Coachella Valley Conservation Commission

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Coachella Valley Multiple Species Habitat Conservation Plan & Natural Community Conservation Plan

Developing an Effective Agassiz's Desert Tortoise Monitoring Program:

Final Report to the Coachella Valley Conservation Commission

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Introduction

Agassiz's desert tortoise (*Gopherus agassizii*) is a conservation-reliant species with populations north and west of the Colorado River protected as threatened under the Endangered Species Act (Averill-Murray et al. 2012). Since it was listed under this category in 1990, a great deal has been learned about the natural history of the species, and it is now one of the best-studied turtles in the United States (Lovich and Ennen 2013). However, the accumulated body of scientific data available for the species has not yet been translated into recovery or delisting of the species. Successful conservation of any species requires knowledge of their natural history and how vital rates affect their ability to maintain stable populations in the face of natural and anthropogenic stresses.

Agassiz's desert tortoises occur from southwestern Utah to near the Mexican border in California – a distance of over 450 km – but population densities vary greatly across this immense landscape (U.S. Fish and Wildlife Service 2015). Tortoises occur in the Sonoran Desert of California, including the eastern and western ends of the Coachella Valley, where it is one of 27 species covered under the Coachella Valley Multiple Species Habitat Conservation Plan and Natural Community Conservation Plan (CVMSHCP/NCCP). The southern portion of Joshua Tree National Park (JTNP) lies within this 1.1 million acre planning area, and was predicted to be an area of low-density tortoise populations using habitat suitability modeling (Barrows 2011). JTNP is near the southern distributional limit of *G. agassizii*, yet very little has been published regarding the ecology of tortoises in the Sonoran Desert of California.

Reproductive output is an important gross measure of the ability of a population to persist. When integrated with data on fertility and survivorship, this information forms a foundation for assessing population status and formulating effective management strategies (e.g., Congdon et al. 1993, 1994), especially for imperiled species. One aspect of the biology of *G. agassizii* that has been particularly well-studied is reproductive output. However, most of what we know about this topic comes from research in the Mojave Desert portion of the species' range (Ernst and Lovich 2009). Comparatively little has been published on the reproductive ecology of populations living in the Sonoran Desert ecosystem of California. Publications by Lovich et al. (1999, 2011, 2012, 2014, 2015) constitute the main body of literature on desert tortoise reproductive ecology in the Sonoran Desert of California, with one study population located at the western end of the CVMSHCP/NCCP area. Collecting data on Agassiz's desert tortoise ecology in the Sonoran Desert ecosystem is important due to significant differences between the two adjacent desert ecosystems, especially the timing and amounts of annual precipitation, and their potential effects on reproductive output (e.g., Lovich et al.

2015). There are also differences in the vulnerability of tortoises to the effects of a warming, drying climate between the two deserts (Barrows 2011; Zylstra et al. 2012).

The overall goal of this study was to collect data on demography, reproductive output, and genetic affinities at a study site in the Sonoran Desert portion of JTNP in the eastern end of the CVMSHCP/NCCP area. Specific objectives included: 1) Collect data to establish baselines on tortoise populations and/or their habitat suitability in core habitat within the CVNCCP area, including biotic and abiotic variables affecting persistence of tortoise populations; 2) Compare and contrast with data collected on desert tortoises at USGS/BLM study site near Palm Springs over 16 years; 3) Support long-term modeling efforts needed to determine tortoise population viability; 4) Refine modeled relationships with identified threats such as fire, invasive species and climate change; and 5) Prioritize adaptive management needs for the desert tortoise in and beyond the CVNCCP area. The data from this study will aid in determining baseline estimates of the desert tortoise population size within the planning area as well as establish a marked population of Agassiz's desert tortoises for future monitoring. Data will be integrated with habitat modeling in order to refine model output. Genetic data will be collected on both the north and south sides of Interstate 10 to determine the potential effects of habitat fragmentation and genetic mixing. Analyses are ongoing and results beyond those presented in this report will be published in peer-reviewed scientific journals following inclusion of additional data collected on the south side of Shavers Valley in 2017-2018.

Methods

Study site

The Cottonwood study site (hereafter Cottonwood) is located in the southernmost portion of JTNP, in an area drained by Shavers Wash (Figure 2). This site was identified as an area of high tortoise densities during surveys of JTNP by Karl (1988). In addition, tortoises were marked there during the period from 1997–1999 by the senior author. The site is characterized by the steep boulder-strewn Cottonwood Mountains that meet gently sloping bajadas and arroyos running southward toward Interstate 10. The study area was unbounded and the area surveyed encompassed approximately 5.75 km^2. Elevations range from 520 m on the bajadas to over 800 m in the adjacent mountains, with tortoises occupying known elevations from 534–780 m. Vegetation is typical of the arborescent Sonoran Desert plant communities of California and is dominated by creosote (*Larrea tridentata*) scrub interspersed with ironwoods (*Olneya tesota*), palo verde trees (*Parkinsonia florida*), smoke trees (*Psorothamnus spinosus*), and ocotillos (*Fouquieria splendens*).

