policies or protocols.*

Permits and Coordination

This document provides guidance on how to translocate desert tortoises in accordance with the three overarching principles described previously in this section. Proponents will need to develop **translocation plans** that meet the specific needs of their projects. Therefore, we used appropriate wording (*e.g.*, “should”) in describing the general components of these plans that will be developed in coordination with the USFWS.

Section 9 of the Endangered Species Act of 1973, as amended, and Federal regulations pursuant to section 4(d) of the Act prohibit the take of endangered and threatened species (fish and wildlife) without special exemption. The definition of “take” is to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct, and the implementation of any translocation activity will necessitate take of desert tortoises in some form. Consequently, activities described by this guidance may be undertaken only when the USFWS has issued: *1) a recovery permit pursuant to section 10(a)(1)(A) of the Act; 2) an incidental take permit pursuant to section 10(a)(1)(B) of the Act; or 3a) a biological opinion that contains an incidental take statement under the authorities of section 7(a)(2) of the Act, if an exemption to the prohibitions against take is granted, and 3b) the action agency has issued their decision document.*

Projects will also need approval from the appropriate State wildlife agency to translocate desert tortoises and permission of the relevant landowners or managers. Failure to obtain the review and approval of all involved agencies is likely to delay the project and potentially incur agency-specific penalties. When planning a translocation, ensure that all appropriate agencies are notified early in the process. The **translocation plan**, after approval by the agencies, would then be incorporated into the project design or included in the terms and conditions of the USFWS's biological opinion or incidental take permit. *Without a recovery permit, activities requiring the handling of tortoises can only be conducted in accordance with an incidental take permit or biological opinion.* Separate permits also may be required by other Federal agencies or by State laws and regulations. Because any given project may have unique circumstances, we recommend project proponents and the lead action agency work closely with the appropriate USFWS field office, State wildlife agency, and the relevant land-management agency early in the planning process to determine which of the components and to what degree each of the following should be included in project-specific **translocation plans**.

Document Organization

- The bulk of this document contains detailed protocols and rationale for aspects of the translocation planning and implementation process. Various terms bolded in the text are defined in the glossary (page ii).
- A flowchart (Figure 1) summarizes various actions, key considerations, and decision points throughout the translocation planning process.
- An example illustrating several of the concepts described herein is provided prior to the Literature Cited.

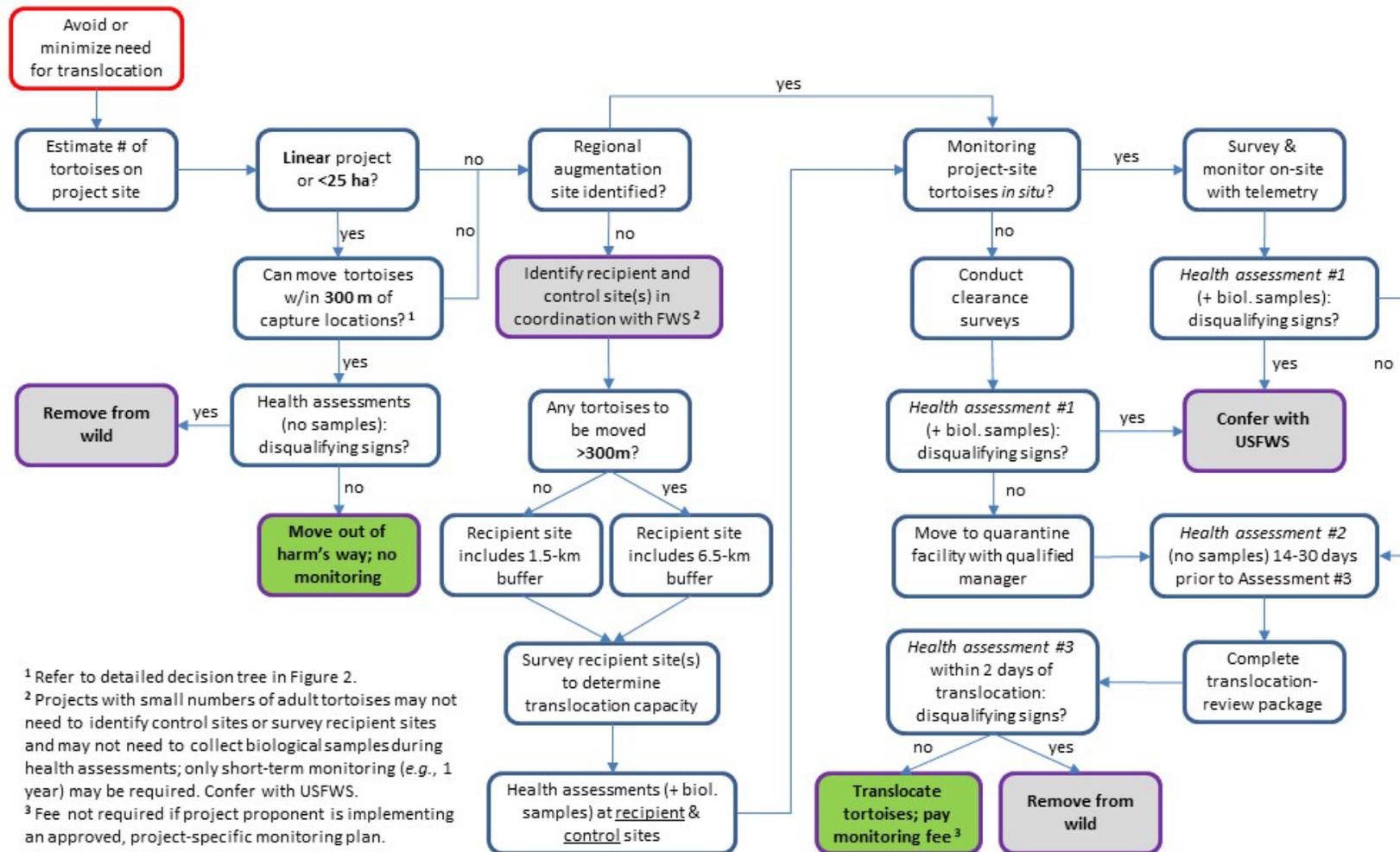


Figure 1. Translocation planning flowchart (see text for details; see also Fig. 2 for more detail on projects moving tortoises < 300 m).

Protocols and Rationale

Development projects within occupied desert tortoise habitat have several required and optional actions to consider. All projects should estimate the number of tortoises on the project site to allow for the development of the most appropriate **translocation plan**. Many projects may be able to move or adjust the **development footprint** to reduce the need to translocate tortoises from harm's way, but two primary scenarios exist for projects with tortoises remaining within the **development footprint**. 1) Preferably, tortoises would be translocated to pre-identified, regional, population-augmentation sites, thereby precluding the need to develop and implement individual translocation and monitoring plans, and specified fees would contribute to post-translocation monitoring. 2) Projects may hire qualified scientists to develop their own translocation and monitoring plan to be approved by the USFWS, including identification of potential **recipient sites**. Details for each of these scenarios follow.

Estimate the number of desert tortoises that will be affected at the project site

Pre-development surveys are important in order to prepare for the three potential outcomes for tortoises located during the ultimate **clearance surveys** (see Figure 1 and **General clearance protocols** for more details):

- a. tortoises remain on-site for *in situ* monitoring until health assessments and translocation (preferred option);
- b. transfer tortoises to quarantine facility for holding (*ex situ*) until health assessments and translocation (≤ 6 months);
- c. transfer tortoises that are ineligible for translocation to an agency-approved facility.

We recommend that you work with the local USFWS office to determine the most appropriate method of estimating the number of desert tortoises on site. For most situations, we recommend that you conduct surveys according to the most recent USFWS **Pre-project Survey Protocol** and include data on all tortoise sign observed. For some situations (*e.g.*, linear and small projects, when no large desert tortoises are found during protocol surveys, *etc.*), estimating density may be the best be done by using the density of desert tortoises from the USFWS's range-wide sampling.

Data collected during the surveys should be standardized according to and compatible with the master database to be provided by USFWS and Bureau of Land Management (BLM). Submit survey data and associated maps and GIS shapefiles to the USFWS. These data will be used to estimate the number of desert tortoises expected to be adversely affected or impacted by the project and to assist in identifying potential **recipient** and **control sites** (see glossary) based on the density estimates (see Example 1). Note: when a project occurs within a "no survey" area identified by California's Desert Renewable Energy Conservation Plan or in any land use plan, the project proponent should confer with the California Department of Fish and Wildlife to determine survey requirements. Such projects should prepare to handle tortoises found on the **clearance** site according to the protocols described below for **Regional population-augmentation sites**.

Regional population-augmentation sites

Given that most development projects will occur outside designated conservation areas or other important habitat, priority is placed on using desert tortoises displaced from development to augment depleted populations specifically identified for their importance to recovery, rather than merely moving them out of the way (*i.e.*, “over the fence”). Note that, hypothetically, habitat adjacent to a development site may be valuable for recovery and may thus qualify as a regional augmentation site. If tortoises from a project qualify for a site in need of augmentation identified by the USFWS, translocation requirements will be simplified to the following four steps:

- At least two health assessments (see *Health assessments*).
- A fee to cover expenses associated with archiving **biological samples** and maintaining the sample bank: \$3000 (2020 dollars; see *Health-assessment protocols* for a description of the archival fee).
- A **translocation-review package** (see *Translocation of desert tortoises following acceptance of translocation-review package*), not including information on **recipient-site** and **control tortoises**.
- Coordinate funding for post-translocation monitoring with the USFWS (see *Post-translocation monitoring*).

Linear and < 25-ha projects

If a regional population-augmentation site is unavailable or the USFWS determines that maintaining tortoises in the vicinity of the project site is desirable (*e.g.*, the project occurs within a population linkage and suitable habitat is available), tortoises may be moved from linear projects, or other projects up to approximately 25 ha (62 acres), up to 300 m from their capture locations into adjacent habitat (Figure 2).¹ Linear projects include infrastructure such as roadways, pipelines, fiber-optic lines, transmission lines, or other buried lines. In these cases, tortoises generally should be moved out of harm’s way into adjacent habitat following **clearance** and handling procedures outlined in Attachment 1 and the current USFWS Desert Tortoise Field Manual. Such tortoises should be moved < 300 m after confirming a lack of significant clinical signs of ill health or recent captivity (*e.g.*, grossly emaciated, severe runny nose, painted or

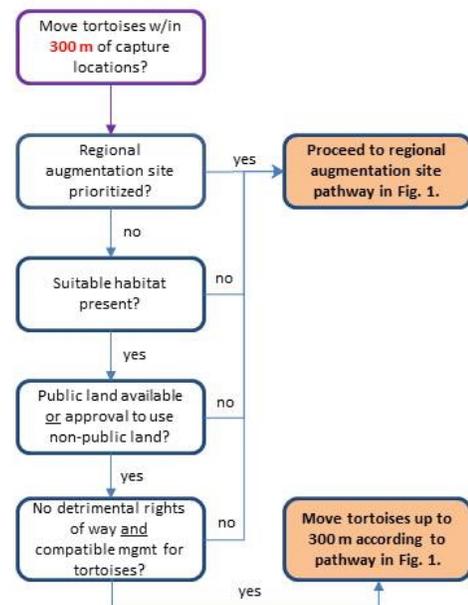


Figure 2. Decision tree for moving tortoises < 300 m on linear or < 25-ha projects.

