GENETIC ORIGINS AND POPULATION STATUS OF DESERT TORTOISES IN ANZA-BORREGO DESERT STATE PARK, CALIFORNIA: INITIAL STEPS TOWARDS POPULATION MONITORING

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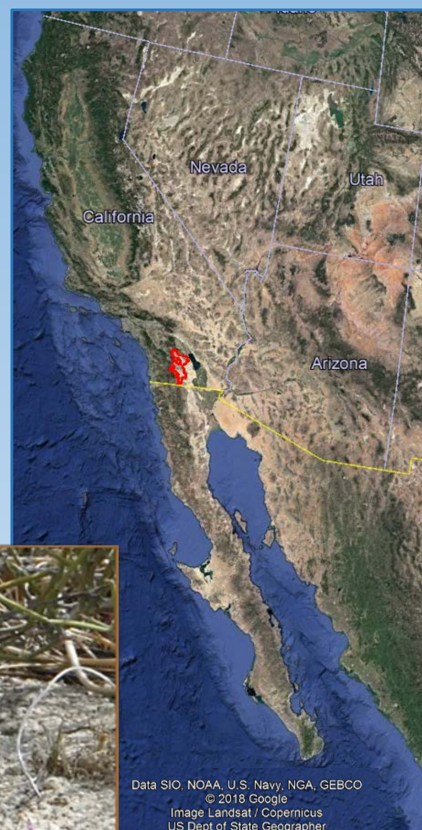

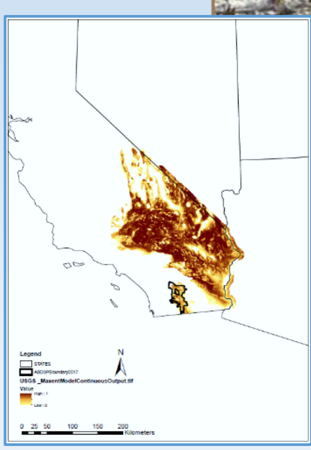

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Genetic Origins and Population Status of Desert Tortoises in Anza-Borrego Desert State Park, California: Initial Steps Towards Population Monitoring

Final Report

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FOREWORD

The Desert tortoise (*Gopherus* sp.) was formally reported to science in 1861, and became the official California state reptile in 1972. Recent studies reveal three species, the Mojave desert tortoise (*G. agassizii*), Sonoran Desert tortoise (*G. morafkai*), and Sinaloan desert tortoise (*G. evgoodei*) (Murphy et al. 2011, Edwards et al. 2016; Figure 1). Range-wide declines in the Mojave desert tortoise population led California to prohibit the collection of this species in 1961. Despite this, it was emergency listed as federally endangered and state listed as threatened in 1989, and subsequently listed as federally threatened in 1990 (Federal Register 55, No 63, 50 CFR Part 17). The population in the Colorado Desert Recovery Unit continued to decline, with a 36% decline (a loss of 37,578 ± 11,006 tortoises) between 2004-2014 (Allison and McLuckie 2018). The Mojave desert tortoise is also considered vulnerable by the International Union for Conservation of Nature (IUCN), and trade controlled under the Convention on International Trade in Endangered Species of Wildlife Fauna and Flora (CITES).

The Mojave desert tortoise is limited to only 4 park units within the California State Park System [exclusively in Red Rock Canyon State Park (Berry and Bailey 2008), Providence Mountains State Recreation Area, Picacho State Recreation Area, and Anza-Borrego Desert State Park (ABDSP)]. Despite the long-held view that ABDSP is situated outside of what is currently considered to be the tortoise's native range (Luckenbach 1976, Patterson 1976, Stebbins, 1985), literature searches and interviews with former park employees conducted by the author reveal the following: (1) paleontological records and photographs and articles from the 1930s suggest that tortoises were present in the area prior to and after the park was established (Wade 1937, Lindsay 2005), and (2) releases of tortoises into the park were documented as early as 1958, with at least 65 individual desert tortoises being released into the park in 1971-72, which mirrors known releases elsewhere across the Mojave Desert. In an effort to identify the current population status and prepare baseline biological information from which to develop a long-term desert tortoise monitoring program, the California Department of Parks and Recreation's Colorado Desert District initiated monitoring and research in 2017.

This document presents the findings from the first two years of desert tortoise research and monitoring in ABDSP, including an in-depth historical account, the establishment and maintenance of an incidental tortoise sightings database, an assessment of habitat suitability, novel genetic testing to determine genetic origins, a framework for population monitoring, estimates of demography, disease prevalence, and reproductive status, as well the identification of possible threats and past and present conservation actions. In line with California Department of Parks and Recreation policy, this document presents scientific information intended to inform resource management. It is written in the passive voice to achieve the thematic structure of highlighting the Department's desert tortoise research and monitoring in ABDSP, rather than highlighting individual author contributions.

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INTRODUCTION

Desert tortoises (*Gopherus spp*.) are long-lived, medium-sized terrestrial reptiles in the Testudinidae family that have occupied the Mojave, Colorado, and Sonoran Deserts of the United States and mainland Mexico for millions of years (Fritts and Jennings 1994, Grover and DeFalco 1995, Berry et al. 2002a). The Desert tortoise was formally reported to science by James G. Cooper during a California Academy of Natural Sciences meeting in 1861, and published in the subsequent Proceedings. Recent studies reveal three species, the Mojave desert tortoise (*G. agassizii*), Sonoran Desert tortoise (*G. morafkai*), and Sinaloan desert tortoise (*G. evgoodei*) (Murphy et al. 2011, Edwards et al. 2016; Figure 1). Range-wide declines in the Mojave desert tortoise population led California to prohibit the collection of this charismatic species in 1961. The desert tortoise became the official California state reptile in 1972. However, this population further decreased by 90% after the 1980s due to various factors, leading to an emergency listing as federally endangered and state listing as threatened in 1989. Subsequently, the Mojave population was listed as federally threatened in 1990 (Federal Register 55, No 63, 50 CFR Part 17), although this population experienced a 37% decline (a loss of 124,050 ± 36,062 tortoises) across its range between 2004-2014 and a 36% decline (a loss of 37,578 ± 11,006 tortoises) in the Colorado Desert Recovery Unit during that same time period (Allison and McLuckie 2018). The Mojave desert tortoise is also considered vulnerable by the International Union for Conservation of Nature (IUCN), and trade controlled under the Convention on International Trade in Endangered Species of Wildlife Fauna and Flora (CITES). The Mojave desert tortoise is limited to only 4 park units within the California State Park System [exclusively in Red Rock Canyon State Park (Berry and Bailey 2008), Providence Mountains State Recreation Area, Picacho State Recreation Area, and Anza-Borrego Desert State Park (ABDSP)]. This document establishes the baseline biological information on desert tortoises in ABDSP, and is intended to inform long-term monitoring and conservation actions.

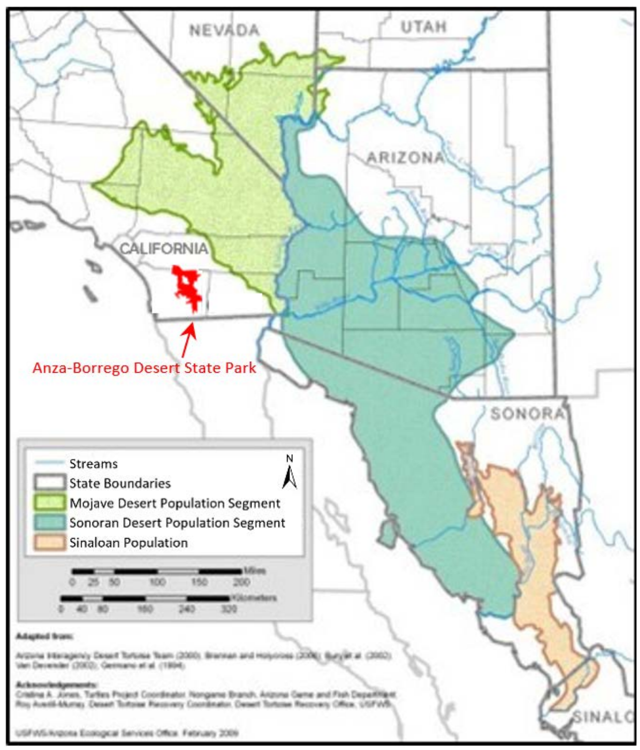


Figure 1. Geographic ranges of North American desert tortoises, and location of Anza-Borrego Desert State Park, California. Adapted from http://www.swparc.org/resources/desert-tortoise/

Adult male and female desert tortoises are sexually dimorphic; adult males have a gular horn located at the anterior end of the plastron, shorter claws, longer and thicker tails, a concave plastron, and pronounced chin glands (Boarman 2002b). Desert tortoises exhibit delayed sexual maturity, with the majority of individuals becoming reproductively active at 12-20 years of age, which is when they are about 8.2 inches (208mm) long (median carapace length, MCL), although some reproduce as young at 12-15 years [7.4 inches (180mm); Turner and Berry 1984, Turner et al. 1986]. Typical lifespan is expected to be 25-35 years in the wild, but can reach 70+ years (Germano 1992, 1994). Females lay ≤3 clutches of hard-shelled eggs per year, with an average clutch size of 4.5 eggs (range 1-8; Turner et al. 1986). Most eggs are laid in sandy or friable soils often at burrow entrances in spring (Apr-Jun in the Mojave Desert) and occasionally in fall (Sep-Oct), and parents do not tend eggs or young. Akin to elephants, whales, and rhinos, the interaction of these K-selected natural history traits cause tortoise populations to recover slowly from population declines (MacArthur and Wilson 1967). Consequently, it is widely recognized that high juvenile survival (75-98%) is required to ensure population stability or growth (Congdon et al. 1993). Interestingly, the sex (gender) of tortoises is environmentally controlled during incubation (i.e., natural soil temperature; Spotila et al. 1994). Incubation (i.e. soil) temperatures >89.3^{o} F (31.8^{o} C) produce female hatchlings, and lower temperatures produce male hatchlings (Spotila et al. 1994). Because of this sensitivity to incubation temperature, Boarman (2002c) postulated that tortoise populations are likely vulnerable to changes in soil temperature due to changes in vegetation cover and warming climatic conditions.

Box 1 – DPR DOM Section 0311.2 General Animal Management Policy

It is the policy of the Department to implement park acquisitions and resource, facility, and visitor use management strategies that foster long-term sustainability of natural animal populations and the processes that influence the dynamics of animal populations. In managing animals and animal habitats, the Department will:

a. Preserve, protect and restore the natural abundance, diversity, dynamics, distributions, habitats, and behaviors of native animal populations and the communities and ecosystems in which they occur, including State and federally-listed threatened, endangered, or otherwise sensitive species;

b. Maintain functional linkages to other natural areas in order to sustain populations;

c. Restore native animal populations in parks where they have been extirpated by past human-caused actions;

d. Minimize negative human impacts on native animals, populations, communities, and ecosystems, and the processes that sustain them while providing opportunities for the public to experience animals native to California; ...

Resource management within the California State Parks System is guided by California law, proclamations, and executive orders, as well as the California Code of Regulations (CCR), California State Park and Recreation Commission, and Department Notices and policies defined within the California Department of Parks and Recreation (DPR) Operations Manual (DOM). The DOM is an official publication of the DPR, with its contents approved and published at the order of the Director, and it contains specific policy for general animal management (Box 1). As stated in Section 5019.53 of the California Public Resources Code, the purpose of California State Parks is to preserve outstanding natural, scenic, and cultural values, indigenous aquatic and terrestrial fauna and flora, and the most significant examples of ecological regions in California. As such, it is important to recognize that not only do some of the desert environments in ABDSP support tortoises, but there is evidence that desert tortoises themselves contribute to the ecology of an area by constructing burrows that are used by many indigenous animals, including the banded gecko, burrowing owl, cactus wren, poorwill, roadrunner, kangaroo rat, cottontail rabbit, and jackrabbit (Woodbury and Hardy 1948).

Figure 2. Anza-Borrego Desert State Park, California.

Each State Park is to be managed as a composite whole in order to restore, protect, and maintain its native environmental complexes to the extent compatible with the primary purpose for which the park was established. Anza-Borrego Desert State Park was established in 1933, and lies within the DPR’s Colorado Desert District (CDD) (Approximate centroid: 33°07’03.83”N 116°19’25.05”W; Figure 2). Containing roughly 1,000 square miles, the purpose of ABDSP is to preserve the unique and diverse natural, cultural, and scenic resources of this Western Colorado Desert Region and to provide opportunities for high quality recreation that supports a healthy natural environment (ABDSP General Plan 2005). Species designated as threatened or endangered of becoming extinct, such as the desert tortoise, not only are important components of healthy natural environments, but also are afforded special protection and warrant special consideration under conservation and management planning.

Anza-Borrego Desert State Park follows various guidelines established in the ABDSP General Plan (2005) to protect the native biota, including the preservation of sensitive species and habitats to encourage their recovery (Guideline – Biota 1a). The park also works towards identifying situations where species are rare or rapidly declining and to develop methods to protect such species and/or their habitats (Guideline – Biota 1b). Despite the long-held view that ABDSP is situated outside of what is currently considered to be the tortoise’s native range (Luckenbach 1976, Patterson 1976, Stebbins, 1985; Figure 1), paleontological records, as well as photographs and articles from the 1930s suggest that tortoises were present in the area prior to and after the park was established (Wade 1937, Lindsay 2005). Of equal interest are documented releases of tortoises into the park as early as 1958, with at least 65 individual desert tortoises being released into the park in 1971-72 (Appendices A, B, and C). It is unknown whether these were *Gopherus agassizii*, *G. morafkai*, and/or *G. evgoodei*, stimulating interesting questions about the origins of the current tortoise population, such as whether it is comprised entirely of introduced individuals or a mixture of introduced and native individuals that originated from a remnant native population. Other than incidental sightings by park visitors, little information about the current status of these desert tortoises in ABDSP is known (Appendix D).

