DESERT TORTOISE PRE- AND POST-TRANSLOCATION MONITORING PLAN

AUGMENTATION OF A
MOJAVE DESERT TORTOISE (*GOPHERUS AGASSIZII*) POPULATION
ELDORADO VALLEY, CLARK COUNTY, NEVADA

CLARK COUNTY
MULTIPLE SPECIES HABITAT CONSERVATION PLAN

13 November 2013

Final
Version 2.0

desert conservation
PROGRAM

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Prepared for:

Prepared by:

SCIENCE ADVISOR
2009-ECO-801D
D60

ACRONYMS AND ABBREVIATIONS

ACEC	Area of Critical Environmental Concern
BACI	before-after control impact
BCCE	Boulder City Conservation Easement
BiOp	biological opinion
BLM	Bureau of Land Management
cm	centimeter(s)
DCP	Desert Conservation Program
DTCC	Desert Tortoise Conservation Center
DTRO	Desert Tortoise Recovery Office
ELISA	enzyme-linked immunosorbent assay
GIS	geographic information system
GPS	global positioning system
HA	health assessment
km	kilometer(s)
m	meter(s)
mm	millimeters
MCL	mid-line carapace length
mi	mile(s)
MSHCP	Multiple Species Habitat Conservation Plan
NEPA	National Environmental Policy Act
NDOW	Nevada Department of Wildlife
PDA	personal digital assistant
QA/QC	quality assurance/quality control
US 95	U.S. Highway 95
USFWS	U.S. Fish and Wildlife Service

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EXECUTIVE SUMMARY

The Mojave desert tortoise (*Gopherus agassizii*) is a priority species for conservation in Clark County, Nevada. It is listed as threatened under the Endangered Species Act and is a covered species under the Clark County Multiple Species Habitat Conservation Plan. Current conservation efforts include the protection of existing populations, monitoring to detect population trends, applied research to assess disease and the factors determining the distribution of desert tortoises, and the translocation of tortoises to augment populations.

Purpose for Monitoring Plan

The Clark County Desert Conservation Program (DCP) has partnered with the United States Fish and Wildlife Service (USFWS) on a project to augment the existing tortoise population in the Eldorado Valley. The purpose for this monitoring plan is to gather the protocols and procedures that will contribute to successful pre- and post-translocation monitoring of desert tortoises for the Eldorado Valley Translocation Project. It outlines expected or anticipated processes and steps to complete the larger translocation project, as well as partner roles and responsibilities. This monitoring plan will be used to develop the DCP's contract, scope of work, and detailed protocols for desert tortoise monitoring activities.

Content of Monitoring Plan

The Eldorado Valley Translocation Project is proposed as a 3-year study (Years 0, 1, and 2) to monitor the short-term effectiveness of translocation. The USFWS plans to translocate tortoises from the Desert Tortoise Conservation Center to the upper Eldorado Valley in Year 0. The DCP plans to collect data in Year 0 prior to the release of the translocated tortoises, and depending on available funding, for 2 years after release (Year 1 and Year 2) to monitor the effectiveness of the project. Data collection and tortoise releases would occur during the April through June timeframe.

This monitoring plan outlines the goals, objectives, and assumptions of monitoring the translocation project. The study design includes two release sites and two control sites. Pre-and post-translocation data include an estimate of abundance, expected visibility, and health assessments of resident tortoises. The data collection teams, data collection methods, and data metrics are described and outlined in the monitoring plan. Data collection for comprehensive health assessments would occur in year 0 (pre-translocation), while surveys for abundance and visibility occur in year 0 and in years 1 and 2 (post-translocation), depending on available funding. The monitoring plan discusses the permits and authorizations needed for the project, and the qualifications of the data collection teams. Data management and the roles and responsibilities of the USFWS and the DCP are described. While the monitoring plan is specific to the USFWS's planned translocation project to the Eldorado Valley, it could be used as a template for monitoring of future translocation projects elsewhere.

Acknowledgements:

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1.0 INTRODUCTION

The Mojave desert tortoise (*Gopherus agassizii*) is a priority species for conservation in Clark County, Nevada. It is listed as threatened under the Endangered Species Act (USFWS 1990) and is a covered species under the Clark County Multiple Species Habitat Conservation Plan (MSHCP) (Clark County 2000). Current conservation efforts include the protection of existing populations, monitoring to detect population trends, applied research to assess disease and the factors determining the distribution of desert tortoises, and the translocation of tortoises to augment populations. The Clark County Desert Conservation Program (DCP) has partnered with the United States Fish and Wildlife Service (USFWS) in the Eldorado Valley Translocation Project to augment the existing population in this area of Clark County.

The USFWS translocation guidance (USFWS 2011b) addresses risks to minimize impacts based on the best scientific information currently available. However, various risks have not been fully evaluated and long-term success has not been demonstrated, therefore, the USFWS guidance is to design translocation projects in a research or effectiveness-monitoring framework so as to address outstanding questions related to the success or impacts of translocation. This monitoring plan is specific to the USFWS's planned translocation project to the Eldorado Valley, but it could be used as a template for future monitoring of translocation projects elsewhere.

1.1 Purpose of Translocation

Translocation to augment natural populations of desert tortoise is considered an important component for species recovery and is one of the primary actions outlined in the Revised Recovery Plan (USFWS 2011a). Moving individuals from areas of disturbance and potential harm to areas managed for the protection of desert tortoise could contribute to the conservation of the species. Although there are few studies that assess the short-term success of desert tortoise translocation (Field et al. 2007, Esque et al. 2010, Nussear et al. 2012), augmenting wild populations is hypothesized to improve their long-term survival in the face of natural and human threats. The short-term studies have focused on tortoise movement after translocation, and survivorship and reproduction over two or three years. No studies have assessed the long-term success of translocation, which would be measured by sustained higher population densities and successful recruitment by translocated tortoises.

1.2 Goals, Objectives, and Assumptions

The goals of augmentation by translocation are not only for survival and reproduction of translocated individuals, but for recovery of the species as outlined in the Revised Recovery Plan. The goals for translocation of tortoises to the upper reaches of Eldorado Valley are to augment the wild population, increase population numbers through reproduction, and eventually establish a more secure population in this area.

The objectives of the joint DCP and USFWS translocation project to Eldorado Valley are to:

- Guide the pre- and post-monitoring of resident and translocated tortoises.
- Provide a baseline health assessment for both the translocated and resident tortoises.
- Use tortoises from the Desert Tortoise Conservation Center (DTCC) to contribute to the conservation of wild (resident) populations.
- Improve the effectiveness of translocation methods and the monitoring procedures of augmented populations.

- Increase the abundance of desert tortoise over a two-year post-translocation period.

The assumptions inherent in a translocation project include:

- Release sites have the resources available to support a larger tortoise population, including habitat suitable for all life stages.
- Release sites do not have major sources of threat to desert tortoises and that known threats are minimized (for example, desert tortoise exclusion fencing along major roads).
- Release site will be managed for the conservation of desert tortoises so that potential threats from future impacts are minimized.
- The translocated tortoises are in good health and that survival is determined primarily by translocation methods, habitat conditions, and current and future climate.
- The majority of the translocated tortoises stay within the translocation (release) sites.

1.3 Translocation Project Location and Description

The USFWS selected the Eldorado Valley to translocate desert tortoises because the area has a low density of resident tortoises, appropriate habitat, and protected status as a Bureau of Land Management (BLM) Area of Critical Environmental Concern (ACEC).

The Eldorado Valley is located southwest of the populated area of Boulder City and extends south to the town of Searchlight (Figure 1). The valley is bisected by U.S. Highway 95 (US 95), which connects these two communities and is bordered by tortoise-proof fencing. Land to the north, east, west, and south is under federal ownership. The BLM manages the land to the crest of the Eldorado Mountains to the east, to the McCullough and Highland ranges to the west, and to Searchlight to the south. Further to the east, the National Park Service manages the land to the Colorado River. The Boulder City Conservation Easement (BCCE) is located at the northern end of Eldorado Valley and is managed by Clark County. No part of the upper Eldorado Valley south of the BCCE is privately owned.

The Eldorado Valley is a closed drainage basin ranging in elevation from 549 to 914 meters (1,800 and 3,000 feet). There is no permanent surface water within the valley. Runoff following large precipitation events drains onto a playa known as Eldorado Dry Lake, located at the lowest elevation at the northern end of the valley.

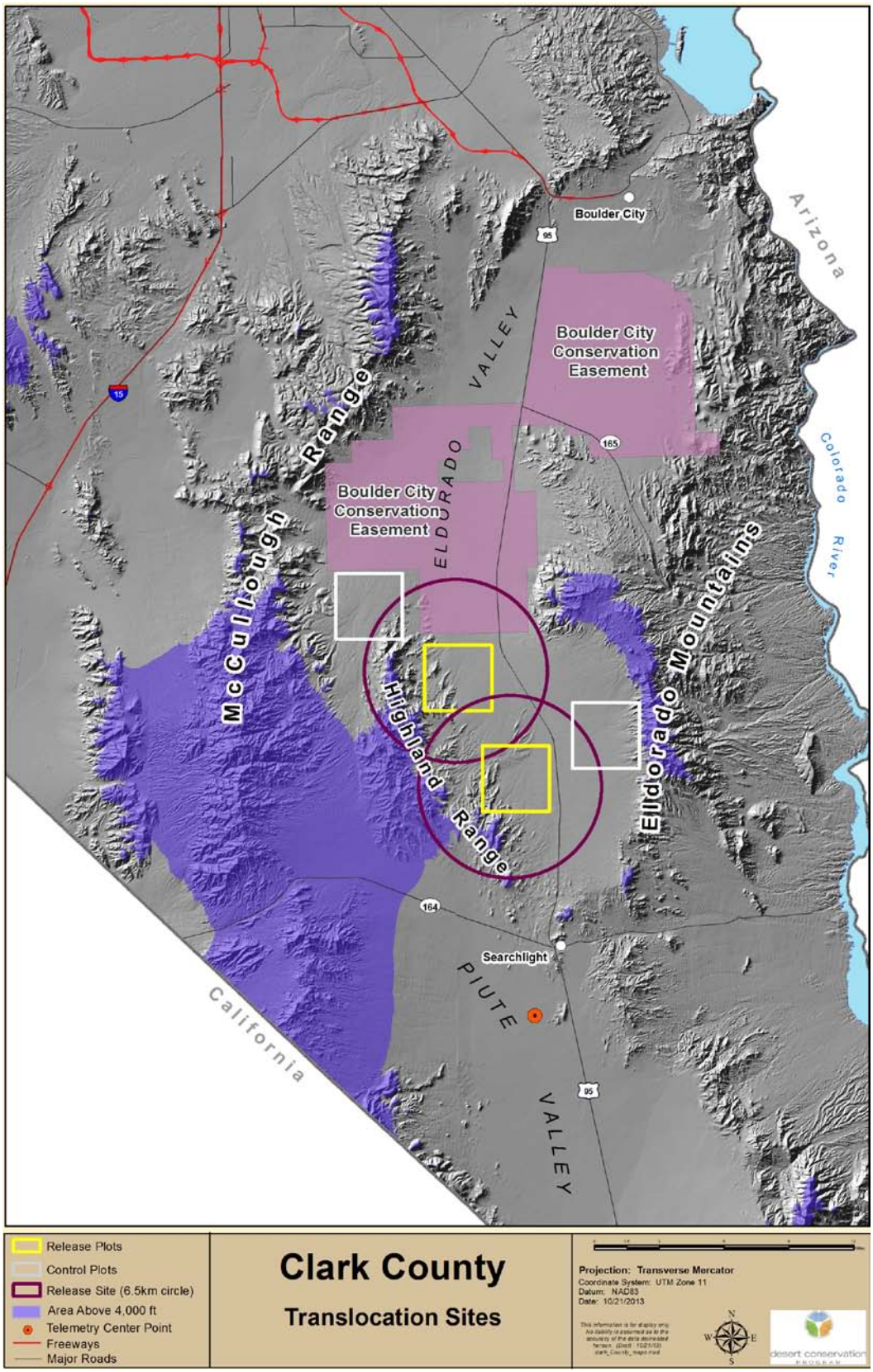
The soils within Eldorado Valley are primarily young alluvial deposits derived from sedimentary and igneous sources (Heaton et al. 2011). These soils are characterized as gravelly and sandy with coarse texture, low organic matter content, and low carbon/nitrogen ratios (O'Farrell 2009). Rock outcrops occur within the valley at the foothills of the McCullough Range and Eldorado Mountains, and where there are basalt flows and intrusions.

The vegetation of Eldorado Valley is mostly Mojave Desert scrub. Most of the valley bottom consists of deep sands and a near surface hardpan, and is dominated by vegetation of creosote bush (*Larrea tridentata*) and white bursage (*Ambrosia dumosa*). Areas with rocky soils are dominated by creosote bush, desert thorn (*Lycium andersonii*), and spiney hop-sage (*Grayia spinosa*) (Clark County 2007).

1.4 Source and Condition of Translocated Tortoises

The DTCC is the primary source for tortoises for translocation to Eldorado Valley for this project. Other sources could include development sites. Tortoises will meet the health criteria developed by the USFWS and the DTCC to be eligible for translocation (USFWS 2013b). The USFWS and the DTCC will develop the release protocol, including carriage from the DTCC to the release sites, release time of day and location, and hydration of released tortoises.

Figure 1-1. Location of Eldorado Valley and the Monitoring Project



2.0 STUDY DESIGN

The selected study design is a before-after control impact (BACI) design. This type of design compares one to several control sites (locations of no planned action) to one to several impact sites (locations of a planned action). The control and impact sites are sampled before and after the implementation of the planned action. This design attempts to separate the effects of the planned action from other sources of spatial and temporal variability (Osenberg and Schmitt 1996). To improve confidence in the results, the control sites should to be environmentally similar to the impact sites. Pairing control and impact sites improves the results of this design. The BACI design is commonly used in environmental monitoring (Osenberg and Schmitt 1996).

For this monitoring plan, the impact sites are referred to as release sites, and the planned action is the translocation of tortoises.

The Eldorado Valley Translocation Project is proposed as a 3-year study (Years 0, 1, and 2) to monitor the effectiveness of translocation. The USFWS plans to translocate tortoises from the DTTC to the upper Eldorado Valley in Year 0. The DCP plans to collect data in Year 0 prior to the release of the translocated tortoises, and depending on available funding, for 2 years after release (Year 1 and Year 2) to monitor the effectiveness of the project. Data collection and tortoise releases would occur during the April through June timeframe.

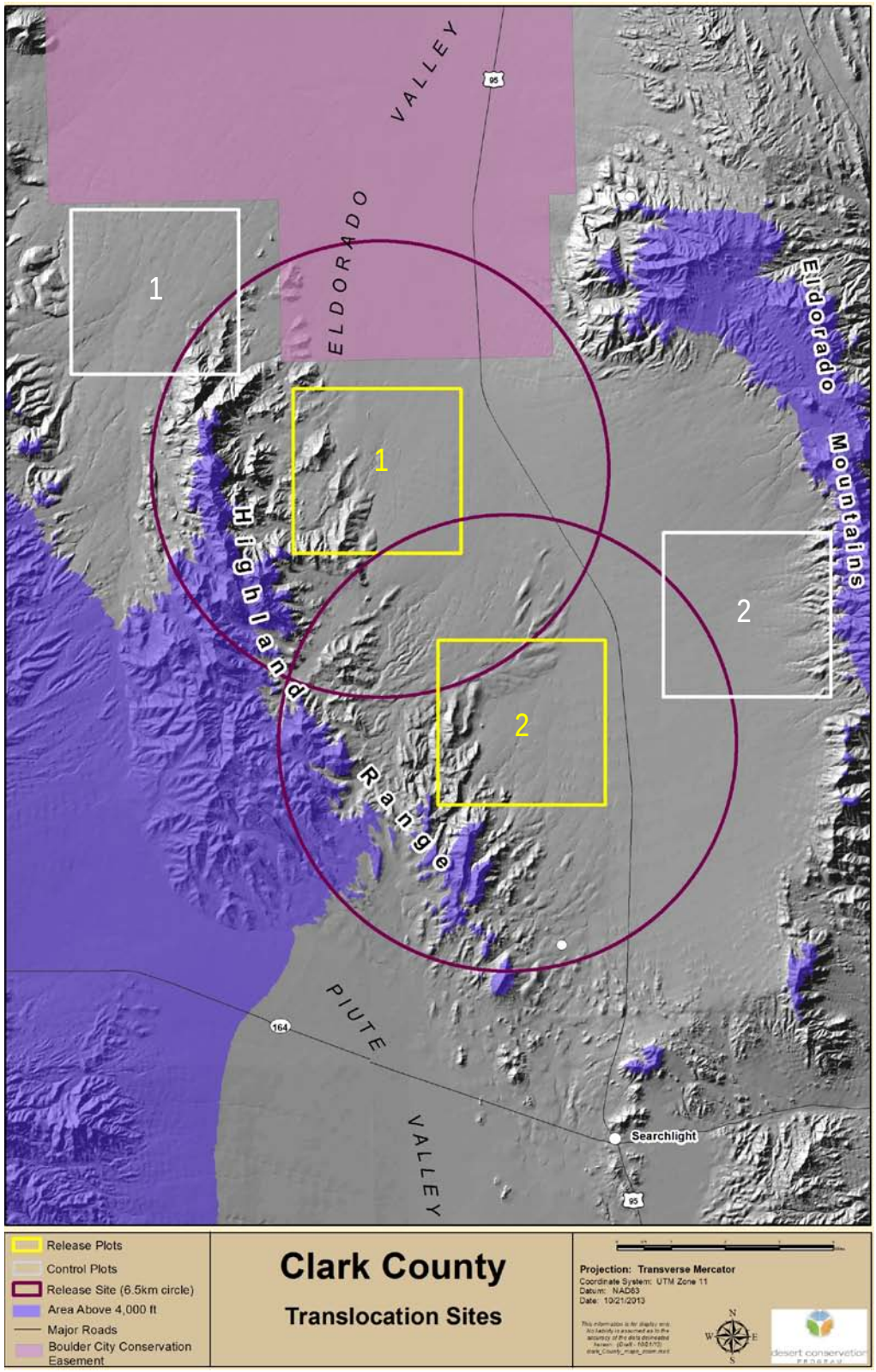
2.1 Release and Control Sites

The design for this project includes two release sites and two control sites. Release sites are defined as a circle with a radius of 6.5 kilometers (km) (4 miles (mi)) around points where tortoises are released. The distance of 6.5 km has been shown to contain 97.5 percent of the first-year dispersal movements of translocated tortoises (K. Field, personal communication). Tortoises are released at selected release points and a grouping of release points is called a release area. The specific location of release points are determined by habitat, access, and the number of tortoises available for release. Control sites are an area of similar size to release sites, defined by a circle with a radius of 6.5 km around a center point.

The two release sites (Release 1 and Release 2) are located from 1 to 8 km (0.6 to 5 mi) west of US 95 and extend for 15 km (9 mi) south of the BCCE in the upper Eldorado Valley (Figure 2). The release sites cover an area of approximately 185 square km (71 square mi). The elevation ranges from 932 to 1,250 meters (m) (3057 to 4,100 feet) with the aspect primarily east to east-northeast. The sites are located just west of a major wash that parallels US 95. The primary landforms are fan aprons and fan remnants with well-developed washes. Soils are primarily extremely gravelly loamy sand and gravelly loamy sand to cobbly coarse sand (Arizo-Peskah-Cosgrain association), with small areas of partial ballenas with a duripan (Peskah-Cosgrain association). The vegetation type is Mojave desert scrub of creosote, bursage, and blackbrush. Potential threats include a few active roads within the release sites and a major powerline that parallel US 95.

The two control sites (Control 1 and Control 2) are located in the same part of the upper Eldorado Valley as the release sites (Figure 2). Control 1 is located east of US 95, approximately 15 km (9 mi) south of the BCCE boundary. Control 2 is located in a mid-valley, southwest extension of the Eldorado Valley, just beyond the BCCE boundary. The elevation ranges from 758 to 1,250 m (2486 to 4,100 feet) for Control 1 and 963 to 1,250 m (3,459 to 4,100 feet) for Control 2. The aspects of the two control sites differ; Control 1 has a west aspect and Control 2 faces northeast.

Figure 2-1. Location of Release and Control Sites



The primary landforms in Control 1 are fan aprons and fan remnants, and the soils are extremely gravelly loamy sand, gravelly loamy sand, and gravelly and clay loam (Arizo-Lanap association). The soils in Control 2 have a surface of very gravelly loamy sand, covering a layer of sand, and then underlain by stratified very gravelly coarse sand to extremely gravelly sand (Arizo association), with well-developed washes. The vegetation type in both sites is Mojave desert scrub. Potential threats include a few roads and one powerline in both sites.

The placement of the control sites does not maximize their similarity with the release sites. It is very difficult, if not impossible, to have control sites completely similar with the release sites. Topographic positions, soils, and past land use history make sites different. The placement of the release sites was based on habitat and lack of threats. The control sites were placed in the nearest similar habitat.

2.2 Survey Plots and Transects

A survey plot is located within each release and control site, for a total of 4 plots. The survey plot is 5 km by 5 km (3.1 mi x 3.1 mi). Each plot has 100 transects for a total of 500 km (100 transects at 5 km each). A transect has a compass bearing of either 90 or 270 degrees. A systematic transect design is used to place transects at 50 m apart in each plot. A random survey order for transects is established for each plot and provided to the Transect Survey Team with the global positioning system (GPS) coordinates of each transect starting point.

All transects within a plot are completely surveyed before the team begins the survey of the next plot. The release site survey plots are surveyed before the control site plots. A pair of neighboring transects and verification of data should be completed per person during a standard work day. Each survey plot should take about 2 weeks to complete with a 5-person team completing 2 transects per day. The USFWS could begin translocating tortoises to the first release site by the first week of May.

Surveys start the second week of April and are completed by the end of May. Transect surveys start at 8:00 a.m. during the first week of the season, with progressively earlier start times in subsequent weeks, but no earlier than 30 minutes after sunrise. Transect start and end times are coordinated with the Telemetry Team.

2.3 Telemetry Site

NOTE: Monitoring at a telemetry site is being considered as part of the translocation project, but it is not certain to occur. The discussions about telemetry in this document describe how it might be incorporated if the DCP decides to include telemetry monitoring.

An established telemetry site in Piute Valley is monitored to assess the visibility of tortoises. The site encompasses an area of approximately 44 square km (17 square mi) and is located approximately 29 km (18 mi) south of the release sites in Eldorado Valley (Figure 1). A subset (8 to 12) of tortoises that are equipped with radio transmitters are selected for monitoring. These tortoises are monitored by the Telemetry Team to estimate the proportion of tortoises that are active or visible during the same time as the transect surveys. Telemetry observational data are used to calibrate transect data from the release and control sites for calculating tortoise abundance.

The transmitter frequency and GPS coordinates of the last known locations of a subset of transmittered tortoises in Piute Valley are provided to the Telemetry Team. Individual tortoises are tracked throughout the survey day using a VHF radio receiver and a directional antenna. The Telemetry Team coordinates monitoring start and end times with the Transect Survey Team. The telemetry site is monitored every day that the Transect Survey Team is in the field.

2.4 Metrics

2.4.1 Tortoise Surveys

The objective of the tortoise surveys is to collect data to estimate tortoise abundance in the survey plots. The Transect Survey Team records data on live tortoises observed above ground and in burrows, and data on carcasses. In addition to collecting the plot, transect, tortoise, and carcass data (metrics) listed in Table 2-1 and Table 2-2, the Transect Survey Team attaches an identification tag and affixes (semi-permanently) a transmitter to each resident tortoise located in the release and control sites (see Section 3.1.2).