¹ The 300-m threshold defines an area within which resources and neighboring tortoises are expected to be familiar to the **translocated tortoise**. The area of a moderate-term home range (ca. 4 years) is 43 ha, the radius of which is 370 m (Vamstad *et al.* 2013; Harless *et al.* [2010] summarized smaller home ranges estimated over shorter durations). Desert tortoises translocated < 500 m disperse shorter distances than tortoises moved > 500 m (Walde, pers. comm., 2015 [updated from Walde *et al.* 2009]) and are thus less likely to encounter tortoises with which they are unfamiliar and have contact with (*cf.* Aiello *et al.* 2014). To be conservative, we apply a 300-m threshold in this guidance. For non-linear projects, 25 ha conservatively approximates the area of a 300-m radius project.

pyramided scutes), without the need to collect **biological samples**. Post-translocation monitoring (and associated fees) is not recommended for these tortoises. Tortoises with significant clinical signs of ill health should be removed from the wild in coordination with the USFWS (see *Translocation of desert tortoises following acceptance of translocation-review package* for information on disposition of sick tortoises). If suitable habitat is not available within 300 m of the tortoises' capture locations or other land ownership restrictions prevent the release of individuals within 300 m (e.g., privately owned land lacking permission), the remainder of this guidance applies.²

Project-specific translocation plan: identify potential recipient and control sites

If a regional population-augmentation site is not available, **recipient** and **control sites** should be identified. Lands to consider as potential **recipient sites** may include designated critical habitat or lands identified as Desert Tortoise Conservation Areas (TCAs) in the revised recovery plan (USFWS 2011), lands outside TCAs that are important for maintaining habitat and population connectivity, or lands where management actions are being tested. The latter factor especially requires qualified scientific oversight in developing the **translocation plan** to avoid confounding effects. If the area surrounding the project site is suitable (appropriate habitat, large enough to contain likely dispersal distances, and meets other requirements in this guidance), all or some of the tortoises may be released into habitat adjacent to the project. We recommend that the proponent conduct surveys in the potential **recipient** and **control sites** according to the most recent USFWS **Pre-project Survey Protocol**. Provide survey data and associated maps and GIS shapefiles to the USFWS.

Projects with a **small number of tortoises** for which a regional population-augmentation site is unavailable need not survey the **recipient site**. Translocating few tortoises will negligibly increase the density of a **recipient site** and will minimally disrupt **resident-tortoise** social dynamics and contact rates. Monitoring a **small number of tortoises** lacks power to evaluate translocation effectiveness, so identifying a **control site** is unnecessary in this situation. Determining precisely what constitutes a “small number” of tortoises will depend on project-specific details, such as the scope and scale of the project, the area across which tortoises will be displaced, and the area within which the tortoises are proposed to be moved.

Recipient-site selection criteria—The following criteria should be addressed when selecting prospective **recipient sites**.

1. The site supports desert tortoise habitat suitable (including accessible land ownership) for all life stages.
2. The site contains a depleted tortoise population (see *Determination of recipient-site size and quality*).
3. There is no evidence of an active outbreak of disease, such as high prevalence of clinical signs of disease or seropositive responses to disease agents. The USFWS will consult

² If the project and potential **release site** occur in an area the USFWS has determined is less important for recovery of the species (e.g., outside of conservation areas, population linkages, and other contiguous, high-value habitat) and a regional augmentation site is unavailable, it may be acceptable to release the tortoise(s) into suitable habitat > 300 m from its capture location.

with wildlife health professionals to determine whether sites with relatively high disease prevalence are appropriate for translocation.

4. Major unfenced roads (*i.e.*, high traffic volumes/speed limits and no desert tortoise **exclusion fence**), highways, or human development that would pose a risk to desert tortoises, are no closer than 6.5 km to the **release area**. Distances from unfenced hazards may be reduced if proposed monitoring or topographic features justifies a shorter distance.³
5. Unless otherwise approved by the USFWS based on **small numbers of tortoises** to be translocated, the **recipient population** should be on the same side of the southern/western boundary of the Eastern Mojave Recovery Unit as the source population.⁴
6. The site has no detrimental rights-of-way or other encumbrances that would pose ongoing risks to successful establishment of **translocated tortoises**.
7. The site will be managed compatibly with continued desert tortoise occupancy.

Determination of recipient-site size and quality—**Recipient sites** include the **release site** and expected area of dispersal of **translocated tortoises**. Data from recent translocations indicate that desert tortoises moved up to 500 m from their capture location are expected to settle within 1.5 km of their release point, and most tortoises (> 97.5%) moved > 500 m are expected to settle within 6.5 km of their release point within the first year of release (unpublished data analyzed by the Desert Tortoise Recovery Office). However, to further maximize the chance that tortoises moved short distances are familiar with the area and will disperse a minimal distance, we apply a threshold of 300 m for “short-distance translocations.” Therefore,

- For tortoises moved > 300 m: the **recipient site** encompasses the area within a 6.5-km radius of the set of potential release points (see Example 1).
- For tortoises moved ≤ 300 m: the **recipient site** encompasses the area within a 1.5-km radius of the set of potential release points.

Some projects may include tortoises moved both < 300 m and > 300 m adjacent to the project site, in which case the **recipient population** is defined by the larger area. In these cases, all tortoises to be translocated short distances still fall within the complete guidance (health assessments, density requirements, monitoring, etc.) and should be included in the project’s tortoise **disposition plan** (see below).

Little information exists on what constitutes carrying capacity for desert tortoise habitat or what specific variables relate to quantifiable measures of habitat quality. However, project proponents

³ Installation of tortoise barrier fencing along a highway adjacent to a proposed **recipient site** may be considered as part of the project’s proposed action.

⁴ The major genetic difference between Mojave desert tortoise populations occurs across the southern and western boundary of the Eastern Mojave Recovery Unit (Sanchez-Ramirez et al. 2018; Shaffer et al. 2017). On either side of this boundary, populations within a 200-km straight-line distance of each other (249 km measured around topographic barriers) tend to be genetically correlated and may be considered single genetic units for management purposes, and the risks of outbreeding depression due to genetic mixing is low (Averill-Murray and Hagerty 2014). See also Frankham et al. (2017), who stated “Disturbingly, evidence of any genetic differentiation among populations typically leads managers to conclude that the populations should be kept isolated, thereby dooming many to eventual extinction.”

should provide a qualitative assessment of prospective **recipient sites** relative to habitat conditions likely to support the **translocated tortoises**, including perennial cover and density, annual forage, native/non-native plant species diversity, and soil conditions for burrowing or significant areas of other shelter types (*e.g.*, caliche dens). Prospective **recipient sites** should be evaluated relative to potential threats that may affect desert tortoises translocated to the area, including those that may originate nearby (*e.g.*, elevated predator populations [domestic or native], proximity to major highways or OHV high-use areas, historic mining sites or other toxicant sources, and proximity to existing and future utility infrastructure).⁵ We recommend that more than one potential **recipient site** be identified during planning in the event that disease status, desert tortoise densities, or other factors prevent the use of a potential site.

Determine capacity of recipient site to hold translocated tortoises—The density of tortoises in the **recipient site** must be low to minimize risk of disease transmission (Rideout 2015), so translocation typically should be applied to depleted populations, which are defined as < 3.9 adult tortoises/km².⁶ For populations not yet below this threshold, we calculate a depleted-population density as that at which the population is estimated to decline within three years to 3.9 adults/km² based on trends estimated in Allison and McLuckie (2018; Table 1). We use the estimate at the 3-year period before a population is projected to reach 3.9 adults/km² as a means to allow **translocated tortoises** to settle into the population before it reaches the most critical level. For regions containing increasing populations, depleted-population density is set at 3.9 adults/km². At these densities, there is minimal risk from disease outbreaks or other impacts if the risk-minimization measures of this guidance fail. Exceptions to this threshold must be supported by scientific justification and monitoring.

In any case, translocation will increase tortoise densities in **recipient populations**, so for a successful translocation, the **post-translocation density** in any area should not exceed the capacity of the surrounding desert. However, as noted above, information is lacking on specific metrics for determining carrying capacity for desert tortoises. From a statistical standpoint, though, densities described by a single standard deviation of the mean tortoise density for a particular area or region are not unusually high for that area (Figure 3). Therefore, assuming appropriate habitat and management exist, projected density of adult tortoises in the **recipient population** after translocation (**residents plus translocated** adult individuals [≥ 180 mm carapace length]) should not exceed 1 standard deviation above the mean density in the respective recovery unit or specific critical habitat unit, as applicable (Figure 3; Table 1). Note that 1 standard deviation above the mean density is still less than the minimum density threshold of 3.9 adults/km² in many regions (Table 1). In these cases, increasing populations to at least 4 adults/km² is encouraged in conjunction with site-specific recovery actions and associated effectiveness monitoring. The USFWS and partner agencies will evaluate such scenarios on a case-by-case basis to increase the probability that the habitat can support a more robust population. Finally, the number of juvenile tortoises (defined here as < 180 mm carapace length)

⁵ Management of documented threats within the **recipient site** should be considered as part of the project's proposed action to reduce threats to the desert tortoise.

⁶ While updated population viability analyses are needed (USFWS 2011), previous analyses indicated that populations at densities lower than 3.9 adult tortoises/km² are not viable in the long term (USFWS 1994:C25). This provides a density metric below which risk of disease transmission or negative population consequences of transmission is low. Areas below this density are also in need of population augmentation to reach viability.

released should not exceed three times the adult limit (*i.e.*, $\leq 75\%$ of the ultimate **translocated** population could be juveniles).⁷ Contact the USFWS for the most current data on desert tortoise densities within each recovery unit.

Table 1. Density (adult tortoises/km²) thresholds for identifying (A) depleted populations eligible to receive tortoises via translocation and (B) maximum post-translocation densities within recipient populations for each recovery unit and monitoring stratum.

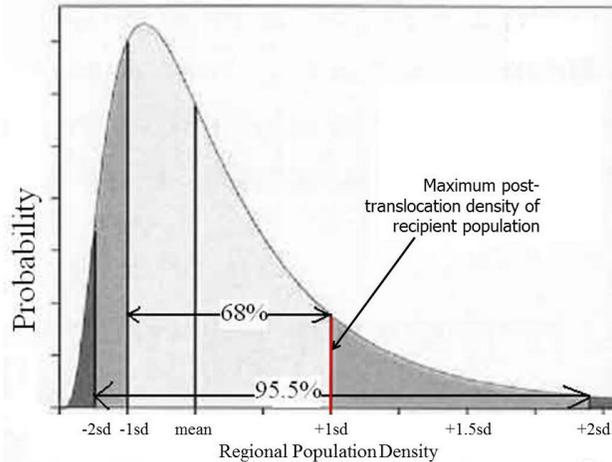
Recovery Unit	2014 Mean Density^a	(A) Maximum Population Density <i>before</i> Translocation^b	(B) Maximum Population Density <i>after</i> Translocation^c
Western Mojave	2.8	3.9	3.6 (4.0)
Fremont-Kramer	2.8	3.9	3.4 (4.0)
Ord-Rodman	3.8	3.9	4.6
Superior-Cronese	2.5	3.9	3.0 (4.0)
Eastern Mojave	1.5	3.9	2.0 (4.0)
Eldorado Valley	1.6	3.9	2.0 (4.0)
Ivanpah Valley	2.5	3.9	3.0 (4.0)
Colorado Desert	3.7	3.9	4.6
Chocolate Mtn	7.6	4.3*	9.1
Chuckwalla	3.6	3.9	4.3
Chemehuevi	2.8	3.9	3.4 (4.0)
Fenner	5.0	4.9*	6.1
Joshua Tree NP	4.1	3.9	4.9
Pinto Mountains	2.7	3.9	3.2 (4.0)
Piute Valley	5.3	3.9	6.3
Northeastern Mojave	4.4	3.9	5.6
Beaver Dam Slope	6.2	3.9	7.9
Coyote Springs	4.2	3.9	5.0
Gold Butte	2.9	3.9	3.5 (4.0)
Mormon Mesa	6.8	3.9	8.2
Upper Virgin River	16.0	4.3*	20.9

^a Mean estimated density from trend analysis through 2014 (from Allison and McLucke 2018).

^b Populations at marked with asterisks are projected to reach 3.9 adult tortoises/km² within 3 years. For regions already below this threshold or that contain increasing populations, depleted-population density is set at 3.9 adult tortoises/km².

^c The upper limit is set at 1 standard deviation above the mean density for that area, as estimated from the range-wide monitoring program (Allison and McLuckie 2018). For regions where the cap is below the minimum viable density of 3.9 adults/km², the population may be increased to at least 4 adults/km² in conjunction with additional on-the-ground recovery actions implemented within the **recipient site** to increase the probability that the habitat can support a more robust population; associated effectiveness monitoring also should be conducted.