Research and monitoring designed to increase understanding of resources and ecological processes are foundational to data driven resource management necessary for achieving the DOM's special animal policies in parks (Boxes 1, 2, & 3). In an effort to identify the current status and prepare baseline biological information from which to develop a long-term desert tortoise monitoring program, the CDD Natural Resource Program began conducting surveys for and targeted research on desert tortoises in select areas of ABDSP in 2017. The overall purpose of these efforts was to identify the species, genetic origins, genetic relatedness, presence of hybridization and/or introgression, distribution, abundance, reproductive status, and disease prevalence throughout the park. An additional goal was to identify potential threats to tortoises, as well as develop a long-term population monitoring approach. The park also engages in recovery actions, including habitat improvement and protection.

Box 2 – DPR DOM Section 0311.5.2.1 Special Animal Policy
It is the policy of the Department to protect species listed under the federal or state endangered species acts that are native to State Park System units. The Department will conserve listed species and avoid detrimental effects by:
a. Participating in the recovery planning process;
b. Working with other agencies to help ensure that any formal delineation of critical habitat, essential habitat, and/or recovery areas on State Park System lands is compatible with State Park System management goals; and
c. Cooperating with responsible state and federal agencies to support the protection and recovery of listed species by maintaining the species and the habitats upon which they depend and reducing negative impacts when feasible.

Box 3 – DPR DOM Section
0311.5.2.2 Knowledge of Special Animal Localities
The Department will strive to maintain a working knowledge of the occurrence of listed and other special species occurring within park units.
... Information on location of sensitive species will not be generally available to the public if the information could lead to disturbance to the animal or increased threat of take, such as through collection. ...

HISTORICAL ACCOUNT

This historical account of desert tortoises in ABDSP stems from searches of museum records (Museum of Vertebrate Field Zoology, San Diego Natural History Museum, Los Angeles Natural History Museum, and the Stout Research Center paleontological records of Anza-Borrego Desert State Park), newspapers, magazines, web-based scientific literature and book search engines, US Fish and Wildlife Service and California Department of Fish and Wildlife records, DPR CDD records, and interviews with retired DPR CDD and ABDSP personnel conducted by the author. These accounts establish that the desert tortoise was present in the vicinity of ABDSP before and after the park was established in 1933.

Before Establishment of Anza-Borrego Desert State Park

Little is known about the status of tortoises in the ABDSP region prior to the park becoming established in 1933. As part of this historical account for the pre-establishment period, paleontological and cultural records were examined to help determine if the environment historically and prehistorically supported tortoises. Albeit paleontological records can inform whether tortoises roamed the ABDSP area millions of years ago, tortoise fossils in the ABDSP area can pose additional interpretative challenges because the southern Californian and northern Baja California regions rest on the Baja Tectonic Microplate, which has been moving northwest along the San Andreas Fault with respect to the stable North American Plate since 6.5 million years ago (i.e., the Miocene; Oskin and Stock 2003; Figure 3). Consequently, at about 8 million years ago, today's ABDSP was south of 32^{o} N and geographically connected to Sonora, Mexico (Figure 3) and what is now considered the Sonoran Desert Tortoise range (Figure 1). By 5.3 million years ago, westward movement away from the North American Plate separated the ABDSP region from Sonora with the Gulf of California. By 3 million years ago, ABDSP had moved north of 32^{o} N and terrestrially reconnected with the North American Plate just west of what is currently considered the Mojave desert tortoise range (Figure 3). Such spatial shifts and distinctly separate geographic connections to two different species' ranges raise fascinating questions about the origins of living and fossil tortoises found in ABDSP today.

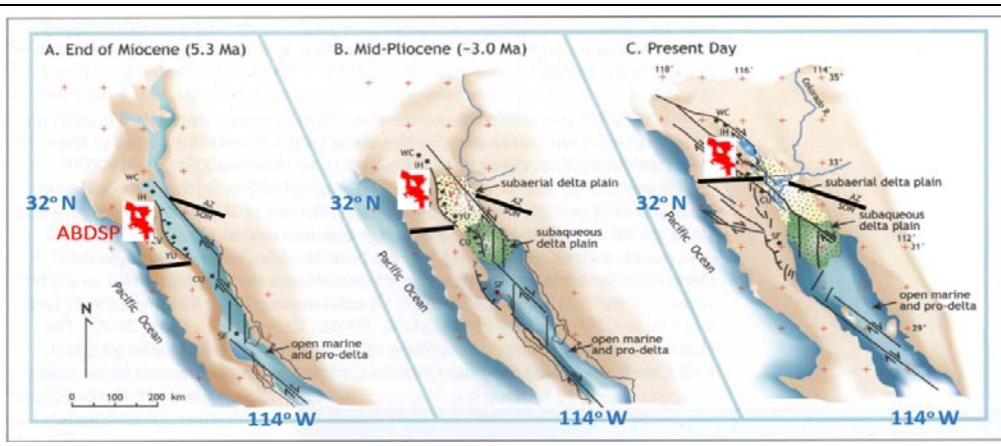


Figure 3. Paleogeographic reconstructions of sedimentary basins and faults in the Salton Trough and northern Gulf of California since the end of Miocene time. Modified from Dorsey (2006).

Paleontological records of fossil tortoises in Anza-Borrego Desert State Park

Lyndon K. Murray, Ph.D., District Paleontologist, California DPR, Colorado Desert District

The North American fossil ancestry of the related large land tortoises of family Testudinidae; subfamily Xerobatinae (Meylan and Sterrer, 2000), extends at least to the early Eocene (47.8-56 Ma). *Hesperotestudo* (a giant tortoise) and *Gopherus* are distinct genera recognized from fossil specimens of the Oligocene (about 23-28 Ma; Biewer et al. 2016, Reynoso and Montellano-Ballesteros 2004). Pleistocene records of one or both tortoises have been reported from the southern parts of Arizona, California, Nevada, and New Mexico, western Texas, and in northern Chihuahua and western Sonora, Mexico (Harris 2008-2016). *Hesperotestudo* became extinct near the end of the Pleistocene (about 0.01-0.24 Ma). The genus *Gopherus* is extant, although genus and species assignments have undergone significant reorganization in recent decades (see introduction of this report for recent species). This is due to an increase in new fossil discoveries, re-evaluation of previously identified fossils based on apomorphic character analyses, and genetic studies of turtle phylogeny.

Turtle bones are ubiquitous in the non-marine fossil-bearing sediment beds of ABDSP. They appear in nearly every depositional environment within the >2,500 m-thick section of terrestrial sediments laid down at the western edge of the Salton Trough, between about 0.5 and 3.8 Ma (Megaannum, million years ago). Extensive exposure and weathering of skeletal materials prior to burial in braided stream channels led to disarticulation, breakage, and scattering of most ABDSP fossil bones. Due to differential shape and internal structure of turtle skeletal elements, identifiable skull and limb fossils are not often found (thin or elaborate shapes break readily into very small pieces) as opposed to more robust shell elements, which are thick and flat. Consequently, turtle fossils are typically found as fragments of individual carapace and plastron bones, which are used to identify tortoise specimens to family or genus levels in ABDSP.

The CDD has an active paleontology program that maintains the Stout Research Center paleontology collections. As with most museum collections, the catalogued fossil specimens contain original documentation (catalogue records dated between 1954 and 2018), with identifications based on the best taxonomic and phylogenetic information available at the time (Murray 2008, Murray and Jefferson 2011). The curatorial process includes preliminary identification of each specimen to some level of taxonomic identity, which is often carried out by assistants-in-training. The confidence of these preliminary identifications is reasonably good because turtle bone in the ABDSP region is visibly distinct from mammal bone, enabling

Hesperotestudo reconstruction, from Fossil Treasures of the Anza-Borrego Desert (Jefferson and Lindsay 2006). Artwork by John Francis.

Excavation (2010) of *Hesperotestudo* fossil ABDSP 3551/V9225. ABDSP District Paleontologist (retired 2011) George Jefferson, paleontology volunteers Linda Gilbert, Sandra Keeley, Robert Keeley. Photo by ABDSP paleontology volunteer Jon Gilbert.

identification to "Testudines" or "Chelonia" (Carroll 1988, Romer 1966). Moreover, bones from adult giant tortoises are readily separated from all other local turtle material based on the significant size difference. Also, the identification of catalogued specimens is sometimes refined when formally studied and published, as is the case with many specimens in the ABDSP collection. For example, visiting tortoise researchers have made preliminary assignments of many of the tortoise fossils to the genera *Hesperotestudo* and *Xerobates* (Jolly, 2000). *Xerobates* is a monophyletic group consisting of *Gopherus agassizii*, *G. berlandieri*, *G. morafkai*, and *G. evgoodei* (Lamb and Lydeard 1994, Meylan and Sterrer 2000, Reynoso and Montellano-Ballesteros 2004), where the genus *Gopherus* includes the desert tortoise (Berry et al. 2002a, Murphy et al. 2011, Edwards et al. 2016).

Phylogenetic tortoise studies since the mid-1990s have established *Xerobates* as a sub-group within genus *Gopherus*, but the name is still used in discussions designating the biogeographic grouping of sister taxa. The informal name is often represented with addition of quotation marks, as "*Xerobates*", or as *Xerobates*-group (Reynoso and Montellano-Ballesteros 2004). This is particularly true of fossil studies where DNA is unavailable and character evaluation can only be made on bone morphologies of incomplete skeletons (Bramble and Hutchison 2014). As such, small tortoise specimens in the Stout Research Center paleontology collections may bear the genus designations *Geochelone*, *Hesperotestudo*, *Gopherus*, or *Xerobates*. However, some catalogued specimens may only have the preliminary designation, which can lead to inflated errors of omission and commission when creating paleontological-based species distribution maps. Despite such shortcomings, maps generated from the current collection may help elucidate general patterns in the historical presence and distribution of tortoises in ABDSP.

Hesperotestudo fossil ABDSP (LACM) 1918/V16217. Photos by ABDSP paleontology volunteer Barbara Marrs.

The CDD Stout Research Center paleontology collections contain 17,500 catalogued vertebrate fossils from 4,500 catalogued terrestrial field localities in ABDSP, of which 776 specimens are tortoises from 462 field localities. Despite the fact that ABDSP tortoise fossils have yet to be studied in detail, a preliminary review of the currently available data suggest the following observations and plausible perspectives:

Hesperotestudo fossil ABDSP 3551/V9225. Prepared by and Photo by ABDSP paleontology volunteer Ron Pavlu.

Hesperotestudo fossil ABDSP (LACM) 1918/V16217. Restored plastron. Photo by ABDSP paleontology volunteer Susie Walker.

1) Two genera have been recognized; *Hesperotestudo* or cf. *Hesperotestudo* (275 specimens from 143 localities) and *Xerobates*, or cf. *Xerobates*, or *Xerobates* cf. *X. agassizii* (38 specimens from 37 localities). All ABDSP fossils formerly identified as *Geochelone* have been included here as *Hesperotestudo* (Bramble 1982, Meylan and Sterrer 2000). Fossil specimens of genus *Xerobates* in ABDSP are identified as and considered to be synonymous with genus *Gopherus.*

   At least two taxonomic groups of tortoises occupied the ABDSP region by at least 3 Ma. The paleontology program determined that the earliest recorded ABDSP specimen of *Xerobates* appears in sediments aged 3.58-4.18 Ma (Figure 4). This was done by linking the geographic locations of fossils to a map of stratigraphic layers that were dated using geomagnetic polarity and volcanic ash studies (Berggren et al. 1995, Cande and Kent 1995, Dorsey et al. 2011, Janecke et al. 2010, Lutz et al. 2006). The next oldest *Xerobates* specimens are dated between 2.581-3.04 Ma, and the genus is present until at least 0.5 Ma. Based on this approach, *Hesperotestudo* first appears in the ABDSP region close to 3 Ma, and declines significantly after 1.8 Ma (Figure 4). A few fragmentary specimens recovered from the Ocotillo Formation (0.5-1.1 Ma) are listed as

Hesperotestudo. The greatest incidence of *Hesperotestudo* localities (n = >30) occurs between 1.95-2.14 Ma, whereas *Xerobates* fossils peak (n = 10) between 1.77-1.95 Ma.

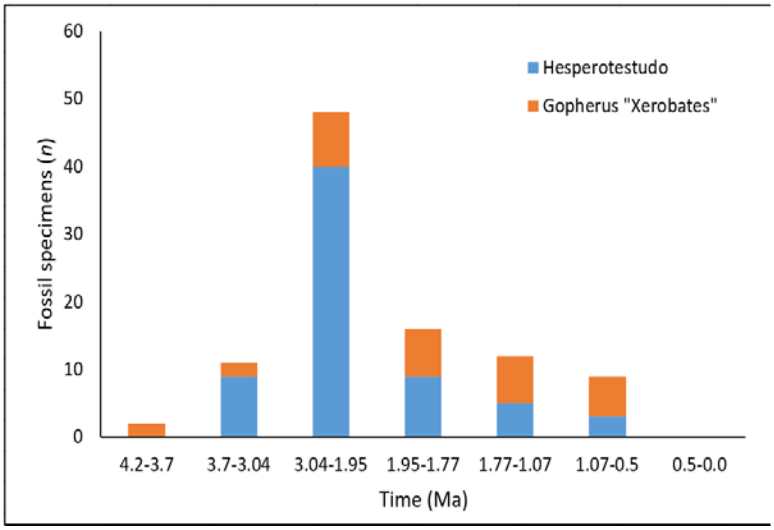


Figure 4. Number of paleontological specimens through time in Anza-Borrego Desert State Park, California.