Table 2-1. Data Metrics – Transect

Metric Category	Metric	Metric Entry
General Information	Plot Number	Release or control site number
	Transect Number	Unique assigned number
	Team Number	Assigned by contractor
	Observer	Transect surveyor name
	Date	Date of data collection
Waypoint	Waypoint Number	Begin with 0, number consecutively
Time	Observation Time	Record time as a.m. or p.m.
GPS Coordinates	GPS – Easting	UTM, NAD 83
	GPS – Northing	UTM, NAD 83
Burrows	Burrow Count	Tick marks for observed burrows of tortoises >180 mm MCL
Transect Obstacles	Transect Interrupted	Yes No
	Transect Shortened	Yes No
	Obstacles if Shortened	Describe
Additional Data	Comments	Describe
	Data Recorded By	Transect surveyor name
	Data Proofed By	Name

Table 2-2. Data Metrics – Live Tortoise and Carcass

Metric Category	Metric	Metric Entry
TORTOISE		
General Information**	Plot Number	Release or control site number
	Transect Number	Unique assigned number
	Team Number	Assigned by contractor
	Observer	Transect surveyor name
	Date	Date of data collection
Tortoise Identifier**	Transect Tortoise Number	Number consecutively
	Observation Time	Record time as a.m. or p.m.
Location Information*	Last Waypoint on Transect	Waypoint location from transect datasheet
	Transect Bearing	0, 90, 180, 270 degrees
	Local Bearing	degrees
	Azimuth	degrees
	Radial Distance	Meters to one decimal place
	Perpendicular Distance	Calculated by database
	GPS – Easting*	UTM, NAD 83
	GPS – Northing*	UTM, NAD 83
Tortoise Observation Data*	Cue to Tortoise	SearchedVeg BodyPart Movement Burrow BurrowApron Audible
	Tortoise Heading	Profile HeadOn TailOn PulledintoShell HeadintoBurrow HeadOutOfBurrow
	Tortoise Location*	Burrow Pallet Open Vegetation Rock
Burrow Observation Data**	Burrow Visibility	Degrees of approach – high, medium, low
	Tortoise in Burrow	Location/visible within burrow – high, medium, low
	Tortoise Visibility	Degrees of approach – high, medium, low
	Distance to Burrow	Estimate; 0 to 100
Temperature**	Temperature	degrees Celsius
	Temperature > 35° C	Yes No
Tortoise Condition**	Sex	Male, female, unknown (if any uncertainty)
	MCL >/= 180 mm	Yes No Unknown
	MCL	millimeters
Tag Information**	Body Condition Score	1 to 9, unknown; reference handbook for pictures to illustrate muscle development and fat deposition evidence to match each score
	Existing Tag Present	Yes No U/R (unreadable) Unknown
	Existing Tag Number	Record number
	Existing Tag Color	Record color

Metric Category	Metric	Metric Entry
	New Tag Attached	Yes No
	New Tag Number	Number assigned by DCP
Transmitter**	Transmitter Serial Number	Record number
	Transmitter Frequency	Record frequency
Voiding**	Tortoise Void	None Urine Feces Both
Additional Data**	Tortoise Not Handled	Describe
	Comments	Describe
CARCASS		
General Information**	Plot Number	Release or control site number
	Transect Number	Unique assigned number
	Team Number	Assigned by contractor
	Observer	Transect surveyor name
	Date	Date of data collection
Carcass Identifier*	Transect Carcass Number*	Number consecutively; record only if at least half the shell (plastron and carapace) is present
	Observation Time	Record time as a.m. or p.m.
Location Information*	Last Waypoint on Transect	Waypoint location from transect datasheet
	Transect Bearing	0, 90, 180, 270 degrees
	Local Bearing	degrees
	Azimuth	degrees
	Radial Distance	Meters to one decimal place
	Perpendicular Distance	Calculated by database
	GPS – Easting*	UTM, NAD 83
	GPS – Northing*	UTM, NAD 83
Condition**	Carcass Condition	Intact D/A (disarticulated)
	Carcass Status	1-fresh or putrid; 2-normal color, scutes attached; 3-scutes peeling; 4-shell bone falling apart, scute rings peeling; 5-disarticulated
	MCL >/= 180 mm	Yes No Unknown
	MCL	millimeters (only if intact)
	Sex	Male, female, unknown (if any uncertainty)
Tag Information**	Existing Tag Present	Yes No U/R (unreadable)
	Existing Tag Number	Record number
	Existing Tag Color	Record color
Additional Data**	Photo Taken	Yes No File Name
	Comments	Describe

* Only the marked metric is recorded for opportunistic observations of tortoise or carcass.
** All the metrics in the category are recorded for opportunistic observations of tortoise or carcass.

2.4.2 Telemetry Surveys

The objective of telemetry surveys is to assess the visibility of tortoises at the Piute Valley telemetry site to calibrate the results of transect surveys in the release and control sites in the Eldorado Valley. For each located tortoise, data are recorded indicating its visibility on the surface, in a burrow, or in vegetation, along with the other metrics listed in Table 2-3.

Table 2-3. Data Metrics – Telemetry Survey

Metric Category	Metric	Metric Entry
General Information	Site	Piute Valley
	Observer	Telemetry tracker name
	Date	Date of data collection
	Photos	Yes, no
	Photo Comments	Landscape, burrows, etc.
Tortoise Identifier	Tortoise Number	Number consecutively
	Observation Time	Record time as a.m. or p.m.
	Tortoise Visible	Yes No
	Tortoise Location	Burrow Pallet Open Vegetation Rock
Burrow Observation Data	Burrow Visibility	Record if tortoise location is in burrow; degrees of approach – high, medium, low, not
	Tortoise in Burrow	Location/visible within burrow – high, medium, low, not
	Tortoise Visibility	Record if tortoise not associated with burrow – high, medium, low, not
	Distance to Burrow	Estimate; 0 to 100
Tortoise Behavior	Behavior	Unk AtRestActive Moving Basking Eating Mating Agonistic Digging
Location	GPS - Easting	UTM, NAD 83
	GPS - Northing	UTM, NAD 83

2.4.3 Health Assessments

The objective of the health assessment (HA) is to record the health condition of resident tortoises located in the release and control sites. The assessment is designed to minimize risks of translocation and is not intended to be an in-depth study into diseases that affect tortoises (USFWS 2013a). The HA and selection of tortoises for translocation are completed by the DTCC and are not included in this monitoring plan. The data (metrics) collected by the Health Assessment Team on resident tortoises are listed in Table 2-4.

Table 2-4. Data Metrics – Health Assessment

Metric Category	Metric	Metric Entry
General Information	Date	Date of data collection; ddmmmyy
	Start Time	Record when tortoise is located; 24 hour
	Plot Number	Release or control site number
	Name Examiner	Record name
	Name Handling	Record name
	Name Observer	Optional; record name
Tortoise Identifiers	Tortoise EV Number	Record tag number
	Check EV Number After Locating	Yes No
	DTCC Number	Record number
	Transmitter Frequency	Record frequency
Location Information	GPS - Easting	UTM, NAD83
	GPS - Northing	UTM, NAD83
Temperature	Temperature	degrees Celsius; measure 5 cm above ground in shade
General Condition	Attitude/Activity	Normal Lethargic/Weak
	Respiration	Normal Abnormal Sounds Increased Effort
	Beak	Normal Abnormal Evidence of Foraging
Naris Condition	Left Naris	Asymmetrical Eroded Occluded
	Left Naris Discharge and Severity	None Serous 1 2 3 Mucous 1 2 3
	Right Naris	Asymmetrical Eroded Occluded
	Right Naris Discharge and Severity	None Serous 1 2 3 Mucous 1 2 3
Eye Condition	Left Eye	Normal Sunken Corneal Opacity Partially-Closed Fully-Closed Serious Discharge Mucous Discharge Periocular Swelling Periocular Redness Conjunctival Swelling Conjunctival Redness
	Right Eye	Normal Sunken Corneal Opacity Partially-Closed Fully-Closed Serious Discharge Mucous Discharge Periocular Swelling Periocular Redness Conjunctival Swelling Conjunctival Redness
Skin Condition	Skin Lesion Location	None Generalized Head Neck L/R Forelimb L/R Hindlimb L/R Axillary Region L/R Prefemoral Region Vent/Tail
	Condition of Skin Lesion(s)	N/A Active Inactive
	Coelomic Cavity Palpation	No Mass L/R Mass Not Done
Shell Condition	Shell Characteristics	Compressible N/A Sunken +/- Scutes Peeling/Flaking Keratin Bone Exposed
	Shell Abnormality Location	Carapace Plastron N/A
	Shell Abnormalities	None Localized Multifocal Generalized
	Condition of Shell Abnormalities	N/A Active Inactive
	Circumstances of Skin/Shell Trauma	N/A Unknown Suspect Canid Bite Vehicle Other

Metric Category	Metric	Metric Entry
Sex	Sex	Male, female, unknown (if any uncertainty)
Initial Weight	Initial Weight	Weigh before much handling to avoid voiding; grams
Body Condition Score	Body Condition Score	1 to 9; reference handbook for pictures illustrating muscle development and fat deposition evidence
Photographs	Photo 1: Front Face and Body	Photo Name
	Photo 2: Left Side Face	Photo Name
	Photo 3: Right Side Face	Photo Name
	Photo 4: Carapace	Photo Name
	Photo 5: Plastron (only if abnormal)	Photo Name
	Photo 6: Abnormalities	Photo Name
Ticks	Ticks Number	0 1-10 >10
	Location	Soft Tissue Seams Scules Eyes Nares Beaks
	Collected	Yes No N/A
	Removed	Yes No N/A
Choana	Choana	Not Examined Normal Pale Reddened
Oral	Tongue and Oral Mucus	Not Examined Normal Pale Reddened Crust Ulcers Plaques Hypersalivation Impaction
	Number of Oral Swabs	Record number, no more than 2
Blood	Time of Blood Draw	Record time of finish/removed needle from vein; 24 hour
	Total Volume (blood, lymph) collected	Milliliters
	Total # of Hep. Tubes (number each)	0 1 2 3 4
	Sample Contaminated with Lymph	Yes No
	Degree of Lymph Contamination	Small (1-10%) Moderate (11-29%) Severe (>30%)
	Blood Processing Time	Record time tubes placed in centrifuge; 24 hour
	Plasma Color	Colorless Red Yellow Green
	UFL Plasma Aliquots	0 1
	USFWS Plasma Aliquots	0 1 2 3
	Total Tubes with RBCs Saved	0 1 2 3 4
Size	MCL	Straight midline length; mm
	Width V3	Width of carapace; mm
	Height V3	Height of carapace; mm
	Plastron	Length of plastron; mm
Voiding	Void During Processing	None Urine/Urates Feces
	Post Void Weight	N/A in grams
	Hydration Method	N/A Epicoelomic Fluid Type Vol in ml
	Post Fluid Weight	N/A in grams
End Time	End Handling Time	Record time processing is completed; 24 hour

3.0 SAMPLING METHODS

The sampling (survey) methods vary by study year; pre-translocation (pre-release) surveys are Year 0 and post-translocation (post-release) surveys are Year 1, Year 2, and so on. The number of post-release surveys depends on available funding, but a minimum of 2 years is anticipated for Eldorado Valley. Data collected for pre-release surveys establish information for the "baseline" population – abundance and general health status of the resident population. Data collected for post-release surveys are to monitor change in abundance and health condition of the resident and translocated population.

3.1 Pre- and Post-Release Survey Transects

Transects in the survey plots at the release and control sites (see Sections 2.1 and 2.2) would be surveyed during the April-May timeframe before tortoises are translocated or released (pre-release survey) and during the same timeframe for two consecutive years following translocation or release (post-release survey), depending on available funding.

Transects are surveyed following a 1-person transect method based on line distance sampling methods (USFWS 2012). The Transect Survey Team consists of 5 persons, each walking a separate transect and recording data separately using a GPS unit and field datasheets. In good field conditions, two adjacent transects (10 km total) should be completed per person during a standard workday.

Vehicle entry points for the plots and transects are located with the geographic information system (GIS) spatial files and coordinates provided to the Transect Survey Team. The team plans their daily vehicle access based on the transect survey order randomly selected for the team.

Transect surveys start at 8:00 a.m. during the first week of the season, with progressively earlier start times in subsequent weeks, but no earlier than 30 minutes after sunrise. Transect start times are coordinated with the Telemetry Team.

3.1.1 Data Collection – Transect

The transect surveyor begins at Waypoint 0, which is the location the surveyor leaves the vehicle. The surveyor completes the initial data fields (plot number, transect number, surveyor, date and time) and records the coordinates of Waypoint 0 on the Transect datasheet (see Appendix A). The surveyor locates the start of the transect using the coordinates provided to the surveyor, and proceeds as follows:

- Using the GPS unit, record the coordinates of the transect start point as Waypoint 1.
- Using a compass with the declination adjusted to true north, start walking on the designated cardinal bearing (to be provided to the surveyor). Walk a straight transect as best possible using the compass and a reference point on the horizon.
- Pull a 25-m length of durable line (dragline) while walking. Pass the dragline over top or directly through vegetation that lies in the transect path. The purpose for the dragline is to facilitate measuring the transect bearing.

- Scan the ground in a radius of approximately 5- to 10-m for tortoises, tortoise carcasses, or burrows. Search vegetation intersected by the transect path. Limit time spent scanning the horizon to maintaining a consistent bearing.
- Leave the transect path to investigate burrows or suspected tortoises or carcasses more closely. Drop the dragline in place to keep the transect path clearly marked before leaving the transect path; avoid drifting the transect toward an observed tortoise.
- Whenever the transect has been paused to collect data, resume walking on the bearing that matches the orientation of the 25-m dragline.
- Record waypoints at approximately 500-m intervals. Between waypoints, maintain the current bearing even if it does not match the designated cardinal (correct) bearing. Recheck the transect bearing at the waypoint and resume walking on the correct bearing.

Obstacles might be encountered that require changes in the standard transect path, such as hazardous rock formations or hills, or washes too steep for safe navigation. The surveyor's safety takes precedence. Field responses to certain obstacles include:

- Paved and unpaved roads – cross safely without interrupting the transect.
- Short but severe obstacles – use a short scramble (≈ 20- to 30-m) to bypass small obstacles, such as a ravine or steep slope. Assess the forward transect path using landmarks before diverting from the transect. After scrambling, carefully assess where the transect ended before the diversion. Resume the transect path following the assigned bearing. This transect is considered non-standard but not shortened.
- Severe obstacles – shorten (interrupt) the transect when more than 100-m of the planned route is not traversable because of cliffs or long steep slopes. Shortened or interrupted transects are non-standard transects.
    - Interruptions internal to transect – enter a waypoint to interrupt the transect at the obstacle and find a safe route around the obstacle. Enter a new waypoint where the transect can be resumed and identify the interruption by including a decimal place with the transect number. For example, the waypoint after the first interruption on transect XXX is numbered XXX.1 and a second interruption is XXX.2. Continue to number the waypoints in sequence, despite starting new transect numbers.
    - Interruptions at beginning or end of transect – the length of the transect that can be continuously traversed is shortened because the planned start point or end point are not safely accessible.

3.1.2 Data Collection – Tortoise

The surveyor completes the data fields on the TranLiveObs datasheet (see Appendix A) and records the same data in the GPS unit for tortoises observed while walking the transect. In addition to recording observational data, the surveyor handles the tortoise to apply an identification tag, and the surveyor or a member of the survey team attaches a transmitter. When a tortoise is observed, the surveyor proceeds as follows:

- Drop the dragline, turn and face the far end of the line, and record the local bearing. The dragline identifies the effective transect.
- Record the azimuth, which is the compass bearing from the surveyor to the tortoise.

- Using a tape measure, record the radial distance to the nearest 0.1-m from the surveyor's location on the transect to the tortoise.
- The perpendicular distance is calculated automatically by the database.
- Record the coordinates of the tortoise location.
- Record tortoise surroundings and activity (location, heading, cue).

The primary purpose of the transect survey is to locate tortoises, with a secondary purpose to collect basic information on the characteristics of the tortoise. Moving and handling the tortoise should be limited to that necessary to attach a numbered tag and a radio transmitter. Data can be collected by observing the tortoise or can be collected by the Health Assessment Team. Minimizing actions that could stress the tortoise and/or cause it to void are of critical importance throughout the data collection process because of the difficulty of rehydrating the tortoise in the expected field conditions. The surveyor proceeds as follows:

- Place non-handling equipment and pack away from the tortoise's sight.
- If the tortoise is in the sun, use body shade to observe the tortoise and collect data. If the tortoise needs to be moved, put on latex gloves to move the tortoise to a shaded area. Approach and pick up the tortoise from behind using both hands, one on each side of the shell. Keep the tortoise close to the ground and in correct orientation and move slowly.
- Record the temperature in the shade approximately 5 centimeters (cm) above where the tortoise is handled. Do not handle the tortoise if the temperature exceeds 35°C (95°F).
- Record basic observations of body condition score.
- Record any existing tag number and color; use a small amount of non-drinking water to clear dust to read the tag. The non-drinking water should not have contact with humans.
- If temperature is below 35°C, set out handling gear and tape measure in the shaded area. Place clean equipment to the left; contaminated items and used equipment are placed to the right of the tortoise.
- Measure the mid-line carapace length (MCL) of the tortoise with calipers and the tape measure. The Health Assessment Team can measure the MCL if doing so by the surveyor is causing stress on the tortoise.
- Determine sex of tortoise by observation only. If the tortoise has to be lifted to view the plastron and tail to determine the sex, do not lift the tortoise but record "unknown" on the datasheet. The Health Assessment Team will confirm the sex of the tortoise.
- Apply a new identification tag, if necessary, and record the tag number and color. Affix a paper tag to the 4th right or left costal scute; epoxy must not touch the margins of the scutes where growth occurs. Sub-adult tortoises with scutes that are too small to safely affix tags are not marked.
- Attach a transmitter on the tortoise. Minimum scute size and attachment methods are provided by USFWS during the 1-day training session. Record the transmitter serial number before attaching it to the tortoise.
- If at any time there is any indication that the tortoise might void, stop handling the tortoise.
- If the tortoise was moved to a shaded area, leave it there.

- Clean up the processing area. Pick up contaminated disposable items in one hand, turning the latex glove inside out as it is removed to contain the trash. Remove other glove and place in disposal bag, taking care to not contaminate skin or the outside of the bag.
- Put on latex gloves to clean and disinfect the handling equipment; allow equipment to air dry in the sun for further ultraviolet disinfection.
- Put trash and gloves in the disposal bag and place in the pack in a compartment separate from the clean equipment.

If the tortoise was not handled, record the reason why it was not (for example, not necessary, not accessible, stress). Do not attempt to remove a tortoise from a burrow, but record whether the tortoise is an adult (>180 millimeters (mm) MCL) or sub-adult (<180 mm MCL). Indicate if the tortoise voided during handling. Rehydration requirements and methods will be discussed during the training session before the field season.

Tortoises observed outside an established survey transect, such as elsewhere in the survey plot, between the vehicle and transect start point, or when returning to the vehicle at the end of the survey work day are referred to as "opportunistic". The same procedures are followed for recording data for an opportunistic tortoise as for a transect tortoise except for transect and local bearings, azimuth, and radial distance. Data is recorded using the OppLiveObs datasheet (see Appendix A).

3.1.3 Data Collection – Carcass

The surveyor completes the data fields on the TranCarcObs datasheet (see Appendix A) and records the same data in the GPS unit for carcasses observed while walking the transect. When a carcass is observed, the surveyor follows the same procedure as for a tortoise for recording the local bearing (dragline), azimuth, and radial distance. If at least half of the carapace remains, the surveyor then proceeds as follows:

- Record the coordinates of the carcass location.
- Indicate the condition (intact, disarticulated) and status (fresh, putrid, peeling, etc.) of the carcass.
- Measure the MCL if the carcass is intact.
- Record the sex if the carcass is intact.
- Record any existing tag number and color.

Carcasses observed outside an established survey transect are referred to as "opportunistic". The same procedures are followed for recording data for an opportunistic carcass as for a transect carcass except for transect and local bearings, azimuth, and radial distance. Data is recorded using the "OppCarcObs" datasheet (see Appendix A). During pre-release surveys, only carcasses observed elsewhere in the survey plot outside established transects are recorded. During post-release surveys, carcasses located elsewhere in the survey plot, between the vehicle and transect start point, or when returning to the vehicle at the end of the survey work day are recorded. This is to locate any translocated or resident tortoises marked during pre-release surveys that might have died.

3.1.4 Field Equipment

Each surveyor on the Transect Survey Team has the same equipment and field kit of supplies. The equipment includes a GPS unit with accuracy of 3 meters or better. Data can be electronically entered into the GPS unit or a

PDA (personal digital assistant) with data forms that match the paper datasheets. The DCP provides one original set of datasheets. The Contractor prepares a data dictionary using the datasheet metrics (see Tables 2-1 and 2-2 and Appendix A) to load on the surveyor's GPS or PDA unit.

The field kit contains transmitters (Holohil Systems LTD, model BD-2), dragline, calipers, mirror, two-way radio (or some form of communication among surveyors), thermometer, clipboard, pens, epoxy, toothbrush, duct tape, latex gloves, bleach (disinfection), cotton swabs, toothpicks, garbage bags, hand sanitizer, alcohol swabs, batteries, and camera. The surveyor might include other items in the field kit as necessary or desired. The DCP provides the identification tags. The Contractor is responsible for all other items in the field kit.

3.2 Telemetry Surveys

The telemetry surveys are conducted at a known telemetry site in Piute Valley, south of the Eldorado Valley release and control sites (see Section 2.3 and Figure 1). Approximately 8 to 12 tortoises are monitored by the Telemetry Team. The Telemetry Team consists of 2 people (trackers).

The telemetry site is monitored every day that transects are surveyed. Telemetry and transect teams begin the field day at the scheduled time (see Section 2.2); however, completion times are variable and are affected by terrain, air temperature, number of tortoises encountered, etc. The teams establish a method for communicating (for example, cell phones) so that the start and end times are the same for both the telemetry trackers and transect surveyors.

3.2.1 Data Collection – Tortoise

The telemetry tracker completes the data fields on the G_0 Start and Obs datasheet (see Appendix B). Telemetry equipment use and techniques (assembling antennas and receivers, entering frequencies, equipment calibration and troubleshooting) are described in the Desert Tortoise Monitoring Handbook (2012), Chapter 6: Radio Telemetry and G_0 Protocols (see Appendix B). The USFWS has the transmitter frequencies and GPS coordinates of the last known locations for the telemetry tortoises. With this information, the tracker proceeds as follows:

- Coordinate survey start time with the Transect Survey Team.
- Enter the radio frequencies of the individual tortoises into the receiver.
- Locate the GPS coordinates of the last known locations.
- When a telemetry tortoise is detected, complete the data fields on the telemetry datasheet (see Appendix B), including tortoise location, visibility, and behavior.
- Continue to enter and track radio frequencies to locate tortoises until notified of the survey end time by the Transect Survey Team.

Telemetry surveys are not conducted during inclement weather when lightning strikes are possible.

3.2.2 Field Equipment

Each tracker on the Telemetry Team has similar field equipment to locate the telemetry tortoises. Specific information on bandwidth and frequency range of the receiver and antenna are described in Chapter 6 of the Monitoring Handbook (see Appendix B). The field equipment includes a GPS unit, receiver (fully charged), Yagi antenna, 2 coaxial cables, replacement batteries, two-way radio (or some form of communication with transect

surveyors), mirror, compass, datasheets, pens, and safety equipment. The tracker might include other items in the field kit as necessary or desired.

3.3 Health Assessments

The 2013 Health Assessment Procedures Handbook (USFWS 2013a) is the version referenced for this plan for monitoring the translocation of tortoises to the Eldorado Valley. The HA consists of a physical exam to evaluate specific clinical signs, and collection and diagnostic testing of biological samples.

The components of the physical exam include an overall assessment of the attitude and activity of the tortoise; an examination of the eyes, nares, beak, oral cavity, skin, muscle mass, and shell; and assignment of a body condition score (BCS). Biological samples include blood and a swab from the oral cavity. The University of Florida conducts diagnostic testing (enzyme-linked immunosorbent assay – ELISA) of blood samples to identify antibodies to the pathogenic species of *Mycoplasma* (*M. agassizii* and *M. testudineum*), which are known to cause upper respiratory tract disease in tortoises. The San Diego Zoo Global conducts the analyses of the oral cavity swabs to identify microorganism presence including herpesvirus and Mycoplasma species.

Although physical examinations are useful, they do not provide conclusive evidence about the overall health of the individual tortoise (USFWS 2013a). The same is true of the presence of the *Mycoplasma* pathogenic species. Mycoplasmosis in tortoises is increasingly considered to be a chronic disease (cf. Berish *et al*. 2010, as cited in USFWS 2013a); therefore, identifying the impacts of such diseases can be a difficult task.

Because tortoise health might influence the success of translocation, health assessments on resident tortoises are conducted to establish a baseline of health conditions. The Health Assessment Team consists of two biologists – an examiner and a handler. Although a third person observer might be useful to assist with recording data, this observer is optional and is not included under this monitoring plan.

3.3.1 Pre-release Health Assessment – Translocated Tortoises

The health assessments of the translocated tortoises are the responsibility of the DTCC and the USFWS. The USFWS, with the assistance of San Diego Zoo Global, developed health criteria (USFWS 2013b) to evaluate a tortoise's eligibility for translocation.

3.3.2 Pre-release Health Assessment – Resident Tortoises

A health assessment is conducted on resident tortoises located during pre-release surveys of the release and control plots. The resident tortoises located during pre-release surveys (Year 0) constitute the sample for the population as a whole. The extent of the pre-release HA includes a physical exam, a BCS, and collection of biological samples (blood draw and oral cavity swab).