⁷ Approximately 74-88% of a wild population consists of tortoises < 180 mm carapace length (Turner *et al.* 1987; Karl 1998), but juvenile desert tortoises have naturally higher mortality rates than adults (Bjurlin and Bissonette 2004). Individuals released in this size category are expected to ultimately add less to the population than, and compete minimally for resources with, adult tortoises.



*Figure 3. Desert tortoise density threshold for **recipient populations** relative to regional estimates from the range-wide monitoring program. The density of most (68%) local populations falls within 1 standard deviation (sd) of the mean density in the surrounding region. Tortoise numbers in the **recipient population** following translocation should not exceed 1 sd above the mean regional density without approved justification (see text).*

Selection and approval of **recipient sites** also depend on the disease status of the tortoises in the potential **recipient population**. If handling tortoises for health assessments has not been approved at this stage, clinical signs of disease that can be observed without handling should be documented. This information might alert you to disease issues at the site that could preclude its approval. See Appendices B and G in USFWS (2019, or more recent) for a datasheet with the clinical signs of interest and an algorithm that identifies the most serious and risky of the clinical signs.

Control-site selection considerations—Potential **control sites** should:

- be similar in habitat type/quality (*e.g.*, level of disturbance), post-translocation population size, and disease status to the **recipient sites**, to the maximum extent possible;
- not have foreseeable development or other impacts precluding tortoise occupancy;
- not have been previously used as a **recipient site** for other projects; and
- be a minimum distance of 10 km away from an unfenced **recipient site** that has no substantial anthropogenic or natural barriers to prevent the interaction of **control**, **resident**, and **translocated desert tortoises**.

Review of recipient and control sites—Once the **recipient** and **control** sites have been surveyed, the information below should be submitted to the USFWS to ensure that the proposed **recipient site** meets the site-selection criteria. This information should be provided as early as possible so the project is not delayed.

- Summary information, including complete health histories, for sampled tortoises (*i.e.*, follow the **disposition plan** template in Appendix H in USFWS 2019 or more recent);
- Photographs of individual tortoises as specified on the health assessment data sheet;
- Health assessment data sheets;
- Density information, *including Table 3 from the USFWS Pre-project Survey Protocol and calculation of post-translocation density*;
- Maps of the **recipient** and **control** sites, including all relevant digital GIS layers, illustrating distribution and health status of **resident tortoises**, proposed **release areas** for **translocated tortoises** and the appropriate radius of post-release movement, and any mitigated or unmitigated hazards (*e.g.*, Example 1).

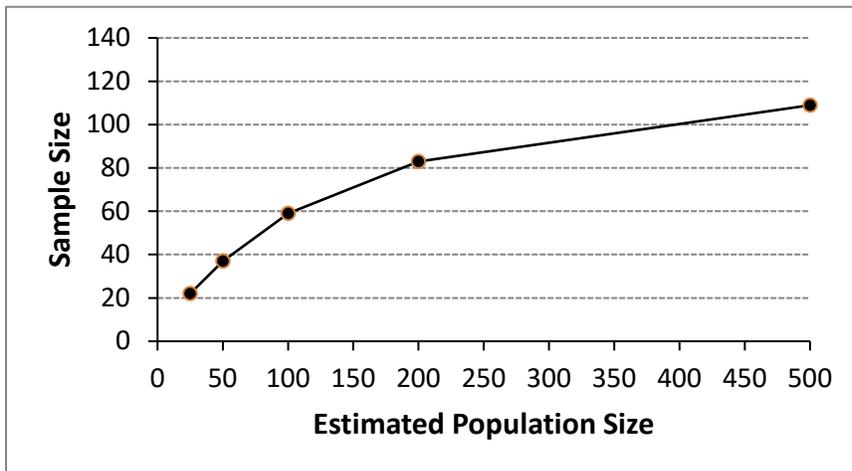
Health assessments

Resident and control tortoises—Unless a regional population-augmentation site is already available, health assessments must be performed on desert tortoises in the **recipient** and **control** populations according to the most recent protocols and under a biological opinion, incidental take permit, or recovery permit issued by the USFWS (see **Health-assessment protocols**). If the USFWS has issued a recovery permit for the project, health assessments conducted in

*Full health assessments must be conducted on **resident and control tortoises** during the same season as translocation; however, **biological sample** collection is not necessary if samples previously were collected within 1 year of the translocation season.*

accordance with the most recent protocols may be performed concurrent with the protocol surveys of the **recipient** and **control** site. Additional health assessments of the **recipient** and **control** tortoises, not including collection of **biological samples** if previously collected within 1 year, should occur during the same season as the translocation. The final assessments will serve as the baseline condition with which to compare post-translocation assessments.

Target sample sizes are estimated as those needed to detect 10% prevalence at the 95% confidence level and 5% precision (Figure 4).⁸ These sample sizes will likely be greater than the minimum number of observations described by the USFWS **Pre-project Survey Protocol** needed to estimate abundance. Project proponents should coordinate with the appropriate USFWS office to determine sample-size requirements based on **recipient-site** abundance estimates.



*Figure 4. Target sample size to detect 10% disease **prevalence** at the 95% confidence level and 5% precision. If the true **prevalence** is < 10%, fewer samples will be needed to reach the desired confidence level and precision.*

⁸ The targeted level of sampling is necessary to precisely document baseline conditions relative to monitoring potential changes in **prevalence** throughout the effectiveness-monitoring period. Sample-size analysis was conducted using the EpiTools epidemiological calculator (Sergeant 2015) and is based on the serological test for *Mycoplasma agassizii*, for which documented sensitivity (0.98) and specificity (0.99) estimates exist (Wendland *et al.* 2007; see also Gray *et al.* 2017).

Translocated tortoises—A minimum of two health assessments must be completed 14–30 days apart prior to translocation (under a biological opinion, incidental take permit, or recovery permit issued by the USFWS). Additional assessments (outside of 30 days) may be conducted, but a narrow window is necessary to discover animals with intermittent clinical signs. The last assessment should occur immediately (within 1–2 days) prior to the translocation date and does not require collection of **biological samples** from tortoises for which samples were previously collected. The final assessments will serve as the baseline condition with which to compare post-translocation assessments and as a final check against the current algorithm determining whether tortoises are suitable for translocation (Figure 5). Any tortoises that were previously approved for translocation, but now show clinical signs that disqualify them according to the algorithm, should not be translocated and their disposition discussed with the USFWS.

*A full **health assessment** must be conducted on each **translocated tortoise** within 14–30 days of the final assessment of release AND within 1–2 days of release; however, **biological sample** collection is not necessary if samples were collected within 1 year of the translocation season.*

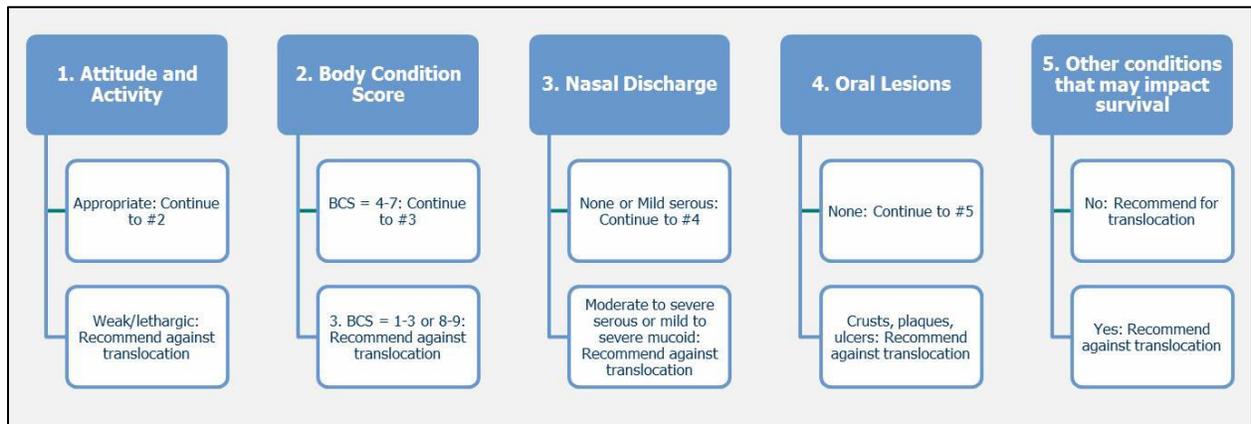


Figure 5. Health assessment algorithm used to determine whether an individual tortoise is suitable for translocation (Appendix G in USFWS 2019). Confirm with USFWS for most recent algorithm.

Health-assessment protocols—Health assessments include a physical inspection and, typically, collection of **biological samples**. Health assessments may only be conducted by individuals approved and permitted by the USFWS. Individuals should inquire with USFWS about opportunities to receive certification. Because of new health-assessment standards and the need for standardized data, certification will not be granted solely on past experience. Training for performing health assessments and collecting **biological samples** is conducted subject to availability of tortoises and instructors.

While health assessments may occur at any time after tortoises in the population generally have emerged from hibernation, it is important that the desert tortoise’s immune system is actively responsive when blood samples are drawn. Blood may be drawn beginning May 15 or, upon specific approval from USFWS, four weeks after the date that the individual of interest left its hibernaculum or was first found active and above ground. The last date for blood sampling is October 31. See (USFWS 2019) for complete details, but all tortoises will have blood collected

to check for antibody presence via **ELISAs** for *Mycoplasma agassizii* and *M. testudineum*. Pathogen detection via **qPCR** will be used to check for the presence of both mycoplasma species and Testudinid herpesvirus 2 on the oral swabs. The results of these tests will provide baseline information for comparison of pathogen **prevalence** over time, but will not determine eligibility for translocation of individual tortoises. *Therefore, results from **biological samples** are valid for 1 year from the date they were collected (for **translocated, resident, and control tortoises**).* A plasma sample and oral swabs should be sent to the labs specified in the health assessment handbook (see Appendix F.7 in USFWS 2019 or more recent) for immediate analysis, and the remainder of the plasma should be banked at the designated sample bank for future use in evaluating the effects of translocation on changes in disease status within individuals or within the **recipient population** at large.

Diagnostic laboratory results are used for monitoring potential changes in disease status or **prevalence** over time rather than disqualifying individual tortoises from translocation because

- a) the likelihood of presence of the target pathogens in source and destination populations is similar (*e.g.*, see Weitzman *et al.* 2017),
- b) the probability that planned translocations is the only avenue of exposure is low,
- c) translocations are targeted toward low-density tortoise populations, and
- d) the estimated cumulative risk of a negative population consequence is low (Rideout 2015).

Furthermore, without known **prevalence** in the population or if the population-level prevalence of a particular pathogen is low, positive test results for individuals may not be reliable (*e.g.*, Wendland *et al.* 2007). Repeated **health assessments** by qualified individuals will screen tortoises currently in poor health.

Given this rationale, projects translocating a **small number of tortoises** that will not be monitored long-term—when a **regional augmentation site** is unavailable—need not collect **biological samples** unless the tortoises are from a population of particular interest (*e.g.*, in the vicinity of a population with a history of a disease outbreak). A physical exam of each **translocated tortoise** will be conducted to screen against disqualifying conditions (Figure 5). When a **regional augmentation site** is available, projects will collect **biological samples** from all tortoises to contribute to the overall baseline at the augmentation site.

Samples should be delivered directly to the appropriate lab and/or the entity hosting the USFWS sample bank (currently the University of California, Los Angeles) for banking/analysis on a monthly basis (see Appendix F.8 in USFWS 2019 or more recent). *A fee to cover expenses associated with archiving **biological samples** and maintaining the sample bank should be included with the shipment: \$3000 in total to cover all fees for the duration of monitoring (2020 dollars). The full archival fee covers costs associated with samples expected to be collected during the pre-translocation and **post-translocation monitoring** periods, including proportional*

numbers of **resident and control tortoises**.⁹ Upon completion of the health assessments for a given season (*i.e.*, spring or fall), a copy of all health data sheets must be submitted to the USFWS, in accordance with the terms and conditions of the biological opinion, incidental take permit, or recovery permit. Additionally, data sheets must be available when **disposition plans** are being reviewed by USFWS.