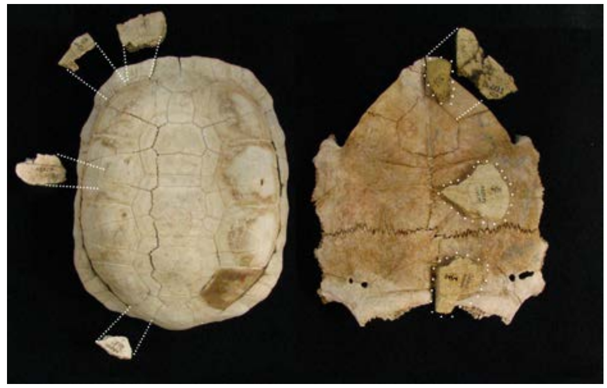
Both taxa appear to have been present in the local environment from at least 3 Ma until the local reduction of *Hesperotestudo* after 1.8 Ma. Based on the frequency of fossil locations, it appears that *Hesperotestudo* was well established on the landscape between about 3 and 2 Ma, and *Xerobates* became the dominant tortoise after 2 Ma. As *Hesperotestudo* declined, *Xerobates* remained relatively stable in the system. This perspective stems from our current state of knowledge, although a close evaluation of taxonomic identifications and fossil locality data may provide minor refinements to the apparent stratigraphic distributions.

Hesperotestudo fossil ABDSP 3551/V9225. Prepared by ABDSP paleontology volunteer Ron Pavlu. Photo by ABDSP paleontology volunteer Charlie Anderson.

2) Several biases affecting the reliability and completeness of local distribution records must also be taken into account when linking tortoise fossils to the geomagnetic polarity framework referenced above. For example, availability of exposed fossil-bearing sedimentary beds is restricted to those areas altered by uplift and tilting due to the onset of faulting and vertical displacement, between 0.9 and 0.5 Ma. That is, older sediments are the ones being brought to the surface and exposed, whereas most of the younger sediments after 0.5 Ma (the main surface of the Borrego Valley) are still flat-lying with fossil layers that remain buried, unexposed, and hidden. Consequently, the current fossil record of tortoises in ABDSP is incomplete, and remains a focus of interest for future research.

3) Environmental conditions influencing the appearance and disappearance of these taxa in the western Salton Trough were likely driven by interactions between global climate and continental and regional tectonics. Based on co-occurrence of large, mammalian

herbivores and tortoises in the same region between 2.6-3.0 Ma, a plausible hypothesis is that winters were warm (above freezing temperatures) and grasslands were expansive during that period. The changing topography and cyclic pluvial conditions throughout the Pleistocene led to episodic filling and emptying lakes and streams in the expanding Salton Trough and local lowlands. This periodically created distinct geographic boundaries between aquatic and terrestrial environments, with tortoises restricted to the latter. Whereas neither tortoise was capable of self-regulating their body temperature, of the two, only *Gopherus* was able to dig and occupy burrows to avoid temperature extremes at the surface. As the Pleistocene progressed, downward trending temperature (Figure 5) may have led to the decline of *Hesperotestudo*, because, like some modern giant tortoises, e.g., Galapagos tortoise (*Chelonoidis*), it may not have been able to burrow and avoid cooler winters (Jolly 2000, Biewer et al. 2016). The continuous uplift of the Peninsular Ranges to the west of what is now ABDSP intensified a rain shadow effect during the last million years, facilitating the onset of modern desert conditions, which may have further degraded habitat suitability for *Hesperotestudo* while improving it for the desert tortoise.



ABDSP Fragments of fossil *Gopherus* "*Xerobates*" bone specimens placed next to or overlaid onto a modern *Gopherus* carapace and plastron. Photo by LK Murray.

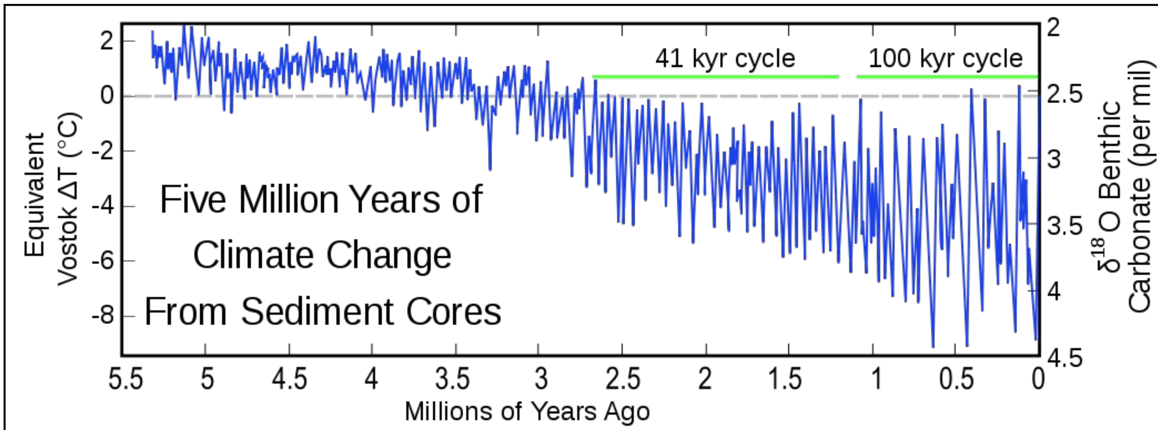


Figure 5. Five million years of climate history based on data derived from Lisiecki and Raymo (2005) and Petit et al. (1999). Source: Robert A. Rohde (https://commons.wikimedia.org/w/index.php?curid=5891468).

Cultural records of desert tortoises in Anza-Borrego Desert State Park

The desert tortoise has captivated human cultures for centuries (e.g., see Gopherus agassizii: a cultural history of tortoises by K. Stringfellow; available at http://mojaveproject.org/dispatches-item/gopherus-agassizii/). Archeological records from the Lower Colorado Desert indicate that the desert tortoise was used by early human inhabitants of the region as early as 9,500 years ago (Douglas et al. 1988). Ethnographic and historic data also reveal that the desert tortoise was economically and ideologically important, and that this importance increased over time (Schneider 1989). Because tortoises are non-migratory, they provided some Native American clans and groups with a year-round source of protein, as well as household and special ceremonial instruments and symbols of art and mythology (Schneider 1996).

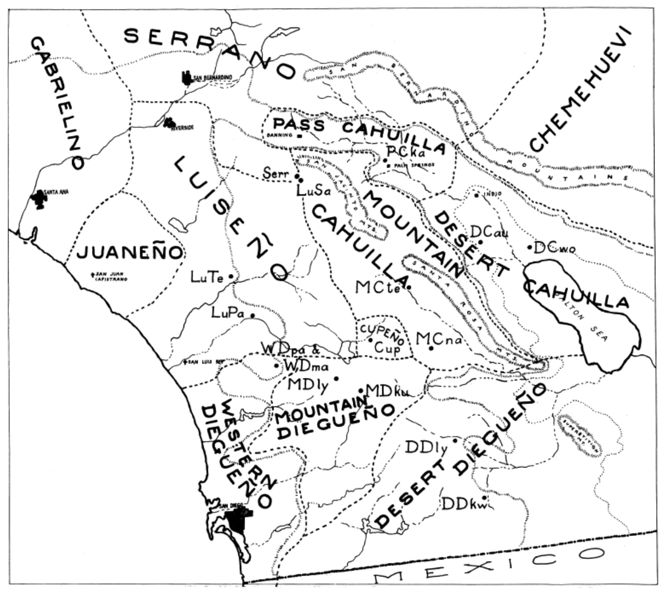


Figure 6. Native American groups represented in Drucker's (1937) element list. ABDSP spans from the Desert Mountain Cahuilla southward to the Desert Diegueño. Source: Kroeber (1937).

To understand the regional differences in cultural elements among southern California Native Americans, Drucker (1937) administered a standardized ethnographic questionnaire to Native American clan and group informants across the region in 1934 and 1935, which included reference to the desert tortoise (Figure 6). Ethnography is a qualitative method aimed at providing an in-depth description of everyday life and practice gained from the perspective of society members and/or their descendants. Of the five clans and groups identified in this work that previously occurred in the vicinity of what is now ABDSP, two reported that desert tortoise were "absent or denied from hunting and fishing," and three reported that desert tortoise was "absent because lacking or impossible in environment inhabited" (Table 1).

This information should be considered with caution when developing our understanding of whether desert tortoise occupied the ABDSP region during the pre-European contact time because verbal communications with a few members of a society may not capture the society's full breadth of knowledge (von Till Warren et al. 1981). For instance, three clans that were situated as close as 15 miles to the east of Coyote Canyon (Desert Cahuilla, Autaatem clan; Desert Cahuilla, Wontcaktamyahwic clan; and Pass Cahuilla, Kauisiktum clan) reportedly utilized tortoises (Drucker 1937), and were believed to gather in the northern Borrego Desert area with other groups from the surrounding regions, including the Coyote Canyon group to trade mesquite products for other resources (von Till Warren et al. 1981). As such, it is reasonable to expect that clans in the ABDSP area would have at least been exposed to tortoises during the trade process, yet clan informants in the ABDSP area reported that tortoises were not used as food or culinary receptacles. Additionally, because much of the basic ethnographic fieldwork from the Lower Colorado Desert dates from the late 1920's and early 1930's, von Till Warren et al. (1981) believed that informant memories extended back only to about 1880, and concluded that the ethnographic sources from that region (e.g., Laird 1976. Bean and Saubel 1963, Bean 1972) do not represent the pre-contact period. If true, this raises questions about whether Drucker's (1937) informants may have based their answer regarding tortoises solely on their own memories and observations since the 1880s. If tortoises were present in the area prior to establishment of ABDSP, it is plausible that they, like bighorn sheep (South 1944c; also see South's writings available @ https://archive.org/details/desertmagazine), may have been overexploited to near extinction by the high influx of human travelers, military personnel, and residents since the mid-1800s, in which case, the informant's answers may have corresponded to a period when tortoises were temporarily absent rather than because it was impossible for tortoises to occupy the environment. In support of this view, the USGS Maxent desert tortoise habitat suitability model predicts a range of suitable habitat conditions in ABDSP today (Nussear et al. (2009), and these conditions likely have remained unchanged over the previous 200 years.

Table 1. Anthropological records with reference to desert tortoise, as presented by Drucker (1937).

	Clan/Group/Party Informant							
Question	DDly*	DDkw*	MCna*	MCte*	Cup*	DCau	DCwo	PCka
Hunting and fishing observations with reference to desert tortoise	0	—	0	0	—	+	+	+

+ -- present
— -- absent from hunting and fishing observations
0 -- absent because lacking or impossible in environment inhabited
* -- geographically situated within current ABDSP boundary

After Establishment of Anza-Borrego Desert State Park

Early publications that reference tortoises

The oldest known written account of desert tortoises in ABDSP since its establishment in 1933 comes from the California Historic Landmark Project Collection created under the Federal Writers' Project of the Works Progress Administration of Northern California (Wade 1937). The California Historic Landmark Collection contains objective, historical typescript essays and monographs written between 1936-1940 on the geography, natural history, and geology of Californian registered landmarks, monuments, and state parks. With impressive detail, Wade (1937) described the park boundary and an abundance of desert tortoises in the park. Specifically, Wade (1937) wrote:

“The Anza Park is west of the central portion of the Colorado Desert. Its boundaries are, roughly: on the north the line which crosses the Santa Rosa Mountains and divides San Diego and Riverside Counties; on the west the Peninsular Range, or the southeastern extension of the San Jacinto Range, which narrows the desert margin considerably toward the south, where it almost touches the Mexican border; on the east the line of the Imperial County up to the parallel of the southern end of the Salton Sea where the park broadens east to that body of water. The number and variety of the fauna of Anza Park are comparable to its wealth of flora. Among those commonly seen are … turtles, … scorpions, and mosquitoes. Desert turtles emerge in the spring from a long hibernation. Armies of them can be seen at that season, but by summer they have selected cool burrows in which they spend most of the day. The Indians can forecast the weather by watching them. Although desert turtles are valuable food, they are not usually eaten by Indians unless food be scarce.”

Written accounts by homesteaders

In 1930, Marshall South and his family established a primitive home in what they referred to as Ghost Mountain in the hills south of Blair Valley (Lindsay 2005). They lived there until 1947; the park acquired that specific inholding in 1958. Mr. South was a writer for Desert Magazine (https://archive.org/details/desertmagazine/), and wrote stories about his family’s activities and wildlife encounters during extensive travels, searches, foraging, exploring, and camping throughout the park during that period. From October 1943 to December 1946, he authored 10 articles about his family’s pet desert tortoises. A total of 8 different tortoises were referenced in the various articles (names: Tiny Tim, Mojave, Dona Antonio, Grandpa Tortoise, General Machado, Monica, Juana Maria, and newly hatched baby; South 1943, 1944a, 1944b, 1944c, 1945a, 1945b, 1946a, 1946b, 1946c, 1946d).

Marshal’s first reference to tortoises was while traveling from Ghost Mountain to Mesquite, Nevada, giving the impression that their initial tortoises were found and brought back from Nevada. The adult tortoise named Monica was given to the family from someone in Santa Fe, New Mexico, and the former owner of Monica told Marshal that she had originally been born in Palm Springs, California (South 1946d). It is unclear from Mr. South’s writings whether Tiny Tim was found and died in Nevada, or that he had been brought to and died at Ghost Mountain (South 1943). According to these articles, the remaining 7 resided at Ghost Mountain, but one adult male (Mojave) died in June 1946 from heat exposure. The other 6 remained there until the family left their homestead in 1947. With great detail, South wrote about observed behaviors, start and end dates of hibernation, diet, accidental deaths, egg laying, and births. The eldest son, Rider South, wrote the following in the Introduction of Lindsay’s (2005) book:

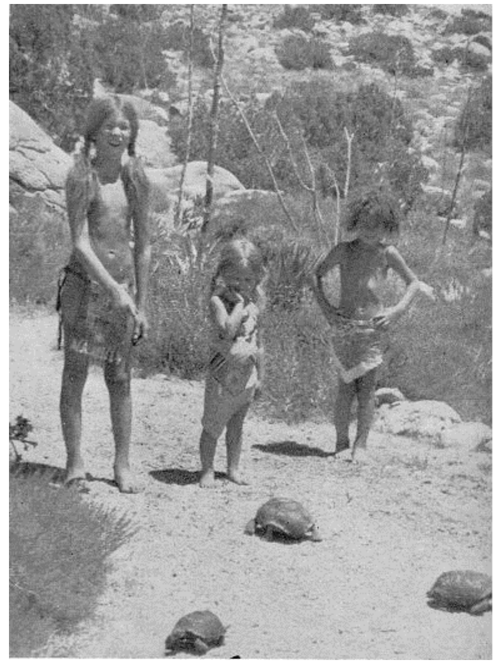
"One day when we were taking a little ride in the car, we saw three tortoises crossing the road. Father put them in the car with us, and we were able to keep them because in 1940 it was before the Endangered Species Act was passed. We placed them in a little cage outside in summer. They were kept in the house in the mouse-proof room in the winter so they would be warmer in their winter hibernation. Because there were three of us children and three tortoises we would each guard our own tortoise when they were eating outside of their cage.