In general, blood samples collected with the intention of detecting an immune response require that the immune system be functioning and active. A desert tortoise's immune response is temperature dependent and thus depressed during periods of winter inactivity. Therefore, it is important to collect samples for ELISA testing after the immune system resumes more typical active-season functioning, which the USFWS has determined to be between May 15 and October 31 (USFWS 2013a). The Health Assessment Team can begin collecting blood samples from resident tortoises in the first release site no earlier than May 15, 2014 (K. Field, personal communication). The team

must complete all health assessments before the end of June 2014 in accordance with the air temperature threshold limitations.

The Health Assessment Team receives radio frequency and transmitter information from the Transect Survey Team (via the DCP), along with GPS coordinates of the last known locations of the resident tortoises. The Health Assessment Team also receives vehicle entry points for the survey plots (Waypoint 0) and transect coordinates from the Transect Survey Team (via the DCP). The team uses this information to locate the tortoises. If a tortoise cannot be located or accessed (i.e., more than 12 inches inside a burrow) after 3 attempts, the Health Assessment Team confers with the DCP for further instructions.

3.3.3 Data Collection – Conducting the Health Assessment

The procedures for conducting the HA are described in detail in the 2013 Health Assessment Procedures Handbook and appendices (USFWS 2013a). The procedures are summarized in this monitoring plan. Some sampling protocols described in the handbook will not be implemented (e.g., nasal lavage and cloacal swab) as noted below. The Handbook is located online at http://www.fws.gov/nevada/desert_tortoise/documents/reports/2013/assess/May2013-Desert-tortoise-health-eval-handbook.pdf. Excerpts/protocols from the Handbook and appendices are included in Appendix D and Appendix E to this monitoring plan.

The HA is only initiated if the air temperature at 5 cm above the ground is 35°C or less.

Disinfection and Sanitation

Caution must be taken whenever handling or sampling desert tortoises to ensure that field personnel do not aid in the spread of infectious microorganisms or contaminate samples.

The Health Assessment Team follows a standard protocol that includes the disinfection of all equipment. Field personnel wear disposable latex or nitrile gloves whenever handling tortoises, and change gloves between individual tortoises. A disinfection protocol is provided in Appendix A of the 2013 Health Assessment Procedures Handbook, which is included in Appendix D to this monitoring plan.

Handling

Handling of tortoises during the HA must occur in a manner that minimizes stress. The tortoise is kept as close to ground level as possible and in its normal spatial orientation. Tortoises can be slightly rotated out of normal spatial orientation for necessary data and sample collection, but should never be turned vertical or upside down. The handler holds the tortoise away from his/her body to minimize risk of contamination from contact or voiding. Handling a tortoise to complete the HA and sample collection must be completed within 30 minutes or less. This time period does not include rehydrating a tortoise that voids.

If the tortoise is located in a burrow, the Health Assessment Team attempts to extract the tortoise, ***only*** if it is accessible within the first 12 inches inside the burrow. The extraction must be without the use of force.

The Health Assessment Team removes the radio transmitter before beginning the HA. The USFWS will provide instructions for removing transmitters during the 1-day training session before beginning the field season. The Health Assessment Team returns the transmitters to the Transect Survey Team.

Notching

The Health Assessment Team marks the tortoise by notching the shell. The USFWS will provide instructions on how and where to notch the shell during the 1-day training session.

Physical Examination

The basic components of the physical exam include an overall assessment of the attitude and activity of the tortoise, an examination of the eyes, nares, beak, oral cavity, skin, muscle mass, and shell. The Health Assessment Team reviews published guidelines for line drawings that show how to examine the eyes and periocular area of desert tortoises (Berry and Christopher 2001), and reviews descriptions and photographs of different health conditions and clinical signs (http://www.fws.gov/nevada/desert_tortoise/documents/reports/2013/assess/clinical-signs-2013d.pdf).

The physical examination procedures and data collection form instructions are in Appendix B of the 2013 Health Assessment Procedures Handbook. The HA datasheet from Appendix B of the Handbook was modified for the translocation project to Eldorado Valley. The modified datasheet is included in Appendix C to this monitoring plan.

As part of the physical examination process, the Health Assessment Team records or confirms data (MCL and sex) that the Transect Survey Team was not able to collect. The tag number, MCL, and sex are listed for each transmitter frequency provided to the Health Assessment Team, who verifies the information and/or records any missing data.

Body Condition Score

Body condition scoring (BCS) is a visual appraisal system that estimates average body energy reserves. Since individual tortoises can vary in size and shape, weight alone is not a good indicator of body condition. The BCS is based on an evaluation of muscle mass and fat deposits in relation to skeletal features. It is an efficient method of evaluating the condition of a tortoise that does not require much handling or intrusive examination. Appendix E to this monitoring plan includes the scoring methodology and pictures for each body condition category for the BCS.

Biological Sample Collection

The biological samples include a blood draw and two oral cavity swabs. Guidelines for sample collection and processing, handling and storage, supply lists, and laboratory shipping information are in Appendix E and Appendix F of the 2013 Health Assessment Procedures Handbook, which are included in Appendix D to this monitoring plan.

If the handler cannot open the tortoise's mouth to collect oral cavity swabs after three attempts, or the tortoise is experiencing stress at any time, this part of the HA is stopped. There are no further attempts to observe and swab the oral cavity at a later time.

A minimum amount of blood is required for ELISA testing. If the minimum amount is not drawn by the third attempt (as defined in Appendix E of the Handbook), the amount is recorded on the datasheet but the sample is not submitted for analysis. This part of the HA is done; there are ***no further attempts*** to collect a blood sample.

The Health Assessment Team may deliver the processed blood samples and swabs to the DTCC for temporary storage pending shipment to the University of Florida or San Diego Zoo Global. When there are enough samples to fill a shipping box, the team ships the box via overnight carrier for next-day morning arrival at the University or the Zoo. The exact process will be determined by the DCP and the Contractor after contract award.

Hydration

Voiding occasionally occurs while tortoises are handled during the HA, data collection, and biological sampling. Loss of urine due to handling-induced voiding may have an adverse affect on long-term survivability based on a previous retrospective study (Averill-Murray 2002). Given the potential for adverse effects on the tortoise's health occurring from voiding urine, the Health Assessment Team administers fluids to replace voiding losses.

The Health Assessment Team rehydrates by injecting epicoelomic fluids. This method has the benefit of providing a known amount of fluid replacement and does not require significant amounts of equipment. The procedures for injectable epicoelomic fluids method for rehydration are described in Appendix F of the Handbook, which is included in Appendix D of this monitoring plan.

Waste Disposal and Safety

The Health Assessment Team prepares a protocol for the safe handling and proper disposal of medical waste, including sharps (syringe needles). The protocol describes the response to accidental exposure to biological samples through needle sticks or other means. The DCP reviews and approves the waste handling and disposal protocol before the team begins the HA.

3.3.4 Post-release Health Assessment – Resident and Translocated Tortoises

The Transect Survey Team records the BCS of translocated and relocated resident tortoises (i.e., baseline population) in Year 1 and Year 2.

Post-release health assessments would be done at 5-year intervals, or when the BCS that are determined by the transect surveyors suggest a decline of health in the population. The post-release HA is done on the translocated tortoises and on the resident tortoises in the release and control sites that had a HA in Year 0. Other resident tortoises located in Year 1 and Year 2 that were not located in Year 0 are not included in the baseline population, and therefore, would not receive a health assessment. The post-release assessment repeats the same procedures described for the pre-release assessment; a physical exam, a BCS, and collection of biological samples (blood draw and oral cavity swab). The HA is conducted after May 15 when the immune system is functioning and active.

3.3.5 Field Equipment

The field equipment and supplies needed to process the HA for a single tortoise are listed in Appendix E of the 2013 Health Assessment Procedures Handbook, which is included in Appendix D of this monitoring plan. The Health Assessment Team provides all the supplies. The HA supplies for individual tortoises are prepared and packaged in zipper-sealed plastic bags to decrease preparation and tortoise handling time in the field. The Research Coordinator at the DTCC (702-526-3436) can provide information to the Contractor for ordering supplies.

4.0 PERMITS, AUTHORIZATIONS, AND QUALIFICATIONS

Translocation to augment wild populations of desert tortoise is a component for the species recovery as outlined in the Revised Recovery Plan (USFWS 2011a). Specific permits and authorizations and staff qualifications and training are necessary to implement species recovery.

4.1.1 Permits and Authorizations

Section 10 of the Endangered Species Act regulates a wide range of activities that affect listed species. A Section 10(a)(1)(A) recovery permit is required for activities that enhance propagation or survival of a listed species. Examples of these activities include abundance surveys, relocations, capture and marking, and telemetric monitoring.

The Desert Tortoise Recovery Office (DRTO) issues permits for strategic elements of the Revised Recovery Plan subject to the terms and conditions of Biological Opinion (BiOp) File No. 2013-F-0273 dated July 3, 2013. The DTRO is the permittee for the translocation project for Eldorado Valley, including the activities of this monitoring plan, and completes any required notice for project review and approval for the recovery permit. The DTRO includes the Contractor(s) for this monitoring plan as a sub-permittee to the recovery permit and ensures compliance with terms and conditions of the BiOp.

The Contractor needs a separate scientific collection permit from the Nevada Department of Wildlife (NDOW) for handling desert tortoises. The Contractor applies to NDOW for the permit and includes the DTRO recovery permit for the translocation project. The NDOW requires four to six weeks for the permit to be reviewed and issued. The permit and fee are for two years; an extension or new permit is required for the second year of post-release surveys.

The translocation of tortoises to land managed by the BLM in Eldorado Valley is a federal action subject to the requirements of the National Environmental Policy Act (NEPA). The DTRO submits the translocation plan to the BLM for review and approval under the Environmental Assessment and Finding of No Significant Impact issued by the BLM on January 31, 2013 for translocation projects throughout the species range in the BLM Southern Nevada District in Clark County.

The activities of the DCP associated with this monitoring plan are covered by the incidental take permit issued to Clark County pursuant to Section 10(a)(1)(B) of the Endangered Species Act.

4.1.2 Qualifications

The DTRO reviews and approves the credentials, education, and training of individuals who will conduct activities under the recovery permit. According to BiOp special terms and conditions for handling, all Contractor personnel must be qualified and implement the most recent USFWS-approved guidance for handling desert tortoises.

The DTRO conducts training sessions for the selected Contractor. The training sessions are intended to be "refresher" sessions to evaluate the individuals' qualifications to participate in transect and telemetry surveys and to conduct health assessments.

Transect Surveyor

The minimum qualification for members of the Transect Survey Team is one year (field season) of line distance sampling experience. The transect surveyor must demonstrate past experience collecting data, attaching radio

transmitters, and handling tortoises associated with line distance sampling as part of the USFWS range-wide monitoring program.

Transect surveyors participate in a 1-day training and practice session on tortoise handling procedures, including a review of methods to attach radio transmitters to tortoises.

Telemetry Tracker

The minimum qualification for members of the Telemetry Team is one year (field season) experience in tracking desert tortoises via radio telemetry. The telemetry tracker must demonstrate past experience in tortoise handling procedures associated with radio telemetry.

Telemetry trackers participate in a 1-day training and practice session on tortoise handling procedures.

Health Assessment Team

The Health Assessment Team consists of two biologists – an examiner and a handler. Because conducting health assessments requires specialized skills, the minimum qualification for the examiner is successful completion of the Health Assessment Training Workshop provided by the USFWS and San Diego Zoo. The examiner must be certified and permitted by the USFWS to conduct all activities involved with the health assessment and demonstrate past experience with marking tortoises by notching the shell, collecting biological samples, and rehydrating tortoises by injecting epicoelomic fluids. The minimum qualifications for the handler are training and demonstrated experience in tortoise handling procedures associated with conducting health assessments and in marking tortoises by notching the shell.

The health assessment examiner and handler participate in a 1-day training and practice session on conducting health assessments and removing radio transmitters. A veterinarian associated with the DTCC evaluates and approves the examiner's skills in completing the health assessment and collecting biological samples.

5.0 DATA MANAGEMENT

An essential component of a successful monitoring project is the quality of the data. Quality data is required for results that meet the objectives of the project. Errors, however, are common in data collection and many projects are compromised by incomplete, poor, or missing data. This section provides guidance for ensuring data collection is complete and of desired quality, available for analysis and sharing, and archived for future use.

These data management guidelines are reviewed and followed by all project staff involved in data collection or data management for the project. All project staff need to understand and implement their roles in data management.

5.1 Assessment of Detail Needed for Data Management

Not all data management sections in monitoring protocols need to contain the same detail, and there is not a "one-size-fits-all" approach to developing a data management section for the wide diversity of long-term monitoring projects. A graded approach is used to identify the level of detail and comprehensiveness needed for project-level data management and quality control. The graded approach assesses the scope and impact of the study, complexity, duration, staffing, project costs, and intended use of the data and determines the appropriate level of detail needed for each data management activity.

The Eldorado Valley Translocation Project is of significant regional and local interest due to the status of the Mojave desert tortoise as a federally listed threatened species and a species covered by the MSHCP. The complexity of the study is high, collecting a range of field and health assessment data. Due to the potential duration of the study (could be resampled in 5- or 10-year period or longer) and the involvement of multiple staff and project leaders, the data management needs to comprehensively cover all aspects of the study. Additionally, because this project is supported by public funds, a high level of accountability is necessary.

5.2 Data Acquisition Quality

The section covers the selected components of the data collection process that require additional emphasis on quality.

5.2.1 Spatial Data

Field collected spatial coordinates are recorded on the field datasheet and obtained from a GPS unit with 3 m or better accuracy. Accuracy less than 1 m is not necessary to record the location of tortoises. Data is recorded in UTM zone 11 projection and NAD83 datum. The spatial coordinates are entered on the field datasheet at the time of data collection.

5.2.2 Photographic Data

Digital photographs (images) are taken of tortoises and habitat within the release and control sites using a digital camera with a minimum of five megapixel capacity. Photographs are associated with a tortoise or carcass, and the data on the photograph are entered on the appropriate datasheet. The purpose of the photographs is to provide documentation of the condition of the tortoise or carcass. Optional photographs may be taken of rare and unique species, examples of biodiversity, and habitat and soil types.

5.2.3 Maintenance and Calibration of Equipment

The Transect Survey, Telemetry, and Health Assessment teams maintain all field equipment in perfect working condition and ensure the GPS units and camera time stamps are synchronized and have a full or adequate charge for each day in the field. Perfect working condition is defined by no field time lost due to equipment issues. The camera clock is synchronized each morning with the date and time of the GPS receiver to assist with data verification.

5.3 Data Completeness and Quality

The overall system of management activities designed to ensure the quality of the data generated by a project or program is commonly known as QA/QC, or Quality Assurance and Quality Control. For the purpose of this data management plan, QA/QC is being described by its two major components, data verification and data validation.

5.3.1 Data Verification

Data verification ensures that all required data have been collected and recorded. This is accomplished by reviewing the paper and electronic datasheets for blank fields. The verification process occurs within a time frame after data collection that allows for accessing the recollection of the data collectors (e.g., Transect Survey, Telemetry, and Health Assessment teams) and, if needed, the potential recollection of data.

The Transect Survey Team reviews the datasheets after the observation of a desert tortoise and at the end of each transect to ensure that all data has been collected. This also occurs at the end of each field day when the Transect Survey Team reviews all paper and electronic datasheets and the photo log, checking data while memories are most accurate. This review includes making notations on paper field datasheets of any corrections made. The Telemetry Team reviews their datasheets after a transmittered tortoise is located and data is collected, and at the end of each field day. The Health Assessment Team reviews the datasheet after the health assessment of a tortoise.

The Contractor enters the datasheets into an electronic database and submits both the datasheets and database to the DCP Translocation Project Manager who conducts data verification (QC). The database is developed by the Contractor and approved by the DCP. Data submission and verification frequency will be determined upon contract award.

5.3.2 Data Validation

Data validation assesses the quality of each data entry, checking its structural integrity and logical consistency. For example, it includes comparing the expected and entered GPS coordinates and assessing the expected density of desert tortoise in the area surveyed. Data validation requires the reviewer to understand the data and their range of values.

The DCP completes data validation. The DCP reviews tortoise detections by transect and size, transect order, and transect start and completion time on a regular basis. The DCP will provide data to the USFWS at intervals yet to be determined. At intervals throughout the field season, the USFWS may complete an assessment, including methodological assessments of tortoise detections by week and plot, tortoise detections by distance from the transect line, transect paths and walk order, transect completion times, and a summary of the G_0 data and a list of date errors and inconsistencies.

Errors in more than two percent of the entries in any data field triggers a review of the data collection and verification procedures and may require additional training of transect surveyors.

5.3.3 Preparing Data for Uploading

The data files are saved using a unique naming convention before uploading to an ftp site maintained by the Contractor. The naming convention identifies the files to the project using "EVTP" for Eldorado Valley Translocation Project as follows for the different source of data:

Transect Survey Data: The data from each field survey day is named according to the following naming convention:

EVTP_Transect_<Plot>_<Transect Number>_<Date>.xls

Where,

- <Plot> is either Release 1, Release 2, Control 1, or Control 2
- <Transect Number> is the number of the transect in the plot
- <Date> is the field survey date as yyyymmdd

The underscore symbol separates the identifying features in the file name. The file extension (.xls) indicates the database uses Microsoft Excel™ software.

Telemetry Data: The data from each field survey day is named according to the following naming convention:

EVTP_Telemetry_<Date>.xls

Where,

- <Date> is the field survey date as yyyymmdd

The underscore symbol separates the identifying features in the file name. The file extension (.xls) indicates the database uses Microsoft Excel™ software.

Health Assessment Data: The data from each field survey day is named according to the following naming convention:

EVTP_HA_<Plot>_<Tortoise Tag Number>_<Date>.xls

Where,

- <Plot> is either Release 1, Release 2, Control 1, or Control 2
- <Tortoise Tag Number> is the number of the tag on the tortoise
- <Date> is the field survey date as yyyymmdd

The underscore symbol separates the identifying features in the file name. The file extension (.xls) indicates the database uses Microsoft Excel™ software.

5.3.4 Uploading Data

The data files (database, GIS data, and digital images) for a complete field day from each of the teams is saved as a single compressed (.zip) file and uploaded to an ftp site maintained by the Contractor. Paper field datasheets and the database are delivered to the DCP at intervals to be determined after contract award.

5.3.5 Version Control

Version control is the process of managing copies of changing files over the course of a project. Any alteration or update to a file is considered a change and is reflected in the complete file name. Version control includes developing file-naming guidelines that include the file name, the QA/QC status, and the date of the file.

Version control is required for the database and the image files. The categories of QA/QC versions are *_fieldverified_* for the data that has been verified by the Transect Survey, Telemetry, and Health Assessement teams, *_DCPverified_* for data that have been verified by the DCP Project Manager, *_DCPvalidated_* for data that have been validated by the Project Manager, and *_master_* for the master compilation of data for which all QA/QC steps have been completed by both the DCP and the USFWS. For example, the validation of the data collected during the transect surveys in Release Site 1 on April 17, 2014 is named:

EVTP_Transect-Survey_Release-Site-1_20140417_DCPvalidated.xls

5.4 Data Description

Clearly written and comprehensive metadata are a key component for successfully (a) managing active data over time, and (b) re-using long-term monitoring/research data for a variety of purposes.

Metadata describe what a data set user needs to know or understand to successfully use a data set – what the data are, why they were collected, how they were collected, data quality checks performed and results, idiosyncrasies of the data set, and who to contact if there are questions about the data. Metadata are particularly useful for long-term data series to help the original team remember what was done and why over time, and to provide crucial orientation information to new team members. In a general sense, metadata may include descriptive text, diagrams, flow charts, images, video, sound files, or other descriptive files. A formal metadata record provides a high level overview of the data set, and references to more detailed information in other sources. Increasingly, formal metadata records are required to be machine readable, using the appropriate software.

The Contractor develops metadata, in coordination with the DCP (and USFWS and BLM, as appropriate), for this project that meets ISO 19115-2. This standard is the most complex and flexible and incorporates the special FGDC Biological Data Profile elements without modification. Most U.S. Government agencies are implementing ISO 19115-2 because of its added support for imagery and gridded data.

The metadata developed during the data collection phase of the project is refined and augmented over the course of the project. This allows the capture of idiosyncrasies of the data set when they happen, rather than having to recall them and their details at a later time. It also allows the team to use questions asked by new team members as insight into what additional metadata content future team members and external data users will find useful.

5.5 Data Analysis

The analysis of the data is the responsibility of the USFWS. Data from the 4 surveyed plots from 3 years of surveys (if available) would be used in a BACI analysis, comparing population changes for the 2 control plots to changes in the 2 release plots (Buckland et al. 2009). The process would incorporate estimates of tortoise availability from bootstrapping telemetry data collected at the time each plot was surveyed, if available.

5.6 Data Interpretation and Reporting

The interpretation of the data and the resulting management responses and reporting are the responsibility of the USFWS and will occur after completion of Year 2. The USFWS report will be shared with the DCP. The DCP and the USFWS will cooperate in any results that suggest changes in the monitoring plan.

5.7 Data Distribution and Publication

The distribution of data and publication of the results are the responsibility of both the USFWS and the DCP.

Decision makers and stakeholders impacted by the results of this study include, but are not limited to the DCP, USFWS, MSHCP Permittees, BLM, and NDOW. The results of the study may be presented in a written final technical report that would receive peer review. Once the outcomes of the peer review are addressed in the final report, it should be shared with the above key decision makers. Other local land managers may also benefit from the outcomes of this study and the technical report should be shared with them. The monitoring plan and data will be part of the public records of Clark County, Nevada, and available for inspection by anyone who requests them. Any technical report or publication may become part of the public records if completed and provided to the DCP. Agencies may seek opportunities to deposit any technical report in open access repositories.

5.8 Data Archives

Data archiving is the long-term (multi-year/multi-decade) management of the data once it is received by the DCP and the USFWS. It acts as a backup to the active datasets managed by the project team during the life of the project, and is the location of the datasets after the project is complete.

The data from monitoring the translocation project will adhere to Clark County records management policy and record retention schedule for archiving data. Data will be stored on lossless and non-proprietary software in multiple locations with appropriate metadata. The DCP will also maintain copies of the paper datasheets. The DCP provides periodic verification of the archived data. Academic archives, such as University of Nevada, Las Vegas, may also be assessed to permanently house copies of the monitoring plan and the data.

All data (scanned copies of the paper datasheets and electronic data products) may also be archived by the USFWS following their procedures.

6.0 ROLES AND RESPONSIBILITIES

The translocation of desert tortoises to the Eldorado Valley and the pre- and post-translocation monitoring involves different agencies, personnel, and contractors. It is important for all involved to understand their roles and specific responsibilities to ensuring acceptable data collection and quality, and management and completion of the project.

- Pre- and Post-Translocation Monitoring Plan and Translocation Plan
    - The development of the monitoring plan for the pre- and post-translocation monitoring is the responsibility of the DCP, with the plan written by the Science Advisor. The Science Advisor worked closely with the USFWS in developing this monitoring plan. The USFWS prepares the Translocation Plan used to fulfill the NEPA requirements of the BLM to release tortoises on BLM managed land.
- Translocation of Desert Tortoises
    - The DTCC and the USFWS are responsible for the translocation of at least 100 desert tortoises from the DTCC to the release points. This process includes the health assessment of tortoises at the DTCC, the transportation of tortoises to the Eldorado Valley, and the transport and release of tortoises at points within each of the two release sites. The DTCC and USFWS are responsible for providing the complete health assessment data on each tortoise translocated for this project and will provide this data to the DCP.
- Survey of Resident Tortoises
    - The survey of resident tortoises in the release and control sites is the responsibility of the Transect Survey Team. This team is contracted by the DCP. The Transect Survey Team surveys for tortoises in the four sites between the second week of April and the end of May. The team is responsible for the collection of all required data and the validation of the data before submitting it to the DCP. The DCP Project Manager oversees the Transect Survey Team in coordination with the USFWS.
- Telemetry of Tortoises in Piute Valley
    - The telemetry of transmittered tortoises within an established telemetry site in Piute Valley is the responsibility of the Telemetry Team. The Telemetry Team is contracted by the DCP. This team assesses the detectability of tortoises between the second week of April and the end of May. The DCP Project Manager oversees the Telemetry Team in coordination with the USFWS.
- Health Assessment of Resident Tortoises
    - The health assessment of resident tortoises is the responsibility of the Health Assessment Team. The Health Assessment Team is contracted by the DCP. This team completes a physical exam, assigns a body condition score, and collects biological samples on located resident tortoises between May 15 and the end of June. The team is responsible for the collection of all required data and the validation and verification of the data before submitting it to the DCP. The DCP Project Manager oversees the Health Assessment Team in coordination with the USFWS.
- Contract and Project Management by DCP
    - The DCP manages the contracts with the Transect Survey, Telemetry, and Health Assessment teams and manages each of the teams during their work in the field. The DCP appoints a Translocation Monitoring

Project Manager to coordinate the project. The DCP maintains the master database submitted by the Contractor during the extent of the fieldwork.

