Desert Tortoise Nesting Study on the BCCE

Final Project Report

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EXECUTIVE SUMMARY

During 2021, the Clark County Desert Conservation Program had contracted with University of Nevada, Reno (Reno, NV) to document reproduction rates of resident and translocated tortoises at the Boulder City Conservation Easement (Boulder City, NV). This project addressed one of the strategic elements listed in the 2011 Revised Recovery Plan for the Mojave Population of the Desert Tortoise (*Gopherus agassizii*): to augment depleted populations of desert tortoises through a strategic program and to monitor those populations to be sure that the augmentation has the desired effects. The University of Nevada, Reno monitored 22 female *G. agassizii* from April–July 2021 for reproduction, monitored nests until hatching, and collected blood samples from all encountered individuals (females, males, juveniles, and hatchlings from known mothers).

INTRODUCTION

DESCRIPTION OF THE PROJECT

The Clark County Desert Conservation Program ("DCP", Nevada) has augmented the Mojave desert tortoise (*Gopherus agassizii*) population of the northeast portion of the Boulder City Conservation Easement ("BCCE", Boulder City, Nevada) by translocating individuals from a captive facility (Desert Tortoise Conservation Center, Las Vegas, Nevada) or from construction sites beginning in 2014. As part of the conservation of this priority species under the Multiple Species Habitat Conservation for Clark County, and in order to monitor the success of the augmentation, DCP contracted with the University of Nevada, Reno (Reno, Nevada) to document reproduction and collect genetic samples during 2021.

Background and Need for the Project

Population augmentation is one method recommended in the 2011 Revised Recovery Plan for the Mojave Population of the Desert Tortoise to ensure persistence of this threatened species. Where population augmentation is performed via translocation of individuals, post-translocation monitoring should assess the relative success of the intervention. If the augmentation is functionally adding to the population, then reproduction of translocated individuals should be able to be documented. The DCP is seeking to document whether reproduction of translocated males and females is occurring at the BCCE, and is interested to compare reproduction rates between translocated and resident individuals.

Management Actions Addressed

The establishment and management of the BCCE is identified in the Clark County Multiple Species Habitat Conservation Plan as a conservation action in part to protect habitat for *G. agassizii*. Past translocations have occurred at the site since 2014, and continued monitoring of the ecology and behavior of the translocated and resident tortoises provide important research data regarding the success of such population augmentation efforts. This project addressed the need to investigate the paternal contribution of translocated tortoises to future clutches at the translocation site as an indicator of fitness of translocated male tortoises.

Goals and Objectives of the Project

The purposes of this project were to determine basic reproductive statistics for tortoises on the BCCE and to collect blood samples from hatchling tortoises for future genetic analyses. Project goals were as follows:

1. To determine clutch frequency and clutch size of translocated and resident female tortoises monitored with VHF radio telemetry on the BCCE in 2021.
2. Collect blood samples from all hatchlings from monitored nests and up to 100 incidentally encountered tortoises to enable them to be used in a genetics and paternity study in the future.

METHODS AND MATERIALS

PROJECT LOCATION

We conducted work in the northeastern section of the BCCE in southern Nevada in 2021. This section is generally located south of Boulder City, NV, east of US Highway 95, and north of State Highway 165, and our particular area within this section consisted of approximately 19,000 ha. (Figure 1). Work was conducted under U.S. Fish and Wildlife Service Recovery Permit TE-50049D-2, Nevada Department of Wildlife Scientific Collecting Permit License Number 40292, University of Nevada, Reno Institutional Animal Care and Use Committee Protocol 20-08-1068, and University of Nevada, Las Vegas Institutional Animal Care and Use Committee Protocol 1730688-2.