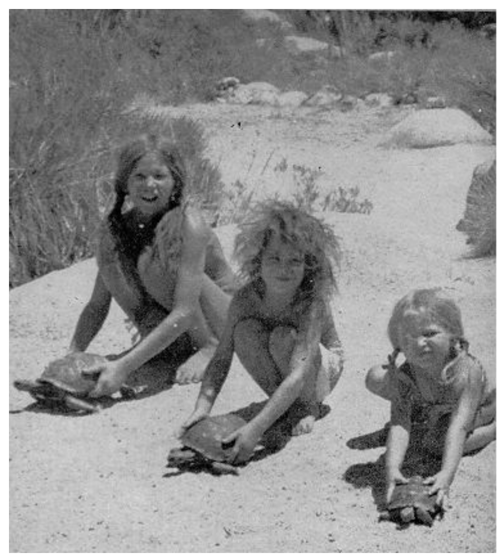
Sometimes we would line them up for a race. We were always frustrated because they would never stay in a straight line for a race but would go their own way.

Somehow they never caught on to the race idea. They were a lot of fun, but they were deep thinkers and didn't have much personality. When we moved to San Diego we gave them to the San Diego Zoo."

The children later reported:

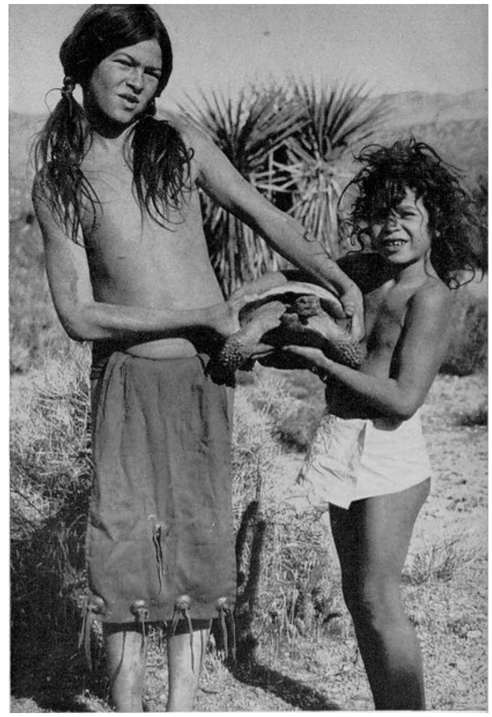
"we never had any tortoises anywhere in the desert near our homestead. I believe the ones we had were all picked up on our trip into Utah or other places, and at least one and possibly more than one were given to us by "others". … Also, if we had ever found remains of any dead tortoises, I would have known about it. They just were not in the vicinity" (Sunbelt Publications, San Diego, California, personal communications, 2018).





Marshall South's children (top: from left to right: Rider, Victoria, and Rudyard) with their pet tortoises on Ghost Mountain, circa 1947. Photos by Marshal South, provided by Sunbelt Publications.

These tortoises were with the South family for 3-4 years, and Rudyard witnessed them breeding at their Ghost Mountain area during that time (Sunbelt Publications, San Diego, California, personal communications, 2018). Of equal interest is the detailed description of one of the adult females (Juana Maria) digging nests and laying a clutch of 2 eggs in September 1946 (South 1946c), followed by the successful hatching of 1 baby tortoise 11 weeks later (South 1946d). This occurred just before the South family vacated their home, and no additional reference about the hatchling or the other egg was reported. Given these adult tortoises were there for multiple years, and that tortoises can lay up to 3 clutches per year, it is unknown whether other eggs may have been laid by this or another one of the females in the vicinity of Ghost Mountain during that 4-year period, raising questions as to whether any animals present today may be descendants of those tortoises.



Marshall South's children (from left to right: Rider and Rudyard) with their pet tortoise 'Mojave' on Ghost Mountain, circa 1944-46. Photo by Marshal South, provided by Sunbelt Publications.

The South family's writings and interviews are of particular value here because the Souths were tortoise enthusiasts familiar with what tortoises looked like, lived in and traveled regularly throughout the region, and wrote copious notes about plants and animals. Thus, because Marshal never referenced free-ranging tortoises in the park or Ghost Mountain, we can conclude with reasonable confidence that desert tortoises were absent or extremely scarce during their stay. Interestingly though, Wade (1937) provided an independent reference to "armies" of tortoises in the park, although the years that this occurred was not mentioned. Moreover, South posited that overexploitation of some wildlife, such as bighorn sheep prior to his arrival led to reduced numbers of some animals, even emphasizing that

> *"although we continued to search every ridge and crest on every desert tramp, we never saw sign of a living bighorn [from 1930-1944]"* (South 1944c).

Thus, it is not implausible to consider that a thriving tortoise population may have existed in the park region sometime before its establishment in 1933, but were overexploited by travelers, settlers, miners, military personnel, and others before the Souths arrived.

Incidental sightings and records of released desert tortoises

There are various sources of desert tortoise sightings in ABDSP. The following is a chronological history of tortoise sightings in the park. In addition, The CDD maintains an incidental desert tortoise sightings database (see next section of this report).

1958-1969

Interviews by the author with retired ABDSP employees Jim Dice, Fred and L. Louise Jee, Mark Jorgensen, Ernie Cowan, Bud Getty, and Paul Johnson in 2017 revealed a rich set of incidental sightings and specific releases of desert tortoises into ABDSP over the past 60 years. Incidental park records indicate that 4 tortoises were released into remote areas of the park between 1958 and 1962 (Figure 7; see next section). Based on the above interviews, these released animals were apparently captive pets delivered to the park from the coastal regions of San Diego, Orange, and Los Angeles Counties rather than from a formalized translocation program.

1970-1973

In 1973, a California law was passed making it unlawful to sell, purchase, harm, take, possess, transport or shoot any projectile at a desert tortoise (Amant 1976). The intent of this regulation was to halt the collecting of wild tortoises. Despite legally acquired tortoises being OK to possess with a state permit, preceding news releases to this new regulation led citizens to voluntarily turn over captive tortoises to the State Department of Fish and Game (Amant 1976). Many of those tortoises were provided to zoos, however, the number of relinquished tortoises was too great for zoos, which led biologists and citizens to find alternatives. Because it was known that releasing tortoises directly into the wild can lead to low survival, one alternative that gained momentum was the development of pens (referred to as the “halfway house”) on Bureau of Land Management property at Fort Soda near Baker, California, (Amant 1976, Amant and Hoover 1978). There, tortoises were held for a year to acclimate to natural desert conditions prior to any effort of releasing them back into the wild. As was the case in other southern California desert areas, citizens began dropping pet tortoises off in ABDSP as early as 1970, where most were presumably relinquished to California State Fish and Game wardens and rangers located at the park headquarters in Borrego Springs (Appendices A and B). According to retired State Park employees Ernie Cowan, Bud Getty, and Paul Johnson (J.A. Manning, pers. comm.), holding pens were established in the park to house these tortoises while state employees developed a solution to address the mounting numbers. California continues to have detailed regulations desert tortoises (Appendix E).

The first cohort of penned tortoises were released into the park in the summer of 1970, followed by two additional releases in the spring and fall of 1971 (Appendix C). Between 1970 and 1972, approximately 65 desert tortoises were reportedly released into remote areas of the park (Luckenbach 1982; Appendices A and B). Few park records detailing these releases exist, and details regarding these efforts and the subsequent status of these animals are largely absent from the scientific literature (e.g., see Murphy et al. 2007; except see Luckenbach 1982). Interviewees shared that both male and female tortoises were released, many of the animals were carried by backpack into remote areas of the park, many were marked with numbers painted in white on the carapace, and periodic monitoring and recording of incidental sightings ensued for an unknown number of years (e.g., Appendix C). According to former California State Park Naturalist Ernie Brown (Appendix C), subsequent site visits revealed signs of activity, digging, and expansion of their range. Interestingly, no incidental sightings were recorded in the park during 1971 and 1972 (Figure 7; see next section), although egg shells were located in one of the release areas soon after. More specifically, Luckenbach (1982) reported:

"*Between 1971 and 1972, 65 tortoises were released in the Vallecito Mountains within Anza Borrego Desert State Park, which is an area outside the natural range of G. agassizii. Some reproduction of these animals has been reported (M. Getty, personal communication).*"

A few (< 4) incidental sightings of tortoises with numbers painted on their carapaces were recorded during subsequent years. This small number, in combination with limited information about the released animals, prevented a retrospective analysis of individual fates of released animals during this current effort.

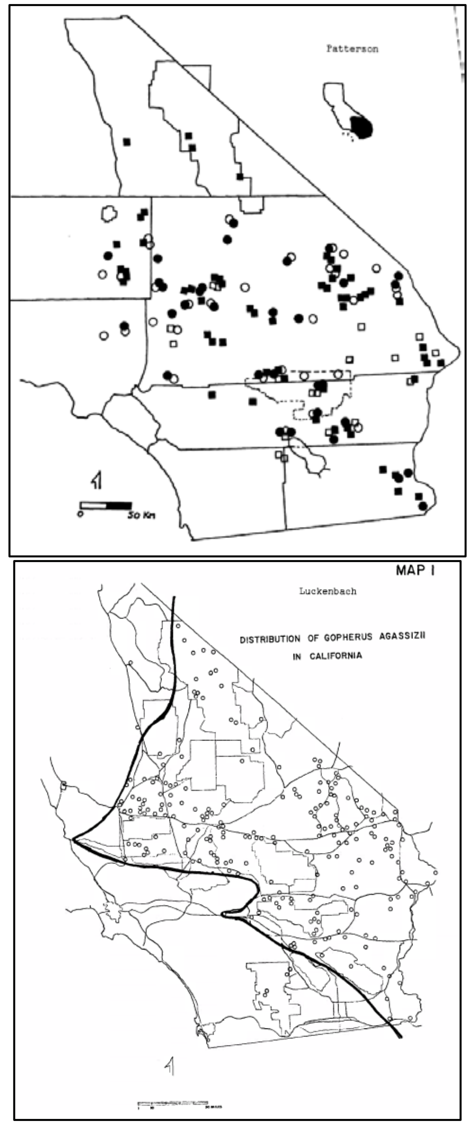


Figure 8. Localities of Mojave desert tortoise in California prior to 1976 from (top) Patterson (1976) and (bottom) Luckenbach (1976).

1974-2017

Patterson (1976) conducted an exhaustive search of museum records and professional, semi-professional, and amateur observations for desert tortoises in California, and reported two tortoise localities in the southern Santa Rosa Mountains in San Diego County (Figure 8). Specific details and dates of those two observations were not provided in the article, but both were located within ABDSP. Patterson (1976) also reported a Museum of Vertebrate Zoology record 0.5 miles south of Palm Springs in Riverside County, which is approximately 17 miles north of the ABDSP boundary.

In the same symposium proceedings that Patterson (1976) present his findings, Luckenbach (1976) presented his work on the distribution of desert tortoises in California, which included and referenced 4 locations inside ABDSP (Figure 8). However, Luckenbach (1976) felt that these were "liberated animals."

The documented tortoise releases prior to 1973 obviously drew attention to tortoises in ABDSP. From 1973 through 2017, 53 animals were reported to have been incidentally seen (ABDSP incidental sightings), with reported sightings rapidly increasing since 2010. The recognition that tortoises occupy the park also appears in park documents. The earliest DPR document found that referenced tortoises in ABDSP was the Initial Study and Checklist for the Coyote Canyon Public Use Plan (CDPR Oct 2 1995), which stated:

"Listed as a federal and state threatened species, desert tortoise occurs in Coyote Canyon, yet its status is unclear. The park is outside the recognized distribution of the tortoise, but the species is found in the mountains east of Salton Sea, forty miles from Coyote Canyon. Most records for the park are from Sheep Canyon in Coyote Canyon and include nine records from 1973 to 1993. An observation during April 1993 of newly hatched tortoises is thought to represent captive individuals released into the park, or perhaps a remnant natural population. In any event, the tortoises in Coyote Canyon are entitled to full protection under the law. Potential impacts include being run over by vehicles or capture and removed by visitors" (CDPR 1995:16).

The ABDSP General Plan (2005:2-56) also references the desert tortoise, stating:

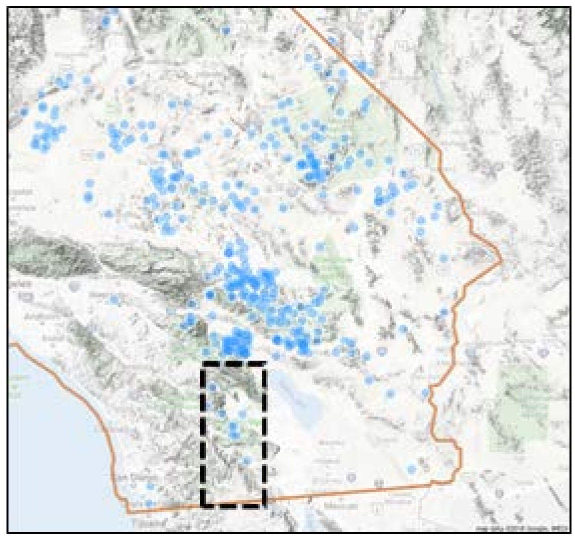


Figure 9. Locations (blue dots) of possible Mojave desert tortoises (animals or sign: tracks or burrows) recorded in iNaturalist in the southern California region. Eight locations are recorded within Anza-Borrego Desert State Park (situated within dashed lines). Data acquired November 6, 2018 via https://www.inaturalist.org.