Determine if desert tortoises on the project site will be held on- or off-site

The **translocation plan** should identify which of the following interim holding/monitoring arrangements will be used for the desert tortoises on the project site. All desert tortoises too small to be transmittered (*i.e.*, transmitter packages weighing no more than 10% of the tortoise's body mass are unavailable; project proponents should procure transmitters with a range of sizes to accommodate sub-adult and juvenile tortoises) should be placed into individual quarantine pens so they can be relocated at the time of translocation.

Option 1 (preferred): In situ monitoring – monitoring desert tortoises on the project site via telemetry—As protocol **clearance surveys** are conducted, health assessments, assignment of unique identifiers provided by USFWS, and affixing transmitters should be performed on each desert tortoise (including juveniles) as it is located. Telemetry monitoring would then be conducted a minimum of once per month with more frequent monitoring under certain circumstances. The USFWS will provide a standardized, minimum dataset to be collected for all projects. This option minimizes potential stress and associated health issues that may result from confinement in pens (Rideout 2015).

Option 2: Ex situ monitoring – construction of individual quarantine facilities—Desert tortoises located during protocol **clearance surveys** would be transferred to a quarantine facility, and these tortoises should be translocated within 6 months of collection. The quarantine facility may be located off-site or within an area on-site not scheduled for development activities until after the translocation. Attachment 2 provides an example for facility design, animal husbandry, and operating protocols, but the USFWS must approve the quarantine facility and operating plan for each project, under the authority of the project's biological opinion, incidental take permit, or recovery permit.

Facilities must be constructed and managed to prevent tortoises from coming into contact with one another, exclude predators, provide ability for appropriate thermoregulation, and allow for necessary husbandry activities by a caretaker qualified to care for captive tortoises and certified to conduct health assessments and collect samples. If this option is selected, quarantine facilities should be constructed to avoid inadvertently capturing any **resident tortoises** within the facility. If suitable USFWS-approved facilities exist in the area, the project proponent may inquire with facility managers about temporary use; however, these opportunities are extremely limited.

⁹ The fee assumes 5 archived tubes/assessment and 30 tubes archived/hour @ \$15/hour (\$15/hour ÷ 6 assessments/hour = \$2.50/assessment [tortoise]). Up-front fees to cover sample archival over the entire monitoring period assume approximately 100 **health assessments** per **recipient/control** site survey * 8 surveys: 800 assessments * \$2.50/assessment = \$2000. An additional \$1000 partially defrays the cost of purchasing a freezer to house the samples.

Construct project fencing and conduct clearance surveys of the project site

General clearance protocols—All desert tortoises, including juveniles, encountered during **clearance** of the project site and associated **perimeter fence** are considered part of the project and should be treated accordingly (e.g., tortoises moved < 300 m and \geq 300 m on the same project all receive the same health-assessment and monitoring considerations). During the initial health assessment, desert tortoises will be assigned a unique identifier (provided by USFWS), and a transmitter will be attached for monitoring purposes. If the desert tortoise is being moved to a quarantine facility, it need not be fitted with a transmitter until it leaves the quarantine facility (if telemetry-based monitoring is necessary).

Data collected during **clearance surveys** (see Attachment 1) will be standardized for all projects and must include detailed information about the exact point of collection. For those desert tortoises that will be monitored *in situ*, these data should be collected again on the day of translocation from the project site. The unique identifiers will allow us to link each individual desert tortoise with data obtained during **clearance surveys** and subsequent health assessments.

The placement of desert tortoises will depend on the health assessment results and the **translocation plan** (i.e., holding/monitoring option) approved under the incidental take permit or biological opinion. Individual tortoises eligible for translocation are those that exhibit appropriate attitude and activity; acceptable body condition (Body Condition Score of 4–7); no mucoid and not more than mild, serous nasal discharge; no oral lesions; and no other condition that may impact its survival (Figure 5; USFWS 2019).

There are three potential outcomes for tortoises located during **clearance surveys**.

- a. Preferred option: tortoises remain on-site for *in situ* monitoring until translocation, pending concurrence with results of health assessments and **disposition plan** (Appendix H in USFWS 2019 or more recent).
- b. Transfer of desert tortoises to quarantine facility for holding (*ex situ*). Final translocation decisions depend on concurrence with results of health assessments and **disposition plan** (see Appendix H in USFWS 2019 or more recent), but tortoises should not be held in the quarantine facilities for > 6 months.
- c. Transfer to agency-approved facility. Project proponents may identify and propose a separate facility to transfer tortoises. This option is only appropriate for individuals deemed ineligible for translocation. Project proponents should expect to pay costs associated with the provision of housing, care, treatment, and other expenses.

Component-specific protocols

Project proponents will require a biological opinion or incidental take permit from the USFWS to implement the following activities. In this section, we provide guidance regarding the implementation of these activities. The USFWS's biological opinion or incidental take permit will describe specific requirements for each project.

Perimeter Fence: Fence construction may be done during any season. Individuals located within the fence-**clearance** area should be moved into adjacent habitat outside the fence line (with the

landowner's approval) in accordance with **clearance** and handling procedures outlined in Attachment 1. These individuals still require a **health assessment**, as described above, should be given a unique identifier, and should be fitted with a transmitter or otherwise monitored to ensure the individual does not move back into the project site before the fence is completed. Shade structures should be installed along the fence line at a minimum 1,000 feet apart to provide cover for any tortoises that pace the fence (Figure 6; USFWS 2018b).



*Figure 6. Shade structure on outside of desert tortoise **exclusion fence**.*

If a desert tortoise that was moved out of the fence alignment moves back into the project site prior to the completion of the fence, the individual should be translocated as identified in the **translocation plan**. If the individual remains on the outside of the fence upon completion, it will be considered a **resident** of the area, and if **resident** individuals in this area do not need to be monitored as part of the **translocation plan**, the transmitter would be removed. However, if the tortoise is observed behaving abnormally (*e.g.*, pacing the fence, not seeking shelter during hot temperatures), the USFWS may direct that additional monitoring with telemetry be conducted long enough to ensure that the tortoise resumes normal behavior and activity. If there is no suitable habitat adjacent to the fence line and the **recipient site** is not yet available to receive desert tortoises from the project site, the individuals should be placed into quarantine pens or fitted with transmitters and placed inside the perimeter fence for *in-situ* monitoring. If **clearance** of the perimeter fence is conducted during the winter, then any desert tortoises located along the fence alignment should be moved as described above, fitted with a transmitter, barricaded into an artificial or empty natural burrow, and monitored as described below.

Non-linear Project Site (*e.g.*, power plant, substation, etc.): **Clearance surveys, health assessments**, and subsequent translocation should be conducted during the tortoise active season. The maximum transect width for **clearance surveys** is 5 m (see Attachment 1). All tortoise scat should be collected during each pass of the **clearance surveys** to facilitate locating tortoises that may have been missed on previous passes.

Linear Facilities (*e.g.*, transmission and buried lines): **Clearance surveys** may be conducted during any season. Any desert tortoises found during **clearance** of linear facilities should be moved out of harm's way as described under **Linear projects**, above.

Translocation of desert tortoises following acceptance of translocation-review package

As previously noted, translocation to regional population-augmentation sites is preferred. However, if a regional augmentation site is unavailable, projects may hire qualified scientists to develop and implement their own project-specific translocation and monitoring plan, which must be approved by the USFWS to ensure that it adequately addresses specific criteria of success (see ***Post-translocation monitoring***, below). In either scenario, a **translocation-review package**, incorporating the penultimate **health assessment** in the month before the scheduled translocation, must be submitted to the USFWS for approval of the proposed disposition of each tortoise from the project site. If **health assessments** are conducted in a season prior to the scheduled translocation date, a tentative **translocation-review package** may be submitted for review subject to consideration of new results from the assessments conducted in the month prior to translocation; this can help expedite final approval of the **disposition plan** (see below). The **translocation-review package** must include the following (information on **recipient-site** tortoises is not required for projects using a USFWS-identified regional augmentation site):

- **Disposition plan** (see Appendix H in USFWS 2019 or more recent available from USFWS), including complete health histories, for the project-site tortoises;
- Complete survey data from the project, **recipient**, and **control** sites (if updated from site selection surveys that were previously submitted);
- Photographs of individual tortoises as specified on the **health assessment** data sheet;
- **Health assessment** data sheets for **resident**, **control**, and project-site tortoises, if not submitted previously;
- Maps of the **recipient site**, showing proposed release points of project-site tortoises;
- Maps of the project site (including all project phases and all relevant digital GIS layers), illustrating distribution and health status of project-site tortoises and proposed **release sites** of tortoises to be moved < 300 m (if applicable); and
- Any other project-specific information that supports or clarifies translocation decisions.

Disposition plans summarize the key health findings from the translocation-suitability algorithm (Figure 5) and describe the proposed fate of each desert tortoise (*e.g.*, translocated to **recipient site** or removed from population due to suspected disease) from the project site and must be completed within the season in which translocation occurs. Project delays that result in translocation occurring in a subsequent desert tortoise activity season than that in which the **disposition plan** was developed also result in the need to complete updated **health assessments** (with the exception that results from **biological samples** are valid for 1 year). A minimum of two weeks should be provided for evaluation of the **translocation-review package**, assuming that the package is complete.¹⁰ Desert tortoises must not be moved prior to acceptance by the USFWS of the evaluated **translocation-review package**.

Once approved, translocation must proceed to the USFWS-approved, final **recipient site(s)** in a manner consistent with existing protocols, this guidance (*including a final health assessment, without biological samples, immediately prior to moving the tortoise*), and the specific translocation and monitoring plan. Some flexibility may exist for individual projects based on the

¹⁰ Note that anomalous results in past projects have required additional review, so we recommend sharing health data as early as possible (*e.g.*, prior to compilation of the full **translocation-review package**) to minimize unexpected delays.

time of year, local/regional weather patterns, actual weather conditions during the proposed release event, and condition of the project sites and final selected **recipient sites** (e.g., degraded or recently burned); the project-specific biological opinion or incidental take permit will provide such instructions. Translocations should occur in spring (April 1 through May 31) or fall (September 1–30), subject to temperature constraints described below. Note that the project proponent must make arrangements to hold any tortoises removed from project sites after the final date of translocation. In addition, the following conditions must be met for translocation to proceed:

- Releases will occur only when temperatures range from 18–30°C (65–85°F) and are not forecasted to exceed 32°C (90°F) within 3 hours of release or 35°C (95°F) within 1 week of release. Additionally, forecasted daily low temperatures should not be cooler than 10°C (50°F) for one week post-release.¹¹

Temperature thresholds for translocation differ from those for handling **resident** and **control tortoises** because **translocated desert tortoises** spend more time above ground subsequent to release as they habituate to unfamiliar surroundings, increasing their susceptibility to stress factors such as temperature extremes.

- Release points will be pre-selected during visits to the **release site** and specified in the **disposition plan** (configuration of release points is project-specific). Release points located in washes may contribute to increased site fidelity (reduced dispersal) within the **recipient site** (Germano *et al.* 2012; Nafus *et al.* 2017).
- Desert tortoises must be transported to their **release sites** in clean, ventilated protective containers. If re-used, these containers must be cleaned and disinfected before being used for another desert tortoise (see Appendix A in USFWS 2019 or more recent, for discussion on disinfection).
- When weather records indicate that desert tortoises likely have not had a chance to drink within the previous or current active season, or clinical signs indicate that a tortoise may be dehydrated, tortoises to be translocated should be hydrated within 12 hours before release. All tortoises that void will need to be hydrated according to existing protocols (Appendix F.5 in USFWS 2019 or more recent).
- Desert tortoises should be released at unoccupied shelter sites. Shelters include unoccupied soil burrows, spaces within rock outcrops, caliche caves, and the shade of shrubs.