The study area is bisected by Cottonwood Springs Road – a paved and moderately trafficked road that provides access to the southern portion of JTNP. From 2 February 2012 to 9 July 2013, mean traffic volume was 355 cars/day (range: 138 in the month of June to 917 in the month of March). Traffic volume was highest from December–May, a time period overlapping peak above-ground desert tortoise activity at our study site (March–June). The highest traffic volume occurred in March, with a mean of 535 and 605 cars/day in 2012 and 2013, respectively with similar values for May. June, July, and August yielded the least amount of traffic daily with July generating the lowest average (190 cars/day in 2013; all traffic data provided by JTNP). According to JTNP statistics, visitation increased significantly in 2015, so these earlier statistics may underestimate more recent traffic volume.

Severe drought occurred in southern California during the time of our study. Drought conditions between 2012 and 2014 constituted the worst drought for central and southern California in the last 1,200 years based on paleoclimate reconstructions (Griffin and Anchukaitis 2014; Mann and Gleick 2015), and that drought continued through 2016. However, precipitation was adequate enough to support good germination of tortoise food plants in 2015 and 2016.

Field techniques

Demographic and reproductive studies at Cottonwood were completed during 2015–2016. Beginning in late March 2015, tortoises were located via semi-systematic surveys. Upon capture, tortoises were marked using a triangular file to notch unique combinations of marginal scutes for future identification (Cagle 1939). A subset of mature male and female tortoises were outfitted with radio transmitters (models R1850, R1860; Advanced Telemetry Systems). Male tortoises are often easier to locate initially and can be used via tracking to aid in locating females. Tortoises were tracked every 10–14 days from April–July, and once per month during the remainder of the year when transmitters were still attached. Mid-line straight-line carapace length (SLCL) was measured using tree calipers (± 1 mm). Weights were taken at every capture using Pesola spring scales (± 10 g). Assessments of health and notes on general appearance were also taken. GPS locations were recorded with a Garmin Oregon 550T. Tortoise sample sizes varied from year to year as in some cases radio transmitters malfunctioned or were lost partway through the season, and we were unable to relocate some of these tortoises.

X-radiography was used to determine the presence of shelled eggs, clutch size, clutch number, egg width, and annual egg production. Female tortoises were X-radiographed (Hinton et al. 1997) in the field approximately every 10–14 days from April–July, and then returned to their capture location, usually within one hour. The period from April to July overlaps the previously known earliest and latest dates of the production of shelled eggs at JTNP (Lovich et al. 1999). X-radiographs were performed in the field using a digital X-ray generator (model TR80; Min-Xray) connected to a Canon X-radiography system. Exposures were taken using the settings described by Lovich et al. (2015) in doses that are considered to be safe for tortoises (Hinton et al. 1997).

While performing surveys or radiotracking tortoises, tortoise sign was often observed. All burrows that appeared active (not collapsed, filled in, or full of cobwebs or debris) were recorded, described, and a GPS location was taken. If whole shells or shell fragments of dead tortoises were located, a GPS location was recorded along with notes of the state of the shell in order to determine approximately how long ago the tortoise died.

Permits and Approved Protocols

Research was conducted under permits and approvals from the U.S. Fish and Wildlife Service (Permit # TE-198910), Bureau of Land Management, and National Park System, and under a California Endangered Species Act Memorandum of Understanding with the California Department of Fish and Wildlife. The Institutional Animal Care and Use Committee of Northern Arizona University reviewed and approved our research procedures on handling, marking, and obtaining blood samples from tortoises (Approved Protocol #16-002).

Clutch phenology

Clutch size and X-ray egg width (XREW) were determined directly from X-rays. XREW was measured at the widest portion of an egg, from the outermost point of each side of the shell, using K-PACS software (version 1.6.0; http://www.k-pacs.net). XREW was determined from the first X-ray in which a clutch of eggs was clearly detectable. X-radiography was also used to determine dates of appearance and disappearance of shelled clutches, and to measure the annual percentage of reproducing females, clutch frequency (number of clutches produced by a female in one year), and annual egg production (AEP) per female (total number of eggs produced/female/year).

Genetic sampling

Blood samples were collected to perform genotyping and population assignment of the tortoises at Cottonwood in order to compare with another population at the western end of the planning area. We used the subcarapacial venipuncture technique described by Hernandez-Divers et al. (2002). This technique has proven to be a safe and effective way to remove small quantities of blood from a diversity of turtle species, including Agassiz's desert tortoise (Drake et al. 2012). Up to 0.5 mL of blood or blood with lymph were obtained from a subsample of the population in Cottonwood. Blood samples were only taken following permitting by the U.S. Fish and Wildlife Service and the California Department of Fish and Wildlife. Blood collection protocols were stringently followed according to guidelines set by the U.S. Fish and Wildlife Service and protocols approved by the Northern Arizona University Institutional Animal Care and Use Committee. Samples were sent to the University of Arizona Genetics Core for processing, and results will be described in a later report due by June 30, 2017.

Model validation

We used GPS points collected for live desert tortoises, active tortoise burrows, and the remains of desert tortoises to validate the occupancy model generated by Barrows (2011) for our study area. His model predicted habitat likely to be occupied by tortoises in JTNP based on historical records of their distribution including both museum records and recent records of tortoise occurrence in the area. Thus, our inclusion of tortoise shells that might be relatively old is not expected to bias the model. We used raster cells 180 m x 180 m based on point location data from Barrows, and raster cell values greater than or equal to 0.7 were considered a good fit to the model and therefore optimal desert tortoise habitat.