- Project Management by USFWS
  - The USFWS manages the translocation of tortoises, participates in training sessions for the Contractor, and leads the efforts to analyze, interpret, distribute, and archive data.
- Data Analysis
  - The USFWS is responsible for data analysis.
- Data Archiving
  - The DCP and the USFWS will archive data.

7.0 DOCUMENT HISTORY

This section summarizes the development, review, and the history of updates associated with the development of this monitoring plan.

7.1.1 Development of Monitoring Plan

The initial version of the Desert Tortoise Pre- and Post-Translocation Monitoring Plan for the Augmentation of a Mojave Desert Tortoise Population in the Eldorado Valley, Clark County, Nevada was developed between July and October 2013. The Science Advisors to the DCP, Robert Sutter and Mary Peters, wrote the monitoring plan, with assistance from Linda Allison and Kimberleigh Field of the USFWS, and with guidance by the DCP. The development of the monitoring plan was done under WAF 003 of the Science Advisor contract 2009-ECO-801D.

7.1.2 Review, Approval, and Updates to Monitoring Plan

This section will be completed after the review of the monitoring plan by the DCP and USFWS. This section will include the date and outline of the approval process, a list of reviewers and comments, the date of revision in response to reviewers' comments, and date of final approval.

Table 7-1. Monitoring Plan History

Revision Date and Version	Author	Changes Made	Reason for Change
13 Nov 2013 Version 2.0	DCP Science Advisor	Telemetry surveys, miscellaneous edits	Response to USFWS and DCP comments

8.0 REFERENCES

Averill-Murray, R.C. 2002. Effects of Survival of Desert Tortoises (*Gopherus agassizi*) Urinating During Handling. Chelonia Conservation and Biology, 2002, 4(2):430-435.

Berry, K.H. and M.M. Christopher. 2001. Guidelines for the field evaluation of desert tortoise health and disease. Journal of Wildlife Diseases 37(3): 427-450.

BLM (Bureau of Land Management). 2013. Desert Tortoise (*Gopherus agassizii*) Translocation throughout the Species Range within the Southern Nevada District and Caliente Field Office, Nevada. Environmental Assessment, DOI-BLM-NV-S010-2012-0097. February 2013.

Buckland S.T., Russell, R.E., Dickson, B.G., Saab, V.A., Gorman, D.N., and W.M. Block. 2009. Analyzing Designed Experiments in Distance Sampling. Journal of Agricultural, Biological, and Environmental Statistics 14:432-442.

Clark County. 2007. Conservation Management Strategy, Piute Eldorado Desert Wildlife Management Area. February 2007.

Clark County. 2000. Final Clark County Multiple Species Habitat Conservation Plan and Environmental Impact Statement for Issuance of a Permit to Allow Incidental Take of 79 Species in Clark County, Nevada. September 2000.

Esque, T.C., Nussear, K.E., Drake, K.K., Walde, A.D., Berry, K.H., Averill-Murray, R.C., Woodman, A.P., Boarman, W.I., Medica, P.A., Mack, J., Heaton, J.S. 2010. Effects of Subsidized Predators, Resource Variability, and Human Population Density on Desert Tortoise Populations in the Mojave Desert, USA. Endangered Species Research. Vol. 12: 167-177.

Field, K.J., Tracy, C.R., Medica, P.A., Marlow, R.W., Corn, P.S. 2007. Return to the Wild: Translocation as a Tool in Conservation of the Desert Tortoise (*Gopherus agassizii*). Biological Conservation 136: 232-245.

Heaton, J. S., Miao, X., Von Seckendorff Hoff, K., Charlet, D., Cashman, P., Trexler, J., Grimmer, A., and Patil, R. 2011. Final Report 2005-UNR-578. Report to Clark County MSHCP 2005-UNR-578:D27.

Nussear, K.E., Tracy, C.R., Medica, P.A., Wilson, D.S., Marlow, R.W., Corn, P.S. 2012. Translocation as a Conservation Tool for Agassiz's Desert Tortoises: Survivorship, Reproduction, and Movements. Journal of Wildlife Management. DOI: 10.1001/jwmg, 390. 16 February 2012.

O'Farrell, T.P. 2009. Management Action Plan for the Boulder City Conservation Easement. Report to the Clark County, Nevada, Desert Conservation Program, Department of Air Quality and Environmental Management. August 2009.

Osenberg C.W. and R.J. Schmitt. 1996. Detecting Ecological Impacts Caused by Human Activities. In: Detecting Ecological Impacts. Edited by R.J. Schmitt and C.W. Osenberg. Academic Press.

USFWS (U.S. Fish and Wildlife Service). 2013a. Health Assessment Procedures for the Mojave Desert Tortoise (*Gopherus agassizii*): A Handbook Pertinent to Translocation. Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service, Reno, Nevada. May 2013.

_____. 2013b. Translocation Plan. Greater Trout Canyon Area, Clark County, Nevada. Revised August 20, 2013.

_____. 2012. Desert Tortoise Monitoring Handbook. Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service, Reno, Nevada. Version: 23 February 2012.

_____. 2011a. Revised Recovery Plan for the Mojave Population of the Desert Tortoise (*Gopherus agassizii*). U.S. Fish and Wildlife Service, Pacific Southwest Region, Sacramento, California. 222 pp.

_____. 2011b. Translocation of Mojave Desert Tortoise from Project Sites: Plan Development Guidance. November 2011.

_____. 1990. Endangered and Threatened Wildlife and Plants; Determination of Threatened Status for the Mojave Population of the Desert Tortoise. Federal Register 55:12178–12191.

Appendix A:
Survey Transect Datasheets

Desert Tortoise Distance Sampling Eldorado Valley: TranLiveObs Form

Plot Num:	Tran Num:	Team Num:	Observer:	Date:

Tran Live #:		**Tortoise location:** Burrow Pallet Open Vegetation Rock		Existing tag present?:	Y N U/R Unk
Last Waypoint:				Existing tag #:	
Observation Time:	am / pm	Burrow visibility:	H M L	Existing tag color:	
Transect Bearing:	0º 90º 180º 270º	Tort in burrow visibility:	H M L	New Tag Attached?	Yes No
Local Bearing:	º	Tortoise visibility:	H M L	New Tag Number:	
Azimuth:	º	Distance to burrow:	m	Transmitter serial #:	
Radial Distance:	m	Temperature:	ºC	Transmitter frequency:	
Perpendicular Dist:	m	Temp > 35?:	Yes No	Tortoise Voided?	None Urine Feces Both
Cue to tortoise: SearchedVeg BodyPart Movement Burrow BurrowApron Audible		Sex:	M F Unk	Tortoise_not_handled_b/c:	
		MCL ≥ 180:	Yes No Unk	Easting:	
Tort heading relative to line when detected: Profile HeadOn TailOn PulledIntoShell HeadIntoBurrow HeadOutOfBurrow		MCL (mm):		Northing:	
		Body condition score: 1 2 3 4 5 6 7 8 9 Unk			

Comments:

Tran Live #:		**Tortoise location:** Burrow Pallet Open Vegetation Rock		Existing tag present?:	Y N U/R Unk
Last Waypoint:				Existing tag #:	
Observation Time:	am / pm	Burrow visibility:	H M L	Existing tag color:	
Transect Bearing:	0º 90º 180º 270º	Tort in burrow visibility:	H M L	New Tag Attached?	Yes No
Local Bearing:	º	Tortoise visibility:	H M L	New Tag Number:	
Azimuth:	º	Distance to burrow:	m	Transmitter serial #:	
Radial Distance:	m	Temperature:	ºC	Transmitter frequency:	
Perpendicular Dist:	m	Temp > 35?:	Yes No	Tortoise Voided?	None Urine Feces Both
Cue to tortoise: SearchedVeg BodyPart Movement Burrow BurrowApron Audible		Sex:	M F Unk	Tortoise_not_handled_b/c:	
		MCL ≥ 180:	Yes No Unk	Easting:	
Tort heading relative to line when detected: Profile HeadOn TailOn PulledIntoShell HeadIntoBurrow HeadOutOfBurrow		MCL (mm):		Northing:	
		Body condition score: 1 2 3 4 5 6 7 8 9 Unk			

Comments:

Tran Live #:		**Tortoise location:** Burrow Pallet Open Vegetation Rock		Existing tag present?:	Y N U/R Unk
Last Waypoint:				Existing tag #:	
Observation Time:	am / pm	Burrow visibility:	H M L	Existing tag color:	
Transect Bearing:	0º 90º 180º 270º	Tort in burrow visibility:	H M L	New Tag Attached?	Yes No
Local Bearing:	º	Tortoise visibility:	H M L	New Tag Number:	
Azimuth:	º	Distance to burrow:	m	Transmitter serial #:	
Radial Distance:	m	Temperature:	ºC	Transmitter frequency:	
Perpendicular Dist:	m	Temp > 35?:	Yes No	Tortoise Voided?	None Urine Feces Both
Cue to tortoise: SearchedVeg BodyPart Movement Burrow BurrowApron Audible		Sex:	M F Unk	Tortoise_not_handled_b/c:	
		MCL ≥ 180:	Yes No Unk	Easting:	
Tort heading relative to line when detected: Profile HeadOn TailOn PulledIntoShell HeadIntoBurrow HeadOutOfBurrow		MCL (mm):		Northing:	
		Body condition score: 1 2 3 4 5 6 7 8 9 Unk			

Comments:

Data Proofed By:

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Desert Tortoise Distance Sampling Eldorado Valley: OppLiveObs Form

Plot Number:	Transect Number:	Team Number:	Observer:	Date:

Opp Live #:		Temp > 35?:	Yes No	New Tag Attached?	Yes No
Time:	am / pm	MCL ≥ 180:	Yes No Unk	New Tag Number:	
Tortoise location:		MCL (mm):		Transmitter serial #:	
Burrow Pallet Open Vegetation Rock		Sex of Tort:	M F Unk	Transmitter frequency:	
Burrow Visibility:	H M L	**Body condition score:**		Tortoise Void	None Urine Feces Both
Tort in Burrow Visibility:	H M L	1 2 3 4 5 6 7 8 9 Unk		Tort not handled b/c:	
Tortoise Visibility:	H M L	Existing tag present?:	Y N U/R Unk	Easting:	
Distance to burrow (m):		Existing tag #:		Northing:	
Temperature:	ºC	Existing tag color:			

Comment:

Opp Live #:		Temp > 35?:	Yes No	New Tag Attached?	Yes No
Time:	am / pm	MCL ≥ 180:	Yes No Unk	New Tag Number:	
Tortoise location:		MCL (mm):		Transmitter serial #:	
Burrow Pallet Open Vegetation Rock		Sex of Tort:	M F Unk	Transmitter frequency:	
Burrow Visibility:	H M L	**Body condition score:**		Tortoise Void	None Urine Feces Both
Tort in Burrow Visibility:	H M L	1 2 3 4 5 6 7 8 9 Unk		Tort not handled b/c:	
Tortoise Visibility:	H M L	Existing tag present?:	Y N U/R Unk	Easting:	
Distance to burrow (m):		Existing tag #:		Northing:	
Temperature:	ºC	Existing tag color:			

Comment:

Opp Live #:		Temp > 35?:	Yes No	New Tag Attached?	Yes No
Time:	am / pm	MCL ≥ 180:	Yes No Unk	New Tag Number:	
Tortoise location:		MCL (mm):		Transmitter serial #:	
Burrow Pallet Open Vegetation Rock		Sex of Tort:	M F Unk	Transmitter frequency:	
Burrow Visibility:	H M L	**Body condition score:**		Tortoise Void	None Urine Feces Both
Tort in Burrow Visibility:	H M L	1 2 3 4 5 6 7 8 9 Unk		Tort not handled b/c:	
Tortoise Visibility:	H M L	Existing tag present?:	Y N U/R Unk	Easting:	
Distance to burrow (m):		Existing tag #:		Northing:	
Temperature:	ºC	Existing tag color:			

Comment:

Opp Live #:		Temp > 35?:	Yes No	New Tag Attached?	Yes No
Time:	am / pm	MCL ≥ 180:	Yes No Unk	New Tag Number:	
Tortoise location:		MCL (mm):		Transmitter serial #:	
Burrow Pallet Open Vegetation Rock		Sex of Tort:	M F Unk	Transmitter frequency:	
Burrow Visibility:	H M L	**Body condition score:**		Tortoise Void	None Urine Feces Both
Tort in Burrow Visibility:	H M L	1 2 3 4 5 6 7 8 9 Unk		Tort not handled b/c:	
Tortoise Visibility:	H M L	Existing tag present?:	Y N U/R Unk	Easting:	
Distance to burrow (m):		Existing tag #:		Northing:	
Temperature:	ºC	Existing tag color:			

Comment:

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Desert Tortoise Distance Sampling Eldorado Valley: TranCarcObs Form

Plot Num:	Transect Num:	Team Num:	Observer:	Date:

Tran Carc #:		Carcass Condition:	Intact D/A	Easting:	
Last Waypoint:		Carcass Status:	1 2 3 4 5	Northing:	
Observation Time:	am / pm	MCL ≥ 180?	Yes No Unk		File name
Transect Bearing:	0º 90º 180º 270º	MCL (mm):		Photo 1? Y N	
Local Bearing:	º	Sex:	M F Unk	Photo 2? Y N	
Azimuth:	º	Existing tag present?	Yes No U/R	Photo 3? Y N	
Radial Distance:	m	Existing tag #:			
Perpendicular Dist:	m	Existing tag color:			
Comments:					

Tran Carc #:		Carcass Condition:	Intact D/A	Easting:	
Last Waypoint:		Carcass Status:	1 2 3 4 5	Northing:	
Observation Time:	am / pm	MCL ≥ 180?	Yes No Unk		File name
Transect Bearing:	0º 90º 180º 270º	MCL (mm):		Photo 1? Y N	
Local Bearing:	º	Sex:	M F Unk	Photo 2? Y N	
Azimuth:	º	Existing tag present?	Yes No U/R	Photo 3? Y N	
Radial Distance:	m	Existing tag #:			
Perpendicular Dist:	m	Existing tag color:			
Comments:					

Tran Carc #:		Carcass Condition:	Intact D/A	Easting:	
Last Waypoint:		Carcass Status:	1 2 3 4 5	Northing:	
Observation Time:	am / pm	MCL ≥ 180?	Yes No Unk		File name
Transect Bearing:	0º 90º 180º 270º	MCL (mm):		Photo 1? Y N	
Local Bearing:	º	Sex:	M F Unk	Photo 2? Y N	
Azimuth:	º	Existing tag present?	Yes No U/R	Photo 3? Y N	
Radial Distance:	m	Existing tag #:			
Perpendicular Dist:	m	Existing tag color:			
Comments:					

Tran Carc #:		Carcass Condition:	Intact D/A	Easting:	
Last Waypoint:		Carcass Status:	1 2 3 4 5	Northing:	
Observation Time:	am / pm	MCL ≥ 180?	Yes No Unk		File name
Transect Bearing:	0º 90º 180º 270º	MCL (mm):		Photo 1? Y N	
Local Bearing:	º	Sex:	M F Unk	Photo 2? Y N	
Azimuth:	º	Existing tag present?	Yes No U/R	Photo 3? Y N	
Radial Distance:	m	Existing tag #:			
Perpendicular Dist:	m	Existing tag color:			
Comments:					

Tran Carc #:		Carcass Condition:	Intact D/A	Easting:	
Last Waypoint:		Carcass Status:	1 2 3 4 5	Northing:	
Observation Time:	am / pm	MCL ≥ 180?	Yes No Unk		File name
Transect Bearing:	0º 90º 180º 270º	MCL (mm):		Photo 1? Y N	
Local Bearing:	º	Sex:	M F Unk	Photo 2? Y N	
Azimuth:	º	Existing tag present?	Yes No U/R	Photo 3? Y N	
Radial Distance:	m	Existing tag #:			
Perpendicular Dist:	m	Existing tag color:			
Comments:					

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Desert Tortoise Distance Sampling Eldorado Valley: OppCarcObs Form

Plot Num:	Transcect Num:	Team Num:	Observer:	Date:

Opp Carc #:		Existing tag present?	Yes No U/R		File name
Carcass Condition:	Intact D/A	Existing tag #:		Photo 1?	Y N	
Carcass Status:	1 2 3 4 5	Existing tag color:		Photo 2?	Y N	
MCL ≥ 180?	Yes No Unk			Photo 3?	Y N	
MCL (mm):		Easting:			
Sex:	M F Unk	Northing:			
Comments:					

Opp Carc #:		Existing tag present?	Yes No U/R		File name
Carcass Condition:	Intact D/A	Existing tag #:		Photo 1?	Y N	
Carcass Status:	1 2 3 4 5	Existing tag color:		Photo 2?	Y N	
MCL ≥ 180?	Yes No Unk			Photo 3?	Y N	
MCL (mm):		Easting:			
Sex:	M F Unk	Northing:			
Comments:					

Opp Carc #:		Existing tag present?	Yes No U/R		File name
Carcass Condition:	Intact D/A	Existing tag #:		Photo 1?	Y N	
Carcass Status:	1 2 3 4 5	Existing tag color:		Photo 2?	Y N	
MCL ≥ 180?	Yes No Unk			Photo 3?	Y N	
MCL (mm):		Easting:			
Sex:	M F Unk	Northing:			
Comments:					

Opp Carc #:		Existing tag present?	Yes No U/R		File name
Carcass Condition:	Intact D/A	Existing tag #:		Photo 1?	Y N	
Carcass Status:	1 2 3 4 5	Existing tag color:		Photo 2?	Y N	
MCL ≥ 180?	Yes No Unk			Photo 3?	Y N	
MCL (mm):		Easting:			
Sex:	M F Unk	Northing:			
Comments:					

Opp Carc #:		Existing tag present?	Yes No U/R		File name
Carcass Condition:	Intact D/A	Existing tag #:		Photo 1?	Y N	
Carcass Status:	1 2 3 4 5	Existing tag color:		Photo 2?	Y N	
MCL ≥ 180?	Yes No Unk			Photo 3?	Y N	
MCL (mm):		Easting:			
Sex:	M F Unk	Northing:			
Comments:					

Opp Carc #:		Existing tag present?	Yes No U/R		File name
Carcass Condition:	Intact D/A	Existing tag #:		Photo 1?	Y N	
Carcass Status:	1 2 3 4 5	Existing tag color:		Photo 2?	Y N	
MCL ≥ 180?	Yes No Unk			Photo 3?	Y N	
MCL (mm):		Easting:			
Sex:	M F Unk	Northing:			
Comments:					

Opp Carc #:		Existing tag present?	Yes No U/R		File name
Carcass Condition:	Intact D/A	Existing tag #:		Photo 1?	Y N	
Carcass Status:	1 2 3 4 5	Existing tag color:		Photo 2?	Y N	
MCL ≥ 180?	Yes No Unk			Photo 3?	Y N	
MCL (mm):		Easting:			
Sex:	M F Unk	Northing:			
Comments:					

Data Proofed By: ______________________

Page ______ of ______

Desert Tortoise Distance Sampling Eldorado Valley **Transect Form**

Transect Num:	Team Num:	Observer:	Date: 2014

Waypoint 0 Time: am pm Easting: Northing:
Comments:

Waypoint 1 Time: am pm Easting: Northing:
Comments:

Waypoint 2 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 3 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 4 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 5 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 6 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 7 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 8 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 9 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 10 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 11 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 12 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Waypoint 99 Time: am pm Easting: Northing:
Burrow ct (fr previous): Tran interrupted? Y N
Comments:

Transect shortened? Y N Obstacles if shortened:
Comments (include waypoint number):

Data Proofed By:

Appendix B:
Telemetry Survey Datasheet, Radio Telemetry and G_0 Protocols

Desert Tortoise Distance Sampling G_0 Start and Obs Form

Site:	Piute Valley	Date:	2014
Observer:			

Photo 1:	Yes No	Photo 1 comments:	
Photo 2:	Yes No	Photo 2 comments:	
Photo 3:	Yes No	Photo 3 comments:	
Photo 4:	Yes No	Photo 4 comments:	

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Comments (include tortoise number):

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Desert Tortoise Distance Sampling G_0 Obs (continued)

Site:	Piute Valley	Date:	2014
Observer:			

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Tortoise Num:		Tortoise location:	Burrow Pallet Open	Behavior:	GPS Location	
Time:			Vegetation Rock	Unk AtRestActive	Easting:	
Tort Visible?:	Yes No	Burrow Visibility:	High Med Low Not	Moving Basking	Northing:	
Dist to burrow (m)		Tort in Burrow Visibility:	High Med Low Not	Eating Mating		
		Tortoise visibility	High Med Low Not	Agonistic Digging		

Comments:

Comments (include tortoise number):

Data proofed by: Page of

6. RADIO TELEMETRY AND G_0 PROTOCOLS

Across the Mojave Desert, several small groups of 8-12 tortoises each have been equipped with radio transmitters and are used to estimate the proportion of tortoises in the local area that are active/visible. Individuals are observed repeatedly throughout the day using a VHF radio receiver and a directional antenna. Each time a tortoise is located, data are recorded indicating its visibility on the surface, in a burrow, or in vegetation. These data allow us to calibrate distance sampling results to account for the proportion of the population that eludes sampling due to fossorial or cryptic behavior. The radio-equipped tortoises are called G_0 tortoises ("gee-sub-zero") for the mathematical term in the density equation that represents tortoise availability.

The primary goal of G_0 training is successful implementation of the G_0 protocol by telemetry crews. This includes correct use of telemetry equipment, understanding G_0 data collection fields, observation of as many radio-equipped tortoises as possible during the day, observing the appropriate focal population for the transects being sampled, and a window of observation that overlaps the day's transect time window for each sampling area. An additional goal is to make related work on transects more understandable to line distance crews.

Objective 1: Locate tortoises and collect activity data.

Standard: G_0 monitors will be proficient in using telemetry equipment to locate tortoises.
Standard: G_0 monitors will be proficient at collecting appropriate data.

Metric: G_0 monitors will use telemetry equipment to locate radio-equipped tortoises and will complete site-day and observation forms correctly. They will demonstrate correct operation of VHF radio receivers. When a tortoise is not immediately detected with a receiver, they will apply appropriate troubleshooting procedures to locate the tortoise.

Refer to **Appendix I** for paper data sheets.

Objective 2: Implement the daily G_0 protocol.

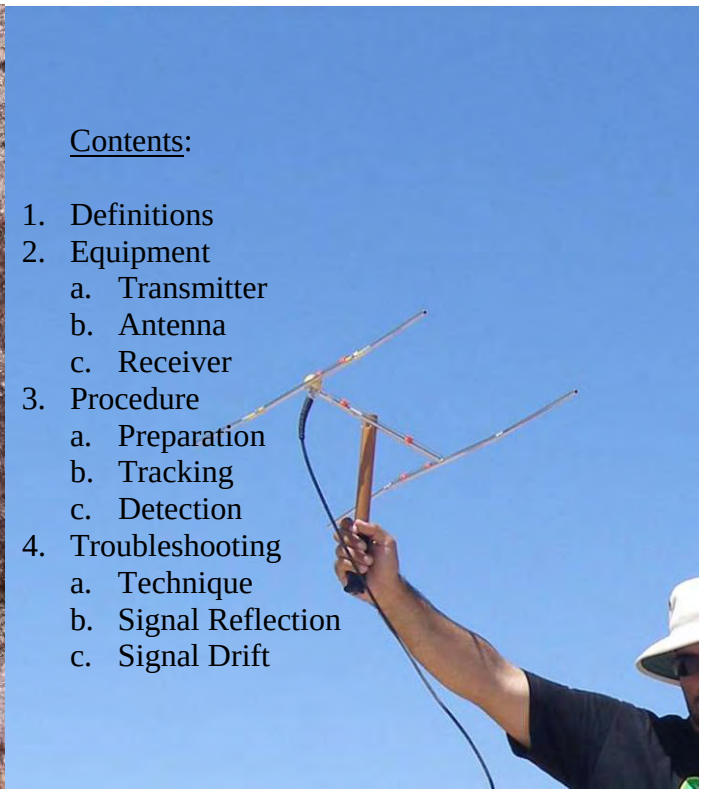
Standard: G_0 monitors will successfully complete daily monitoring activities related to schedule coordination with line distance crews and with one another to collect sufficient daily observations on each tortoise.

Metrics: At an actual G_0 site, monitors will locate and record G_0 data at a rate equivalent to that required to sample 10 tortoises at least 3 times a day (the length of which is defined by the time between typical start and end times for transect monitoring), and bounding the transect sample period as assigned by trainers. G_0 monitors will coordinate with their team leaders to schedule their activities while data are collected on transects.