Short-term data indicate that mortality does not differ between **translocated tortoises** and **residents** during drought (Esque *et al.* 2010; Nussear *et al.* 2012). However, drought can induce

¹¹ Forecasted maximum temperatures recorded at nearby weather stations are typically lower than those near the ground (*i.e.*, closer to tortoise level at 5 cm) at the **release area**. Even though Brand *et al.* (2016) found that **translocated tortoises** had higher average maximum daily temperature and spent more time above 35°C than **resident** and **control tortoises** in the first month after translocation and smaller effects through the fourth month, these differences in the initial months had no effect on survival. These results, combined with comparable survival between **translocated** and non-translocated tortoises observed elsewhere, therefore indicate that this release criterion successfully minimizes the relevant risks to **translocated tortoises**.

latent disease to manifest, and **translocated tortoises** may disperse widely during the first year after release, thereby potentially causing increased transmission of disease (*cf. Aiello et al.* 2014). The risk of spreading disease to **resident tortoises** will already be minimized by selecting a **depleted population** as the **recipient population**, so risks of spreading disease among drought-stressed **translocated tortoises** should be weighed against the risk of holding them in pens if project timelines are inflexible.

Desert tortoises determined to be infectious or unhealthy must be sent to an agency-approved facility where they will undergo further assessment, treatment, euthanasia, and/or necropsy. Such facilities may include a local veterinary clinic, research institution, or other facility with expertise in reptile medicine. Coordination with the USFWS and the proposed facility must be initiated when **clearance surveys** commence to facilitate prompt and humane transport of unhealthy desert tortoises, as necessary.

Compile and synthesize data

Data must be entered into the USFWS/BLM-provided master database, according to standardized format and conventions, to ensure consistency among projects across the desert tortoise's range. Minimum requirements will include data related to observations recorded during surveys, telemetry-based monitoring, and **health assessments**.

Post-translocation monitoring

Post-translocation monitoring must be designed to document the effectiveness of translocation as determined by specific criteria for success at four stages over an approximately 30-year period (Table 2).¹² The USFWS will oversee monitoring of regional augmentation sites, so projects moving tortoises to these sites need to coordinate funding for this monitoring with the USFWS. Projects hiring qualified scientists to develop and implement their own project-specific **translocation plan** must secure the approval of the USFWS for those plans and monitoring programs to ensure that they adequately address the criteria of success. Anticipated monitoring components include project management; field labor for surveys, **health assessments**, and environmental sampling (*e.g.*, annual and perennial vegetation, temperature, rainfall), including supervisory and technical personnel; travel; and supplies, including transmitters to relocate females for X-radiography.

When a regional augmentation site is not available and few tortoises need to be translocated, long-term monitoring of only a **small number of tortoises** lacks statistical power to inform questions about biological effectiveness or contribution to recovery. In addition, such projects conducted under the protocols described herein pose negligible risks of negative population-level effects. Therefore, we do not recommend long-term monitoring in these

¹² A review of reptile and amphibian translocations considered translocations to be successful when a) substantial new recruits were added to the adult population due to successful reproduction at the translocation site, and b) the site was monitored for at least as long as the time to reach maturity (13–20 years for desert tortoises). Success of prior translocations is often uncertain due to inadequate monitoring time (Germano and Bishop 2009). Given the time to reach maturity in desert tortoises and low and variable survival rates of juveniles, it may require 30 or more years to detect substantial recruitment to the adult population. Thirty years is commensurate to the duration of development permits for which this guidance was developed and is less than the duration of direct project impacts, which are essentially permanent.

instances. Instead, telemetry-based monitoring of 1 year may be appropriate to document short-term survival of **small numbers of translocated tortoises**.

The specific monitoring plan, including sample sizes and sampling intervals, should be developed by a qualified scientist working on the project and should be designed to address the success criteria in Table 2. However, for translocations to a regional augmentation site or other large translocations, a basic monitoring program generally is envisioned to consist of tracking each **translocated tortoise** (and a sample of **resident** and **control tortoises**) with radio telemetry for the first 5 years of the program. Telemetry monitoring during this period will allow evaluation of Stage 1 and Stage 2a in the success criteria (Table 2) and validate that there are no unforeseen complications with the **recipient site** that need to be corrected before continuing the translocation/augmentation program. **Health assessments** will also be conducted during this period to compile a solid baseline of health status in the **recipient** and **control** populations for longer-term monitoring comparison.

Subsequent to the telemetry-based monitoring, the remainder of the 30-year monitoring period could consist of periodic surveys (*e.g.*, biennial or triennial) of the **recipient** and **control** populations to evaluate the longer-term stages in the success criteria (Table 2). Environmental sampling and **health assessments** should be conducted in conjunction with alternate population surveys. Monitoring scheduling and approaches will be adapted based on initial results, scientific input, and the rate of translocation into a particular **recipient site**. Monitoring other site-specific measures that could influence the metrics listed in Table 2 will also be conducted depending on site-specific factors, which potentially could result in the need to re-evaluate prior stages of the success criteria. For example, the effectiveness of translocation relative to the contribution of **translocated** male tortoises to reproduction in the **recipient population** should also be evaluated in light of the results of Mulder *et al.* (2017).

Table 2. Success criteria for desert tortoise translocations (cf. Miller et al. 2014; Bell and Herbert 2017). Evaluation of each stage is contingent on success of the previous stage(s).

Stage	Indicators/metrics	Time frame (post-translocation)
1. Survival and growth of released and resident individuals	a. Survival within 20% of controls ¹³ b. Increase in CL since release (tortoises released at <180 mm CL) ¹⁴	a. 5 years b. 5–6 years
2. Evidence of reproduction in released and resident individuals	a. Female reproductive output is similar to controls ¹⁵ b. Juvenile segment of the size-class distribution is increasing ¹⁴	a. 5 years b. 9–18 years
3. Population growth	Increasing trend in adult population size ¹⁴	15–20 years
4. Viable population	Adult density >> 4/square km, excluding founders ¹⁴	20–30 years

¹³ Measured via radio telemetry. Survival within 20% of **control tortoises** is specified as a Stage 1 metric of success, even though much lower survival rates have been considered successful in translocations of other species, because high survival is necessary to achieve a self-sustaining population within 20–30 years (Stage 4).

¹⁴ Measured via periodic (*e.g.*, triennial) mark-recapture surveys

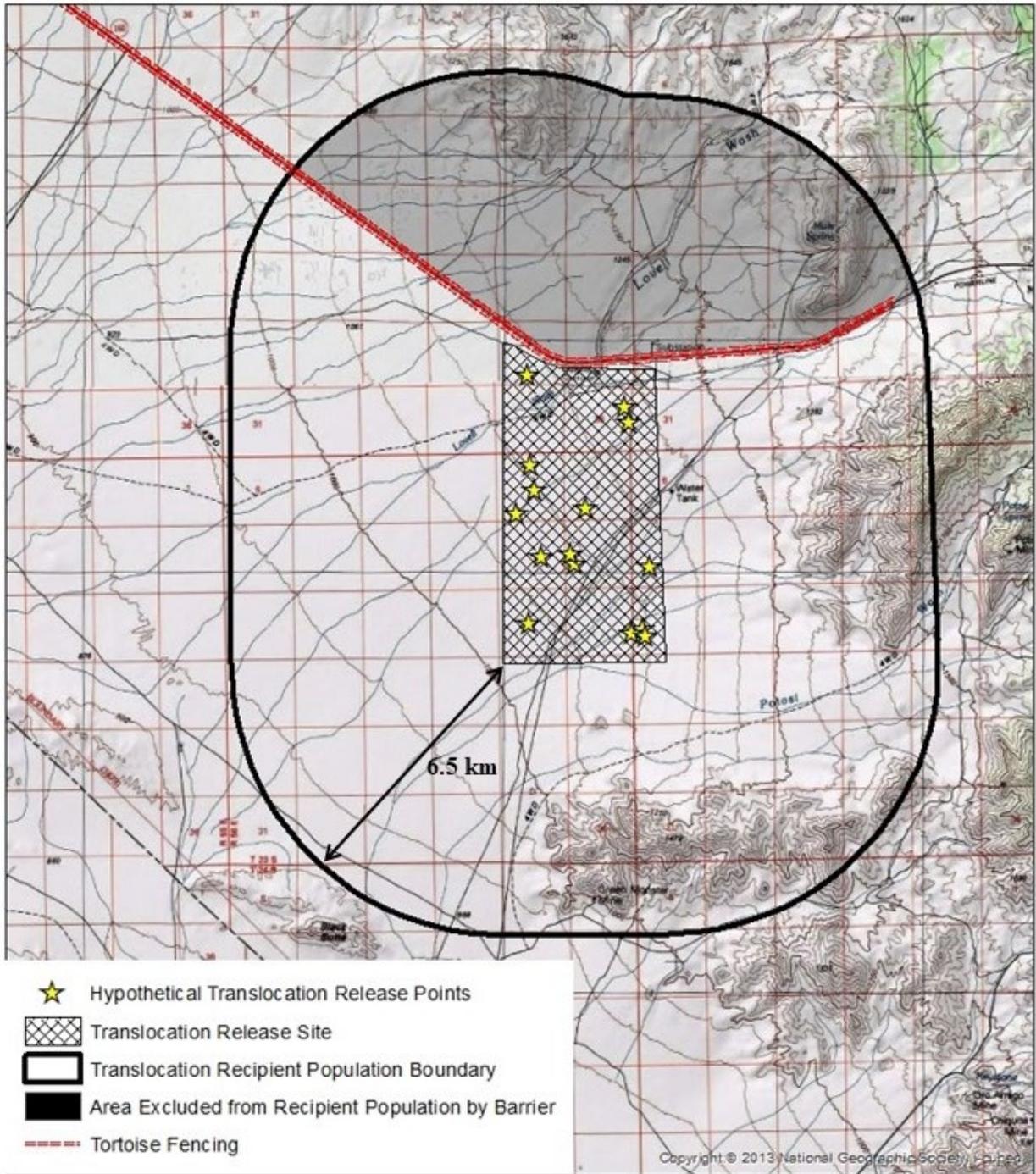
¹⁵ Measured via radiographic examination of females during the telemetry-based monitoring

Example 1. Illustration of key aspects of selecting a **recipient site** for translocation.

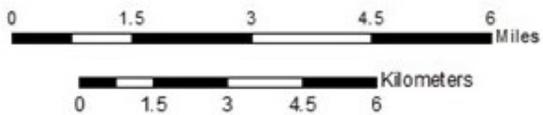
If on-site impacts to desert tortoises cannot be avoided, the emphasis for translocation projects is to contribute to tortoise recovery by augmenting **depleted populations** rather than merely moving them off-site to expedite development. Check with your local USFWS office to determine whether an augmentation site has already been identified that is relevant to your project.

- The **recipient population** should be low density (see *Determination of recipient-site size*) to minimize risk of disease transmission, whether inadvertently introduced by **translocated tortoises** or facilitated by increased tortoise interactions and contacts following the translocation (Rideout 2015).
- One or more potential sites should be identified within which tortoises would be released (hatched polygon).
- **Translocated tortoises** tend to move long distances and are likely to encounter tortoises with which they are unfamiliar (*cf.* Aiello *et al.* 2014). Therefore, habitat, **resident tortoise** densities, and **resident tortoise** health should be evaluated within a 6.5-km radius of the potential **release site(s)** (heavy black outline; distance based on data from the 2008 Ft. Irwin translocation: Berry *et al.* 2009; Drake *et al.* 2009).¹⁶
- Shading in the example illustrates how barriers to tortoise dispersal, such as tortoise exclusion fencing along a highway, may limit the total area requiring evaluation, although such eliminations should be confirmed with the local USFWS office (*e.g.*, to review the potential permeability, or lack thereof, of the putative barrier).

¹⁶ If the **recipient site** is adjacent to the project site, the **recipient site** has not already been surveyed for density and health, and all **translocated tortoises** will be moved <300 m, then an area within only 1.8 km need be evaluated. A 1.8-km radius surrounding the project footprint includes up to 300 m for potential releases and 1.5 km for dispersal.