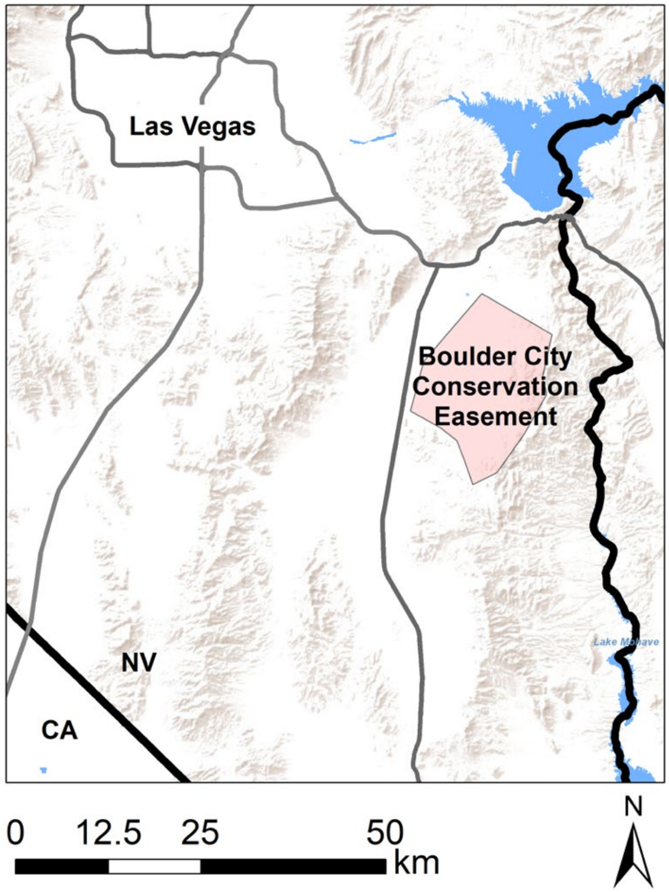

Figure 1. Location of field site within the northeastern section of the Boulder City Conservation Easement for Mojave desert tortoise (*Gopherus agassizii*) research within Clark County, Nevada in 2021.

Study Animals

When field work for this project commenced in April 2021, *G. agassizii* study animals with transmitters were actively being monitored by colleagues at the Great Basin Institute (GBI). We encountered all live study animals at least once during the 2021 field season, and regularly monitored a subset of females for reproduction (N = 22). We also processed tortoises encountered incidentally for blood sample collection. Successfully located clutches (N = 2) were transferred to the University of Nevada, Las Vegas Animal Facility (UNLV) where they were incubated until hatching, and the hatchlings were released at their nest site. The sex of adult tortoises was determined by external morphometric characteristics. Any unmarked tortoises (i.e., not previously encountered and marked by any previous researchers) were uniquely marked with a paper ID tag during the encounter.

MONITORING AND SAMPLING

Monitored female *G. agassizii* had already been outfitted with a VHF radio transmitter by GBI. After discussion with GBI, we located and incorporated an additional three resident adult female *G. agassizii* into our study to increase the number of monitored resident females. We attached a VHF radio transmitter to these individuals and a unique ID tag. For all adult females monitored for reproduction, we attached an aluminum casing that held an archival GPS datalogger (iGotU, MobileAction, New Taipei City 231, Taiwan) that recorded locations every 10–30 min. These loggers were replaced during relocations as needed until the reproductive season ended in July, at which point they were removed with the casing. We performed ultrasounds (Rostal et al. 1994) or coelomic cavity palpations every 10–20 days to detect the presence of shelled eggs. Once shelled eggs were detected, we revisited and weighed tortoises daily until mass loss indicated egg laying. We then searched for the nest using the GPS logger data and caged the nest. We allowed 60 days to elapse so as to ensure that embryonic sex had been determined, then excavated the eggs and transported them to UNLV for incubation until hatching. Eggs that were determined to be nonviable by candling were necropsied and liver samples collected, frozen, then later thawed for drying for genetic samples. Hatchlings that successfully emerged from the egg were held up to seven days to allow time for processing before being returned and released at the nest site.

In order to provide blood samples for future paternity analyses, we collected a blood sample via venipuncture of the subcarapacial cranial plexus (Bonnet et al. 2002, Hernandez-Divers et al. 2016) from study animals and from all incidentally encountered tortoises. Blood samples (0.05–0.3 mL, without liquid heparin) were collected onto GenSaver 1.0 cards (GenTegra LLC, Pleasanton, CA) and temporarily stored at the U.S. Geological Survey Henderson Field Office (Henderson, Nevada) until all samples were compiled and transferred to DCP. At the time of sample collection, we performed a visual health assessment (USFWS 2016) and took photographs to document condition and any apparent disease symptoms or injuries.

Carcasses of young juveniles likely to have hatched after translocation were collected and stored dry for later paternity analyses. Any individual that voided its bladder was rehydrated via soak for 30 min.

Statistical analyses

All analyses were performed in R version 4.1.1 (R Core Team 2021). We set the Type I error rate (α) at 0.05 for all statistical tests. A Fisher exact test was used to compare the proportion of resident versus translocated females that possessed follicles. The same test was used to compare the number of translocated versus resident individuals with shelled eggs. Similarly, the lack of any clutches from resident females precluded any comparison of hatching success between translocated and resident females.

RESULTS AND EVIDENCE OF THE RESULTS

There was no difference in the proportion of translocated versus resident females that possessed follicles (N = 12/14 translocated with follicles, N = 6/7 resident with follicles; *p*-value = 1; Table 1). There was no difference between the number of translocated versus resident females that produced shelled eggs (N = 2/14 translocated with shelled eggs, N = 0/7 resident with shelled eggs; *p*-value = 0.533; Table 1). We did not observe any individual lay a second or third clutch.