Objective 1: Locate Tortoises and Collect Activity Data

Contents:

1. Definitions
2. Equipment
   a. Transmitter
   b. Antenna
   c. Receiver
3. Procedure
   a. Preparation
   b. Tracking
   c. Detection
4. Troubleshooting
   a. Technique
   b. Signal Reflection
   c. Signal Drift



1. Definitions

● **Radio Telemetry** involves data transmission over a distance. In this case, the observer uses a receiver to detect a signal emitted from a transmitter attached to a desert tortoise.

● A **Radio Transmitter** radiates a regularly timed signal at a very specific frequency.

● The **Frequency** is built into the battery-powered transmitter and is a specific band within the electro-magnetic spectrum (in this situation 164-168 MHz).

● Approximately once per second, the transmitter emits a "beep" at one precise frequency; this **Signal** travels in a wave over a specific distance (its **Range**). To detect this signal, the observer needs to be within this range (generally 500 - 900 meters).

● The transmitter signal's frequency is not auditory to humans. Thus a radio **Receiver** is used, allowing the observer to hear the signal when s/he sets the correct frequency into the receiving unit.

● The **Directional Antenna** boosts the receiving power and, because it is tuned to be loudest in only one direction, allows the observer to follow the sound to the signal's source.

● Increasing **Gain** increases the receiving unit's amplification of the signal, but sometimes also increases noise. The gain differs from **Volume**, which only changes the noise intensity coming from the receiver's speaker.

2. Equipment

The equipment used to conduct radio telemetry on desert tortoises is typical of that used on many types of animals. The distance from which a signal can be detected is a result of many interacting factors involving the power of the transmitter, the quality of the receiver and the specificity and gain of the receiving antenna. In addition, your ability to track will be influenced by outside factors such as climate, terrain, and obstructions or interfering structures (e.g. power lines).

Transmitter:

Many tortoises in the Mojave Desert are subjects of research or monitoring programs and carry a radio transmitter. Comprising the transmitter are a battery, a frequency emitter, and a

whip antenna. While size, design and location on the tortoise may vary, the transmitter's basic operation remains the same.

Epoxy binds the transmitter to a scute (segment) on the tortoise's carapace (shell). The whip antenna is affixed to one or more scutes as an additional measure to prevent the transmitter's accidental removal. In the event that this antenna is damaged or severed, the tracking range becomes severely limited, typically less than 50 meters. While this antenna may be completely attached, more commonly it extends loosely behind the tortoise, reducing the epoxy mass and making the unit less cumbersome (see Figure 1).

Figure 1. Examples of typical VHF transmitter attachment to desert tortoises.

Transmitters currently in use for LDS broadcast in the VHF frequency range, between 164-168 megahertz (MHz). Each tortoise has a unique frequency, for example, one tortoise may have the frequency 164.236. Yet the signal will "bleed" into neighboring frequencies, so this tortoise may be heard at 164.234 or even 164.238 depending on the transmitter's accuracy and the receiver type. Nearby tortoises must possess sufficiently dispersed frequencies (i.e. 20 or more Hz) to ensure the ability to track the correct tortoise.

Antenna:

A directional antenna amplifies the signal from a transmitter and allows the observer to aim toward the source. Commonly, the "H" shaped 2- element Yagi antenna (Telonics RA-2AK) is used, but other multi-element Yagi antennas exist. Each antenna is specifically tuned to a 2K MHz range of frequencies (e.g. 164-166 for the antenna in Figure 2), but may also receive frequencies outside of this range, albeit with lower efficiency.

These fragile antennas are costly and should be treated with care.
The two-element Yagi depicted in Figure 2 requires assembly, while other models may unfold into the correct operating configuration. For the H-style Yagi, each "kit" should include the main body (A), the arms (B and C) and the handle (D). The arms vary in length and must connect into the correct port on the antenna body. The yellow colored tape on the arms matches that on the body. When assembled, the shorter arms screw into the antenna's forward portion.

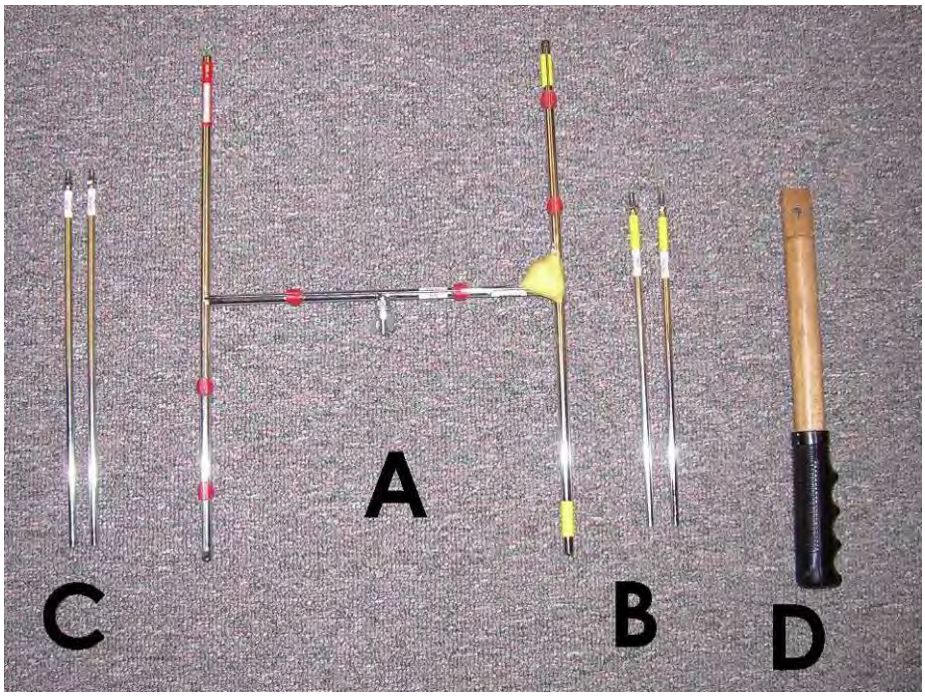


Figure 2. Telonics RA-2AK VHF 2 element Yagi antenna.

A small sticker on the antenna body indicates the “front” (the part to point toward the transmitter) and generally is where the antenna cable attaches. A coaxial cable with BNC connectors on both ends connects the telemetry receiver to the antenna.

Each crew should carry an operational spare cable in the event that the first fails.

Receiver:

Telemetry receivers are radios capable of receiving in the VHF bandwidth. Several companies manufacture receivers specifically used for telemetry (e.g. Telonics, ATS, Lotek, etc.), but other multi-band receivers can be used as well (e.g. Icom). Technical specifications and control layout differ among various receivers, but similar concepts govern their operation.

The tracker enters the tortoise's individual frequency into either a number pad or a series of dials. Some models require entry of all six digits (XXX.XXX) to enter the frequency, while others require the entry of only a portion of the whole frequency (e.g. X.XX) where another knob or button adjusts the frequency by small increments (usually 0.001 MHz, or 1 KHz).
In multi-band receivers, the "Band" or "Mode" button alternates the various modes to AM, FM, WFM, LSB, USB and CW. For tracking, CW mode is often used, but you should track using the band that allows for the best auditory clarity of the signal. The "best" mode for use may change while tracking a tortoise as the distance to the source decreases.

All receivers allow manipulation of both "Volume" and/or "Gain" to tune the directionality and auditory expression of the transmitter signal during tracking. As the tracker approaches the animal or requires additional directionality, reduction in the Gain (and/or the combined Gain and Volume control) aids in the signal's attenuation.

> **Volume** on a receiver matches that on any radio; it simply adjusts the signal's amplification to the speaker. The tracker need only set the Volume at a comfortable level.
>
> **Gain** refers to the receiver's amplification of the signal. Increasing Gain increases the distance from which the observer can detect the signal, but it also increases background noise and reduces directionality. Lowering the Gain reduces noise and increases directionality, but also diminishes the signal's detection range.

Figure 3. Examples of three typical VHF receivers used in radio telemetry. The Icom IC-R10 (top left) is a multi-band VHF receiver, while the Telonics TR-5 (top right) and Telonics TR-2 (bottom) operate in more limited frequency ranges.

Table 1. Comparison of three typical VHF receivers used in radio telemetry: the Icom IC-R10/R20, the Telonics TR-2, and the Telonics TR-5. Information collected from: www.icomamerica.com, www.telonics.com.

Comparison	**Icom IC-R10/R20**	**Telonics TR-2**	**Telonics TR-5**
Control Panel	Digital, clear	Dials, intuitive	LCD, Digital, often difficult to read
Frequency entry	Enter every digit, including the decimal	The 16 is assumed, the four other numbers are then entered in sequence. For the frequency 164.236, you would see 423 on the dials, and the frequency knob would be turned to 6.	Enter every digit including the decimal, and then press enter.
Knobs / Controls	R-10: The Volume and Gain knob are the same: R-20: The Volume and frequency knob are the same. For both, hold down the button labeled "RF GAIN" until the screen reads "Set RF Gain," use the knob to adjust Gain. When it says "max" or "10", press the "RF GAIN" button a second time to return it to Volume or frequency control.	A single knob combines both Gain and Volume, and they cannot be changed separately.	Up/Down arrows allow Gain and frequency changes with a separate knob for Volume.
Receiving Power	Low (Often need to be within 300-500 meters)	Medium- High (Often need to be within 500-900 meters)	High (Often need to be within 700-900 meters)
Charge	11 hours	10 hours	>16 hours
Size	Small 0.32 kg 6 x 14.2 x 3.48 cm	Medium 0.86 kg 11.7 x 5.1 x 18.0 cm	Large 1.2 kg 17.8 x 11.1 x 6.2 cm
Portability	Handheld or shirt clip	Leather case with shoulder strap	Blue cloth case with shoulder strap
Recharge	Wall charger	Wall charger	8 AA batteries
Frequency Band	Extensive, multi-band VHF receiver: SSB, CW, AM, FM, and WFM. 0.150-3304.999 MHz CW: 118.000-146.999 MHz	Limited: SSB and CW 2K or 4K MHz range: set at factory e.g. 164.000-166.999	Limited 4K MHz range:set at factory 163.000-167.999
Pros	Light, easy to use, relatively inexpensive changeable or rechargeable batteries	Intuitive to use, lightweight, manual frequency scanning accomplished easily	Powerful, water resistant, changeable batteries Freqs can be programmed
Cons	Limited range, less effective under powerlines, wrong buttons easily pressed , modes confusing	Batteries cannot be changed, recharge only, non-programmable. Gain and volume coupled.	Heavy, difficult to use, display difficult to read, Gain change takes time, frequency adjustment awkward.

3. Procedure

Preparation:
Assemble the necessary equipment:

GPS
PDA
Two-way radio
Charged receiver
Yagi antenna
2 Coaxial cables
Sufficient replacement batteries
A list of past known locations and frequencies for each tortoise
Mirror
Compass
Paper datasheets
Writing implements
Safety equipment
Water

Check the tortoise frequencies and bring the appropriate receiver and antenna prior to heading into the field.

Never track when lightning may strike.

Tracking:

1. Follow the provided GPS coordinates to the tortoise's last known location; start as close as possible to that site.
   (Note: Listening to the tortoise's frequency while traveling to its last location, and even scanning for other nearby tortoises as well, is recommended. Tortoises move frequently during the activity season, increasing the likelihood that the tortoise you seek will be found in a different location than the last. You will save time if you listen for each animal while traveling between locations.)
2. Assemble the antenna and connect it to the receiver using the coaxial cable.
3. Turn on the receiver and enter the correct frequency.
4. Set Gain to maximum.
5. Listen for the signal:
   a. Stand a few meters away from vehicles or structures.
   b. Hold the antenna high at arm's length.
   c. Orient the antenna in a Vertical position (see Figure 4). The Vertical orientation provides more range but less directionality.
   d. Listening for the transmitter beep, rotate your body in a slow, ***complete*** circle.
   e. Determine the direction from which the signal sounds strongest.

Figure 4. The Vertical antenna position on the left (handle parallel to the ground) provides more range but less directionality in detecting the signal, while the Horizontal antenna position on the right (handle perpendicular to the ground) allows better directionality while lessening range.

6. Follow the signal and adjust:
    a. Walk toward the loudest sound.
    b. Continue listening while walking, sweeping the antenna from side to side to confirm a strong signal directly forward.
    c. If the signal weakens, repeat Step 5 and continue in the new direction.
    d. Reducing Gain while approaching the source also aids in narrowing the loudest signals direction.
7. Improving Directionality:
    a. When you hear the signal clearly (about 300 meters or so), orient the antenna in a Horizontal position (see Figure 4). This provides less range, but better directional certainty. Once you hear the signal using this orientation, the Vertical position becomes unnecessary.
    b. Repeat Step 5 using the Horizontal orientation.
    c. Continue lowering the Gain as you approach the tortoise until the signal sounds loud at minimum Gain.

Detection:

1. Final Location:
    a. When very close, the beeping becomes loud enough to sound distorted and omnidirectional. Continue walking and sweeping until the signal weakens or sounds louder behind you to triangulate to the correct location.
    b. Upon determining a small area with the strongest signal, point the antenna perpendicular to that area and walk in a circle around it to confirm the correct location; this may take time. At close range the signal may sound equally strong directly behind as well as in front of you.
    c. At this point, begin scanning visually for the tortoise or burrows.
2. Burrow:
    a. If the signal seems to emanate from a burrow, make a visual inspection to locate the tortoise. The tortoise may be deep inside; you should use a mirror to reflect sunlight into the burrow.
    b. If the tortoise cannot be seen, two things can be done to detect its exact location:

Be careful: do not step on or collapse the burrow while performing these.

1) Remove the cable and antenna from the receiver. If beeps can still be heard, the tortoise is very close.
2) You may "fish" for the tortoise. Remove the cable from the antenna, but leave it connected to the receiver. Dangle the antenna over the burrow and move it around to the loudest point – the tortoise's most likely location. To avoid getting dirt in the BNC connector, do not let the cable bottom touch the ground.

To avoid stepping on the antenna, place it up high or in a creosote bush.

    c. If you see a tortoise, make sure it is the one you seek by checking for a transmitter and even a tortoise number on a floy tag glued to the shell.
3. Record Location:
    a. Record the Tortoise Number, Frequency, Northing, Easting, Date, Time, Observer, and any other necessary information.
    b. Use your PDA to record required information as well.

4. Troubleshooting

Technique:

Antenna. Understanding the antenna's performance may assist in tracking the signal. In figure 5, the antenna's listening pattern, the sound intensity appears loudest when the antenna points directly at the signal's origination (0 degrees). **Backfeed** may be heard when the antenna receives a signal from the opposite direction as well. An alternative exists to determine the correct direction when following the loudest signal is not possible. Holding the antenna horizontally, identify the "signal nulls," or the sections in which the beeping disappears completely, as opposed to the "signal peaks," while turning in a complete circle. Walking in the direction exactly opposite to the middle of the null areas forms a viable option.

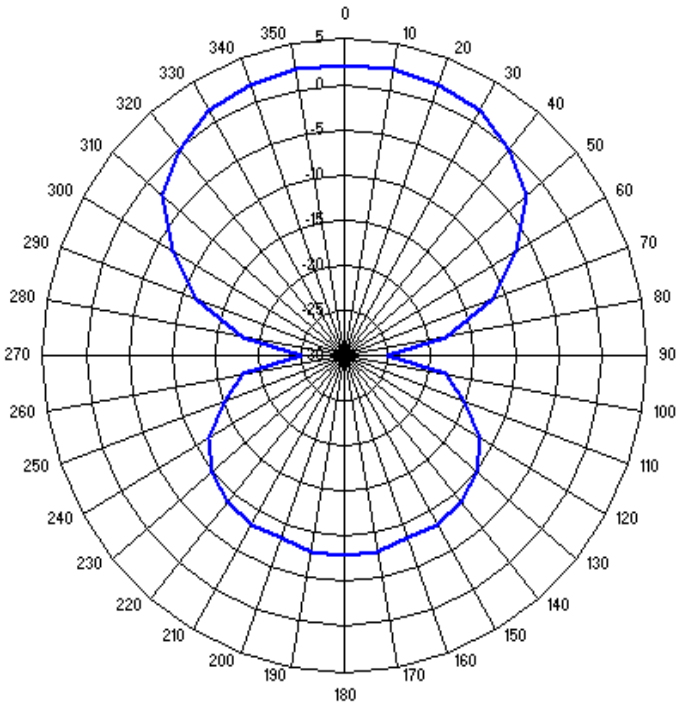


Figure 5. The listening footprint for an antenna held horizontally; northward (0°) sets the signal's source.

Receiver. At minimum Gain, the sound may occur at the same intensity from all sides. This phenomenon makes locating the tortoise at close range particularly difficult. Shifting the frequency slightly up or down may provide a difference in beep intensity. Lowering the frequency until a "thump" sounds or raising it until a very high-pitched beep occurs may provide some direction.

Signal Reflection:

Many factors may distort the signal's direction including frequencies from other transmitters, complex terrain, powerlines, and even nearby fences or vehicles. In all cases, the tracker should move away from obstructions to allow for open space through which the signal may travel.

Terrain. Rocky terrain, hills, washes, cliffs, and even mountains may obscure or redirect signals. Height above the ground, obtained by climbing hills or up to ridge-tops, increases a signal's range. The signal may be lost when dropping into a canyon or wash. If a signal does become lost, a tracker should climb to a high place near the tortoise's last known location to listen for the signal or walk the ridge above a canyon while following the sound.

Echoes. As some tortoises do tend to live in washes and cliffside caliche caves, echoes may sound louder than the true direction. For example, a tortoise may be on a mountaintop while the tracker walks below, between mountains; rocks may obscure the signal's source but the echo resonates clearly bouncing off the opposite mountain. In this case, it is best to climb to a higher point and listen.

Powerlines. Powerlines distort or mask signals, making them difficult to acquire or follow. The tracker should walk at least 100 m away from the lines to listen. As an aside, some people have received mild electrical shocks while tracking under the lines.

Burrow Substrate. The signal may become dampened or distorted if the tortoise rests deep within its burrow. Caliche seems to mask the signal the most, requiring that the tracker be very near to detect the signal or in a direction corresponding with the orientation of the burrow opening. Other substrates may impede the sound as well.

Signal Drift:
A transmitter's frequency may "drift" up or down with changes in climate and over time. Other factors affecting the emitted frequency and transmitted signal include terrain, temperature, humidity, wind speed, the receiver, and the antenna.

If, while in the field, a tracker cannot detect the provided frequency, s/he should first check that the inputted frequency matches that of the desired focal tortoise. When the correct frequency does not yield a signal, the tracker should listen in a complete circle while adjusting the frequency up and down at least 6 KHz away from the original incrementally in both directions. If this does not yield results near the last known location, the tracker may choose to listen from an elevated location, such as a mountain or hilltop. While this may seem like extraneous effort, finding the tortoise immediately represents the best option as it may move even further away if already distant.

Finally, if searching for at least 30 minutes yields no signal, the tracker should proceed to the next tortoise and continue searching for the missing animal while moving among the other animals in the observer's tracking schedule. This animal may be encountered associated with another as tortoises seem to aggregate at times.

Objective 2: Implement the Daily G_0 Protocol

Optimizing monitoring to coincide with tortoise activity
One of the adaptations tortoises have for living in the desert is to restrict surface activity to fairly narrow windows of time during the year. In general, tortoises predictably emerge from deep within shelters (burrows) from mid-March through mid-May and then again (less predictably) in the fall. These periods coincide with flowering of their preferred food plants (in spring) and with annual mating cycles (in fall). Both periods also represent the most likely times to find water in plants or on the surface. The annual range-wide monitoring effort is scheduled to match the spring activity period for tortoises.

During this season, all tortoises are not above ground or visible in burrows. In order to encounter as many tortoises as possible, monitoring is scheduled for early in the day and to be completed before the hottest time of day. Because we are using vision to find tortoises, monitoring is restricted to daylight hours. Based on past experience, we expect tortoises to become most active after 7am at the beginning of April (it is usually too cool before this time), but to emerge earlier and earlier until their optimal activity period is around sunrise by the beginning of May. In May, we also expect daytime temperatures to limit tortoise above-ground activity as the morning

progresses to afternoon (see “Line Distance Protocols, Objective 2: Start and Complete Transects to Optimize Tortoise Detections”).

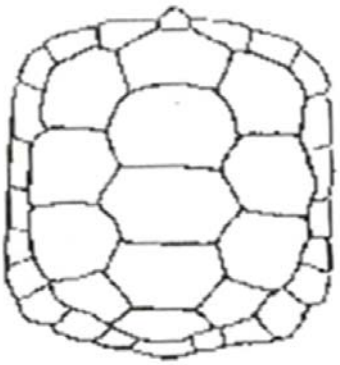
Coordinating start and end times with transect crews

Field crews on transects are working to complete transects during this optimal period each day, so crews are likely to arrive at transects at similar times on a given morning. The role of telemetry crews is to provide data to estimate daily tortoise activity during the period when transects are walked. Telemetry and transect crews are responsible for beginning the field day at the scheduled time. However, completion times will be more variable, and will be affected by terrain, air temperature, number of tortoises encountered, etc. Each field group will use their own method to communicate between transect and telemetry crews so that telemetry crews monitor until all transects have been completed for the day. In general, at the end of the day, when each crew has finished their transect and is back at the truck, they make contact with the logistics coordinator for the group. After this coordinator has heard from everyone that day, the telemetry crew is notified. Telemetry crews cease monitoring once they hear from the logistics coordinator or it is 4pm, whichever comes first.

Appendix C:
Health Assessment Datasheet

Desert Tortoise Translocation Eldorado Valley: Health Assessment Form

Date (ddmmmyy):	**Start time (24h):**	**Plot Number:**	**Tortoise EV #:**	**Checked EV # after locating ?** Yes No	**DTCC # read from tortoise:**	**Transmitter freq:**
UTM easting:	**UTM northing:**	**Biologist name (examiner):**		**Biologist name (handling):**	**Biologist name (observer):**	
Temp (ºC)	**HA start time:**	**Attitude/activity:** Normal Lethargic/Weak		**Respiration:** Normal Abnormal sounds Increased effort	**Beak:** Normal Abnormal Evidence of foraging	
Left naris: Normal Asymmetrical Eroded Occluded		**Left naris discharge and severity:** None Serous: 1 2 3 Mucous: 1 2 3		**Right naris:** Normal Asymmetrical Eroded Occluded	**Right naris discharge and severity:** None Serous: 1 2 3 Mucous: 1 2 3	
Left eye: Normal Sunken Corneal opacity Partially closed Fully closed Serous discharge Mucous discharge Periocular swelling Periocular redness Conjunctival swelling Conjunctival redness				**Right eye:** Normal Sunken Corneal opacity Partially closed Fully closed Serous discharge Mucous discharge Periocular swelling Periocular redness Conjunctival swelling Conjunctival redness		
Skin lesion location: None Generalized Head Neck L / R forelimb L / R axillary region L / R hindlimb L / R prefemoral region Vent / tail			**Condition of skin lesion(s):** N/A Active Inactive	**Coelomic cavity palpation:** No mass L / R mass Not done	**Shell characteristics:** Compressible N/A Sunken +/- Scutes Peeling/flaking keratin Bone exposed	
Shell abnormality location: Carapace Plastron N/A		**Shell abnormalities (describe below):** None Localized Multifocal Generalized		**Condition of shell abnormalities:** N/A Active Inactive	**Circumstances of skin/shell trauma:** N/A Unknown Suspect canid bite Vehicle Other_____	
Sex: M F Unk	**Initial weight (g):**	Body condition score: 1 2 3 4 5 6 7 8 9		**Photo 1: Front face and body** *Photo name:*	**Photo 2: Left side face** *Photo name:*	
Photo 3. Right side face *Photo name:*		**Photo 4. Carapace** *Photo name:*		**Photo 5: Plastron (only if abnormal)** *Photo name:*	**Photo 6. Abnormalities** *Photo name:*	



Label and describe trauma, anomalies, lesions, missing body parts, and identifying features Indidate notches with a "V" and location of DTCC ("D") or EV ("E") tags.