Created By: Roy C. Averill-Murray
 Map Date: August 19, 2015
 Source: USFWS files



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Attachment 1: Clearance Survey Protocol for the Mojave Desert Tortoise

CLEARANCE SURVEY PROTOCOL FOR THE MOJAVE DESERT TORTOISE

1. Objectives

- Locate as many desert tortoises as possible within the project site.
- Remove all desert tortoises encountered from the project site.
- Safely excavate, collect, and rebury desert tortoise eggs.

2. Applicability of Clearance Surveys

For projects located in occupied desert tortoise habitat (as evidenced by presence of live tortoises or recent sign), especially those projects with a permanent or linear disturbance (*e.g.*, pipelines, roads, transmission lines), a clearance survey may be required as part of the conditions of a biological opinion or incidental take permit. This survey is intended to reduce the likelihood that desert tortoises are killed or injured as a result of the proposed action. Clearance survey methods may include temporarily penning desert tortoises within the area surrounding its burrow, moving desert tortoises out of harm's way a short distance from the impact area, or translocating desert tortoises to a designated area outside its home range in accordance with a USFWS-approved translocation plan. Note that it is the project proponent's responsibility to obtain all other necessary authorizations from the applicable State wildlife agency and land management agency.

3. Methodology

Clearance surveys require 100% coverage of the project area, with a focus on locating all desert tortoises above and below ground within the project area. This survey should be conducted immediately prior to surface disturbance at each site within the project area or following construction of a desert tortoise-proof fence encompassing the project area that would ensure that tortoises cannot enter the project area after clearance surveys are completed.

Clearance surveys at the project site must consist of at least 2 consecutive surveys of the site, the second walked in a perpendicular direction to the first. Surveys will involve walking transects less than or equal to 15-ft (5-m) wide under typical conditions. In areas of dense vegetation or when conditions limit the ability of the surveyor's to locate desert tortoises, transects should be reduced in width accordingly. Clearance surveys should be conducted when desert tortoises are most active (April through May or September through October). The ultimate number of surveys required may depend on the scale of the project and the number of tortoises found on the last survey.

Desert tortoises often pace along new fences attempting to gain access to the other side or return to areas from which they were removed. After the desert tortoise exclusion fence has been installed, the fencing should be checked several times a day when temperatures are expected to exceed 95°F (35°C) to ensure tortoises are not trapped within the fence or are fence-walking; these animals may be exposed to lethal temperatures. If a suspected hyperthermic tortoise is found along a fence and temperatures are above 95°F (35°C), the tortoise should be moved to a climate-controlled area and re-hydrated.

All methods used for handling desert tortoises during the clearance surveys must be in accordance with the USFWS Desert Tortoise Field Manual or project-specific guidance contained in a biological opinion or incidental take permit. Anyone that handles desert tortoises during clearance activities must have the appropriate authorizations from USFWS.

During the clearance surveys, desert tortoises in burrows may be removed through tapping (Section 4) or careful excavation. Multiple visits may be necessary if desert tortoises are inaccessible in deep caves or burrows. During all handling procedures, desert tortoises will be treated in a manner to ensure that they do not overheat or exhibit signs of overheating (*e.g.*, gaping, foaming at the mouth, etc.), or are placed in a situation where they cannot maintain surface and core temperatures necessary to their well-being. Desert tortoises will be kept shaded at all times until it is safe to release them. Ambient air temperature will be measured in the shade, protected from wind, at a height of 2 in (5 cm) above the ground surface. All clearance activities must be conducted under appropriate temperatures specified in the translocation guidance.

The area cleared and number of desert tortoises found within that area must be reported to the local USFWS and the appropriate State wildlife agency. The report should be made in writing, either by mail or email. Notification must be made in accordance with the conditions of the biological opinion or incidental take permit.

If a desert tortoise is encountered after clearance surveys have been completed, process the tortoise according to the methods described above.

4. Extracting Desert Tortoises from Burrows

Before touching a desert tortoise or using any instrument that comes into contact with a desert tortoise, implement procedures described in the USFWS Desert Tortoise Field Manual (Chapter 7). Examine the burrow for other occupants (*e.g.*, snakes, spiders, scorpions, wasps, Gila monsters, etc.). Firmly, pound the soil at the side of the “apron” or soil mound at the entrance of the burrow 5 to 6 times with an open hand then listen for desert tortoise movement; wait 30 seconds and repeat several times if repeat several times if the tortoise does not readily emerge. Avoid disturbing or pounding the center of the apron or entrance of the burrow where desert tortoises typically dig nests and lay their eggs. If the desert tortoise is visible deep in its burrow, the observer can gently tap the carapace 3 to 4 times with a stick (Medica et al. 1986). The observer should then remove the stick and move away from the burrow entrance. If tapping is successful, the desert tortoise will emerge, usually to the burrow entrance. If desert tortoise movements are not heard within a few minutes, discontinue tapping.

If the desert tortoise is within arm’s reach, firmly grasp the gular, plastron, or posterior edge of the carapace and gently pull the tortoise towards the burrow entrance. If the desert tortoise resists to the point where moderate pulling effort is unsuccessful, stop pulling while maintaining a grip on the tortoise; resume when the tortoise relaxes. Never use a hook or other instrument to remove a desert tortoise from a burrow or otherwise compromise the integrity of a burrow if the desert tortoise will remain in the project area.

If the area is to be cleared of all desert tortoises, excavate the burrow as described in Section 5. If the tortoise is in a deep caliche cave which cannot be excavated without potentially harming the desert tortoise, record the location and coordinate with the USFWS for further instructions.

5. Excavating Burrows

Desert tortoise burrows should be excavated only if they occur within a proposed disturbance area. As an alternative to excavation in certain circumstances, the immediate area surrounding a burrow occupied by a desert tortoise may be temporarily penned, if authorized by the USFWS and the appropriate State wildlife agency (Section 9).

Before excavation, feel for desert tortoise eggs by gently probing the soil in front of the burrow opening (*i.e.*, the mound) with a blunt instrument (*e.g.*, knitting needle) or similar instrument, and along the floor of the burrow as you excavate the burrow. The purpose of probing is to locate areas of excavated soil that are less compacted and may indicate a nest. Eggs have been found up to 6 ft (1.9 m) in front of burrow openings and up to 6 ft (1.9 m) within the entrance of a burrow; they may also occur in the mound at the burrow opening. To avoid crushing eggs, do not scrape the shovel across the bottom of the burrow, but continue to probe the area with your fingers as you proceed. Removal of the top 10 in (25 cm) of soil (or until a hard layer of soil is encountered) will typically ensure that you find any desert tortoise eggs. Be particularly careful from late April to mid-October when eggs are most likely present. If found, follow the USFWS's egg handling protocol (Section 6).

Excavators should wear leather or cloth gloves during burrow excavation to avoid being bitten or stung by venomous animals. Use blunt-nosed shovels or garden trowels. The preferred method involves two individuals, each with a shovel, to excavate a burrow. Place a shovel in the burrow entrance, or garden trowel for small burrows, and slice away the ceiling with the second shovel or trowel. Remove the soil with the first shovel or trowel as excavation proceeds and repeat. Excavate the burrow slowly and carefully and stop often to see if a desert tortoise is within reach. Do not collapse the burrow ahead of the shovel or trowel inside the burrow. You should feel the shovel contact the other shovel with each stroke to avoid striking a desert tortoise. It may take several minutes or several hours to excavate a desert tortoise burrow, depending on its length and other characteristics.

Always excavate the burrow to its absolute end(s), and then excavate an additional foot-or-so (0.3 m) of harder soil beyond the suspected end to ensure that a desert tortoise is not behind a dirt plug or mound. Search all side tunnels within the burrow for desert tortoises, especially in kit fox dens. If a desert tortoise is found, do not assume that it is alone. After removing the first desert tortoise encountered, return to the burrow and continue to excavate it looking for additional desert tortoises. After excavating the burrow, leave it collapsed so that no desert tortoise may reuse it easily.

When excavating a burrow, stop digging when a desert tortoise is encountered. If during the less-active period for desert tortoises (*i.e.*, during July - August, and November - February; in Arizona the less-active period may begin in late May or June), relocate the desert tortoise to

another suitable burrow and contact the USFWS for additional guidance and coordination. If it is during the most-active period (*i.e.*, when desert tortoises are most likely above ground; March - June, and September - October), place the desert tortoise in the shade of a shrub or near other natural cover sites (Section 7).

6. Nest and Egg Handling Protocol

Desert tortoises may lay eggs during the months of May through July and usually hatch July through October. Some eggs may not hatch, or hatchlings may not emerge until the following spring. Because desert tortoise eggs are also protected by the Endangered Species Act, examine burrows with evidence of digging in the burrow floor to look for eggs. Desert tortoise eggs will be moved to a similarly constructed artificial nest in the wild or to a USFWS-approved facility. If you encounter unemerged hatchlings, release into an unoccupied burrow in the wild unless otherwise directed by the USFWS.

Any nest that is found will be carefully excavated by hand at a time of day when the air temperature 6 in (15 cm) above the ground is approximately equal to the soil temperature at egg level. Excavate suspected nests by hand. Disposable rubber or latex gloves must be worn when marking and handling eggs. Before disturbance of nest contents, each egg will be gently marked with a small dot on the top using a felt-tipped pen to establish the egg's orientation in the nest. In handling nest contents, eggs must be maintained in this orientation at all times. Because egg shells become extremely fragile in the last few weeks before hatching, special care will be taken with eggs found from August to mid-October. Because the egg is very fragile, it may break during handling; this will be lethal to the developing tortoise inside. Broken eggs will be buried nearby and left in the field, or the contents preserved and made available for research projects. Report broken eggs to the USFWS and appropriate State wildlife agency as required for tortoise mortalities.

Measure and record the depth of the nest below the soil surface and the position of the nest relative to the burrow entrance (or other shelter cover). Place approximately 1 in (2.5 cm) of soil from the nest area in a bucket and carefully transfer the eggs to the bucket, maintaining egg orientation. Gently cover the eggs with soil that is free of cobbles and pebbles, to a depth equivalent to that of the original nest.

A nest will be prepared at the release site with the same depth and location in relation to the burrow entrance as the original nest. The eggs will be transferred to the new nest, maintaining their original orientation. The eggs will be replaced so that they touch one another. Gently cover with soil from which cobbles and pebbles have been removed so that all the air spaces around the eggs are filled.

7. Constructing Artificial Burrows

Constructing artificial burrows generally is not necessary when translocating desert tortoises, based on past experience demonstrating minimal use of such burrows by translocated tortoises. However, in the infrequent case that an artificial burrow may be needed (coordinate with USFWS), the information below provides a general description of the methods for constructing

artificial burrows taken from Tortoise Group's adoption and care pamphlet (www.tortoisegroup.org).

Create an artificial burrow that is the same orientation and size as the burrow from which the desert tortoise was taken. The burrow for a juvenile desert tortoise should be 3–4 ft (0.9–1.2 m) long, and an adult tortoise burrow should be 5–6 ft (1.5–1.8 m) long. Burrow construction involves digging a three-sided shelf upon which plywood will be placed to serve as the roof of the burrow. A channel is dug below the level of the shelf which approximates the width of the tortoise and functions as the actual burrow.

Determine the width and length to dig the shelf, and place the plywood on the ground. Use corner stakes and twine to delineate the perimeter. Dig the burrow in a downward slant 15–20° below the horizontal line of the ground (Figure 1). Place the plywood onto the shelf. Fit the plywood snugly and then remove it. Next, dig the channel and loosen the soil along the floor of the channel to a depth of 6 in (15.2 cm) to allow a tortoise to dig its way out should the plywood sag and possibly trap or pin it in the burrow. Replace the plywood and shovel dirt on top. Place rocks along the eave of the burrow roof, above the opening (Figure 1). Mound the dirt so that rain water will not puddle on top of the finished burrow.