We successfully located the nests from the two translocated individuals that possessed shelled eggs (Table 1). One nest contained one egg that ceased development at a late stage. The second nest contained six eggs, of which four hatched. One egg in this nest ceased development at a late stage and was missing its right forelimb with distinctly underdeveloped pectoral muscles. The other failed egg in this nest displayed no development and was likely unfertilized. Clutch size for translocated females was 1–6 eggs, and observed hatching success was 0–67%.

We collected blood samples from 51 individuals. We found one individual dead, and notified GBI of this find so that they could report the incident to the appropriate permitting parties. GBI notified us that they found two of our regularly monitored females dead (Table 1). We collected eight juvenile carcasses for later genetic analyses.

Table 1. Reproduction by resident and translocated adult female Mojave desert tortoises (*Gopherus agassizii*) at the Boulder City Conservation Easement in Clark Co., NV in 2021. Asterisk (*) indicates individual was found dead during the nesting season.

Tortoise ID	Residency	Follicles	Shelled eggs	Clutch size
CC0111	translocated	yes	no	-
CC0157	translocated	no	no	-
CC0602	resident	yes	no	-
CC0603	resident	yes	no	-
CC0605	resident	no	no	-
EV3834	translocated	yes	no	-
EV3951	translocated	yes	no	-
EV3966	translocated	yes	no	-
EV4064	translocated	yes	yes	1
EV4184	translocated	no	no	-
EV4238	translocated	yes	no	-
EV4242	translocated	yes	no	-
EV4243*	translocated	yes	no	-
EV4249	translocated	yes	yes	6
EV4257	translocated	yes	no	-
EV4259	translocated	yes	no	-
EV4269*	resident	yes	no	-
FW7816	resident	yes	no	-
FW9425	translocated	yes	no	-
FW9721	resident	yes	no	-
FW9995	resident	yes	no	-

EVALUATION/DISCUSSION OF RESULTS

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All of Clark County had entered moderate (“D1”) drought conditions by August 18, 2020, with over 75% of the county experiencing at least severe drought (“D2”). By September 15, over 70% of the county was experiencing at least extreme drought (“D3”), and 100 % of the county was categorized as experiencing exceptional drought ("D4") by December 15, 2020. This situation continued until the last week of July 2021 (North American Drought Monitor, Lawrimore et al. 2002). Drought conditions during vitellogenesis in the fall have previously been shown to affect the reproductive output of females in the spring and summer of the subsequent year (Lovich et al. 2015, Wallis et al. 1999), and indeed, we observed an unusually low number of individuals that we confirmed to have had shelled eggs. Our results appear even more extreme than previously observed, however, as *G. agassizii* are considered to practice a "bet hedging" strategy in which individuals will likely produce eggs despite suboptimal conditions, although the number of eggs produced in a drought year is likely to be lower. Our results show that when extreme drought occurs during vitellogenesis followed by exceptional drought extending during spring emergence and through the nesting season, individual *G. agassizii* may abandon the bet hedging strategy and instead forgo nesting entirely.

CONCLUSION

We were able to meet our first goal to document clutch frequency and clutch size of *G. agassizii* at the BCCE in 2021. The fall of 2020 and the nesting season of 2021 were unusual in that the site experienced extreme to exceptional drought conditions. Thus, we cannot consider our results from this year to be "typical" of individuals at this site in any given year. However, it is worth noting that under such unusual conditions, both translocated and resident females appeared to respond similarly in terms of the proportion of individuals in either group that possessed follicles and that reproduced.

We were only partially able to meet our second goal to collect blood samples for future paternity analyses. Our sample size of 2021 hatchlings was extremely low because of the aforementioned unusual drought conditions, and, even if supplemented by genetic analysis of several juvenile carcasses from clutches from previous years, cannot be used to make any conclusions about the relative fitness of translocated versus resident males. We were successful, however, in collecting blood samples from all known 2021 mothers and all other monitored translocated and resident females, as well as all live monitored males and additional incidentally encountered individuals. Depending on the chosen analysis for paternity, 100% sampling of potential translocated fathers and sampling a high percentage of the potential resident fathers is vital for confidence in paternity assignment (with the ability to eliminate mothers informed by similar representative sampling of females).

RECOMMENDATIONS

We recommend the use of GPS dataloggers using a ≤15 min recording interval on females as a method to locate nests. The shorter recording interval improves fix accuracy and permits better filtering of potential nest locations using the duration of time that the tortoise spent at any one spot.

The low number of genetic samples from young of this year will not yield sufficient sample size to make conclusions about fecundity of translocated versus resident males. Although these paternity analyses are not part of this project, we advise that the DCP consider an additional year of nest monitoring in order to improve the ability to form conclusions related to its broader research goals at this site. We have banked many samples from tortoises with and without transmitters at the site; these banked samples should reduce the need for more intensive collection efforts from resident tortoises if future nest monitoring is performed.

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