Results

Population monitoring

A total of 33 tortoises were captured and registered at the Cottonwood study site from 2015–2016, not including one unmarked immature tortoise (Table 1). The immature tortoise was too small to notch its shell safely with our equipment. Additionally, three adult tortoises observed in burrows were inaccessible and therefore not captured or marked. A frequency accumulation curve of the number of unique registered tortoises located over time suggests that our team located the majority of tortoises within the study area footprint in the first year, but there was a spike in the number of new tortoises located in the second year (Figure 3). From April–July 2015, nine tortoises (four males, five females) were outfitted with radio transmitters for relocation. Two of these tortoises were subsequently lost; one of the radios from a lost tortoise was recovered after it detached from the shell but the tortoise was never relocated. The other tortoise was never detected again even though the radio remained attached. From April–July 2016, ten tortoises were outfitted with radios (three males, seven females), including seven that were monitored the previous year. Blood samples were taken from this subset of ten desert tortoises (Table 2) to compare to the genetic affinities of a population of *G. agassizii* at the western end of the CVMSHCP/NCCP area located near Palm Springs (manuscript in preparation).

A total of three juvenile tortoises were located. The smallest was a juvenile (1–2 years old), and the other two were estimated to be 3–4 years old. One juvenile had a California barrel cactus (*Ferocactus cylindraceus*) spine impaling its right anterior axillary area, with 27.2 mm of the spine (total length of 63 mm) inserted into the body (Smith et al. 2015). The spine was removed as the protruding portion inhibited the juvenile tortoise's movements. Because the juvenile was located in rocky, upland habitat, it is possible the tortoise tumbled on the slope or was dropped (i.e. by a bird) causing the impalement.

In April 2015, a large adult male tortoise that had been previously registered and noted as outwardly healthy was found dead. The observations associated with the carcass (partially decapitated, eviscerated from a prefemoral pocket, intact appendages, overturned onto carapace, lack of chew or scratch marks) potentially indicated a badger was the predator. A badger was subsequently detected on a trail camera placed in the same wash where the male tortoise was originally located (see Smith et al. 2016). This was the only carnivore documented by the trail camera in 78 days of monitoring.

A total of 17 shells were found in the study area. Of these, seven were bleached, disarticulated shell fragments suggesting a deterioration process of greater than 20 years *post mortem*. We also located seven mostly intact, adult shells with scutes attached or beginning to peel, suggesting that death occurred within the last five years. Three juvenile shells were located, all of which exhibited signs of predation or scavenging that may have been weathering for ten years or less. A total of eight shells (adults and juveniles) showed evidence of predation or scavenging via bite marks or bones which were cracked or broken off of the suture lines.

Burrows and Habitat

A total of 208 active burrows were located, and of these, 100 were observed being used by tortoises. Co-habitation at a burrow was observed on only one occasion, involving a male tortoise that was sitting on the burrow apron with a female tortoise inside the burrow. Some burrows were used at separate times by more than one tortoise. We recorded ten burrows that were used by two tortoises and one burrow used by three different tortoises. Occasionally, tortoises would be found in a burrow that had been previously marked via GPS and considered active, but no tortoise had been present. Tortoises were located outside of burrows a total of 74 times.

Tortoises used both upland (mountainous, steeply sloped, rocky substrate) and lowland (gently graded, loamy or sandy soil, bajadas) habitats (Table 3). Out of the 33 tortoises in the marked population, 13 were located at least one time in rocky, upland areas. This includes three juveniles, each of which were located a single time, found in areas surrounded by boulders. Six of the adult tortoises using upland habitat were radiotelemetered and were found using upland areas as much as 92% of the time (Table 3). Of the 20 tortoises located using solely lowland habitat, six were radiotelemetered. Although these six tortoises were never located in upland habitat, half of them were located within 0.5 km of upland areas (Table 3). This would be considered a reasonable distance for a tortoise to move within its home range based on our experience at the site. There were three tortoises located south of the Colorado River Aqueduct (including one outfitted with a radio transmitter) just outside of the JTNP boundary. These tortoises were greater than 2 km from upland areas, and it would be unlikely for them to move to upland areas. We did record one female moving up and over the aqueduct between burrow locations, indicating the high berm did not act as a barrier to tortoise movement.

None of our radioed tortoises were observed to cross the paved Cottonwood Road during our studies. In fact, none of the tortoise locations were closer than about 150 m to the road (the majority were much farther away) suggesting road avoidance on this heavily-traveled entrance into the JTNP (manuscript in preparation).

Habitat suitability model

For locations where tortoises were found but not associated with a particular burrow, about 2/3 of our points were in areas predicted by the Barrows (2011) model (Table 4). The other third of our tortoise observations were outside of the optimal desert tortoise habitat model. For burrows that were considered active, similar ratios were observed, with the majority located in areas predicted to be occupied. Tortoise shells found during our study exhibited higher classification accuracy with over 75% being located in areas considered suitable for tortoises under the model.