"Although the desert tortoise occurs naturally within 50 miles of the park, current data suggest that they are not native to ABDSP, but were brought here by people in the last 50 years. An unknown number of park visitors have released unmarked previously captive tortoises into the park, thus making potentially native wild individuals impossible to distinguish from former pets. Dozens of sightings have been documented within ABDSP and this species is known to reproduce at the mouth of Sheep Canyon. The habitat within ABDSP is not thought to be critical to desert tortoise and its importance to the survival of the species should be evaluated. General threats to the desert tortoise includes off-road vehicle use, ravens, development, grazing animals, and diseases contracted from illegally released captive tortoises."

iNaturalist provides further information on the presence of tortoises in ABDSP, with 8 records in the park as of Nov 6, 2018; see Figure 9).

ANZA-BORREGO DESERT STATE PARK DESERT TORTOISE PROGRAM

Anza-Borrego Desert State Park lies south of the current distribution of desert tortoise recovery units and has long been considered outside the native range of desert tortoises (Luckenbach 1976, Patterson 1976, Stebbins, 1985, USFWS 1994). However, ABDSP supports 1,000 square miles of native Colorado Desert vegetation, much of which appears suitable for desert tortoises (Nussear et al. 2009). Based on its size alone, the park naturally meets Condition 4 of the Desert Tortoise Recovery Plan, which states "each reserve should contain a minimum of 1000 mi^2 (1610km^2) of tortoise habitat" (USFWS 1994). Moreover, as described in the previous sections, desert tortoises have occurred in the park region prior to and subsequent to its creation in 1933, and documented introductions of tortoises occurred in the 1970s like elsewhere across the Mojave Desert (e.g., see Murphy et al. 2007).

Despite the park not being a recovery unit to date, the author began to develop a desert tortoise program in the park in 2016 in response to increased incidental sightings and interest of desert tortoises by visitors. As part of this program, the park initiated the following steps:

1) Formalize the incidental sightings database.
2) Identify habitat suitability and conduct validation of a desert tortoise habitat model in ABDSP.
3) Conduct a baseline reconnaissance survey for live tortoises and evidence of presence.
4) Determine the genetic origins and genetic diversity of the park's tortoises.
5) Establish a framework from which to develop long-term population monitoring protocols.
6) Establish baseline estimates of demography and disease. Prevalence.
7) Identify threats.

Permits and Authorizations

All activities, including the handling and marking of desert tortoises, are conducted under United States Fish and Wildlife Service permit TE27242C-0 and a California Department of Fish and Game MOU, with the District's Senior Environmental Scientist as the Principle Officer of those permits. This work complies with California law, proclamations, executive orders, CCRs, California State Park and Recreation Commission, and the DPR DOM.

Incidental Sightings Database

The park has maintained records of incidental desert tortoise sightings since 1958. These records are from sightings reported by visitors and park employees that are incidental to (and not part of) standardized tortoise surveys, studies, or monitoring. L. Louise Jee (retired DPR CDD Research Analyst) developed the database during her career, and maintained it until her retirement in June 2017. The author and Mrs. Jee conducted interviews with former park employees and searched the park's archived records to collect additional incidental sightings. These efforts led to a formalized incidental sightings database. To date, the database contains 74 records of incidental desert tortoise sightings (Figure 7).

The presence of incidental sightings in this database is driven by several hierarchical factors: 1) the presence of a tortoise at a location, 2) the presence of a person at that same location, 3) the detection of the tortoise by that person, 4) the reporting of that detected tortoise by (or on behalf of) that person to park staff, and 5) that park staff forward that incidental sighting information to the park employee maintaining the incidental sightings database. Because all 5 steps must occur for the presence of a tortoise to be recorded, the resulting database portrays only a minimum count of tortoise presence records in the park. Moreover, the probabilities associated with any one or combination of these factors is expected to vary over time, limiting our ability to interpret overall spatial and temporal patterns in these data.

Despite its limited scope, records of incidental sightings can still help managers gain an understanding of the minimum number of tortoise sightings recorded over time since record keeping began 60 years ago. To maintain consistent and comparable annual incidental sightings data to that collected from 1958 to 2017, the recording of incidental sightings are planned to continue into the foreseeable future. As a source of presence-only data, this database provides opportunities to investigate a variety of topics, including species distribution modeling (Pearce and Boyce 2005, Royal et al. 2012). No formal advertising to park visitors to report tortoise sightings has been conducted, but this form of citizen science could prove beneficial for acquiring continued presence-only data, as well as maintaining public interest in this charismatic species.

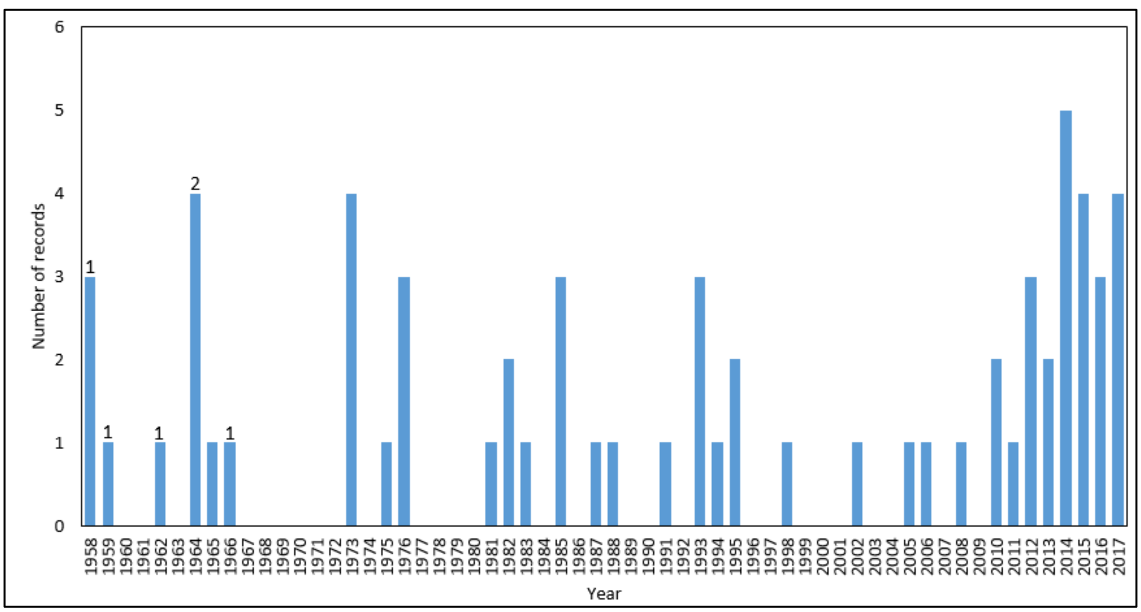


Figure 7. Number of incidental records of desert tortoise sightings (of live and dead) in Anza-Borrego Desert State Park, California, 1958-2017. Numbers on top of bars are number of known released animals in the incidental sightings database. Records of additional releases (see next section) are not included.

Habitat Suitability and Validation of a Desert Tortoise Habitat Model in ABDSP

Obtaining accurate assessments of wildlife habitat suitability is an important step towards achieving the DPR's goal of data driven resource management (Boxes 1, 3, & 4). Likewise, identifying species-specific natural history traits and behaviors is fundamental to understanding habitat suitability. In line with this, Boarman (2002c) provides a detailed description of desert tortoise habitat requirements, and Weinstein (1989) lists major topographic features as including flats, valleys, bajadas, and rolling hills generally from 2000 to 4100 ft above sea level. Importantly, desert tortoise activity patterns, as well as demographic and growth rates have been shown to be correlated with ephemeral vegetation and rainfall (Nagy and Medica 1986, Zimmerman et al. 1994, Medica et al. 2012). Tortoises eat primarily annual forbs, and some perennials (e.g., cacti and grasses) (Jennings 1993). Annual precipitation in the Colorado Desert occurs in both summer and winter, providing green up of annual forbs and perennials, as well as water for tortoises during years with precipitation. Tortoises in this region appear to be active in late March through June, with a secondary activity period in September and October (J.A. Manning, pers. obs.). They may also be active during mild weather or rain conditions throughout the year. When active, tortoises spend nights and hot daytime periods in subterranean burrows, caliche caves, or granitic rock cavities, under a shrub, or in a shallow burrow (pallet) (Marow 1979, Nagy and Medica 1986). During periods of inactivity (approximately 98% of the time), tortoises hibernate, aestivate, or rest in their burrows described above (Marlow 1979, Nagy and Medica 1986). Importantly, tortoises use an average of 7-12 burrows at any given time to survive local environmental conditions (Barret 1990, Bulova 1994). Environmental conditions are also anticipated to influence the phenology of egg production and oviposition, affecting survival and development of neonates, offspring, and adult fitness (e.g., Lovich et al. 2017a,b). As such, habitat models for desert tortoises generally include numerous environmental factors.

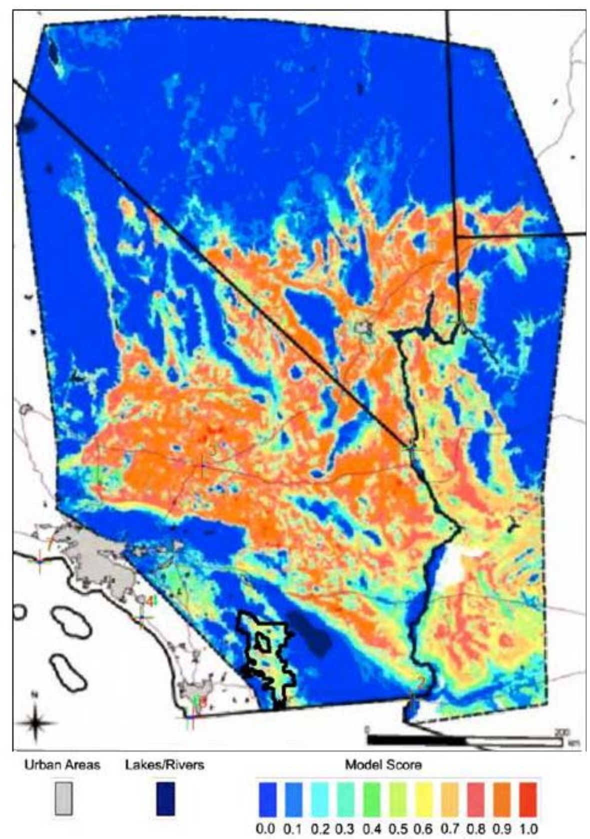


Figure 10. Predictive Maxent probability model of desert tortoise habitat potential based on presence-only data, with Anza-Borrego Desert State Park outlined in black. Brown depicts high potential (probability >0.6) of habitat suitability. From Nussear et al. (2009): https://www.fwspubs.org/doi/suppl/10.3996/022015-JFWM-013/suppl_file/022015-jfwm-013.s5.pdf

To date, a local model of desert tortoise habitat in ABDSP has not been developed. However, Nussear et al. (2009) developed quantitative habitat model in the Mojave and parts of the Sonoran Deserts of California, Nevada, Utah, and Arizona that has been extrapolated to the lower Colorado Desert region where ABDSP lies (Figure 10). Applying a Maxent algorithm (Phillips et al. 2006), they used 16 environmental variables and an extensive set of field-collected desert tortoise presence data to predict and validate potential tortoise habitat in the Mojave and parts of the Sonoran Deserts (Figure 10). When extrapolated across the Lower Colorado region of southern California, nearly the entire ABDSP region emerged as an island of suitable habitat tenuously connected to suitable (and occupied) habitat to the north (Figure 10). To the east are unsuitable areas of the Coachella Valley and Salton Sea, and to the west is the Peninsular Range (Figure 10). Here, 74 live desert tortoise sightings from the park's incidental sightings database were used to assess and validate Nussear et al.'s (2009) model in ABDSP.

Methods

The 1-km^2 resolution rasterized output from Nussear et al.'s (2009) Maxent model provided a map of the statistical probability of habitat potential extrapolated across the park. The park boundary was used to clip out the geographic area of the raster that occurred within ABDSP, and the probability of habitat potential in each raster pixel formed the basis for this analysis (Figure 11).

The expected frequency (i.e., collected availability) of pixels in each category was also calculated, and a Chi-Square Goodness of Fit test of habitat use vs availability was performed. This test of first-order habitat selection (Johnson 1980) followed a design of "collective use vs. collective availability" identified by Thomas and Taylor (2006), where collective refers to use by an entire population (e.g., ABDSP tortoise population) and collective availability pertains to habitat available for all animals in the population). A classic description of applying this test to a wildlife-habitat-selection study is provided by Neu et al. (1974). The probability of habitat potential (0 – 1.0) was then grouped into 11 categories (Figure 11), incidental

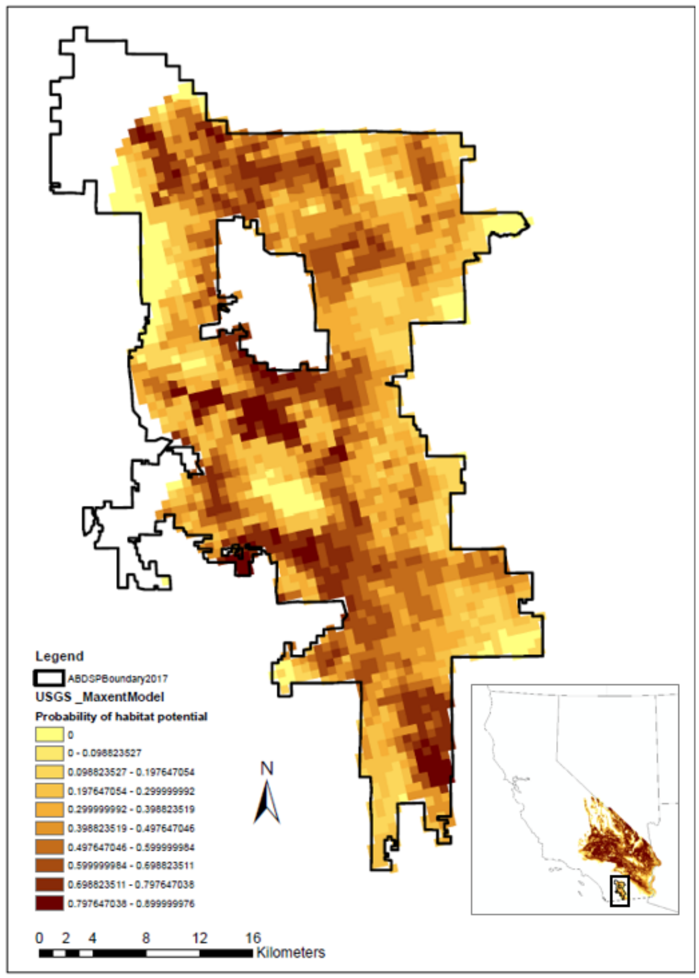


Figure 11. Predictive Maxent probability model of desert tortoise habitat potential in Anza-Borrego Desert State Park. Modified from Nussear et al. (2009); https://databasin.org/maps/385dd726e56b4a7eac8b46ccb3389e24/active.

tortoise sightings were overlaid onto the raster depicting the probability of habitat potential, and each sighting location was classified according to the probability of habitat potential in the corresponding pixel. This constituted a measure of observed frequency (i.e., collective use) of tortoise sightings in each category, which was converted to proportional use.