Ticks: 0 1-10 >10 **Location:** Soft tissue Seams Scutes Eyes Nares Beaks **Collected?** N/A Yes No **Removed?** N/A Yes No		**Choana:** Not examined Normal Pale Reddened	**Tougue and oral mucosa:** Not examined Normal Pale Reddened Crust Ulcers Plaques Hypersalivation Impaction		**# oral swabs collected:** _____
Time of blood draw (24h):	**Total sample volume (blood and lymph) collected:** _____ml	**Total # hep tubes (number each):** 0 1 2 3 4	**Is the sample contaminated with lymph?** Yes No **Estimate the degree of lymph contamination:** Small (1-10%) Moderate (11-29%) Severe (>30%)		**Void during processing:** None Urine/Urates Feces
Post void weight: N/A _____g	**MCL:** _____mm	**Width V3:** _____mm	**Height V3:** _____mm	**Plastron:** _____mm	**Hydration method:** N/A Soak Nasal-oral Epicoelomic Fluid type:_____ Vol: _____ml	**Post fluid wgt:** N/A _____g
End handling time (24h):	**Blood processing time (24h):**		**Plasma color:** Colorless Red Yellow Green	**UFL plasma aliquots:** 0 1	**USFWS plasma aliquots:** 0 1 2 3	**Total tubes with RBCs saved:** 0 1 2 3 4

Total number of tubes/vials collected:_____

Revised September 9, 2013

NOTE: The Health Assessment datasheet has been modified for the Eldorado Valley Translocation Project. Not all data fields described on the following pages are included on the modified datasheet.

Description of data fields on the data collection form

Date (ddmmmyy) – 2 digit day, 3 letter month, and 2 digit year (e.g. 04Mar11).

Start time (24h) – The time when the tortoise is located. This is not the time at which you begin assessing or touching the tortoise, but instead, it's the time at which you visually locate the tortoise. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Project name – Name and phase of the project.

~~**Site description/current pen #** – If the tortoise is newly caught from the wild, provide a description of the capture location, or if the tortoise was already in captivity, provide the quarantine pen number.~~

Tortoise ID # - Each tortoise will be assigned a unique identification number from a series of numbers assigned by USFWS for that project. The tortoise may have been numbered prior to the health assessment or you may need to number it (refer to numbering protocols provided by the USFWS).

~~**Transmitter frequency** – Numbers of the radio transmitter frequency, if applicable. Usually includes 3 digits, a decimal, and 3 more digits (e.g. 164.020)~~

GPS datum – Make sure your GPS is set to UTM, WGS 1984. Datum is the model used to match the location of a feature on the ground to the coordinates of the feature on a map.

UTM zone – In the UTM system, the earth is divided into 60 zones of 6 degrees of longitude wide. Your GPS displays zone as 2 digit number (e.g. 11, 12 that may be followed by N denoting north of the equator).

UTM easting – 6 digit number displayed on your GPS. Easting is measured from the central meridian of the zone, which is given the value of 500,000 m, and increases as you travel east.

UTM northing - 7 digit number displayed on your GPS. Northing is measured relative to the equator, which is assigned a value of 0 in the northern hemisphere, and increases as you travel north.

Temp °C – Temperature in degrees Celsius measured 5 cm above the ground in the shade and protected from wind where you set up your equipment to process the tortoise, or as specified in the most recent USFWS Desert Tortoise Field Manual.

Full name of biologist(s) - First and last names of the biologists handling the tortoise and recording the data. List the Authorized Biologist primarily responsible for conducting the health assessment and biological sampling as the first name, followed by the name of the person assisting by handling the tortoise, then the name of person recording the data, if applicable.

HA start time – Health assessment start time. This is the time at which you approach a tortoise and get within 2 meters of it to begin assessing its condition, even without touching it or moving it.

Attitude and activity – Circle one.

Normal – Tortoise paddles its forelimbs when held, attempts to escape, and repeatedly retracts into its shell when handled; or is shy and tends to remain retracted into its shell when being handled, but has normal strength. During cold temperatures, activity may be minimal, but is considered normal for the temperature or time of year.
Lethargic/weak – Forelimbs may hang limp when tortoise is lifted, tortoise appears weak, is slow to respond to stimuli, and/or does not resist gentle tugging on the limbs.

Respiration – Circle all that apply.

Normal - No sound or a very faint, high-pitched whistle when expelling air out of their nares.
Abnormal sounds - Includes wet, crackling, or gurgling sounds associated with congestion.
Increased effort – Tortoise pumps forelimbs up and down symmetrically when breathing, may indicate lower respiratory disease or compromised lung volume. Open-mouthed breathing or neck extended while breathing may indicate increased respiratory effort, but this must be distinguished from occasional normal gaping.

Beak – Circle all that apply.

Normal – Usual shape, size, color, and texture. May have pieces of food-related debris or dirt stuck on it.
Abnormal – Unusual shape, size, color, or texture. Describe in area provided.
Evidence of foraging – Presence of food or coloration associated with food, usually green or black, but may be more colorful (e.g. pink) in spring depending on food sources.

Left/right naris – Circle all that apply.

Normal – Usual shape and/or size.
Asymmetrical - One naris is larger and/or wider than the other.
Eroded - Loss of scales and skin around naris opening.
Occluded – Plugged or reduced size of naris opening.

Left/right naris discharge – Circle one.

None – No discharge present.
Serous - Clear, watery discharge present.
Mucous – Cloudy, thick discharge present.

Severity of naris discharge – Circle one.

1. Mild - Moisture present around nares.
2. Moderate - Discharge coming out of the nares, but not running far from the nares themselves.
3. Severe - Discharge coming from nares that is running down the beak.

Left/right eye – Circle all that apply.

Normal – Usual shape, size, and color.
Sunken – Eye recessed within the orbit.
Corneal opacity- Eye is cloudy, hazy, or there is a loss of transparency of the cornea.
Partially closed – Eye is not fully open.
Fully closed – Eye is not open at all.
Serous discharge - Clear, watery discharge present.

Mucous discharge - Cloudy, thick discharge present.
Periocular swelling – Area around the eye is swollen.
Periocular redness – Area around the eye is abnormally pink or red.
Conjunctival swelling - Membranes around the eye are swollen.
Conjunctival redness – Membranes around the eye are abnormally pink or red.

Skin lesion location – Circle all that apply, describe in area provided, and draw on diagram.
None – No skin lesions.
Generalized – Widespread lesions in many locations all over the body.
Head
Neck
L/R forelimb – Circle left, right, or both.
L/R axillary region – Circle left, right, or both.
L/R hindlimb – Circle left, right, or both.
L/R prefemoral region – Circle left, right, or both.
Vent/tail

Condition of Skin lesions – Circle one, but for multiple lesions circle all that apply and describe in area provided.
N/A – Not applicable.
Active – Very recent or unhealed lesion.
Inactive - Old or healed lesion.

Coelomic cavity palpation – Circle one.
No mass – The tortoise was palpated but no masses were detected.
L/R mass – Circle left, right, or both.
Not done – The tortoise was not palpated.

Shell characteristics - Circle all that apply and describe in area provided. Note that these characteristics may not always be considered abnormalities, as in the case of sunken scutes on an older tortoise. Therefore, it is possible to circle a characteristic here, but record N/A in the section labeled "Shell abnormality location."

Compressible - Capable of being flattened by pressure or pressed into a smaller space.
N/A – There are no unusual shell characteristics.
Sunken – Scutes are sunken lower than the seams of the shell.
+/- Scutes – Circle (+) if there are more scutes than usual and circle (–) if there are fewer scutes than usual on the carapace and/or plastron.
Peeling keratin – Scutes are peeling on the carapace or plastron.

Shell abnormality location - Circle all that apply, describe in area provided, and draw on diagram. Note that it is possible to circle a "Shell characteristic" above, but record N/A in this section since not all characteristics that could be noted there are considered abnormal, as in the case of sunken scutes on an older tortoise. However, if you record something in the section "Shell abnormalities" below, then you must circle a location in this section.
Carapace
Plastron

N/A – There are no shell abnormalities.

Shell abnormalities (describe below) - Circle one, but for multiple abnormalities circle all that apply and describe in area provided.

None – No shell abnormalities.
Localized – Abnormalities are restricted to a particular place or area on the body.
Multifocal – Abnormalities found on two or more distinct places or areas on the body.
Generalized - Widespread lesions in many locations all over the body.

Condition of shell abnormalities – Circle one, but for multiple abnormalities circle all that apply and describe in area provided.

N/A – Not applicable.
Active – Very recent or unhealed abnormality.
Inactive - Old or healed abnormality, or genetic alteration, such as extra or too few scutes.

If present, circumstances of skin/shell trauma – Circle one.

N/A – Not applicable
Unknown – There is no way to determine the cause of the trauma.
Suspected canid bite - Suspected or known predation by coyote, dog, or other canid. Provide details, if known, in the area provided.
Vehicle – Trauma is suspected or known to be caused by a vehicle, including but not limited to a car, truck, military tank, ATV, dirt bike, dune buggy, etc. Provide details in the area provided.
Other _____ - Provide details if the other options do not provide an adequate description of the circumstances of the trauma.

Sex – Circle one.

M – Male
F – Female
Unk – Unknown sex

Initial weight (g) - Weigh the tortoise prior to extensive handling to avoid the risk of the tortoise voiding. Spring scales or electronic balances may be used. When using a spring scale, weigh the tortoise with the smallest scale appropriate for the individual to get an accurate weight.

Body condition score – Circle one. Scores range from 1 (emaciated) to 9 (morbidly obese). Descriptions of each body score are included in Appendix D.

Photos (take all*) – Photos should be taken with a good quality digital camera. Prior to taking photos of the tortoise, take a photograph of the data collection form clearly showing the Tortoise ID number, date, site, UTM location, biologists, etc. Whenever possible, take photos using a macro lens to show detail, such as those taken to show trauma and signs of disease.

Front face and body - Show nares, forelegs, gular, and anterior shell.
Left side face – Full frame close up.
Right side face – Full frame close up.
Carapace – Full frame close up.

Plastron (*take when measuring, but only if abnormal) - Take this photo when you are doing the plastron measurement (after sample collection) and only if the plastron is abnormal. Abnormalities – Full frame close up.

Label and describe trauma, anomalies, lesions, missing body parts, and identifying features – On the line drawings of a tortoise carapace and plastron, draw in any trauma, anomalies, lesions, and identifying features of the tortoise, and circle or point an arrow to the location of missing body parts. Draw in the shape of the gular on the picture of the plastron. Provide a written description in the space provided for details about the tortoise that may require clarification.

Ticks (*Ornithodoros spp*) – Circle one. See Appendix F.2 for details regarding tick collection.

0 – No ticks were observed.
1-10 – 1 to 10 ticks were observed.
>10 – More than 10 ticks were observed.

Collected? - Circle one.

N/A – No ticks were observed so no ticks were collected.
Yes – Ticks were collected as per the tick collection protocols.
No – Ticks were present but not collected.

Removed? – Circle one.

N/A – No ticks were observed so there were no ticks to remove.
Yes – Ticks were removed as per the tick collection protocols.
No – Ticks were present but not removed.

Location – Circle one. Location where ticks were observed.

Soft tissue – Skin, including limbs, vent, and tail.
Seams – Areas between the scutes on the carapace and plastron.
Scutes – Keratinized plates of the carapace and plastron.
Eyes
Nares
Beak

Choana – Circle one. See Appendix F.3 for details regarding the oral cavity examination.

Not examined – An oral cavity examination was not conducted or it was conducted but the choana was not observed.
Normal – Pale pink or pink.
Pale – Lacking pink coloration, white, or slightly yellow
Reddened – Very dark pink to red.

Tongue and oral mucosa – Circle all that apply. See Appendix F.3 for details regarding the oral cavity examination.

Not examined – The oral cavity examination was not conducted or it was conducted but the tongue and oral mucosa were not observed.
Normal – Pale pink or pink.
Pale – Lacking pink coloration or white
Reddened – Very dark pink to red.
Crust - An outer layer of solid material formed by the drying of a bodily exudate or secretion.
Ulcers - Localized defects or excavations of the surface of a tissue, usually produced by sloughing of necrotic inflammatory tissue.

Plaques - Localized patches or flat areas in the oral cavity. Plaques tend to have a white or yellow appearance.
Hypersalivation - Increased saliva in or around the oral cavity.
Impaction – Lodgment of something, such as food or debris, in the oral cavity

 # oral swabs collected – Write the number of oral swabs collected. You can collect 0, 1, or 2 oral swabs. See Appendix F.3 for details regarding oral swab sample collection.

Time of blood draw (24h) – This is the time that you finished collecting the blood and removed the needle from the vein. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Total sample volume (blood and lymph) collected – Total volume in milliliters of each blood collection attempt. Up to three attempts to collect total maximum volumes are allowed. See Appendix F.5 for details regarding blood sample collection**.**

Total # hep tubes (number each) – Circle one. You can have 0, 1, 2, 3, or 4 tubes containing whole blood and/or lymph samples.

Is the sample contaminated with lymph – Circle yes or no.

Estimate the degree of lymph contamination - Small (1-10%) Moderate (11-29%) Severe (>30%)

Void during processing – Circle all that apply.
None – The tortoise did not discharge bladder or digestive tract contents.
Urine/urates – Waste material that is secreted by the kidney as a clear fluid or as a semi-solid salt of uric acid that may be white, yellow, or brown
Feces – Bodily waste discharged through the digestive tract; firm excrement

Post void weight – If the tortoise voided during processing, weight the tortoise again at this point before rehydrating. Record the weight in grams. If the tortoise did not void, circle N/A.

Hydration Method – If the tortoise voided urine/urates, circle one, or if multiple rehydration methods were employed, circle all that apply and describe in the area provided. See Appendix F.6 for details regarding rehydration techniques.
N/A – The tortoise did not void urine/urates, so it was not provided with fluids.
Soak – The tortoise was placed in a plastic tote with shallow fresh water for 30 minutes.
Nasal-oral – The tortoise was offered fresh water ad lib with a syringe through the nares and mouth.
Epicoelomic – The tortoise was given an epicoelomic injection of sterile saline.

Fluid type – Fill in blank with fluid(s) used. If multiple rehydration methods were employed, describe in the area provided. See Appendix F.6 for details regarding rehydration techniques**.** If the tortoise did not void, skip this field.
Water – Fresh drinking water.
Saline – 0.9% sodium chloride.

Vol - Amount of fluids in milliliters injected into the tortoise or offered ad lib. Do not provide a volume if you used the soaking method. If the tortoise did not void, skip this field.

Post-fluid weight – If the tortoise voided, weight in grams after administration of fluids. If the tortoise did not void, circle N/A.

MCL – Straight midline carapace length measured in millimeters by holding the calipers directly over the center line of the tortoise and measuring from the center of the outer edge of the nuchal scute to the most caudal aspect of the carapace. Note that the most caudal end of the carapace may not be the edge of the supracaudal scute.

Width V3 - Width of the carapace measured in millimeters by holding calipers directly over the center line of the tortoise crosswise in the middle of the 3rd vertebral scute. Keep the calipers level with the ground.

Height V3 - Height of the carapace measured in millimeters by holding the tortoise so that one arm of the calipers is held across the center of the 3rd vertebral scute on the carapace and the other arm of the calipers is held in the same position across the plastron so the tortoise is between the arms of the calipers. The arms of the calipers should extend all the way from one side of the tortoise to the other while being held level against the tortoise's carapace and plastron.

Plastron – Length of the plastron measured in millimeters measured by having a handler tip the tortoise to one side (the tortoise's left or right side) so the examiner can see the plastron. The examiner then uses calipers to measure from the notch between the gular scutes to the notch between the anal scutes. If the calipers do not fit into the notch, then hold them outside the notch on the plastron where the notch comes to a V to get the most accurate measurement. Do not tip the tortoise too far to the left or the right and do not put it on its carapace to take this measurement.

End handling time (24h) – The time at which you complete the processing of the tortoise and return it to its original location (point of capture, pen, etc). Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Disposition - Circle one.

Wild capture location - For tortoises that were caught in the wild and are being placed back at their site of capture immediately following processing (i.e, the tortoise is not being brought into captivity after processing).

Same pen – For tortoises that were already in captivity at the time of processing and are being placed back in the same pen.

New pen – Provide the number of the new pen where the tortoise is being placed after processing, regardless if it was newly captured from the wild, or if it was already in captivity but moving to a new pen.

Other- Describe the new location.

Blood processing time (24h) – The time at which samples were removed from the cooler and placed in the centrifuge. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Plasma Color – Circle one. This is the plasma color of the sample you will use for the "Hep plasma UFL" cryovial. This should be the best sample you collected for each tortoise, meaning the one with the least lymph contamination. Choose from colorless, red, yellow, and green.

UFL plasma aliquots – Circle one. You can have 0 or 1 aliquots of plasma for UFL. See Appendix F.7 for details regarding sample processing**.**

USFWS plasma aliquots - Circle one. You can have 0, 1, 2, or 3 aliquots of plasma for USFWS. See Appendix F.7 for details regarding sample processing**.**

Total tubes with RBCs saved – Circle one. You can have 0, 1, 2, 3, or 4 heparinized microtainers with RBCs in them to save. All of the plasma should have been pipetted out of these microtainers into the UFL or USFWS plasma and plasma/lymph tubes prior to storage, leaving only RBCs in the microtainers. See Appendix F.7 for details regarding sample processing**.**

Total number of tubes/vials collected – Count the total number of tubes/vials containing samples from the individual.

Appendix D:
Excerpts – 2013 Health Assessment Procedures Handbook

The following pages are excerpts from the May 2013 version of "Health Assessment Procedures for the Mojave Desert Tortoise (*Gopherus agassizii*): A Handbook Pertinent to Translocation" prepared by the U.S. Fish and Wildlife Service, Desert Tortoise Recovery Office (http://www.fws.gov/nevada/desert_tortoise/documents/reports/2013/assess/May2013-Desert-tortoise-health-eval-handbook.pdf .)

Not all of the sections and pages of the Handbook are applicable to this plan for pre- and post-translocation monitoring in Eldorado Valley. Therefore, the page numbers and appendix numbers included herein are not all inclusive or are out of sequence or are crossed-out. If conflicts and/or contradictions arise between this monitoring plan and the Handbook, this monitoring plan takes precedence over the Handbook. The DCP should be contacted first for any necessary clarifications.

Appendix A: Disinfection and Sanitation

Caution must be used whenever handling or sampling desert tortoises to ensure that pathogens (i.e., disease causing microorganisms) are not introduced to a field site through contaminated equipment. Equally important, field personnel must take necessary precautions to ensure that they do not aid in the unintended transmission of pathogens among the individual tortoises sampled or contaminate the samples that have been collected. Therefore, development and implementation of a step-by-step disinfection protocol is essential for field studies. An effective disinfection protocol must address the microorganisms being targeted, the characteristics of the disinfectant, and the impact a disinfectant may have on the environment. The health and safety of field personnel and desert tortoises are of vital importance.

A number of products are available for disinfection/sanitation purposes. The most common antimicrobial products fall within one of the following classes: alcohols (i.e., hand gels), chlorine (i.e., bleach), iodine/iodophors (i.e., povidone, iodine), chlorhexidine (i.e., Nolvasan), oxidizers (i.e., VirkonS), phenols (i.e., Lysol), quaternary ammonia (i.e., Roccal), and aldehydes (i.e., Wavicide). Each has varying effectiveness for different classes of microorganisms, and the reader is referred to the University of Nebraska - Lincoln Extension website entitled, 'Selection and Use of Disinfectants' for more information about disinfectants.[1] The effectiveness of any disinfectant or antiseptic is determined by the amount of time that the cleaning agent is allowed to contact the surface, concentration of the product used, the organic load (amount of dirt/debris), the level of microorganism contamination, the condition of the object being cleaned (cracks, crevices, wood vs. plastic surfaces), ambient temperature, and sometimes the environmental pH. In field conditions where high organic loads are almost always present, effective antisepsis and disinfection are not possible without first cleaning to remove excessive debris. Therefore, regardless of the agent used, an initial cleaning is required.

The protocol listed below describes methods to be used when working with desert tortoises. Certainly, other protocols may be developed to meet the needs of specific projects or management goals, upon approval from the U.S. Fish and Wildlife Service. Users should follow local and state regulations for transport and disposal of disinfectant solutions.

Disinfectant solutions

Solutions should be stored in dark bins or in opaque bottles and should be made fresh regularly (i.e., daily or weekly depending on storage conditions). Take care to keep all solutions off of skin and out of eyes. Gloves should be worn when using disinfectants. Appropriate choices as disinfectants include those listed below.

Trifectant®

This product is sold as a powder or tablet and is effective against viruses, bacteria (including mycoplasma), and fungi. It is also fairly resistant to inactivation by hard water and organic matter. Once mixed, the solution is stable for seven days. Contact time for disinfection is 5-10 minutes and metal instruments should not be soaked for more than ten minutes. A 1-2% solution should be mixed according to instructions on the packaging.

[1] Available through URL: http://www.ianrpubs.unl.edu/epublic/live/g1410/build/g1410.pdf

Chlorhexidine diacetate (e.g. Nolvasan®)
This disinfectant is sold as liquid and is effective against viruses, bacteria, and fungi. It should be prepared according to the instructions on the packaging.

Bleach
While no longer the preferred option, a standard bleach solution may be used (1:20 dilution of 5 percent household bleach in water). Bleach should be purchased in small bottles or dispensed into small bottles to minimize deterioration from opening/closing the lid. In the event of a high organic load, thoroughly remove or wash the organic material off before using bleach to disinfect to prevent the bleach from being inactivated. Bleach solutions are also readily inactivated by sunlight.

Protocol for cleaning and disinfecting equipment and sanitizing personnel

All equipment must be thoroughly cleaned and disinfected prior to arriving at a field site. Additionally, all equipment and work surfaces (if not on natural ground) must be cleaned before and after handling each tortoise. Efforts should be made to first remove organic debris (i.e., dirt, feces, etc.) by rinsing the area with water, brushing debris off with paper towels, or cleaning the equipment/work surface with the chosen disinfectant solution and wiping with paper towels. The equipment should then be thoroughly moistened with disinfectant at appropriate dilution and appropriate contact time and dried or allowed to air dry. Heat and direct UV light exposure will improve the disinfection process.

Gloves are to be worn and changed between handling individual tortoises to prevent the transfer of pathogens from one tortoise to another. Additionally, wearing gloves will protect field personnel from the effects of potentially irritating disinfectants and pathogens. Contaminated gloves should be removed after handling and cleanup and prior to the disinfection process. New gloves should be used while disinfecting equipment. All items touched with contaminated gloves should be considered contaminated (e.g. pens, clipboards, clothing). Skin must also be sanitized between tortoises, if contact with a tortoise and/or its excretions occurs. Acceptable sanitizing solutions include soap and water (if available) or a hospital grade ethyl alcohol hand wash (minimum alcohol concentration of 60%). Example product: Alcare Foamed Alcohol Scrub (Steris Co.). Follow manufacturers' recommended contact times for all sanitizing products. Removal of organic debris is essential for proper sanitation; therefore, a water rinse before using the product will be more effective when hands are extremely dirty. Alcohol hand washes should be allowed to air dry while rubbing hands vigorously in order to appropriately distribute the product.

Any individual that has broken skin as a result of a needle stick should notify their supervisor. Supervisors should have appropriate protocols in place and review them with all field personnel prior to the initiation of any fieldwork.

Appendix B: Completing the Health Assessments and Data Collection Form

This appendix provides an overview for conducting a physical examination of a desert tortoise and completing the data collection form, but it is not a substitute for appropriate training and hands-on experience. A thorough and accurate physical examination can only be performed if the individual performing the exam has knowledge of normal tortoise physical appearance and behavior. Prior training in assessing desert tortoise health is required. Physical examinations provide valuable information about the health of individual tortoises and insight into population level issues. Because the desert tortoise is a long-lived species, it is extremely important to use standardized techniques for data collection so that information can be compared over time.

This appendix provides a description of how to conduct a basic physical examination of a desert tortoise and follows the order of the provided data collection form. A minimum of two people will be needed in order to conduct a full health assessment that includes the collection of biological samples. One person will serve as the examiner and will also complete the data collection form. This person should be able to stay clean (i.e. not touch the tortoise or contaminated items) until he/she begins to collect the biological samples. The other person (handler) will handle the tortoise such that the examiner can complete the examination and collect samples. If a third person is available, this person (rather than the examiner) may record the data to further reduce contamination risks. It is important to complete certain observational aspects of the physical exam first, so that potential clinical signs are not affected by handling of the animal. For example, head restraint may cause eye bulging or serous discharge so it should be done after initial evaluation. Most of the health assessment can be conducted with minimal, if any, handling of the tortoise. We recommend using the order described below for a systematic health evaluation of every animal. Generally, the tortoise will be evaluated from a distance before direct contact (far to near), beginning at the head and working towards the tail (head to tail), and least invasive procedures will be conducted prior to more invasive procedures (least to most). This approach also helps field staff remember to collect all of the data consistently because they fill out the form as they proceed with the exam. Be sure to carefully describe all anomalies such that comparisons can be made with subsequent examinations. Handling a tortoise for health assessment and sample collection must be completed within 30 minutes or less. This time period does not include rehydrating tortoises that void.