Clutch phenology

At the Cottonwood study site, 80% of females (4 of 5) produced eggs in 2015. All four of these females produced two clutches (Table 5) for a total of 29 eggs produced during the 2015 reproductive season. Of these 29 eggs, 51.7% were produced in first clutches. In 2016, 86% of females (6 of 7) produced at least one clutch of eggs, and five of these females also produced a second clutch (Table 5) for a total of 43 eggs produced during the 2016 reproductive season. Of these 43 eggs, 48.8% were produced in first clutches. In both years, the female tortoise that did not reproduce was female #11. Despite being above the size threshold for being reproductively active (> 20 cm SLCL) and found within the vicinity of multiple males, female #11 never produced a clutch of eggs. It is possible that this female is infertile.

Of the females that reproduced, mean SLCL among years ranged from 21.9 cm in 2016 to 22.7 cm in 2015, (Table 6). X-radiography revealed visible shelled eggs from early April through mid-June. The overall earliest date of detection of shelled eggs was 6 April (2016), and the latest date of a visible first clutch was 16 June (2015) when a double clutch of eggs was likely oviposited simultaneously (see description in following paragraph). The earliest date of appearance of second clutches was 9 May (2015), and the latest date of detection of a second clutch was 16 June (2015). In 2016, one female produced a first clutch consisting of a single egg that appeared approximately one month after all other reproducing females' first clutches disappeared, and the disappearance of this first clutch on 14 June marked the latest date of disappearance of any clutch in 2016. Overall, shelled eggs appeared approximately two weeks earlier than many studies report farther north in the Mojave Desert (Turner et al. 1986; Wallis et al. 1999; McLuckie and Fridell 2002). This is likely due to the fact that average temperatures at the Cottonwood study site are warmer than in the Mojave Desert, allowing for earlier egg production.

Clutch size ranged from 1–6 eggs. One female had overlapping clutches of a total of nine eggs that included a first clutch of four eggs appearing early, with a second, thinly shelled clutch of five eggs appearing later in the season (Figure 4). We assume these two clutches were oviposited at the same time as they disappeared from the X-ray simultaneously. Overlapping clutches have been observed previously in *G. agassizii* (Turner et al. 1986). The overlapping clutches we observed were treated as two separate clutches for statistical purposes due to the fact that they were shelled at separate times with over a month of visibility of the first clutch before the second clutch appeared. Mean overall annual clutch size across both years at Cottonwood was 3.79 ± 1.36 eggs/clutch (Table 6). Mean XREW of all clutches was 36.5 ± 1.6 mm (Table 6). Among all reproductive females, mean clutch frequency was 1.9 ± 0.3 clutches/female/year. A total of 6 (75%) females produced nine second clutches from 2015 to 2016 (Table 5), and one (12.5%) female never produced any visible eggs during the time it was monitored. Over both years, first and second clutches accounted for an equal amount of the total number of eggs produced (50% each). Total annual egg production (AEP) among reproductive females ranged from 1–12 eggs/female/year, and mean AEP over both years was 6.0 ± 3.8 eggs/female/year (Table 6). The year 2016 had the highest and lowest individual AEP with one female producing a total of 12 eggs and another female producing only a single egg.

Discussion

When we began searching for an aggregation of tortoises to study in the eastern end of the CVMSHCP/NCCP area in the spring of 2015, tortoise densities appeared to be lower than expected, especially since the Shaver's Valley area is designated as Critical Habitat for the tortoise. This observation was based on transects walked with the help of volunteers on the north side of Interstate 10 from Chiriaco Summit to Cottonwood Springs Road. Previous experience by the senior author with tortoises marked in the mouth of Cottonwood Canyon from 1997–1999 led us back to that area where we found enough tortoises to establish a new baseline for research and monitoring. Even though we registered 31 new tortoises during our study, the area still has a relatively low population density. We can estimate population density based on the approximate area of the minimum convex polygon of our study area (5.75 km^2) shown in Figure 1 and all the tortoises (34) in Table 1 as 5.9 tortoise/ km^2. This figure is greater than the mean density estimate for JTNP, overall, of 3.7 adults/ km^2 presented in U.S. Fish and Wildlife Service (2015).

Earlier surveys for tortoises in the JTNP identified the Cottonwood area as one of the highest density sites for tortoises (Karl 1988) with 8–29 tortoises/ km^2. Our figure is slightly below Karl's lower estimate. Karl's estimate is difficult to compare to ours as the methods were very different. If our lower figure represents a true reduction in tortoise density over time, it seems unlikely to be the result of a large die-off of tortoises in the area since we found comparatively few shells during our surveys compared to live tortoises. Such was not the case in the nearby Pinto Basin of the JTNP where large numbers of shells (64) and a small number of live tortoises (14) were reported based on surveys in 2012 (Lovich et al. 2014). The die off in the Pinto Basin was attributed to the effects of drought and predator prey-switching with low levels of estimated survival being coincident with low three-year moving average precipitation trends. The location of several juvenile tortoises during our study at Cottonwood indicates that recruitment is occurring, but it would require further monitoring to determine whether recruitment is high enough to offset mortality in the population. The location of juveniles in boulder piles may confer a survival advantage due to the cover provided (Nafus et al. 2017).