Results and discussion

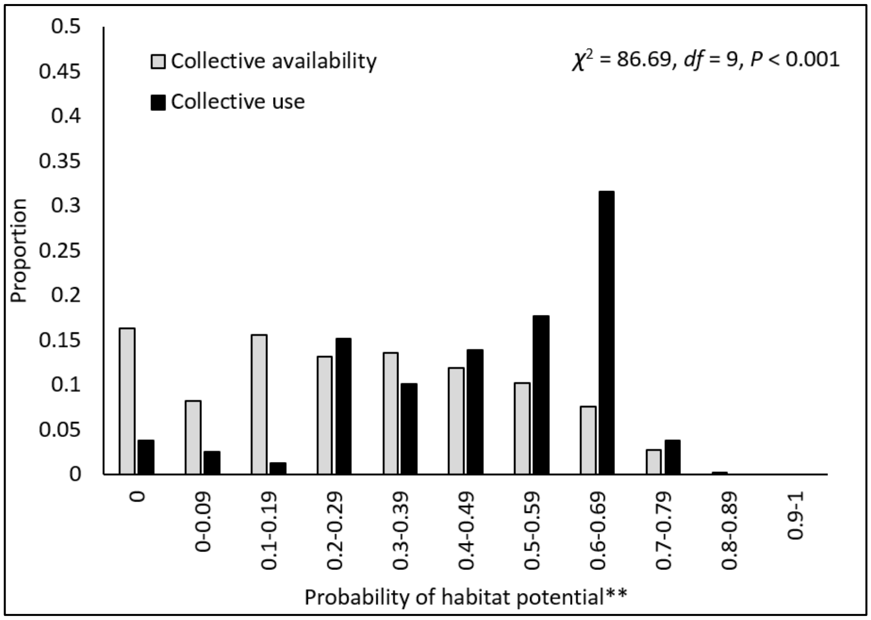


Figure 12. Chi-Square Goodness of Fit test of desert tortoise habitat use vs availability in Anza-Borrego Desert State Park, California. Tortoise data were incidental sightings from 1958-2018 (n = 74), categories and collective availability were obtained from Nussear et al.'s (2009) Maxent model of habitat potential extrapolated into study area. See text for details.

Despite the issues that arise with incidental sightings data (see Incidental Sightings Database section above), 96% of the sightings were located in areas of suitable habitat (prob > 0), revealing that this validation against incidental tortoise sightings data yielded reasonable classification accuracy of the Nussear et al. (2009) model in ABDSP (Verbyla and Litvaitis 1989).

The Chi-Square Goodness of Fit test revealed that tortoise sightings were disproportionately distributed among the habitat categories compared to that available (($\chi^2_{df=9}$ = 86.69, P < 0.001). An interesting pattern of lower than expected occurrence in low habitat potential areas compared to that expected and disproportionately greater numbers in areas of high habitat potential also emerged (Figure 12). Moreover, this pattern indicates the possibility that tortoises may be selecting or surviving in greater numbers in the higher quality habitats in ABDSP in accordance with the ideal free density-dependent habitat selection theory (Fretwell and Lucas 1970).

Initial Reconnaissance Survey

Identifying a preponderance of suitable desert tortoise habitat in the park and compiling and reviewing 60 years of incidental live tortoise sightings provided clear evidence that desert tortoises and their habitat have been present in the park for a long time. The next logical step was to conduct a reconnaissance survey in select areas to determine if multiple age classes were present and whether animals were widely distributed or formed spatial clusters. Thus, Dr. Manning designed an initial tortoise reconnaissance survey in the park, and Rachel Woodard was contracted to conduct the field activities in late spring, 2017. The goal of this survey was to locate as many individual tortoises as possible within the allotted time period (see methods). An additional goal was to record locations of all desert tortoise sign, including burrows, scat, tracks, bones, and carcasses.

Methods

Five separate zones were selected in the park based on records in the incidental sightings database and information acquired during my interviews with former park employees who were present during the 1970-72 releases. Independent tortoise biologist Rachel Woodard was contracted to implement field activities and survey the preselected zones. Ms. Woodward and Dr Manning reviewed and further refined the geographic scope of each search area.

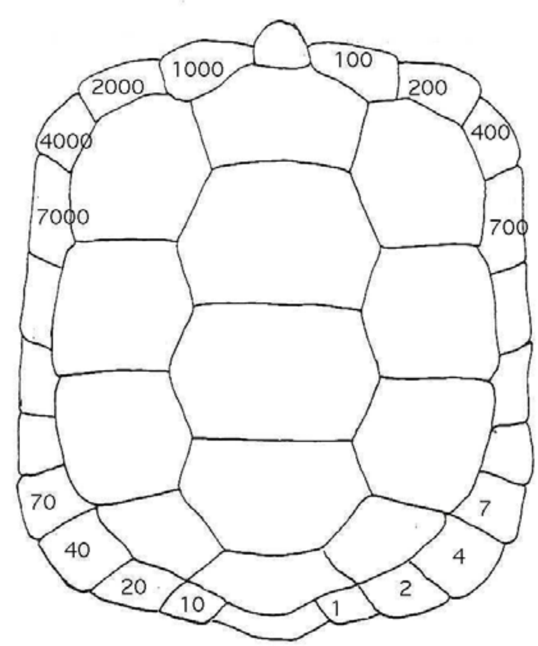


Figure 13. Modified Honegger System for marking tortoiess at Anza-Borrego Desert State Park, California.

Surveys were conducted by 7 qualified biologists (see acknowledgements) for 3 consecutive days in the 5 separate zones in the park; survey effort was not equally distributed among the 5 zones. Pedestrian-based transects were performed in select areas within these zones where tortoises were anticipated to likely occupy. Combined, approximately 20 person days were spent engaged in searching for tortoises in these zones. Capture, handling, and health assessments were carried out on live animals following USFWS (2016) and Hernandez-Divers et al. (2002), as permitted under the park's USFWS Recovery and State Fish and Wildlife Permits. Standard measurements of each animal were also recorded. Live tortoises were uniquely marked with epoxy dots attached to marginal scutes according to the modified Honegger system (Figure 13) and in accordance with the methods described in the *2015 Desert Tortoise Monitoring Handbook* (https://www.fws.gov/nevada/desert_tortoise/documents/reports/2015/2015_Monitoring_Handbook.pdf). A numbered "license plat" tag was also attached to the 4th right or left costal if the animal was large enough to support it (see Figure 24).

Results and discussion

This reconnaissance survey resulted in locating and marking 12 live desert tortoises. Shells, bone fragments, active burrows, scats, and tracks were also detected (Figures 14, 15, 16, 17, and 18), and burrow dimensions recorded (Table 2). The numbers of live animals and tortoise sign, as well as detected presence of various age classes (Figures 19, 20, and 21) indicated that an established population comprised of multiple age classes that show signs of reproduction and mortality was established in the park. These data and suitable habitats identified during this effort formed the initial basis for pursuing further questions about genetic origins, disease prevalence, population viability, and other relevant management and conservation issues.

The appearance of the shell remains in Fig 14 (with the majority of the carapace broken open and the plastron still intact) is similar to that depicting predation by a wild felid (Emmans 1989, Adams et al. 2006). Felid predation on tortoises has been reported elsewhere in the Mojave (e.g., P.A. Meica and P.D. Greger: https://www.osti.gov/servlets/purl/961548).

Figure 14. Carcasses 1-1 (genetics sample 024) and 1-2 (genetics sample 028), Anza-Borrego Desert State Park, California. March 2017. Photo by J.A. Manning.

Figure 15. Carcasses 3-16 and 3-17, Anza-Borrego Desert State Park, California. March 2018. Photo by J.A. Manning.

Figure 16. Various active desert tortoise burrows, Anza-Borrego Desert State Park, California, 2017. Photo by J.A. Manning.

Figure 17. Desert tortoise scat, Anza-Borrego Desert State Park, 2017. Photo by J.A. Manning.

Figure 18. Adult-sized desert tortoise track. Anza-Borrego Desert State park, California, 2017. Photo by J.A. Manning.

Figure 19. At 51 grams, tortoise AB6343 was the second smallest tortoise recorded in 2017-2018 (the smallest was AB6315 @ 38g). Photo by J.A. Manning.

Figure 20. At 4,500 grams, male tortoise AB6354 was the heaviest recorded in Anza-Borrego Desert State Park, California, 2017-2018. Photo by J.A. Manning.

Figure 21. Male tortoise AB6304, Anza-Borrego Desert State Park, California, 2017. Photo by J.A. Manning.

Table 2. Dimensions of desert tortoise burrows in Anza-Borrego Desert State Park, 2018. Data from 2017 initial survey were not included in this table.

Tortoise burrow		Width		Length	
condition	*n*	$\bar{X}$	SD	$\bar{X}$ [1]	SD
Active	15	276.47	116.05	667.78	220.10
Fair	5	254.00	41.59	430.00	220.45
Good	21	270.71	101.32	855.56	325.31
Poor	7	238.86	48.33	649.29	390.52
combined	48	266.13	94.84	728.29	327.63

[1] Four active burrows had unspecified lengths >200, >1000, >1000, and >3000 (not included in calculations).

[1] Four good burrows had unspecified lengths >500, >1500, and >2500 (not included in calculations).

Genetic Origins of Anza-Borrego Desert State Park's Desert Tortoises

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Introduction

The genetic origins of desert tortoises in ABDSP have long been a topic of interest among tortoise experts and conservation biologists. Such challenging questions extend beyond the park, as alluded to in Murphy et al.'s (2014) article poetically titled "The dazed and confused identity of Agassiz's land tortoise, *Gopherus agassizii* …." Most assume that tortoises in the park were introduced, in part because of a complex history of releases, escapes, and commercial transport across the range of the Mojave desert tortoise (Murphy et al. 2007; Figure 22). For example, captive tortoises from the Los Angeles Basin were apparently released in various areas of the Mojave, including the Desert Tortoise Research Natural Area and Joshua Tree National Park (Murphy et al. 2007; Figure 22). Additionally, in his estimates of tortoise populations in California, Luckenbach (1976) stated that

> *"the line [north of the Santa Rosa Mountains] represents what I feel to be the natural limits of their range and is based largely on locality records and elevational and vegetation information. Localities outside this line such as those in … the Santa Rosa Mountains in Anza Borrego State Park, represent liberated animals."*

Paleontological records and published writings from the California Historical Landmark Project Collection (Wade 1937) reveal that desert tortoises were present in the park area prior to its creation in 1933, and prior to the documented releases, escapes, and commercial transports described in earlier sections of this report. The park's position only 17 miles south from where there are believed to be native tortoise populations, ethnographic evidence of tortoises included in Native American cultures in those same areas, and suitable tortoise habitat in the park provides a preponderance of evidence for the potential of a naturally colonized population in ABDSP sometime in the past.

Additionally, although Murphy et al. (2007) do not reference information or data from ABDSP, there are grey literature articles and park records describing releases of desert tortoises into the park (see historical account in this report). Of equal interest is the South family's adult female desert tortoise that reportedly laid a clutch of 2 eggs south of Blair Valley in September 1946, producing one hatchling 11 weeks later (South 1946c,d). Questions regarding the fate of that juvenile and whether other clutches may have been laid by that or another of their female tortoises over the 4-year period the animals were there remain unknown, but the possibility of an inadvertent human-mediated translocation is real. Additionally, releases into ABDSP between 1958 and 1972 have also been documented (see historical account in this report), although it is unknown whether these were *G.agassizii*, *G. morafkai*, and/or *G. evgoodei*. Over 70 incidental sightings of live tortoises have been recorded by the park since 1958, some of which occurred south of Blair valley. This complex information raises important questions and challenges regarding the genetic origins and conservation status of the desert tortoises in ABDSP, and whether the animals alive today are released or escaped animals (or descendants of such), descendants of a relic population of native animals that naturally colonized the park region from nearby populations to the north sometime in the past, or a mixture of both.