We recommend that you cover the opening of the artificial burrow with rocks or wood for 2 or 3 days to ensure that the tortoise remains within the burrow and out of harm's way, or that it resumes hibernation or aestivation. Alternatively, the tortoise and its burrow may be temporarily penned (Section 8).

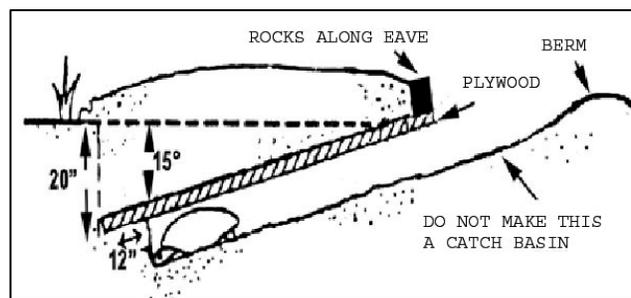


Figure 1

8. Temporarily Confining Desert Tortoises

Occasionally, desert tortoises may be found on the periphery of the project area that may need to be temporarily penned to prevent them from moving onto the project site and then needing to be physically moved. These situations should be coordinated with the USFWS, but tortoises will not be penned in burrows during extreme high temperatures (*i.e.*, above 95°F [35°C]), and construction activity will be carefully monitored in the area around the penned tortoise. The methodology for penning desert tortoises (U.S. Department of Defense 2005) is adapted from a methodology developed by Gilbert Goodlett (EnviroPlus Consulting, Ridgecrest, California). Generally, desert tortoises should not be penned in areas of moderate or heavy public use. Penning will be accomplished by installing a circular fence, approximately 20 ft (6 m) in diameter to enclose the tortoise/burrow. The pen should be constructed with durable materials (*i.e.*, 16 gauge or heavier) suitable to resist desert environments. Fence material should consist of ½-in hardware cloth or 1-in horizontal by 2-in (2.5 by 5.0 cm) vertical, galvanized welded wire. Pen material should be 24 in (50 cm) in width. Steel T-posts or rebar (2–3 ft or 0.6–0.9 m) should be placed every 5–6 ft (1.5–1.8 m) to support the pen material. The pen material should extend 18 in (45.7 cm) aboveground. The bottom of the enclosure will be buried 6–12 in (15–30 cm) or bent inward (towards the burrow), soil mounded along the base, and implement other

measures to ensure zero ground clearance. Care will be taken to minimize visibility of the pen by the public. An Authorized Biologist or Desert Tortoise Monitor will check the pen at least daily and ensure that the desert tortoise is in the burrow or pen, the desert tortoise is okay, and the pen is intact. All instances of penning or issues associated with penning will be reported to the USFWS within 3 days.

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***Attachment 2: Temporary Captive Care of Wild Mojave Desert Tortoises:
Examples of Protocols Used at the Desert Tortoise Conservation Center***

The logo for San Diego Zoo Global, featuring a blue oval shape with the text "SAN DIEGO ZOO GLOBAL" inside in white, uppercase letters.

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Temporary Captive Care of Wild Mojave Desert Tortoises: Examples of Protocols Used at the Desert Tortoise Conservation Center

Updated Oct 12, 2010

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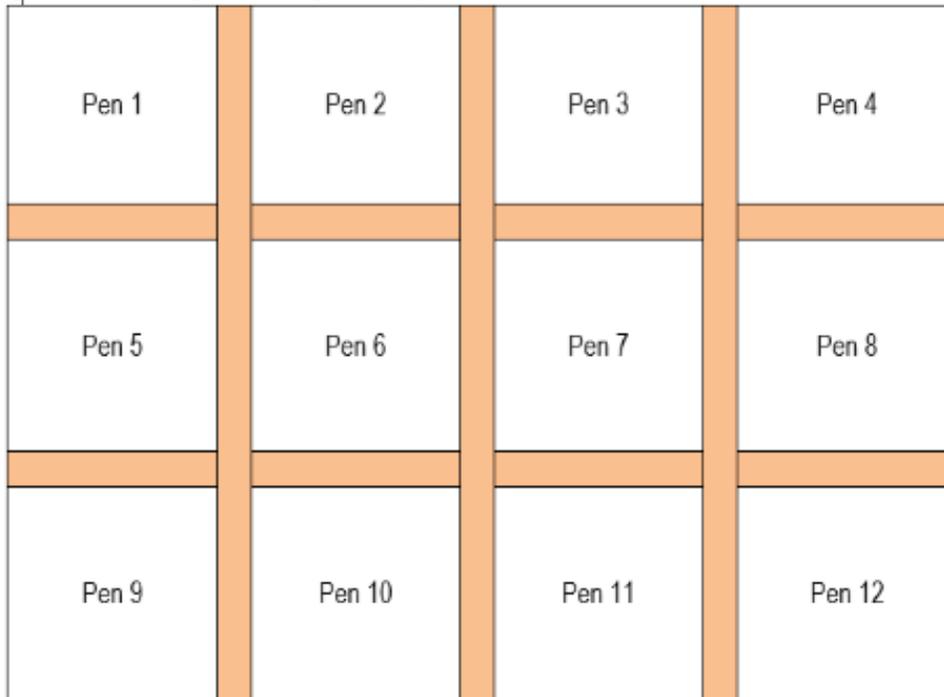
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I. Housing

A. Individual Quarantine Enclosures

Desert tortoises (DTs) at the DTCC are housed outdoors in escape-proof pens, and if under 100mm are maintained in escape-proof, predator-proof pens. Each DT is housed individually to prevent potential disease transmission. The sites where pens are constructed have ample vegetation that is minimally disturbed during construction, and the soil is appropriate for tortoises to dig their own burrows. Pens are constructed with tortoise fencing with at least 60cm of fencing above the ground and at least 30cm buried below ground to ensure that they cannot escape (note that the fencing at the DTCC differs slightly from FWS-approved desert tortoise exclusion fencing). The fencing flares out 25cm at the bottom to ensure that if tortoises try to dig out from the inside or if predators try to dig in from the outside, they will hit fencing. Ideally, each pen has ample vegetation such as creosote bush, yuccas, ephedra, and bursage to provide shade, and other plants like globemallow to serve as food sources. In order to access each pen without the potential for contamination (see the section on Aseptic Techniques), walkways are constructed between individual pens and between each row of pens. If we need to walk inside any pen for any reason, we always disinfect our shoes while moving out of a pen and into a walkway.

Figure 1. Pen Design, Including Walkways



Wild DTs are housed individually to prevent potential disease transmission. Larger DTs (MCL > 124mm) that are less likely to be predated are ideally housed in pens that are 6m x 6m, while smaller DTs (MCL < 125mm) that are more likely to be predated are ideally housed in pens that are 2m x 2m. It is particularly important to us to make sure there are ample native food sources growing in the pens for smaller tortoises. In addition, all pens for smaller tortoises have a lid or cover, and all parts of the enclosure are predator proof, not just against large animals, but against smaller animals such as ground squirrels, which are able to squeeze through ½ inch x ½ inch fencing. We use ½ inch x ¼ inch fencing, but not anything smaller, because smaller fencing can result in small animals, like lizards and snakes, getting stuck in the fence and attracting potential predators. The most critical part of constructing a predator-proof enclosure is making sure that the entry points (the places where we open and close the fencing or lid) are completely predator-proof when they are in the closed position. As an example, here at the DTCC our predator proof enclosures are constructed of

cement block and mortar with a steel diamond-pattern lid painted with reflective paint (picture A). We place shade cloth in the middle of the lid during summer to provide extra shade. Another type we use is a large walk-in predator-proof enclosure with 2m x 2m pens inside it (picture B). Other walk-in enclosures have been constructed at FWS approved head-starting and research facilities, and those facilities may be able to provide example designs and protocols.

Picture A: Hatchling pens with lids



Picture B: Hatchling pens, walk-in structure



B. Burrows

At least one artificial burrow is provided in each pen to fit the particular tortoise it will house. The burrow width is 5-8cm larger than the total length of the tortoise to ensure that the tortoise does not get stuck inside the burrow when it turns around. We measure from the longest anterior and posterior points on the tortoise's shells to get the correct measurement. Similarly, the height of the burrow is 3-5cm taller the height of the tortoise's shell to accommodate movement through the burrow. We do not provide burrows that are more than 10cm larger than the tortoise because tortoises naturally create burrows to fit their size - we are concerned that extra space in a burrow could be detrimental over the winter because it could allow more air flow when temperatures are too low for tortoises to tolerate. Also note that tortoises with special needs, such as those with missing limbs and other conditions that impair movement, are provided with a bigger burrow than they need because they are more likely to become stuck in what would normally be considered a proper size burrow for their body size.

At the DTCC we use PVC pipes to create artificial burrows. We cut them in half lengthwise and use the half pipe as the top of the burrow. The length of an artificial burrow is about 2 meters. To construct a burrow, we dig a trench at a 30-45 degree angle, and 2 meters long. The burrow is approximately 60cm deep at the chamber end. We lay the PVC half pipe in the trench to form the burrow with the front end of the pipe resting 10-15cm higher than the ground to serve as the mouth of the burrow. We sometimes add dirt in the front to make this possible because it's very important to raise the mouth of the burrow to prevent possible flooding. The outer sides of the PVC are lined with large rocks and the entire length of the PVC is covered with rocks and dirt – the rocks help prevent the dirt from blowing away in the wind. We pack at least 30cm of dirt and rocks on top of every part of the PVC. Dirt is moistened daily for 3 to 4 days to promote compaction. It is important that the PVC remains completely covered all the time because without the added layers of dirt and rock, it provides no protection and can actually become an oven in summer and an ice box in winter – tortoises that try to use a burrow that is not properly covered are at a high risk for mortality. Once the burrow is complete, we look inside with a flashlight or mirror to make sure the entire path down the burrow is clear of debris and

ready for a tortoise to enter. We check the temperature at the mouth and chamber of the burrow during the coolest and hottest parts of the day twice each week to ensure that temperatures inside the burrow are within a normal tolerable range for tortoises (15-30C).

Picture C: Artificial burrow



If you will be housing tortoises for an undetermined amount of time, another option for constructing more permanent artificial burrows is offered on the Tortoise Group website (<http://www.tortoisegroup.org/pamphlet.php>) on pages 10-14 of their Tortoise Adoption and Care Handbook. Please note that the San Diego Zoo is not affiliated with Tortoise Group, but offers this as a viable option for the construction of long term tortoise burrows because the method has been used extensively and is shown to be effective. However, the rest of the handbook specifically addresses long term care of captive pet desert tortoises, not wild tortoises, so we do not endorse any of their other material for use with wild tortoises.

We inspect and maintain all burrows, both natural and artificial, nearly every day. Table 1 shows our burrow maintenance schedule.

Table 1. Burrow Maintenance Schedule

Season	Maintenance
March-April	After most tortoises emerge from hibernation, remove remaining berms from burrows where DTs have not emerged. Check burrows daily and add dirt and rocks when necessary
May-October	Check burrows daily and add dirt and rocks when necessary
November-March	After most tortoises have bermed themselves in, berm in the remaining tortoises. Check burrows weekly and add dirt and rocks when necessary

After heavy rains, we check every burrow by looking inside with a mirror or flashlight. If burrows collapse, tortoises can become entombed so the burrow must be dug out immediately. For more information regarding what we do when we find a tortoise entombed after rains, see the Surveillance section.

In order to prevent disease transmission, tortoises are not moved among the pens, or newly placed in a pen where another tortoise was living without first disinfecting the burrow. For more information on how to disinfect burrows and pens, see the Aseptic Techniques section.

C. Irrigation

Every individual pen at the DTCC receives water on a regular basis. For most pens we use a drip system from a central well that leads to a drip head in every pen. We point the drip head toward the downward slope of each pen to avoid flooding the burrow if something goes wrong with the irrigation system. We dig out the earth below the drip head to make a catch basin or we use plant trays to catch the water so the tortoises will have plenty of time to drink before it evaporates.

A second option is to use a row of sprinklers connected to water tanks that are operated with generators and pumps. If you allow a 360 degree spray pattern, then many pens can be watered at once and it promotes the growth of native vegetation.