Barrows (2011) suggested that climate change would lead to reductions in the distribution of tortoises, especially in the Sonoran Desert region of JTNP. Under a scenario with warming/drying of +2°C/ -50 mm precipitation, he predicted an 88% reduction of occupied desert tortoise habitat in that region. Maps of his modeled results show reductions in tortoise habitat in the Cottonwood area. Based on their research in the nearby Pinto Basin of the JTNP, Lovich et al. (2014) concluded that their results may be early signal of Barrow's modeled predictions. The location data for live desert tortoises, active tortoise burrows, and the remains of desert tortoises at the Cottonwood study site from 2015–2016 was used to validate the classification accuracy of the tortoise distribution model presented by Barrows (Table 4). The broad congruence of these categories with model predications suggests that the model has utility at the local scale of our study. Additionally, as more location data for tortoises, tortoise burrows, and tortoise shells are integrated into the model, predictions for habitat suitability will be refined and reflect changes due to pressures from issues such as climate change.

Continued monitoring will be required to determine if the Cottonwood population of tortoises is stable or declining as a possible result of climate change. The severity of the recent drought in California was reduced with high winter precipitation in 2016–2017. It remains to be seen if that will have an effect on tortoise populations, especially their reproductive output and survival (Lovich et al. 2014, 2015). The protected nature of the habitat within JTNP boundaries makes it a good location for long-term study of a population of *G. agassizii* that is less impacted by anthropogenic activities than other sites in Shaver's Valley.

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Table 1. List of all tortoises captured and registered at the Cottonwood study site in Joshua Tree National Park. Last known locations are given in UTM, Zone 11, NAD 83. M = male, F = female, J = juvenile. Straight-line carapace lengths listed indicate the most recent measurement. All radios attached have been removed as of July 2016, unless indicated otherwise in the Notes.

ID	Date of first capture	Date of last capture	Sex	Radio attached (Y/N)	No. of captures	Straight-line carapace length (cm)	Notes
1	03/31/15	07/12/16	M	Y	27	25.6	Made large movements between the bajadas and Cottonwood mountains. Observed copulating with tortoise #2 in April 2015.
2	03/31/15	07/12/16	F	Y	29	21.3	Only mature female observed carrying overlapping clutches (2015).
3	03/31/15	07/12/16	F	Y	11	21.3	Maintained burrows on both sides of the aqueduct (just south of the Park boundary), indicating tortoises can cross over aqueduct.
4	04/01/15	04/13/15	M	N	2	29.6	Found dead on 04/13/15. Likely killed by American badger.
5	04/01/15	04/01/15	M	N	1	24.6	
6	04/01/15	04/01/15	F	N	1	20.8	
7	04/13/15	07/12/16	F	Y	26	22.2	Tortoise originally monitored by NPS. Radio left attached and tortoise transferred to NPS staff.
8	04/13/15	07/12/16	M	Y	24	26.6	Tortoise originally monitored by NPS. Radio left attached and tortoise transferred to NPS staff.
9	04/14/15	07/23/15	F	Y	11	25.0	Lost. Radio fell off tortoise and was located. Fell off cleanly with all epoxy. Tortoise was never relocated despite checking known burrow locations and scouting area.
10	04/14/15	08/25/15	M	Y	10	21.1	Lost and never relocated. Checked known burrow locations and scouted area. Possibilities are dead radio, predation, or extended movement to a new location.
11	04/15/15	07/12/16	F	Y	24	21.7	Only mature female X-rayed that never produced eggs
12	04/16/15	07/11/16	F	Y	26	22.8	
13	04/16/15	07/11/16	M	Y	25	28.0	
14	05/27/15	05/27/15	M	N	1	24.6	
15	06/16/15	06/16/15	M	N	1	24.1	
16	08/25/15	04/06/16	M	N	3	25.8	
17	08/25/15	08/25/15	M	N	1	31.2	Largest tortoise in study population
18	09/22/15	09/22/15	M	N	1	24.8	

ID	Date of first capture	Date of last capture	Sex	Radio attached (Y/N)	No. of captures	Straight-line carapace length (cm)	Notes
19	09/22/15	09/22/16	M	N	1	23.9	
20	06/03/15	06/03/15	J	N	1	8.9	Immature tortoise with barrel cactus spine impaling right axillary area. Approximately four years old.
21	02/25/16	02/25/16	M	N	1	26.0	Observed engaged in a fight with male tortoise #22.
22	02/25/16	02/25/16	M	N	1	29.1	Observed engaged in a fight with male tortoise #21.
23	02/26/16	02/26/16	M	N	1	24.1	
24	02/26/16	02/26/16	M	N	1	27.7	Located south of aqueduct.
25	03/08/16	03/08/16	M	N	1	28.0	
26	02/26/16	02/26/16	M	N	1	29.6	Located south of aqueduct.
27	03/09/16	03/09/16	M	N	1	27.5	
28	04/06/16	07/12/16	F	Y	7	21.4	
29	04/07/16	04/07/16	J	N	1	7.8	Immature possibly 3-4 years old.
30	04/21/16	04/21/16	M	N	1	28.8	
31	04/21/16	07/11/16	F	Y	6	22.8	
188	03/31/15	03/31/15	M	N	1	29.3	Previously registered in 1999 by Jeff Lovich
194	09/22/15	09/22/15	M	N	1	28.9	Previously registered in 1999 by Jeff Lovich
-	06/03/15	06/03/15	J	N	1	6.0	Yearling was too small to mark with notches

Table 2. Data summary of blood collection from tortoises captured in the Cottonwood area of Joshua Tree National Park in 2016. Samples sent to University of Arizona Genetics Core for analysis.