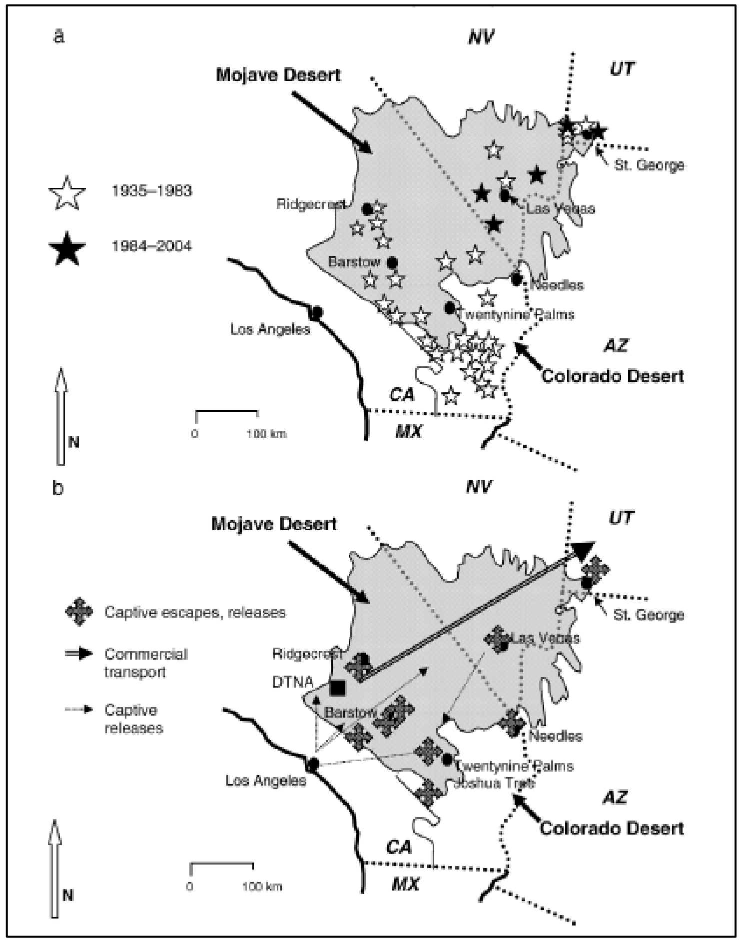


Figure 22. Previously published (a) locations of captive desert tortoises (*G. agassizii*) released by state wildlife agencies or others and (b) locations where captives escaped or were released outside of desert towns. There were also large-scale commercial transfers of tortoises. Note that information regarding ABDSP is absent. From Murphy et al. (2007).

After 60 years of incidental sightings and documented releases of tortoises, the initial 2017 reconnaissance survey provided evidence that a population of tortoises comprised of multiple size-classes occupied ABDSP. As several studies elsewhere have successfully detected evidence of translocated tortoises in genetic datasets from samples collected in the wild (Clostio et al. 2012, Fujii and Forstner 2010, Murphy et al. 2007, Schwartz and Karl 2005), a reasonable next step was to conduct a genetic study to determine which species was present, and the genetic origins and relatedness in this newly discovered population. Such information is needed to assess the status of this population that, to the best of our knowledge, may originate in part or entirely from one of the oldest known human-mediated desert tortoise translocation efforts. It is also critical in order to help the DPR inform its data-driven resource management efforts. To this end, Dr. Manning was awarded a research grant from the Desert Tortoise Council and the DPR provided matching funds to investigate the genetic origins of tortoises in the park.

The primary goal of this study was to gain baseline biological and genetic information on the tortoise population, with the following objectives:

Objective 1. Determine the origin(s) of ABDSP tortoises (i.e. lineage sources from across the desert tortoise range).

H 1. A single tortoise species occupies the park and no hybrids are present.
H 2. Tortoises within the park comprise a single genetic unit and fit an isolation-by-distance model with nearest neighbor populations outside the park, indicating natural origins.

Objective 2. Determine genetic relatedness among ABDSP tortoises

H 1. Genetic relatedness of tortoises within park areas will fit an isolation-by-distance model, indicating dispersal and gene flow (vs. human-mediated releases and/or admixture with other *G. agassizii* individuals from different genetic units).
H 2. Juvenile-size tortoises will have genotypes consistent with that expected of offspring of the adult-size tortoises within the associated group in the park.
H 3. Full and half-sibling groups will be geographically clustered within sampling sites rather than among sites consistent with that expected of naturally dispersed siblings (vs. human-mediated introductions that may disperse individuals within sibling groups among sites across the park).

Methods

Field sampling

Field surveys took place in March 2018 in 9 separate sites across the park. Areas were selected according to frequency of incidental sightings and observations from the 2017 initial reconnaissance survey. Capture, handling, health assessments, and blood sampling methods followed USFWS (2016) and Hernandez-Divers et al. (2002), as permitted under the park's USFWS Recovery and State Fish and Wildlife Permits. The DPR contracted with Rachel Woodard to implement captures, handling, and blood collection. This resulted in capturing and drawing blood from 36 free-ranging tortoises of all sizes (3 of these were recaptures from 2017), and recording standard measurements of each animal (Figure 23).

Blood was collected from captured tortoises to extract DNA and disease testing. New captures were marked with epoxy-dot and filed notches on marginal scutes corresponding to the modified Honegger system (Figure 13) and in accordance with the methods described in the *2015 Desert Tortoise Monitoring Handbook* (https://www.fws.gov/nevada/desert_tortoise/documents/reports/2015/2015_Monitoring_Handbook.pdf).

Epoxy dot marks on recaptures from the 2017 survey were also file-notched into their marginal scutes. A numbered "license plate" tag was placed onto the 4th right or left costal if the animal was large enough (Figure 24). Animals that were too small for attaching a license plate were marked with an indelible ink pen that was coated with clear epoxy, clear epoxy dots and/or filed notches on their marginal scutes (Figure 24). Marking protocols followed that approved in the USFWS permit and clarified during a telephone conversation with the Desert Tortoise Recovery Coordinator, Roy Averill-Murray on March 23, 2017.

Figure 23. District Environmental Scientist Jeff Manning measuring male desert tortoise AB6328. Photo by ABDSP science volunteer Sarah Teed.

All tortoise scats incidentally encountered during tortoise surveys were collected and georeferenced. Based on physical condition, scats that were <12 months old (*n* = 53) were swabbed following protocols developed by the UA Genetics Core (T. Edwards, pers. comm; Figure 25). Each swab was placed into lysis buffer in separate o-ring tubes, individually labeled, and shipped the UA Genetics Core for analysis. Tissue samples from 3 old carapaces and 1 frozen carcass (collected in 2017) were also submitted to the UA Genetics Core.

Figure 24. Desert tortoises captured and marked during the 2018 genetic origins study in Anza-Borrego Desert State Park, California. Photos by J.A. Manning.

Analyses

Identifying species -- To determine which of the 3 desert tortoise species were present in the park, each blood an scat sample was first genotyped using a 16-locus short tandem repeat (STR) panel (Murphy et al. 2007) and compared genotypes to a database of 1,258 *Gopherus* samples, including *G. agassizii* from throughout its range (n = 656). Other *Gopherus* samples in the database included: *G. morafkai* collected in Arizona (n = 348) and central Sonora, Mexico (n = 35); *G. evgoodei* (Edwards et al. 2015) collected in southern Sonora and Sinaloa, Mexico (n = 41); *G. berlandieri* collected in Texas (n = 118; Fujii and Forstner 2010); and *G. flavomarginatus* collected from Chihuahua, Mexico (Morafka et al. 1994) and from captivity (n = 60). For the *G. agassizii* samples, the database was partitioned into ''Mojave'' and ''Northern Mojave'' populations. This division complements the geographic distribution of the two primary mtDNA lineages [haplogroups MOJ_A and MOJ_B assessed by Murphy et al. (2007) and Edwards and Berry (2013)]. The stringency for assigning a tortoise to a species was assessed with the log of the odds ratio (LOD) selection criterion, which is calculated from the 2 most likely source species identified in the analysis. Assignments with a LOD selection criterion ≥2.0 have ≤0.01 chance of a type I error (i.e., a very low probability of mis-assigning an animal to an incorrect species).

Figure 25. District Environmental Scientist Jeff Manning swabbing desert tortoise scats for DNA samples. Anza-Borrego Desert State Park, California, 2018.

Identifying genetic source populations -- For samples confirmed to be *G. agassizii*, a 25-locus STR genotype was compared to a reference database of 657 *G. agassizii* samples collected from throughout the species' range (California, Nevada, Arizona, and Utah); these reference sample sites were previously designated into 15 populations within 8 genetic units (GUs) according to Murphy et al. (2007). The 25 previously described STRs that were used were Cm58 (FitzSimmons et al. 1995); Goag03, Goag04, Goag05, Goag06, Goag07, Goag32 (Edwards et al. 2003); Test56 (Hauswaldt and Glenn 2003); GP15, GP19, GP30, GP55, GP61, GP81, GP96, GP102 (Schwartz et al. 2003); ROM01, ROM02, ROM03, ROM04, ROM05, ROM07, ROM10 (Edwards et al. 2011); and ROM08, ROM09 (Davy et al. 2011). All PCR, fragment analysis, and data analyses followed procedures outlined in Edwards and Berry (2013). Additionally, an approximate 1,100 base pair portion of the mtDNA for selected samples was sequenced to determine variability and if this locus was informative to the study.

Using these 25 STRs, each tortoise sample was assessed for population association using the assignment test in program WHICHRUN (Ver. 4.1; Banks and Eichert 2000). This program calculates the likelihood of an individual tortoise originating from ≥2 of the previously identified candidate tortoise populations on the basis of its multilocus STR genotype. The LOD selection criterion was used to assess the stringency of assigning each tortoise to a population (see above for description of the LOD selection criterion).

Genetic diversity -- A series of genetic diversity indices were calculated using the 21 of the 25-locus STR set that were polymorphic (Table 3 under results). Specifically, observed heterozygosity, expected heterozygosity, standard deviation of randomization tests for Hardy–Weinberg equilibrium, and the F_{IS} inbreeding coefficient were calculated (Weir & Cockerham, 1984, Excoffier et al. 2007). Program FSTAT (Goudet 1995) was used to estimate richness, diversity, and F_{IS}. All other diversity and richness metrics were calculated with program Arlequin.

Genetic relatedness and family groups -- The same 21 locus STR dataset from the genetic diversity and richness testing were used to construct genetic pedigrees (i.e., family trees) in two park management units that contained adequate sample sizes (Coyote Canyon and Pinyon Ridge; see next section on monitoring and management units in this report).

We used Program Colony (Wang and Santure 2009, Jones and Wang 2010) to construct pedigrees by determining the best maximum likelihood-based configuration of relatedness, probabilities of parent pairs, and probabilities of full sibling families and half sibling dyads. The following options were selected when running Program Colony: female mating system = polygyny, inbreeding = yes, probabilities that a mother or father was included in the parent candidates = 0.5 (i.e., a 50-50 chance of a parent being present in our sample), and the number of siblingships = no limit. A single replicate was ran for each final analysis because all results were consistent among several prior runs.

We constructed pedigrees separately in each of the two management units and then ran one global dataset using all the viable samples from the park. Two separate analyses were ran for the Coyote Canyon management unit. The first analysis included generational structure [n = 31 individuals comprised of 6 adult females, 5 adult males, and 20 putative offspring (19 blood & 1 frozen carcass tissue sample from individuals with MCL <180mm; Turner and Berry 1984, Turner et al. 1986)]. No scat samples were available or used in these analyses. The second analysis included only the 5 adult males and 6 females to assess whether any of these were full or half siblings.

The Pinyon Ridge management unit contained a much smaller sample (n = 10; 2 adult females, 1 adult male, and 7 scats of unknown age or sex) that warranted a slightly different set of analyses and assumptions. The first analysis for this area used all 10 samples, and assumed that all 7 scats were from putative offspring. A second analysis also used all 10 samples, but assumed no age or sex structure to determine if any of the scats, adult females, or adult males were full or half siblings.

The park-wide analysis tested for full and half sibling groupings among sampling sites (Table 7; see page 53 in this report). By examining for intra-full sibling and/or intra-half sibling fragmentation among sampling sites, an indirect test for a genetic signal of human-mediated introductions of siblings into the park was conducted. This was done because tortoises have been reported to travel up to 7.3 km (4.5 miles) over periods as long as 5 years (Berry 1986, Duda et

Table 3. Preliminary descriptive statistics for Anza-Borrego Desert State Park desert tortoise samples (35 blood, 9 scat, and 1 frozen carcass samples; Data run - 30 Nov2018).

Locus	*n*	# alleles	Allelic range	Allelic Richness	Gene diversity	Obs Het	Exp Het	P-value	s.d.	F_{IS} (W&C)	*p*
GP96	*	1				0.00					
GP61	35	6	20	5.36	0.26	0.26	0.26	0.14	0.00	0.02	0.56
GP19	33	2	10	1.85	0.03	0.03	0.03	1.00	0.00	0.00	1.00
GP102	33	9	20	8.65	0.77	0.76	0.77	0.15	0.00	0.02	0.49
GP30	36	2	3	2.00	0.43	0.33	0.43	0.24	0.00	0.23	0.18
GP55	36	7	11	6.55	0.76	0.81	0.76	0.20	0.00	-0.06	0.81
GP26	28	2	1	2.00	0.32	0.32	0.32	1.00	0.00	0.00	0.73
GP15	36	9	30	8.45	0.79	0.81	0.79	0.00	0.00	-0.02	0.61
GP81	30	7	7	6.80	0.62	0.70	0.62	0.13	0.00	-0.13	0.91
Goag3	*	1				0.00					
Goag32	*	1				0.00					
Goag4	31	11	17	10.52	0.77	0.55	0.77	0.00	0.00	0.29	0.00
Goag5	45	2	1	2.00	0.13	0.13	0.13	1.00	0.00	-0.06	1.00
Goag6	29	18	41	17.65	0.89	0.93	0.89	0.00	0.00	-0.05	0.90
Goag7	43	5	5	4.64	0.61	0.56	0.61	0.00	0.00	0.09	0.24
Cm58	*	1				0.00					
ROM01	32	3	15	2.88	0.28	0.13	0.27	0.01	0.00	0.55	0.00
ROM07	31	5	7	4.81	0.67	0.65	0.67	0.37	0.00	0.04	0.42
ROM08	33	5	6	4.83	0.58	0.70	0.59	0.71	0.00	-0.20	0.98
ROM04	41	6	15	5.89	0.75	0.56	0.75	0.00	0.00	0.25	0.01
Test56	36	10	24	9.32	0.84	0.69	0.84	0.00	0.00	0.18	0.01
ROM03	39	9	16	7.98	0.54	0.36	0.54	0.00	0.00	0.34	0.00
ROM02	33	2	1	2.00	0.44	0.39	0.44	0.69	0.00	0.11	0.39
ROM05	42	3	2	2.67	0.52	0.57	0.52	0.53	0.00	-0.11	0.83
ROM09	32	15	26	14.10	0.86	0.88	0.86	0.00	0.00	-0.02	0.72
Mean		6.57	13.24			0.53	0.56			0.06	
s.d.		4.41	10.83			0.26	0.25				

* This locus is monomorphic: no test done.