Unless tortoises are being moved into a quarantine holding facility for their health assessment, they should not be moved far (<20 m, if possible) from the location at which they are found in the field. Before the handler touches the tortoise, find a flat shaded area with ample work space where you will not disturb a tortoise's shelter sites. If shade is not available, it can be created by placing a lightweight cloth over vegetation. Be sure to record the temperature where you plan to work to ensure that it falls within the requirements established by USFWS for handling tortoises. If the temperature is appropriate, lay out equipment in the order that it will be used and place it out of the reach of where you will place the tortoise. Depending on the substrate, the top few centimeters of soil can be cleared away, such that the tortoise is in contact with cooler soil while you work. Prepare all the biological sampling equipment and supplies and set them up in an orderly manner for easy access during the health assessment. An area on one side of the examiner should be designated for clean supplies and equipment and an area on the other side for contaminated items. After taking pertinent initial data (date, start time, project name, site description, tortoise ID, transmitter frequency if applicable, GPS location, temperature, and full names of the biologists), record the time at which you approach the tortoise and are within 2 meters of it. Take care not to

startle the tortoise as you approach it. While wearing gloves, the handler should gently lift the tortoise a short distance off the ground by grasping the sides of the shell and slowly carry it to the shaded area while maintaining it close to the ground in its normal spatial orientation.

While placing the tortoise on the ground in the work area, be sure that the tortoise does not touch any of the supplies or equipment. The examiner should perform as much of the visual observation portion of the health assessment as possible before the handler holds the tortoise again. Note that when completing the data collection form, right and left refer to the tortoise's right and left sides (not right and left from the examiner's perspective if looking at the tortoise head-on). Descriptions of how to complete each data field on the data collection form follow the presentation of the actual form below.

Appendix E: Biological Sampling Supplies

Supplies for individual tortoise sampling

The following supplies will be needed to process a single tortoise. You can prepare zipper sealed plastic bags containing these supplies for individual tortoises to decrease preparation time in the field. It is also important to carry extras of all of these supplies in case of contamination or other unforeseen circumstances. We recommend laying out all the supplies from this bag before touching the tortoise to prevent potential contamination while processing the tortoise.

- (4) Alcohol or diluted povidone iodine wipes
- (2) Sterile cotton tipped plastic stick swabs for oral swabs
- (1) Sterile cotton tipped swab for cloacal swab
- (1) Small trash bag
- (1) Zipper sealed plastic bag to hold completed sample tubes
- (1) 20cc or 60cc luer lock syringe with (1) 22G 1.5", 22G 1", or 25G 1" needle for injectable epicoelomic fluid administration – syringe and needle sizes depend on size of tortoise
- (3) 1cc or 5 cc slip tip (luer) syringe with (3) 23G 1", 23G 1.5", or 25G 1" needle for blood sampling - syringe and needle sizes depend on weight of tortoise
- (1) 2.0ml snap top microtainer labeled "Ticks"
- (2) 2.0ml snap top microtainers labeled "Oral swab"
- (1) 2.0ml snap top Microtainer labeled "Cloacal swab"
- (4) 1.3ml heparinized snap top microtainers labeled "RBC"

~~*If collecting nasal lavage samples*~~

- ~~(6) Pieces dry gauze~~
- ~~(1) Plastic disposable pipet for nasal lavage sample processin~~
- ~~(1) 50ml sterile conical tube for nasal lavage~~
- ~~1cc, 3cc, or 5cc slip tip (luer) syringe with 22G 1.5" needle for~~ depends on weight of tortoise

NOTE: Nasal lavage samples are not included in the health assessment for the Eldorado Valley Translocation Project.

Supplies to keep in the cooler (must be with you while processing a tortoise)

The following supplies should be kept in a cooler for use with multiple tortoises.

- Frozen gel packs or dry ice
- (1) bottle liquid sodium heparin
- 1.8ml O-ring cyrovials with aliquots of LB in a zipper sealed plastic bag - 4 vials per tortoise
- (2) 250ml sterile saline bags encased to protect them from damage

General supplies to keep in the field kit

The following supplies should be kept in your field kit for use with all the tortoises that you process. Remember to keep your field kit free from contamination by laying out your supplies before touching a tortoise.

- Gloves
- Hand sanitizer
- Disinfectant in a spray bottle
- Sharps container
- Ultra fine permanent markers
- Tweezers, hemostats, and/or mutli-tool
- Bottle of water (to soak tortoise for rehydration or to clean tortoise face and nares)

- Paper towels
- Clipboard
- Data collection forms
- Extra packaged syringes – 1cc, 3cc, and 5cc slip tip (luer), 20cc and 60cc luer lock
- Extra packaged needles - 22G 1.5“, 22G 1”, 23G 1.5“, 23G 1”, 25G 1”
- Extra 1.3ml heparinized snap top microtainers labeled “RBC”
- Extra plastic disposable pipets
- Camera
- Calipers

Supplies to keep in your vehicle and/or lab
The following supplies and equipment should be kept in your vehicle and/or lab, in addition to extras of all the supplies listed above.

- Disinfectant
- Big cooler with dry ice
- Inverter
- Portable centrifuge
- Plastic disposable pipets
- 0.5ml O-ring cryovial labeled “Hep plasma UFL” – 1 vial per tortoise
- 1.8ml O-ring cryovials labeled “Hep plasma” – up to 2 vials per tortoise
- 1.8ml O-ring cryovials labeled “Hep plasma/lymph” – up to 2 vials per tortoise

The DTCC’s Research Coordinator, Angie Covert (702-526-3436), may be able to provide information on ordering supplies.

Appendix F: Protocols for Biological Sampling, and Sample Processing, Storage, and Shipment

These protocols describe the collection of biological samples from the tortoise; handling, aliquoting, and storage of the samples; and shipment of samples to the appropriate laboratories. Teams of two biologists (an examiner and a handler) will work together to collect samples, with at least one (the examiner) being certified and permitted to conduct all of the activities involved with sample collection. The handler must be trained in handling tortoises. Ideally, a third person will record data on the data form, but a third person is not required.

Conduct a physical examination of a tortoise prior to taking any samples. Follow the order of data collection as presented on the data form and as described in Appendix B. Prior to handling the tortoise, your sampling equipment should be removed from your field pack and/or cooler and placed in the order that you will need it. Avoid re-entering your field pack and supply bags while you work on an individual tortoise, but if you need to do so, remove your gloves to enter the pack and put on clean gloves before continuing with processing. If you forget to remove your gloves before entering your pack, disinfect everything you touched and put on clean gloves to continue. During the processing of a tortoise, you can avoid contaminating supplies and equipment by using the one-handed glove technique in which you wear gloves on both hands, but the glove on your dominant hand is one size too big so you can easily slide your hand into and out of it as needed to avoid contamination while handling samples, recording data, etc. In addition to preparing and laying out all of the necessary supplies and equipment prior to handling the tortoise, label all the sampling tubes you will be using for that animal with the tortoise ID and date. Appendix F.1 describes all of the preparations that should be made for sampling tortoises prior to handling the animal.

In general, samples collected with the intention of detecting an immune response (e.g., plasma for ELISA tests) require that the immune system be functioning and active. Because a desert tortoise's immune response is temperature dependant, thus depressed during periods of winter inactivity, it is important to collect such samples after the immune system resumes more typical active-season functioning. While individual activity does vary across the range of the desert tortoise, we consider blood samples to be used for ELISA tests valid when collected between 15 May and 31 October. Upon specific approval from USFWS, exceptions to the start date may be made. For example, a sample may be considered valid when collected four weeks after the date that the individual of interest left its hibernaculum or was first found active and above ground (not in association with a shelter site).

Appendix F.1: Preparing for Biological Sampling (Before Handling the Tortoise)

Label cryovials and microtainers

- Prepare cryolabels in advance (before going into the field) using a laserjet printer. Following is an example of the information to include on the label:

GoAg ID _____

Site _____ PI _____

Contents _____ Date _____

- Labels should always display GoAg to indicate that the sample came from a desert tortoise (*Gopherus agassizii*).

- Print your own site name and last name of your PI on the labels using a laserjet printer to avoid having to write these in the field.

- In place of the word "Contents", you will print ONE of the following contents on each label with your laserjet printer. Remember that you will be using multiple tubes for some of these samples so you will need to print more than one label for those samples. Example: You could need 4 RBC tubes for an individual tortoise so you will need to print 4 labels for RBCs and place one on each 1.3ml heparinized microtainer. Also be sure to print extra labels in case of unforeseen circumstances in the field

Contents	Number of tubes per tortoise	Type of tube
RBC	4	1.3ml heparinized microtainer
Hep plasma UFL	1	0.5ml O-ring cryovial
Hep plasma	3	1.8ml O-ring cryovial
Hep plasma/lymph	3	1.8ml O-ring cryovial
Nasal LB	3	1.8ml O-ring cryovial
Oral swabs	2	2.0ml snap top microtainer
Ticks	1	2.0ml snap top microtainer
Cloacal swab	I	2.0ml snap top microtainer

- While working in the field, you will record the tortoise ID next to GoAg ID using an ultra fine black permanent marker. Samples with unreadable or questionable ID numbers may be discarded when they arrive at the UFL or DTCC labs so be sure to record the ID clearly.

- Print the word "Date" using the laserjet printer but leave the space after it blank so you can record it while in the field using a black ultra fine permanent marker. The format of the date should read DDMMMYY.

- Complete the fields for “GoAg ID” and “Date” in the field with an ultra fine black permanent marker when you find a tortoise, but BEFORE approaching it. Complete these fields when you prepare and set out all of your supplies and equipment for processing.

- Note that if you purchase your biological sampling supplies from the DTCC, your sample tubes will be labeled for you, including the fields for your site and PI, but you will still need to complete the tortoise ID and date in the field.

Heparinize needles for blood sampling

In order to keep blood from coagulating when it is drawn, the needles and syringes need to be treated with the anticoagulant liquid sodium heparin. Prepare needles and syringes on the day that they will be used and if prepared before going into the field, store them in a zipper sealed plastic bag in a cooler at all times.

Supplies for heparinizing needles

- Bottle of liquid sodium heparin (to be kept in cooler)

- (3) 1cc or 3cc slip tip (luer) or luer lock syringe with (3) 23G 1”, 23G 1.5”, or 25G 1” - syringe and needle sizes depend on size of tortoise

Tortoise weight	Maximum blood volume to collect	Syringe size	Needle size
100-199g	0.25 ml	1cc	25G 0.5” or 1”
200-249g	0.5 ml	1cc	25G 0.5” or 1”
250-499g	0.75 ml	1cc	25G 1”
500-999g	1.5 ml	3cc	23G 1” or 1.5”
1000-1999g	2.0 ml	3cc	23G 1.5”
2000g+	3.0 ml	3cc	23G 1.5”

Procedure for heparinizing needles

- Take a bottle of liquid sodium heparin out of the cooler and use an alcohol swab to wipe the top of it.
- Attach the appropriate size needle and syringe for the tortoise you are sampling according to the table, and push and pull the plunger to loosen it.
- Remove the cap of the needle and insert the needle into the bottle of heparin.
- Tip the bottle upside down and draw in the heparin, ensuring that the bevel of the needle is in the liquid.
- When the syringe is full, keeping the bottle upside down, inject the heparin back into the bottle.
- Do not push the heparin out of the needle completely because you want a little heparin to remain in the hub.
- Remove the needle from the heparin bottle and recap it using the one-handed recapping technique (protocol below).

Safely discard heparinized needles and syringes that are not used on the day they were prepared. Note that heparinizing needles and syringes in advance instead of heparinizing them in the field as you need them can save you time in processing a tortoise. However, this could cause you to heparinize a large number of syringes that you may not need, and you will ultimately have to dispose of unused heparinized syringes and needles at the end of the day.

NOTE: The nasal lavage is not included in the health assessment for the Eldorado Valley Translocation Project.

Draw saline for nasal lavage

Supplies for drawing saline for nasal l

- 250ml bag of sterile saline (0.9% sodium chloride – to be kept in cooler). Note – Use a marker to put a date on the saline bag. Discard fluids within 30 days after first usage, if they become discolored, or if the non-injection port parts of the bag are punctured with a needle.

- 1ml, 3ml or 5ml slip tip (luer) syringe with 22G 1 ½“ needle for nasal lavage - syringe size depends on size of tortoise

Tortoise weight	Syringe size	Total volume of sterile saline	Volume of sterile saline per naris
100-249g	1cc	1.0ml	0.5ml
250-499g	3cc	2.0ml	1.0ml
500-999g	5cc	4.0ml	2.0ml
1000g +	5cc	5.0 ml	2.5 ml

Procedure for drawing saline for nasal lavage

- Select a syringe based on tortoise size (see table for proper size) and attach a 22G 1.5˝needle to the syringe.

- Use an alcohol swab to wipe the port on a 250ml bag of sterile saline, and insert the needle into the port. Be sure to insert the needle directly into the port and be careful not to puncture any part of the bag. If the bag becomes punctured, dispose of it, as well as the needle and syringe, and start fresh with sterile supplies.

- Draw up the total volume of sterile saline required for the tortoise’s weight (see table for volume).

- Carefully withdraw the needle from the port and either use a tool (*e.g.* multi-tool or hemostat; not your hand) to remove the needle from the syringe, or use the one-handed recapping technique to recap the needle and then remove the capped needle with your hand. Dispose of the needle in the sharps container.

- Place the bag of sterile saline back into the cooler and leave the syringe containing saline out such that the saline is not too cold when you are ready to inject into the nares.

One-handed needle recapping technique

Only use this technique to recap needles that have NOT been used on an animal (i.e., after heparinizing syringes and needles or after drawing up saline for nasal lavages).

- While holding the open needle on a syringe in your dominant hand, use your other hand to lay the cap of the needle on a solid surface with the open end of the cap facing you.

- Remove your hand and guide the needle into the cap without your hands touching it. You can use a solid object to hold the cap in place to make it easier to guide the needle in.

- Once the needle is inside the cap, continue moving your hand forward against the capped needle and syringe until the cap is pushed against a solid surface.

- Then angle the end of the syringe closest to you upwards with the needle inside the cap facing downward and push the needle to secure it inside the cap. Make sure it clicks closed.

- If you are heparinizing a syringe prior to going into the field, you may then place it in a zipper sealed plastic bag in a cooler for use that day.

- If you are drawing up saline for nasal lavage, remove the capped needle and place the saline-filled syringe with your clean supplies to be used while processing the tortoise.

Note that you must NEVER recap a needle that has been used on an animal, but you may use the one-handed recap technique to recap needles that are being ***prepared*** for use on an animal.

Appendix F.2: Ticks

Equipment and supplies

- 2.0ml snap top microtainer labeled “Ticks”
- Tweezers or hemostats

Ticks procedure

For all tortoises (translocatees, residents, and controls)

- Examine the entire body of the tortoise including the soft tissue and shell.
- If ticks are seen, record the number and locations on the data collection form.
- Collect up to 20 ticks in a snap top microtainer labeled “Ticks”.
- Recheck that the ID and date are correctly labeled on the tube.
- Put the microtainers in a zipper sealed plastic bag on ice in the cooler. They must remain cold until they are placed directly in the freezer.
- Record the number of ticks you collected on the data form.

For tortoises that are being translocated

- Carefully remove the remaining ticks with a solid object, such as a stick or rock and let them fall to the ground.
- Be sure that all visible ticks are removed to prevent translocating them to another field site along with the tortoise.
- If there are some ticks that are difficult to remove, use tweezers, hemostats, or even a toothbrush to scrub them off.
- Record the number of ticks you removed on the data form.

For tortoises that are NOT being translocated (residents and controls)

- Do not remove the remaining ticks.

Check your skin, clothes, and equipment for ticks that may have landed there or walked there after being removed from the tortoise, and remove any that you find. WEAR GLOVES. DO NOT HANDLE TICKS WITH YOUR BARE HANDS.

Appendix F.3: Oral Cavity Exam and Oral Swab

Equipment and supplies

- Sterile cotton tipped plastic stick swabs
- 2.0ml snap top microtainers labeled “Oral swab”

Oral swab procedure

- The handler is the only person that will touch the tortoise during this procedure. The examiner will wear gloves, but will not touch the tortoise.
- The handler holds the tortoise with one hand and places the other hand behind its head so that its neck is extended.
- The handler uses their thumb and middle finger to stabilize the head, leaving the index finger free.
- When stabilizing the tortoise’s head, the handler holds the head behind the jaw at the neck, and avoids putting pressure on the jugular vein to minimize the chance of trauma.
- The handler uses the free index finger to gently push down on the mandible to make the mouth accessible.
- If you can’t get the tortoise’s head out or you can’t open the mouth after a few attempts, and you believe the tortoise is experiencing stress, do not proceed. You can attempt to collect the sample during subsequent interactions with the tortoise. Another attempt may be made as soon as 24 hours from the failed attempt, if venipuncture was not conducted on the day of the failed attempt. If venipuncture was done on the day of the failed attempt, the next attempt should occur no sooner than 7 days later to allow for soreness associated with the venipuncture to resolve.
- Once the mouth is open, the examiner looks inside the oral cavity paying particular attention to the choana, tongue, and mucosa, and records the observations on the data form.
- The examiner gently inserts two cotton tipped swabs inside the oral cavity. Move them around the entire oral cavity to sample the choana, the tongue, the inside of the beak, and all mucosal surfaces; however, do not attempt to swab beneath the tongue. In addition to moving around the oral cavity with the two swabs, gently rotate the swabs as well to cover all surfaces of the swabs, and spend extra time swabbing abnormalities.
- The handler should not release the tortoise’s head yet because the nasal lavage procedure will immediately follow.
- After collecting the samples, the examiner quickly places each swab in its own microtainer labeled “Oral swab”. Put the cotton tipped end of the swab inside the microtainer so the plastic stick is pointed outward. Be sure not to contaminate the swabs by touching other surfaces or by touching them with your hands.

- Place these samples to the side, uncapped, in an area that you have designated for collected samples so you can quickly move on to the cloacal swab.

- Note: If the samples will not be safe if left uncapped (risk of blowing away, etc), you can follow the procedure for capping them in Appendix F.7.

Cloacal swab procedure

NOTE: Cloacal swabs are not included in the health assessment for the Eldorado Valley Translocation Project.

This should be the last sample permitted to collect cloacal swabs.

- Insert a cotton-tipped swab into the cloaca. Gently turn the swab and remove.

- Place the swab in the microtainer labeled “Cloacal swab”.

- Put the cotton tipped end of the swab inside the microtainer so the plastic stick is pointed outward. Be sure not to contaminate the swab by touching other surfaces or by touching it with your hands.

- Place the sample to the side, uncapped, in an area that you have designated for collected samples so you can quickly move on to the nasal lavage.

- Note: If the sample will not be safe if left uncapped (risk of blowing away, etc), you can follow the procedure for capping it in Appendix F.7.

Appendix F.5: Blood Sampling

Equipment and supplies

- Alcohol or povidone iodine swabs, or 10% povidone iodine solution
- Gauze or cotton
- 1.3ml snap top heparinized microtainers with labeled "RBC"
- Appropriate size heparinized syringes and needles

Blood sampling volumes

The following table shows the maximum whole blood volume to collect based on tortoise weight and collection device. Note that blood is only drawn on tortoises over 100g and always with heparinized syringes and needles.

Tortoise weight	Maximum blood volume to collect	Syringe size	Needle size
100-199g	0.25 ml	1cc	25G 1"
200-249g	0.5 ml	1cc	25G 1"
250-499g	0.75 ml	1cc	25G 1"
500-999g	1.5 ml	3cc	23G 1" or 1.5"
1000-1999g	2.0 ml	3cc	23G 1.5"
2000g+	3.0 ml	3cc	23G 1.5"

Blood sampling procedure

- Blood will be drawn from the subcarapacial vein as described in Hernandez-Divers et al. (2002), and it is recommended that all individuals using this technique should read the publication.

- The subcarapacial venipuncture site is located underneath the carapace, posterior to the nuchal scute and beneath the anterior portion of the 1st vertebral scute, cranial or anterior to the 8th cervical vertebra.

- The site is accessed by inserting the needle a few millimeters posterior to the junction of the skin-carapace junction and then angling the needle dorsally toward the 8th cervical vertebra.

- While the handler restrains the tortoise's forelimbs, the examiner palpates the subcarapacial area by inserting a finger along the midline posterior to the skin-carapace junction and feels for the depression anterior to the 8th cervical vertebra. This allows you to estimate the proper location of the injection site, as well as determine the angle at which to bend the needle (see next step).

- The examiner removes the appropriate sized heparinized needle and syringe from the cooler. Prepare the needle by bending it upwards with the bevel facing up (using the cap, not your fingers!) to a position that will allow it to easily puncture the subcarapacial vein. It

should be bent at a slight angle to correspond with the angle of the dome between the nuchal and 1st vertebral scute.

- The examiner cleans the venipuncture site with alcohol or 10% povidone iodine solution using swabs, gauze, or cotton prior to insertion of the needle. Be careful not to get alcohol or povidone iodine in the tortoise's eyes.

- Before inserting the needle, the handler should position the tortoise at a level and angle that allows best access to the subcarapacial venipuncture site. The appropriate angle is likely to vary by size and doming of the carapace, but an upright position with the head approximately 75 degrees to the horizontal surface provides a good access point for the needle is often easier to see than when tortoises are held in other positions, especially on very large adults.

- Do not allow the needle to touch the head of the tortoise, which may be moving. With one finger on the beak, gently press the head in and downward toward the plastron and hold it in place, being careful not to touch or place pressure on the eyes.

- Insert the needle through the skin before pulling up on the plunger.

- Pull up on the plunger just slightly to locate the vein, pushing the needle in gently and slowly. You can move the needle very slowly and gently inward and outward with tiny motions along a single path of entry and exit. The needle should not be moved side to side or up and down.

- During the process of drawing blood, be careful not to push the plunger down in the syringe.

- When you see a flash of blood in the syringe, draw the maximum amount of blood indicated in the table for the size tortoise you are working with.

- If you cannot get the full amount of blood on the first or second try, you can try again, but you can only make 3 attempts to draw blood on a single tortoise in one day. ~~If after 3 attempts, you cannot draw the minimum amount of blood required for ELISA testing (0.5ml), you can try again in seven days.~~

- Lymph
    - In order to be useful, blood should have as little lymph contamination (clear or yellow fluid) as possible and not be coagulated.

    - If the sample you collect is contaminated with lymph, the needle has likely been placed posterior to the subcarapacial venipuncture site.

    - Put the lymph-contaminated sample in a heparinized microtainer and be sure to note it on the label with an "L" so the sample can be transferred to the hep plasma/lymph cryovial after centrifuging.

- If you have not used the maximum number of attempts, insert the needle anterior to the location of the previous stick and try to get a clean sample.

- When placing a subsequent clean sample in a heparinized microtainer, do not combine the clean sample with the first lymph-contaminated sample.

- After removing the needle from the venipuncture site, if bleeding occurs, apply pressure with a cotton tipped swab and 10% povidone iodine solution for at least 30 seconds or until bleeding has stopped.

- Check the time so you can record it on your data form (Note: the person doing the venipuncture should wear a wristwatch, so that the time can be checked without touching anything).

- **DO NOT recap the needle**. Unglove one of your hands and remove the needle using a disinfected multi-tool or hemostats (not your hand). Immediately dispose of the needle directly into a sharps container.

- Use the clean hand to pick up a heparinized microtainer labeled "RBC". Use your gloved hand that is holding the syringe to slowly transfer the whole blood into the heparinized microtainer.

- Close the snap top lid with your clean hand and gently invert the microtainer two or three times to mix in the heparin to prevent coagulation and place the tube down temporarily in a location that has not been contaminated.

- If you collected too much blood to fit in a single heparinized microtainer, use as many heparinized microtainers as you need, always using your clean hand to hold the microtainer, cap it, and invert it.

- Dispose of the syringe in the trash bag and remove your other glove – both hands should now be ungloved and not contaminated.

- Recheck that the ID and date are correctly labeled on the tube.

- Record all the necessary information on the data collection form.

- Place the microtainers of blood in a zipper sealed plastic bag in a cooler for later processing. It is best if you can place a barrier, such as newspaper, between the zipper sealed plastic bag and the ice to prevent the cells in the sample from lysing.

<u>Multiple attempts to collect blood</u>

- An attempt is considered one full motion inward and outward with the needle, even if the needle does not leave the injection site. Any repositioning of the needle is considered another attempt and should not be done with the needle inside the skin.

- After each attempt to collect blood (with a maximum of 3 attempts in one session), if there is fluid in the syringe, follow the protocol above to transfer the fluid into a heparinized microtainer labeled “RBC”, and label the microtainers in the order in which their contents were drawn (1, 2, or 3). Also include on the label the letter “L” if the sample in that tube is contaminated with lymph.

- When completing the data collection form, record the total sample volume of all of the attempts combined, and circle the total number of tubes (microtainers) containing blood-related samples. There can be up to 4 tubes in total because in the case that you collect a clean sample of blood in your last attempt, you may have collected too much blood during that attempt to fit in a single microtainer so that sample will be divided into 2 tubes.