ID No.	Date	Sex	Volume (ml)
1	05/03/2016	M	0.4
2	05/03/2016	F	0.5
3	06/14/2016	F	0.5
7	05/02/2016	F	0.5
8	05/16/2016	M	0.5
11	05/02/2016	F	0.5
12	05/03/2016	F	0.5
13	07/11/2016	M	0.5
28	05/02/2016	F	0.5
31	05/03/2016	F	0.5

Table 3. Summary of habitat use by radiotelemetered tortoises. Uplands are categorized as having a steep slope with a rocky substrate (i.e. mountainous areas). Lowlands are categorized as having a slightly graded or flat slope with a sandy, loamy substrate (i.e. bajadas). The number of times a tortoise was located in the uplands or lowlands is listed, along with the corresponding percent of time in parentheses. The closest distance to uplands is listed for tortoises that were never located in upland areas. This distance represents the closest location of a particular tortoise to upland habitat.

Tortoise ID	Sex	Total number of captures	No. times located in uplands (%)	No. times located in lowlands (%)	No. times located in burrow/ shelter	Closest distance to uplands (km)
1	M	27	14 (51.9%)	13 (48.1%)	19	-
2	F	29	2 (6.9%)	27 (93.1%)	18	-
3	F	11	0 (0%)	11 (100%)	8	2.09
7	F	25	0 (0%)	25 (100%)	18	0.64
8	M	24	0 (0%)	24 (100%)	17	0.64
9	F	11	5 (45.5%)	6 (54.5%)	6	-
10	M	10	0 (0%)	10 (100%)	8	0.13
11	F	24	0 (0%)	24 (100%)	20	0.48
12	F	26	22 (84.6%)	4 (15.4%)	17	-
13	M	25	23 (92.0%)	2 (8.0%)	18	-
28	F	7	0 (0%)	7 (100%)	6	0.24
31	F	6	3 (50.0%)	3 (50.0%)	6	-

Table 4. Classification accuracy of a tortoise distribution model for Joshua Tree National Park (Barrows, 2011) validated against our location data for live desert tortoises, active tortoise burrows, and the remains of desert tortoises found at the Cottonwood study site. According to the model, any locations with a value greater than or equal to 0.7 within the model are considered to be in optimal desert tortoise habitat. Locations with values less than this fall outside optimal desert tortoise habitat and are assigned as -9999, or no value.

Classification type	Correctly classified (≥ 0.7)		Incorrectly classified (-9999)		Total	
	No.	%	No.	%	No.	%
Tortoises	119	64%	68	36%	187	100%
Burrows	127	67%	63	33%	190	100%
Tortoise shells	13	76%	4	24%	17	100%

Table 5. Clutch sizes for female Agassiz's desert tortoises captured and X-rayed in Cottonwood study area. Dashes indicate no clutch was produced.

ID	Year	Size 1st Clutch	Size 2nd clutch	Difference in clutch size*
2	2015	4	5	+1
	2016	5	2	-3
3	2016	1	-	-
7	2015	3	3	0
	2016	3	5	+2
9	2015	5	3	-2
11	2015	-	-	-
	2016	-	-	-
12	2015	3	3	0
	2016	3	5	+2
28	2016	3	4	+1
31	2016	6	6	0

*Difference in size of second clutch from size of first clutch

Table 6. Summary statistics (mean ± SD) of reproductive characteristics of female *Gopherus agassizii* inhabiting the Cottonwood study area. Carapace length, clutch frequency and annual egg production are calculated only from females that reproduced.

Year	*n**	Carapace length (cm)	Clutch size		Clutch frequency	Annual egg production	XREW in mm		Percent Reproductive females	CV clutch size/ XREW†
			First	Second			First Clutch	Second Clutch		
2015	5	22.7 ± 1.64 (4)	3.75 ± 0.96 (4)	3.50 ± 1.00 (4)	2.00 ± 0 (4)	7.25 ± 1.50 (4)	35.85 ± 1.08 (15)	35.59 ± 1.04 (14)	80	0.25/ 0.03
2016	7	21.9 ± 0.75 (6)	3.50 ± 1.76 (6)	4.40 ± 1.52 (5)	1.83 ± 0.41 (6)	7.17 ± 3.54 (6)	37.07 ± 1.90 (21)	36.94 ± 1.39 (22)	86	0.42/ 0.04

Annual egg production indicates the mean total number of eggs produced by reproductive tortoises within a year. Sample sizes used in statistical analyses are indicated in parentheses. Coefficients of variation (CV) are given for both clutch size and XREW.
**n* indicates the number of female tortoises who were located and X-rayed during the reproductive season.
†Coefficients of variation calculation for all clutch sizes and all egg sizes within a year.

Figure 1. Habitat Suitability Model for desert tortoise in the eastern CVMSHCP Desert Tortoise and Linkage Conservation Area. Inset image depicts location within the state of California.