A final option that we use to irrigate some of our pens is to carry it to the pens manually. We put a large shallow terra cotta or plastic dish in each pen, and sink it into the ground so it is level. We only use shallow dishes so smaller tortoises won't drown if they flip over into it. We carry water to the pens and pour it directly into the dishes.

D. Site Security

It is important that the site where the tortoises are kept is secure from both predators and humans. The DTCC is surrounded with a chain link fence buried at least 30cm into the ground with the bottom flared outward to prevent digging in from the outside. Our fence is topped with 2 to 3 rows of razor wire. It is not recommended that lights be installed, even if they are on motion sensors, because they will likely come on frequently during the night, which can be disturbing to wildlife. If you believe you may have an issue with security at night, you could consider installing motion sensor cameras with night vision in key locations.

Picture D: Perimeter fence



II. Aseptic Techniques

The primary disinfectant we use for surfaces, totes, equipment, instruments, PVC burrows, footbaths, and potentially contaminated clothing and shoes is Trifectant™ (www.amazon.com). This product is a broad spectrum virucidal, bactericidal (including against *Mycoplasma spp*), and fungicidal product. It is effective on porous surfaces such as wood, in the presence of organic matter, in hard water, and at low and high temperatures. Unlike bleach, it is not inactivated by UV light. In addition, it has low toxicity and is biodegradable, and the required contact time is less than 5 minutes.

Trifectant™ is stored as a powder, dissolves quickly in water, and remains stable for 7 days. As per the instructions, we use a 1% solution using warm water - 0.325 oz (1/4 scoop) per quart or 1.3 oz (1 scoop) per gallon. We spray Trifectant™ on all potentially contaminated field equipment and allow it to air dry.

We always wear gloves when handling tortoises, regardless of their health condition, and we change gloves between handling different tortoises. To make sure that tortoises do not come into contact with our clothes, we wear reusable aprons that we disinfected with Trifectant™ after each use and between handling different tortoises. All equipment and supplies that touched a tortoise or that we touch with a gloved hand is disinfected. To prevent contamination of data sheets and some field equipment, we use the one glove technique for handling tortoises and recording data in which one hand remains ungloved and can therefore touch writing utensils, clipboards, field equipment, etc, without potential contamination. If the second hand is needed to help hold or manipulate the tortoise, we slide on a glove one size bigger than the proper fit so we can slide into it and out of it as needed to use equipment and record data.

In order to prevent disease transmission across the site, tortoises are not moved among the pens, or newly placed in a pen where another tortoise was living without first disinfecting the burrow. To disinfect a burrow, we remove the tortoise from the pen and pull the PVC burrow out of the ground. We spray both sides of the PVC with Trifectant™ and expose it to direct sunlight with no rain or moisture for a minimum of 5 days. In addition to sterilizing the burrow, we rake out the area where we pulled the burrow out of the ground and we spray the entire area with Trifectant™. We leave the pen unoccupied (with the burrow removed) and exposed to direct sunlight with no rain or moisture for a minimum of 5 days. However, if there is any moisture in the dirt when we rake it the first time, we spray the area with Trifectant™ and continue to rake and spray it every day until the area appears dry. We then begin the 5 day UV exposure period.

III. Food

Native vegetation and some produce (kale, collards, dandelion greens, etc) are the best sources of nutrition for desert tortoises. However, with large numbers of tortoises in captivity, SDZ Nutritionists and Veterinarians recommend supplementally feeding DTs Mazuri Tortoise Diet 5M21 (<https://shop.mazuri.com/mazuritortoisediets.aspx>). As per the product's feeding instructions, we give healthy tortoises 10 kibbles (10g)/kg of body weight at each feeding during the active season. The food comes in pellet form so we add water until it just reaches the top of the pellets and let it sit for 5 minutes before mixing it up with a gloved hand. If so much water is added that it can be poured off the top after 5 minutes, the food can lose water soluble nutrients so it is important not to overwater the kibble. For tortoises that need to gain weight, we provide additional moistened pellets at each feeding. When feeding the DTs at the DTCC, we walk outside the perimeter of each pen (not inside the pens) and place the approximate amount of food in a ball in front of or near the tortoise. If the tortoise is not out, we toss the ball of food near, but not in, the burrow. Before or during feeding, we remove all old food left behind from the last feeding, disinfecting our shoes when moving from pen to pen. It is important to note if you consistently do not see a tortoise out or if there is consistently food left behind at the next feeding.

Picture E: Mazuri Tortoise Diet soaking



Picture F: DT eating Mazuri Tortoise Diet



Table 2. Feeding Schedule

Season	Frequency
March/April-August	2x/week
September	1x/week
October-March/April	Do not feed

We feed the tortoises at the DTCC at the time of day when they are most active, usually as early in the morning as possible. In summer, we start feeding just before sunrise, with slightly later feeding times in spring and fall. In the spring, we begin feeding tortoises small amounts of food (3 kibbles/kg) for a week or two after they come out of hibernation, then proceed to the normal feeding schedule. In the fall, it is important that tortoises not go into brumation with food in the gastrointestinal tract so we stop feeding after the last week of September, even if temperatures are still relatively warm. It can take up to a month for DTs to digest and pass their food so it's best not to risk feeding so late in the season.

IV. Water

We provide the tortoises with water throughout the active season until the time they enter hibernation. Table 3 shows the frequency for watering captive tortoises using a drip system.

Table 3. Watering Schedule for Drip System

Season	Frequency
March-April	3 days/week 2x/day for 15 minutes
May-August	4 days/week/ 2x/day for 15 minutes
September-October	3 days/week 1x/day for 15 minutes
October-March	Do not water*

* Note: While water is not provided to tortoises during the winter, continued watering of the pens in the winter months using a spray type of irrigation will increase the germination of annual plants in the spring.

We begin providing water during the warmest part of the day as soon as tortoises come out of brumation. Once comfortable spring temperatures set in and tortoises are more active in the morning, we provide water early in the day and evening. During the summer, we provide water during the coolest times of day. We check the irrigation system once a week throughout the season to make sure all drippers and/or sprinklers are functioning properly. When most of the tortoises have gone into brumation in October or November, we shut off the irrigation system and blow out all the water lines to prevent breakage over winter, which can lead to flooding in the burrows.

V. Surveillance

Captive DTs, their burrows, and their enclosures need to be examined consistently throughout the year, not just during the active season. It is particularly important to check on tortoises several times each day (morning, noon, and evening) for at least 2 weeks following their transfer to captivity because it is common for tortoises that are newly placed in captivity to pace the perimeter of the enclosure and not use the burrow provided. This can result in mortality from overheating or other complications related to exposure. To minimize the potential stress and effects of extreme temperatures, it is strongly recommended that tortoises not be removed from the wild and/or introduced to a captive environment when daily low temperatures are below 15C or daily high temperatures are above 30C on the day of and for 3 days following the move. It is also recommended that tortoises not be removed from the wild and/or introduced to a captive environment from Oct 1 to the time that brumation begins since this is a physiologically critical time for desert tortoises. Furthermore, tortoises should never be disturbed from brumation for any reason unless they are in mortal danger.

A. Keep a Daily Record of Tortoises

From spring emergence through winter hibernation, we try to get a visual of every tortoise held in individual quarantine pens every day, and record it in a field notebook and/or on an electronic spreadsheet. It is best to do this in the early morning when tortoises are active. We try to get a good look at their faces and record if they are showing any signs of disease or if they appear lethargic. This is important information in the future if their condition deteriorates.

For tortoises that are in burrows when we locate them, we record how deep they are in the burrow, and which direction they are facing. If they have not moved in 3 days, we either coax them out or tap them gently with a stick to make sure that they are alive and that they are not stuck. If a tortoise doesn't move from its burrow for 5 consecutive days during active season, we gently pull him from the burrow to assess his condition. We do this because we have had cases in

which we saw a tortoise in its burrow facing away from us, and it appeared to be fine, but after tapping it, we realized that it was lodged in the burrow and could not escape on its own.

During the winter months, tortoises are checked weekly and are not disturbed from their brumation. We do not pull them from their burrows unless they are sick, in distress, or dead.

i. Sick, Injured, and Dying Tortoises

We keep daily records of tortoises during the active season because it is helpful in identifying sick and injured tortoises, but our surveillance also includes tortoise checks during the hottest part of the day in warm months and during the coldest part of the day in cool months to check for tortoises that are improperly thermoregulating. If we find a tortoise that is sick, injured, or dying, we immediately remove it from the enclosure and place it in a clean tote. If it is injured or extremely ill, veterinary attention is sought immediately. If the tortoise appears mildly to moderately sick, we soak it in a tote of water with the water level just below the tortoise's chin. The water should be tepid to cool in warm months, and tepid to slightly warm in cool months. Often times a good soak to rehydrate is all a tortoise needs to feel better. Once the tortoise's condition has improved, if the temperatures are 15-30C and will remain in that range for at least 3 days following release, we place the tortoise back in its pen and monitor it 3 times each day for 7 days. If temperatures are outside that range, the tortoise is maintained indoors in a temperature controlled environment until outdoor temperatures are appropriate for release. The indoor housing consists of a very large, clean, dry bin or penned area with Timothy hay for bedding and a mercury halide light in a ceramic fixture on a timer for heat and UV light (ZooMed 160 watt PowerSun UV lamp). If tortoises are brought indoors and not allowed to hibernate due to their condition, we feed and water them weekly.

It is strongly advised that you make arrangements in advance with a veterinarian that specializes in desert tortoises in case an emergency occurs. Most veterinarians do not have desert tortoise or even reptile experience, but it is critical to find one that does. Your local reptile rescue organization may be able to direct you to the nearest desert tortoise veterinarian in your area.

ii. Dead Tortoises

If you find a dead tortoise, take photographs before removing the carcass and follow instructions provided by US Fish and Wildlife Service. If it is a recent death and your permit allows transfer of the carcass to a research institution, place the carcass in a plastic bag in the refrigerator so the tissues will remain viable for necropsy, and contact that institution immediately (for necropsies to be conducted by the San Diego Zoo, contact Dr. Josephine Braun, Pathology Postdoctoral Fellow 760-291-5470 or Dr. Paula Kahn, Conservation Program Manager at the DTCC 702-885-7640).

B. Check on Tortoises After Rains

Tortoises can become entombed in both artificial and natural burrows during the rainy seasons. During and/or after a rainstorm, we shine a light inside every individual burrow to make sure that the tortoises are safe. Many tortoises come out for a drink while we are inspecting the burrow, but we still look inside their burrows to make sure they have not collapsed. For those burrows that we can't see to the back, we use a burrow scope to check them.

If we find that a burrow has collapsed with a tortoise in it, we dig it out as quickly and as carefully as we can. It can be useful to put a hose, shovel handle, or other type of placeholder inside the burrow so we can find the tunnel in case of a collapse while we are digging. Once we remove the tortoise, we keep it in a safe place until we are able to re-dig the burrow. We have found that sometimes hatchlings and small juveniles will dig further back and sometimes upwards in their burrows during rainstorms, so we are particularly careful when digging in a pen with a small tortoise in it.

C. Burrows

Every burrow must be covered completely to effectively protect tortoises from harsh weather conditions so during routine surveillance of tortoises, we also check the burrows in each pen, looking for proper coverage over the top of the burrow, as well as inspecting them for cave-ins toward the back of the burrow. We carry a shovel to put more dirt on top of marginally covered burrows to ensure there is at least 25cm of dirt on top. We use rocks to hold down the dirt and sprinkle the dirt with water to promote compaction. If we consistently see a problem with a specific burrow, we dig it up and start over, digging it deeper or placing it in a different area of the pen.

D. Enclosures and the Perimeter Fence

We check the enclosures and perimeter fence weekly to make sure there are no holes, slides, or other evidence of breaches or escape routes. Tortoises can climb low fences to escape from their pens so if the dirt inside the pen is piled up too high near the fencing, we dig some of the dirt out and place it away from the fence. We also repair any holes or other issues immediately.

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