- Also record on the data collection form the estimated volume of lymph contained in each microtainer.

After blood sampling, collect all of the required morphological measurements. Record the end handling time when you have finished processing the tortoise and are returning it to its point of capture or placing it in a pen. If the tortoise voided during processing, continue to the rehydration protocol in Appendix F.6.

Appendix F.6: Rehydration

Options for fluid replacement include injectable epicoelomic fluids, nasal-oral offering of fluids, and soaking in a shallow water bath. Injectable epicoelomic fluids and nasal-oral administrations are the preferred two of the three methods of fluid replacement that the USFWS may authorize.

Fluids for rehydration are not prepared during pre-handling preparations for processing tortoises because only tortoises that void will be rehydrated. You can wait until you are ready to rehydrate the tortoise (i.e., after you have conducted all of the biological sampling) to prepare these fluids. However, before you rehydrate the tortoise, record a post-void weight on the data collection form.

Injectable epicoelomic fluid administration

Supplies for epicoelomic fluid administration

- 250ml bag of sterile saline (0.9% sodium chloride - to be kept in cooler)
- 20cc or 60cc luer lock syringe with 22G 1.5“, 22G 1”, or 25G 1” needle – syringe and needle sizes depends on size of tortoise

Tortoise MCL	Syringe size	Needle size	Total volume of sterile saline
150-180mm	20cc or 60cc	25G 1”	20ml/kg
180-200mm	60cc	22G 1.5” or 22G 1”	
200mm +	60cc	22G 1.5”	

Procedure for epicoelomic fluid administration

Epicoelomic describes a location between the coelomic cavity/membrane and the shell. This is a potential space similar to subcutaneous areas. The benefits of using this location versus other subcutaneous locations is that fluids in this area do not appear to affect mobility or the ability of the tortoise to withdraw its limbs into its shell prior to complete absorption of the fluids. This procedure has the benefit of providing a known amount of fluid replacement to the tortoise and does not require a lot of specialized equipment. It does, however, require advanced training and permitting by USFWS, if you would like this method to be among your options.

- Put on a fresh pair of gloves and select a syringe and needle based on tortoise size (see table for proper size). The needle size selected should go no further than the caudal aspect of the pectoral scute upon insertion.

- Use an alcohol swab to wipe the port on a 250ml bag of sterile saline, and insert the needle into the port. Be sure to insert the needle directly into the port and be careful not to puncture any part of the bag. If the bag becomes punctured, dispose of it, as well as the needle and syringe, and start fresh with sterile supplies.

- Draw up the total volume of sterile saline required for the tortoise’s size (see table for volume).

- Carefully withdraw the needle from the port and use a tool (not your hand) to remove the needle from the syringe. Dispose of the needle in the sharps container.

- The location of fluid administration is on the lateral aspect of the plastron anywhere from the caudal area of the pectoral scute to the cranial area of the abdominal scute, just inside the bridge.

- The handler should position the tortoise presenting the axillary fossa to the examiner. The handler can either restrain the forelimb into the fossa where the fluids are to be given or pull the forelimb outward from the fossa, forward and towards the head.

- Insert the needle bevel down in the lower outer aspect of the axillary fossa (above the ridge of bone at the skin junction) at approximately a 45˚ angle, directed caudally. When contact with the plastron bone is felt, move the needle caudally and parallel to the plastron and bridge.

- Pass the needle directly along the bones of the plastron to its appropriate depth (*i.e*. caudal aspect of pectoral scute).

- Pull the plunger out just slightly to ensure negative pressure occurs, and that no blood enters the hub. If there is no negative pressure or blood is seen in the hub, remove the needle from the tortoise, remove it from the syringe, and replace it with a new needle. Attempt to insert the needle into the tortoise again, aiming the needle more closely toward and in contact with the plastron.

- Once you have negative pressure in the syringe when pulling the plunger, steady the needle and inject the saline.

- Heavy pressure on the plunger may be needed to inject the fluids if a small needle size is used.

- If flow stops, slightly redirect the needle and continue administering the fluids.

<u>Nasal-oral fluid administration</u>

This procedure is easy to carry out with minimal equipment, but may not work for all tortoises. In such a case, use one of the other rehydration methods.

Supplies for nasal-oral fluid administration

- Syringe (any size, no needle)
- Fresh drinking water (20ml/kg)

Procedure for nasal-oral fluid administration

- The tortoise can be placed on the ground if it is not very active or the handler can hold the tortoise facing the examiner, near to and parallel with the ground, without restraining its legs.

- The examiner holds the water-filled syringe (no needle) facing downward just in front of the tortoise's face, very close to the nares and slowly pushes the plunger allowing the water to enter the nares and/or mouth.

- The tortoise will sometimes actively accept and drink the water, but if the tortoise refuses, do not force the tortoise to take in the water; use one of the alternate rehydration methods.

Water bath

Supplies for water bath rehydration

- Plastic tote slightly longer and wider than the tortoise
- Fresh drinking water

Procedure for water bath rehydration

- Place the tortoise in the tote and add water until it reaches the level between the skin-plastron junction and the chin.

- Allow the tortoise to soak for 30 minutes.

- Tortoises may drink water through their nose or mouth or absorb it through their cloaca.

- Tortoises may void more urine while being soaked in an effort to eliminate urine with high solute levels to replace it with the fresh water it is taking in. If a tortoise voids in its soaking tub and additional fresh water is available, the water should be discarded and replaced with fresh water until the completion of the 30 minutes. Otherwise the tortoise can be soaked for 30 minutes in the water that contains the voided urine and urates. At the end of the soak time, rinse the tortoise with fresh water to remove urine odor on the limbs and shell, which might attract predators.

After rehydrating the tortoise

- Complete the fields on the data collection form for hydration method, fluid type, and volume. Then weigh the tortoise again and record the post fluid weight.

- Record the end handling time now if you have finished processing the tortoise and are returning it to its point of capture or placing it in a pen.

Appendix F.7: Processing Biological Samples (After Handling the Tortoise)

Note: Change your gloves before processing the following samples to be sure they do not become contaminated.

Processing oral and cloacal swab samples

- Once you have completed all the biological sampling and rehydration (if necessary), cap the oral and cloacal swab samples.
- Hold the cotton tipped end of the swab inside the microtainer lifted just slightly off the bottom.
- Bend and break off the plastic stick so it will fit inside the vial when it is closed. You can also use a disinfected multi-tool to clip the stick.
- Close the snap tops tightly, make sure the vials are labeled properly, and place them in a zipper sealed plastic bag in the cooler.
- Recheck that the ID and date are correctly labeled on the tubes.
- Record on the data form the number of tubes containing oral and cloacal swab samples.

Processing nasal lavage samples

NOTE: Nasal lavage samples are not included in the health assessment for the Eldorado Valley Translocation Project.

Note: The nasal lavage conical tub[illegible]nd while touching it and use your free hand [illegible]ee from contamination.

- Swirl the conical tube and take two LB cryovials from the cooler.
- Record on the data form the total amount of nasal lavage flushed and the estimated total collected after flushing.
- Make 2 aliquots by pipetting samples from the conical tube into the nasal LB cryovials at a 1:1 dilution
    - Pipet 0.75ml of the sample into the 1.8ml O-ring cryovial that contains LB (it has 0.75ml LB in it already so you can just add sample until the total amount of fluid in the vial is 1.5ml).
    - If you have extra sample fluids in the conical tube, pipet 0.75ml of the sample into the other 1.8ml cryovial that contains LB.
    - If you have a total of <0.75ml of sample in the conical tube after conducting the nasal lavage, pipet LB ***out of the cryovial*** into a trash bag until the amount of LB remaining in the cryovial equals the sample amount in the conical tube. Then pipet the sample into the cryovial.

- Secure the lids on the cryovials and recheck that the ID and date are correctly labeled on the tubes.

- Put the cryovials with the samples in a zipper sealed plastic bag on ice in the cooler. They must remain cold until they are placed directly in the freezer.

- Tighten the cap on the conical tube that contains the remaining flush and throw it away in a zipper sealed plastic bag. Put this in a trash bag, not the cooler, to make sure you don't confuse it with your samples later.

- Change gloves since the conical vial was considered contaminated.

- Record on the data form the total number of LB cryovials you collected with nasal lavage samples in them.

<u>Processing whole blood samples (at your vehicle or lab)</u>

Supplies for processing whole blood samples

- Centrifuge (with inverter, if at vehicle)
- 0.5ml O-ring cryovial with label for "Hep plasma UFL"
- 1.8ml O-ring cryovials with label for "Hep plasma"
- 1.8ml O-ring cryovials with label for "Hep plasma/lymph"
- Pipets

Procedure for processing whole blood samples

If you will be centrifuging blood samples in the field, test run the centrifuge from the vehicle's cigarette lighter using the inverter before your first day in the field. The centrifuge/inverter setup might not work on some older or smaller vehicles. Always try to keep the centrifuge cool and shaded.

- Centrifuge the blood as soon as possible after collecting the sample, and always within 4 hours, especially if the temperature is higher than 90F (32C). The longer the blood sits before centrifuging, the more likely it is to clot, which will render the sample useless.

- Before starting the centrifuge, ensure that the centrifuge is properly balanced by placing samples with the same amounts across from each other. If you do not have matching sample amounts, use tubes with water in the same amounts of the samples to balance the centrifuge.

- Record on the data form the time that you placed the samples in the centrifuge to spin (blood processing time).

- Spin the blood for the amount of time required by your centrifuge. This could be 3-10 minutes.

- While the blood is spinning, record the ID and date on the labels of the cryovials that you intend to use for each sample (one for "Hep plasma UFL", one to three for "Hep plasma" and/or "Hep plasma/lymph").

- When the centrifuge stops, record on the data form the plasma color of the sample you will use for the “Hep plasma UFL” cryovial. This should be the best sample you collected for each tortoise, meaning the one with the least lymph contamination.

- Use a pipet or syringe to draw out the plasma (clear layer on top of the red blood cells), being careful not to touch the red blood cells with the pipet. If you drop the sample with the lid closed or you touch the red blood cells with the pipet, re-centrifuge the sample before pipetting to ensure that no red blood cells are removed during the process. It is important to draw up only plasma with no red blood cells in it at all.

- Transfer 0.5ml of the plasma into a cryovial labeled “Hep plasma UFL”.

- Transfer the remaining plasma using the same pipet to cryovials labeled “Hep plasma” (or “Hep plasma/lymph” if applicable), and save the red blood cells in the original microtainer labeled “RBC”.

- Recheck that the ID and date are correctly labeled on all of the tubes (microtainers and cryovials).

- Record on the data form for each tortoise how may cryovials of plasma there are for UFL (UFL plasma aliquots), the total number of other cryovials containing plasma and plasma/lymph (USFWS plasma aliquots), and the number of heparinized microtainers you are saving that contain RBCs (all plasma should have been transferred out of these so only RBCs are left behind).

- Check the lids on the microtainers and cryovials several times to make sure they are closed tightly before placing them in a zipper sealed plastic bag.

- Freeze the samples immediately. If you do not have access to a freezer on site, place the samples in a cooler with dry ice and either transfer them to a freezer while they are still frozen or keep them on dry ice.
    - Note: If the freezer you will be using is a self-defrost freezer (like most residential freezers), be sure that it does not self-defrost at any time when there are samples inside it. Also remember not to leave samples in any location where they can become warm at any time and do not hold samples for more than a few seconds with warm fingers.

Appendix F.8: Shipping Samples

The samples that you collect will be shipped to two labs. If diagnostic tests will be performed as part of your study and they are not related to the required samples, please contact laboratories well in advance to determine if they have expertise performing the specific diagnostic tests you need, if they can accommodate your samples, and how they would like the samples to be collected, stored and shipped, as their protocols may differ from the ones presented here.

Shipping plasma samples to University of Florida for ELISA testing

- This protocol is only for the heparinized plasma samples in the 0.5ml cyrovials labeled "Hep plasma UFL".
- Samples should only be shipped to UFL Monday through Thursday.
- Call (352) 294-4086 or (352) 294-4071 before shipping to make sure someone will be in the lab to receive the samples the next day.
- Complete the UFL Mycoplasma Lab submission form.
    - All samples will be analyzed for antibodies to *Mycoplasma agassizii* and *Mycoplasma testudineum*. Mark the check boxes for ELISA *Mycoplasma agassizii and Mycoplasma testudineum*. Culture/PCR will not be done at this time.
    - If you complete the form by hand, you will need to scan it into a computer so you can email it to the lab after shipping the samples.
    - If you complete the form in Word or Excel, or on UFL's electronic form, you will also need to print it to include it in the shipment.
- If there is a time sensitive issue, such as relocation or temporary holding of animals, note it on the submission form so the lab can expedite your request whenever possible.
- Put the completed submission form inside a zipper sealed plastic bag.
- Put the samples in a separate zipper sealed plastic bag, making sure all caps are secure and cryovials are clearly labeled.
- Place the zipper sealed plastic bag with the samples in a small cooler with frozen gel packs. DO NOT use ice or any other substance that can melt into liquid. Note that you may ship the samples on dry ice.
- Place the zipper sealed plastic bag with the submission form in the cooler on top of the frozen gel packs and samples before sealing the package.
- Tape up the cooler or box. Note that hard sided coolers can usually be shipped without an additional cardboard box.

- Take the package to a FedEx office. Choose Priority Overnight for the delivery service. Sample shipments should always be shipped overnight to arrive at the lab no later than 11am unless otherwise instructed.

- Send to:

  Dr. Mary Brown
  University of Florida Mycoplasma Laboratory
  Room V2-234
  2015 SW Archer Rd
  Gainesville, FL 32608
  (352) 294-4086

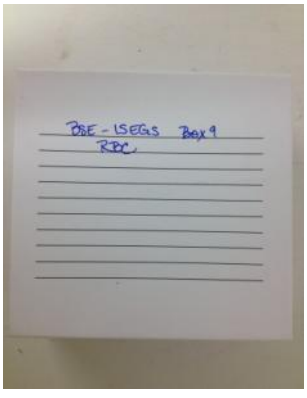
- After you ship the package, email the tracking number and a copy of the submission form as soon as possible to ALL of the following email address: mbbrown@ufl.edu, amburne87@gmail.com

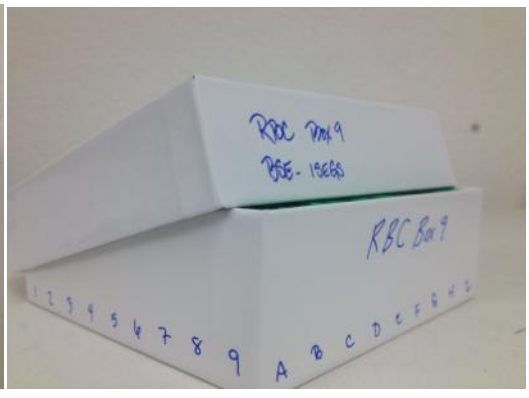
Shipping plasma, oral swab, nasal lavage, tick, cloacal swab, and RBC samples to the USFWS

- This protocol is for ALL samples EXCEPT the heparinized plasma samples in the 0.5ml cyrovials labeled "Hep plasma UFL".
- Samples should only be shipped to USFWS Monday through Thursday. Alternatively, arrangements can be made to drop off samples at the DTCC call 702-338-0104 to coordinate.
- Fill out the sample submission form (below).

Preparation for banking samples into freezer boxes

- Make sure all caps are secure and tubes are clearly labeled.
- As you are recording samples, place each sample in a labeled freezer box with 81 place dividers, organized by date, then by numerical order.
- The box should say the name of the project, the sample type it holds, and what number box it is if all the samples will not fit into one box.
- Box label should be on both the top of the lid and 1 side of the lid.
- The bottom of the box, when facing you will be lettered "A"-"I," and 1-9 when turned to the left, creating a grid "ID" for each slot used. See below





To bank all sample types:

- The sample log must be used to record all samples.
- Sample logs must have the following on each line for each sampled tortoise:
    - Bank location (bank location is the box number and box grid example: 1.1A-1.1I, 2.2A, etc).
    - ID #
    - Date sample was collected
    - Sample type
    - Project name on each line.

- Organize samples by date, then by numerical order.
- Record samples of the same type together, i.e. RBC with RBC, in a log for that sample type.
- Then, place the samples in the appropriate slots in the appropriate boxes.

- Place the banking boxes with the sample information in it on top of the gel packs and samples before sealing the package.

- Tape up the cooler or box. Note that hard sided coolers can usually be shipped without an additional cardboard box.

- Take the package to a FedEx office. Choose Priority Overnight for the delivery service. Sample shipments should always be shipped overnight to arrive at the office no later than 11am unless otherwise instructed.

- Send to:

    US Fish and Wildlife Office
    4701 N. Torrey Pines
    Las Vegas, NV 8913
    ATTENTION Desert Tortoise Conservation Center

- After you ship the package, call the DTCC Hotline at 702-488-8422 to inform staff that a package will be arriving at the USFWS office the following morning and provide the tracking number.

Sample Submission Form
USWFS Samples for Desert Tortoise Conservation Center

Use this form to submit up to 15 samples.

Project name			Site		BO #
PI name			Contact person name		
Contact phone			Contact email		
# plasma samples	# RBC samples	# nasal samples	# oral samples	# cloacal samples	# tick samples

Record each sample tube on a different line. For example, if Tortoise ID 001 has 3 sample tubes, then that tortoise ID should be recorded on 3 lines.

Desert Tortoise ID	Sample Date	Sample Type (plasma, RBC, oral, nasal, ticks)

Ship frozen samples overnight delivery (Mon thru Thur) in cooler to US Fish and Wildlife Service Field Office, 4701 North Torrey Pines, Las Vegas NV 89130, ATTENTION Desert Tortoise Conservation Center. Call the DTCC Hotline to inform them that you shipped the samples 702-488-9422.

Sample Submission Form for Tortoise Mycoplasma Testing
Mycoplasma Research Laboratory, University of Florida

Species of animal(s): _____

County and state of origin (free-ranging only): _____

Date of sample(s): _____

Identification #(s): _____

_____

Is this animal awaiting relocation? ☐ **Yes** ☐ **No** ☐

NOTE: Price discounts (20%) are available for larger numbers of samples (>20) that are submitted for testing or for large volume contracts. Large volume costs are reduced; discounted costs are $28/assay; $11 for dilution curve.

Test requested (please check appropriate box):

↑ ☐ **ELISA serology for *Mycoplasma agassizii*- $35 each.** This is the most commonly used test to detect exposure to *M. agassizii*, a cause of URTD in tortoises; submit serum or plasma.

↑↑☐ **ELISA serology for *Mycoplasma testudineum*- $35 each.** This test detects exposure to a second species of mycoplasma that can be associated with URTD and eye infections; submit serum or plasma. Please note that this test has not been extensively validated at this time.

↑↑☐ **ELISA serum dilution curves - $14 each**

↑ ☐ **Culture/PCR- $35 each.** To avoid false negative results, this test is recommended primarily for clinically ill animals with active nasal discharge; submit nasal lavage sample.

↑ ☐ **PCR sequence if amplicon obtained and RFLP suggests new species- $35.**

Person submitting sample(s): _____

Report results to: _____

Phone: _____ **Email** _____ **Fax:** _____

Preferred method of getting results (check one)↑ ☐ **Email** ↑ ☐ **Fax** ↑ ☐ **Mail**

Preferred method of billing (check one)↑ ☐ **Email** ↑ ☐ **Fax** ↑ ☐ **Mail**

Name & address of person to be billed:

_____

_____

_____

Email contacts: mbbrown@ufl.edu; amburne87@gmail.com

Please ship samples from Monday to Thursday only:

Dr. Mary Brown
University of Florida
Dept. of Infectious Disease and Pathology
2015 SW 16th Ave
Room V2-234/232
Gainesville, FL 32608
Telephone: (352) 294-4071 or 294-4086
Fax: (352) 392-9704

*****Please label tubes with investigator's name, tortoise species, tortoise identification name/number, and date sample collected.**

*****NOTE: Federal Express is the only carrier that delivers directly to our laboratory. Other carriers deliver to the VMTH, and samples may take 1-2 days to be delivered to our lab. In such cases, the quality of the samples may be compromised.**

For laboratory use only

Date Received:_____ Samples cold: ☐Yes ☐ No

No. ELISA: Comments:

Appendix E:
Body Condition Score

BODY CONDITION SCORES FOR DESERT TORTOISES

Nadine Lamberski, DVM, Dipl. ACZM

San Diego Zoo Safari Park, 15500 San Pasqual Valley Road, Escondido, CA 92027 USA

Introduction

Body condition scoring is a visual appraisal system that estimates average body energy reserves without using scales, calipers, or calculators.[1,2] Since individuals can vary in size and shape, weight alone is not a good indicator of body condition. The body condition score (BCS) is based on an evaluation of muscle mass and fat deposits in relation to skeletal features and has been adapted to the desert tortoise (*Gopherus agassizii*). This score is dynamic and should improve if the animal is eating and body energy reserves increase. Conversely, the score will decrease if inanition persists or body energy reserves are depleted.[3]

Adapting BCS for Tortoises

BCS ranges from one to nine, with one being emaciated and nine being extremely obese. Assigning a BCS is a two-step process. The numbers are divided into 3 groups.

STEP 1: Choose the grouping that best describes the tortoise at the current point in time.

a) Under condition (1-3): best assessed by degree of temporalis muscle atrophy and prominence of the sagittal crest;

b) Good condition (4-6): best assessed by degree of temporalis muscle development;

c) Over-condition (7-9): best assessed by degree of subcutaneous fat deposition.

STEP 2: More accurately identify the score by selecting one of the three numbers in the given ranges. Choose the best fit for that individual at this current point in time.

Fig. 1: BCS 1, severe atrophy of the temporalis muscle, visible sagittal crest.

Fig. 2: BCS 2, atrophy of the temporalis muscle, visible sagittal crest.

Fig. 3: BCS 3, slight atrophy of the temporalis muscle, sagittal crest visible.

Fig. 4: BCS 4, no obvious atrophy of the temporalis muscle, sagittal crest not visible, flat appearance to the top of the head.

Fig. 5: BCS 5, developed temporalis muscle itha bi-lobed appearance to the top of the head.

Fig. 6: BCS 6, very well-developed temporalis muscle with an almost rounded appeance to the top of the head.

Fig. 7: BCS 7, subcutaneous fat present in pre-femoral and cervial regions.

Fig. 8: BCS 8, moderate amount of subcutaneous fat pinched between fingers in the axillary region.

Fig. 9: BCS 9, obese animal with large amount of subcutaneous fat especially evident in the cervical and axillary regions.

Fig. 1-9: Body Condition Scores (BCS) 1-9 for Desert Tortoises. BCS 1-3 are tortoises in poor or under-condition, BCS 4-6 are in good condition, and BCS 7-9 are obese or in over-condition.

	BCS 1	BCS 2	BCS 3	BCS 4	BCS 5	BCS 6	BCS 7	BCS 8	BCS 9
Sagittal crest visible	extreme	yes	slight	no	no	no	no	no	no
Sagittal crest palpable	yes	yes	yes	slight	no	no	no	no	no
Temporalis muscle developed	no	no	no	slight	yes	yes	yes	yes	yes
Muscle atrophy (limbs)	extreme	yes	yes	slight	no	no	no	no	no
Subcutaneous fat (pre-femoral space)	no	no	no	no	no	slight	yes	extreme	extreme
Subcutaneous fat (limbs and tail base)	no	no	no	no	no	slight	slight	yes	extreme

Table 1. BCS scores are assigned based on an evaluation of muscle and fat deposits relative to skeletal features.

Conclusion and Relevance

A tortoise's body condition will change with life stage, stage of reproduction, season of the year, drought, food availability, and disease. Therefore, this management tool can be used to monitor and compare populations over time.

Acknowledgements

The author gratefully acknowledges the contributions of the staff of the Desert Tortoise Conservation Center, Las Vegas, Nevada, in the development of this protocol.

Literature Cited

1. Bewley, J. M., and M. M. Schutz. 2008. Review: an interdisciplinary review of body condition scoring for cattle. The Prof. Anim. Sci. 24: 507-529.

2. Stevenson, R. D. and W. A. Woods, Jr. 2006. Condition indices for conservation: new uses for evolving tools. Integr. Comp. Bio. 46: 1169-1190.

3. Lamberski, N., Braun, J., Witte, C., Christopher, M., Field, K., Averill-Murray, R., Keener, L., Robbins, P., Johnson, J., Covert, A., Walsh, A., and B. Rideout. 2012. Identifying key clinical signs and validating body condition scores to minimize disease spread and maximize individual survival durin desert tortoise translocations. Proc. Joint Ann. Meeting Wildl. Dis. Assoc. and European Assoc. Wildl. Dis. Lyon, France.