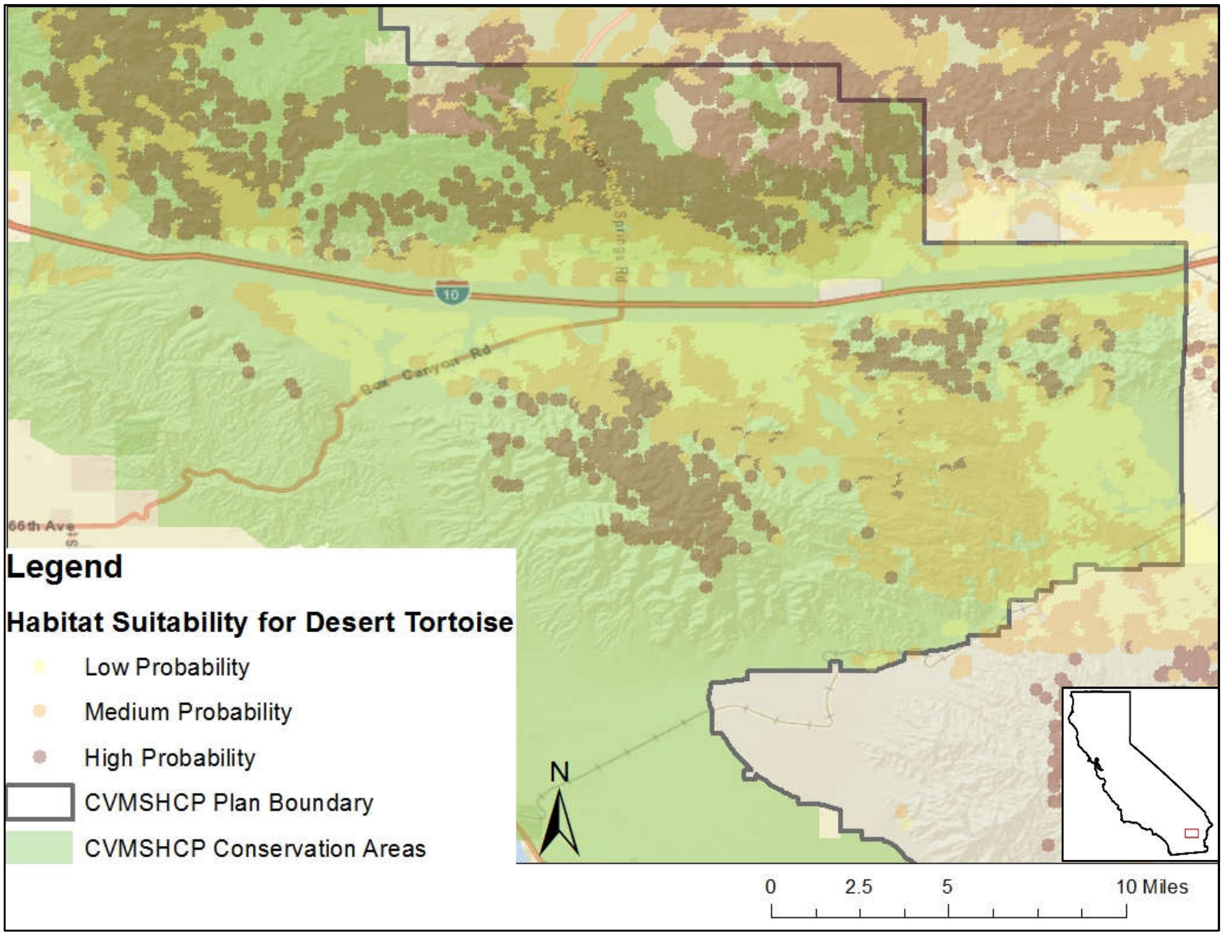

Figure 2. Map of southern Joshua Tree National Park. Polygon depicts approximate footprint of Cottonwood study area, and green border approximates the southern boundary of Joshua Tree National Park. Inset image depicts location within the state of California.

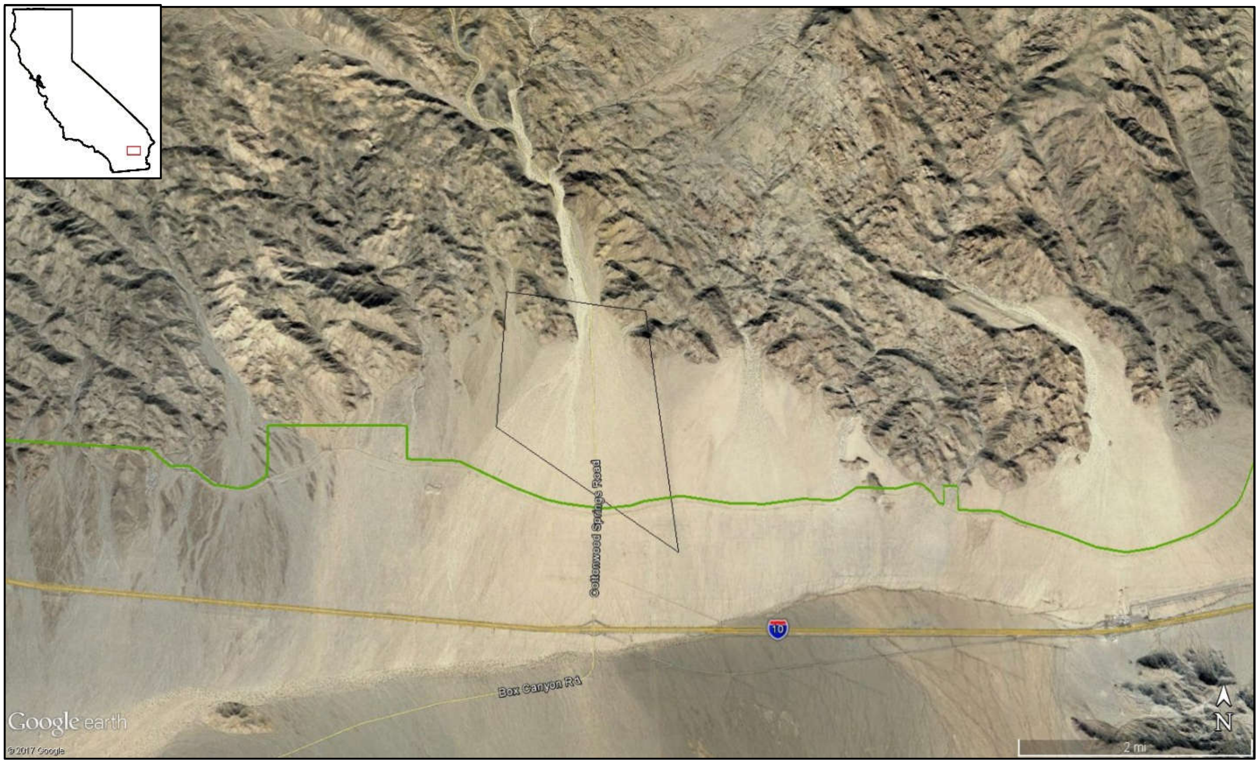

Figure 3. Frequency accumulation curve of the accumulated number of tortoises located over time. Time is measured in days from 30 March 2015 (the first day spent surveying for desert tortoises at Cottonwood), inclusive. The logarithmic curve shows that the number of new tortoises located over time begins to taper, suggesting that more time spent at this study site would not continue to yield the location of many additional new tortoises.

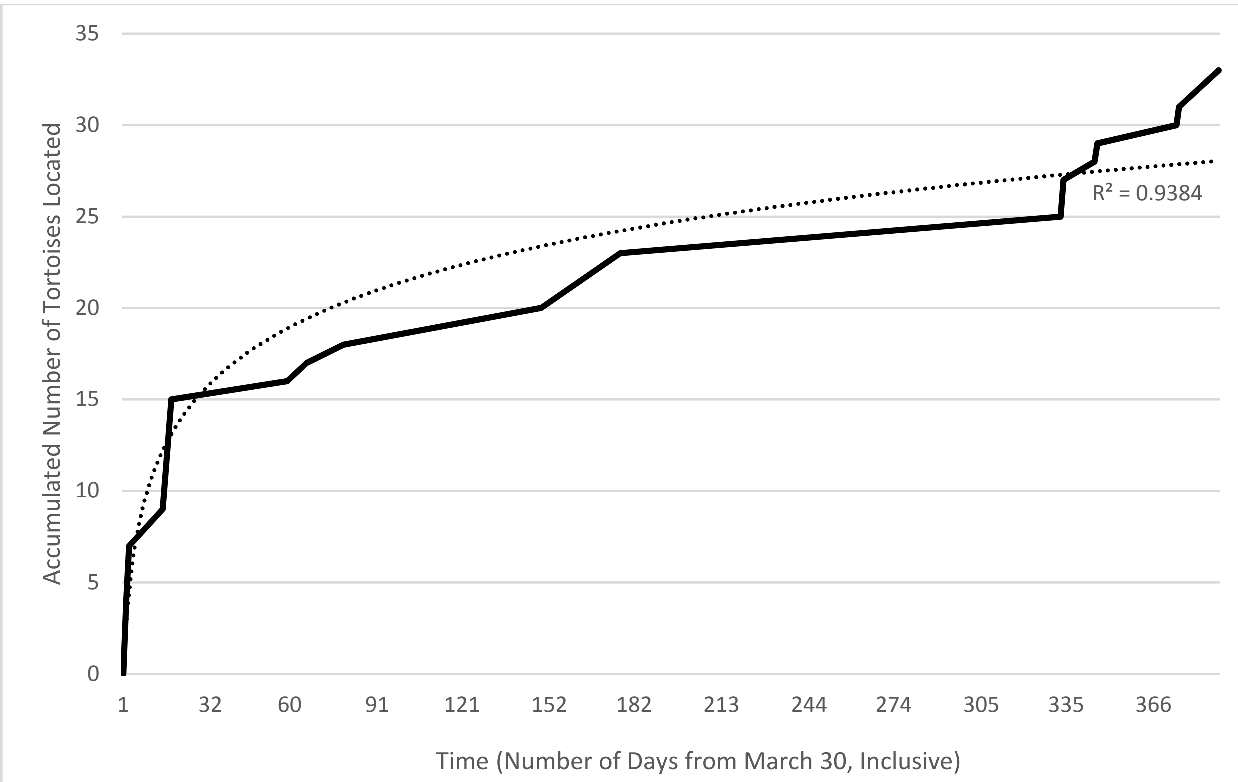

Figure 4. X-radiograph taken on 3 June 2015 showing overlapping clutches forming in female #2. The four thickly shelled eggs in the center were first seen on 14 April, with the five thinly shelled eggs on the periphery appearing over one month later on 27 May. The bright white attachment to the upper right of the tortoise's carapace is the transmitter and antenna. A penny is located at the upper left corner of the X-radiograph for size calibration.