Note: Diversity indices for 21 microsatellite (STR) loci: n, number of individuals genotyped; Obs Het, observed heterozygosity; Exp. Het, expected heterozygosity; s.d., standard deviation of randomization tests for Hardy–Weinberg equilibrium; and F_{IS}, inbreeding coefficient (Weir & Cockerham, 1984). Richness, Diversity and F_{IS}, estimated using FSTAT. All other metrics calculated with Arlequin.

al. 1999), and human assistance would be needed for siblings to successfully travel the greater distances occurring between these 3 sites within the park.

Ongoing testing -- Additional analyses may include using BOTTLENECK (Piry et al. 1999) to test for evidence of historical changes in effective population sizes. This test assumes that a population with recent reductions in effective population size would show an excess of heterozygosity over that expected under mutation-drift equilibrium (Cornuet and Luikart 1996). The program calculates the deviation from expected heterozygosity under a mutation model for each locus, and then averages these across all loci. Program STRUCTURE v.2.3.4 (Pritchard et al. 2000) will also be used to define populations within the park.

Results

Analyses are continuing at this time; the following results are preliminary. Final results will be provided upon completion, and added as an addendum to this report.

Identifying species

Thirty-five of the 36 blood samples, 9 of the 53 scat samples, and the frozen carcass tissue had high enough amplification success that they could be used in the species-level analyses. These data were examined for duplicates, assuming no false alleles, and found that scats ABDSPScat011 and ABDSPScat023 had matching genotypes. Scat011 exhibited more allelic dropout, and was removed. Scats were also examined for matching genotypes with blood samples (n = 0). Based on the 16-locus STR set, the genotypes from these 44 samples were consistent with being *G. agassizii* from the Mojave Desert (Table 4). No other desert tortoise species were detected. The analysis of mtDNA corroborated that the genetic origins of these samples were of the *G. agassizii* Moj_A haplotype that Murphy et al. (2007) described as being broadly distributed across the California portion of the Mojave (Figure 26).

Identifying genetic source populations

We used the same 44 samples as above to carry out the population assignment test. Based on the 25-locus STR test and 15 known reference populations within Murphy et al.'s (2007) and Edwards and Berry's (2013) 8 GUs, preliminary results indicated that 77% (n = 34) of the ABDSP samples had a high probability of correctly assigning tortoises to their wild populations of origin (all LOD selection criteria >2.0). All of these samples were consistent with originating from 6 of the 15 reference populations [Murphey et al.'s (2007) populations 1, 2, 3, 4, 6, and 7], which lie within the southwestern most GUs (Western Mojave, Central Mojave, and Southern Mojave; Table 5; Figure 26). The remaining 10 samples with LOD selection criteria values <2.0 were also assigned to one of the reference population sizes within these 3 GUs (Table 5).

Genetic diversity

Overall, there appears to be low genetic diversity in the ABDSP tortoise population (Table 3). Analyses for understanding the genetic diversity and structure within the park are continuing at

Table 4. Preliminary desert tortoise species assignment test results using 16 STRs, ABDSP, 2018. For details, see text, Murphy et al. (2007), and Edwards and Berry (2013).

Sample	Species 1	Species 2	LOD
ABDSP001	Mojave	North Mojave	>2.0
ABDSP002	Mojave	North Mojave	>2.0
ABDSP003	Mojave	North Mojave	>2.0
ABDSP004	Mojave	North Mojave	>2.0
ABDSP005	Mojave	North Mojave	>2.0
ABDSP006	Mojave	North Mojave	>2.0
ABDSP007	Mojave	North Mojave	>2.0
ABDSP018	Mojave	North Mojave	>2.0
ABDSP020	Mojave	North Mojave	>2.0
ABDSP021	Mojave	North Mojave	>2.0
ABDSP022	Mojave	North Mojave	>2.0
ABDSP023	Mojave	North Mojave	>2.0
ABDSP024	Mojave	North Mojave	1.37
ABDSP031	Mojave	North Mojave	>2.0
ABDSP032	Mojave	North Mojave	>2.0
ABDSP033	North Mojave	Mojave	>2.0
ABDSP034	Mojave	North Mojave	>2.0
ABDSP035	Mojave	North Mojave	>2.0
ABDSP037	Mojave	North Mojave	>2.0
ABDSP038	Mojave	North Mojave	>2.0
ABDSP039	Mojave	North Mojave	>2.0
ABDSP041	Mojave	North Mojave	>2.0
ABDSP042	Mojave	North Mojave	>2.0
ABDSP043	Mojave	North Mojave	>2.0
ABDSP044	Mojave	North Mojave	>2.0
ABDSP045	Mojave	North Mojave	>2.0
ABDSP051	Mojave	North Mojave	>2.0
ABDSP052	Mojave	North Mojave	>2.0
ABDSP053	Mojave	North Mojave	>2.0
ABDSP054	Mojave	North Mojave	1.12
ABDSP056	Mojave	North Mojave	>2.0
ABDSP057	North Mojave	Mojave	1.33
ABDSP058	Mojave	North Mojave	>2.0
ABDSP059	North Mojave	Mojave	>2.0
ABDSP060	Mojave	North Mojave	>2.0
ABDSPScat011	Mojave	North Mojave	>2.0
ABDSPScat023	Mojave	North Mojave	>2.0
ABDSPScat031	Mojave	North Mojave	>2.0
ABDSPTissue01	Mojave	North Mojave	1.71
ABDSPScat035	Mojave	North Mojave	>2.0
ABDSPScat036	North Mojave	Mojave	1.45
ABDSPScat040	Mojave	North Mojave	>2.0
ABDSPScat042	Mojave	North Mojave	>2.0
ABDSPscat052	Mojave	North Mojave	>2.0
ABDSPscat053	Mojave	North Mojave	>2.0

LOD = log of the odds ratio selection criterion (see text).
Species 1 = most likely, Species 2 = second likely.
ABDSPScat011 = duplicate, deleted. ABDSPTissue01 from tortoise AB6306.

Table 5. Preliminary desert tortoise population assignment test results using 25 STRs, ABDSP, 2018. For details, see text, Murphy et al. (2007), and Edwards and Berry (2013).

Sample	Population 1	Population 2	LOD
ABDSP003	3	1	>2.0
ABDSP004	3	6	>2.0
ABDSP005	6	3	>2.0
ABDSP006	6	3	>2.0
ABDSP007	7	6	>2.0
ABDSP018	1	3	>2.0
ABDSP020	3	6	>2.0
ABDSP033	2	6	1.25
ABDSP038	3	1	>2.0
ABDSP039	6	7	>2.0
ABDSP043	3	2	>2.0
ABDSP051	6	3	>2.0
ABDSP001	7	3	>2.0
ABDSP002	3	2	>2.0
ABDSP021	3	1	>2.0
ABDSP022	3	6	>2.0
ABDSP023	3	6	>2.0
ABDSP024	1	7	>2.0
ABDSP031	6	7	>2.0
ABDSP032	3	2	>2.0
ABDSP034	2	3	>2.0
ABDSP035	6	5	>2.0
ABDSP037	6	3	1.15
ABDSP041	3	6	1.59
ABDSP042	3	2	1.04
ABDSP044	6	7	>2.0
ABDSP045	3	5	>2.0
ABDSP052	3	6	>2.0
ABDSP053	1	2	>2.0
ABDSP054	6	2	1.95
ABDSP056	3	2	>2.0
ABDSP057	6	3	>2.0
ABDSP058	3	2	>2.0
ABDSP059	6	4	1.72
ABDSP060	3	2	>2.0
ABDSPScat011	7	6	1.74
ABDSPScat023	7	6	>2.0
ABDSPScat031	7	6	>2.0
ABDSPTissue01	6	5	>2.0
ABDSPScat035	1	2	>2.0
ABDSPScat036	4	6	>2.0
ABDSPScat040	6	4	1.56
ABDSPScat042	7	3	>2.0
ABDSPscat052	6	7	1.21
ABDSPscat053	6	7	>2.0

LOD = log of the odds ratio selection criterion (see text).
15 known Mojave desert tortoise populations (Murphy et al. 2007).
Population 1 = most likely, Population 2 = second likely.
ABDSPScat011 = duplicate, deleted. ABDSPTissue01 from tortoise AB6306.

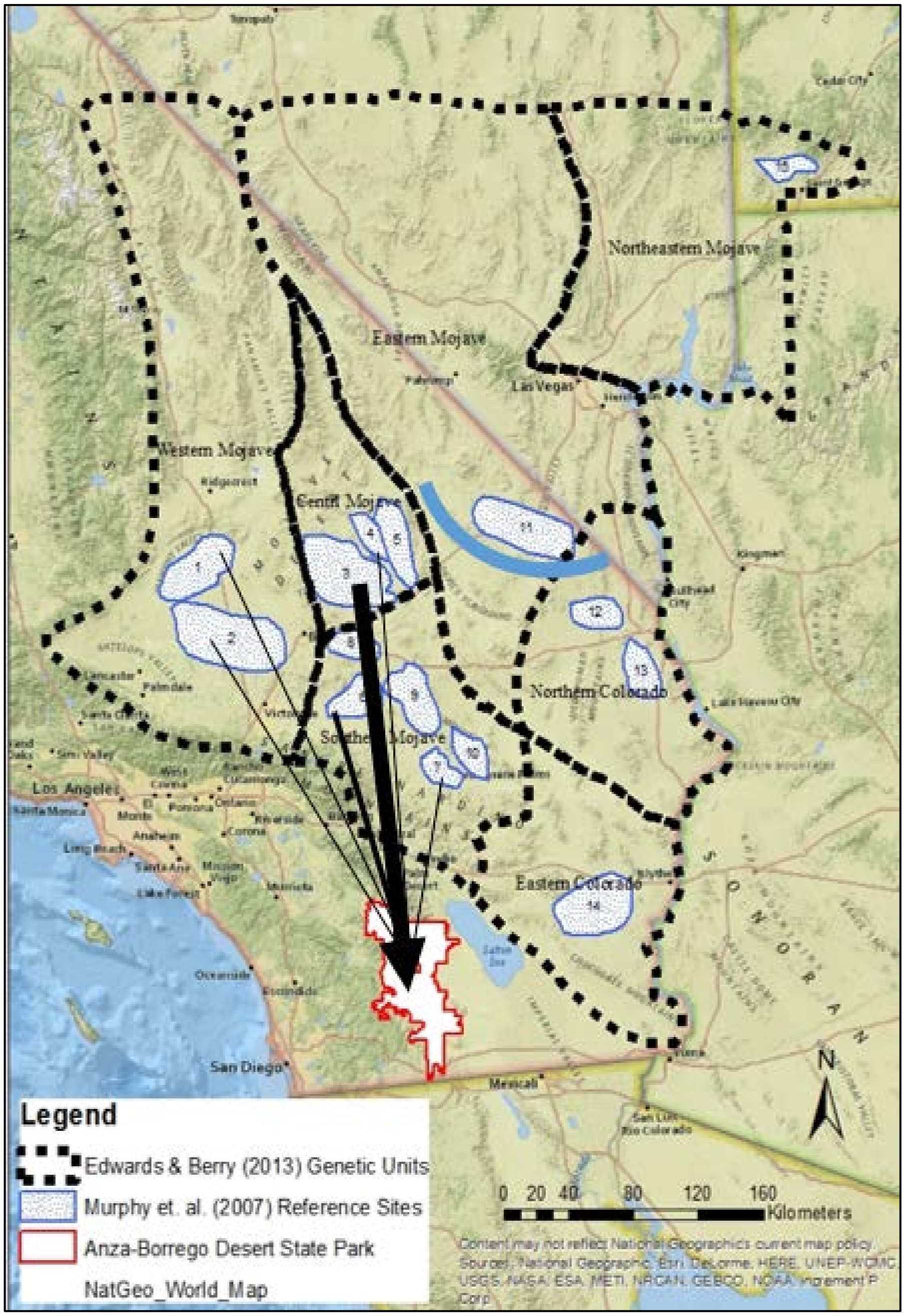


Figure 26. Population assignment test results of 44 Mojave desert tortoises in Anza-Borrego Desert State Park, California in relation to 15 reference populations. A previously defined haplotype gradient (blue curved line) separates the broadly distributed Mojave mtDNA haplotype (Moj_A) in the south from the Northern Mojave haplotype (Moj_B) to the north (Edwards and Berry 2013). Arrows originate from genetic source populations and depict the relative number out of 44 tortoise samples (thicker line = more samples) with a log odds ratio >2.0 from population assignment tests in this study (see text for details). Modified from Murphy et al. (2007) and Edwards and Berry (2013).

this time. Although not a focus of these genetic analyses, an interesting deformity was observed (Figure 27). Further communications with tortoise experts and geneticists will hopefully shed light as to whether this deformity is genetic or related to trauma, although despite its appearance, the tortoise appeared relatively healthy and agile.


Figure 27. Female tortoise AB6331 with deformed scutes, Coyote Canyon DTMU, Anza-Borrego Desert State Park, California, March 2018.

Genetic relatedness and family groups

Coyote Canyon management unit -- The most probable configuration of relatedness for the 31 samples from this management unit identified 3 of the adult males as fathers, 2 of the adult females as mothers (Figure 28), and 5 additional fathers and 6 additional mothers as parents that were not included in the sample dataset. Adult male AB6357 (MCL=178mm, Wt.=1120g; sample ABDSP056) and adult female AB6361 (MCL=251mm, Wt.=2700g; sample ABDSP005) were identified as the parents of offspring AB6314 (MCL=77mm, Wt.=107g; sample ABDSP038) (probability=0.94). Female AB6361 was also identified as the mother of offspring AB6360 (MCL=143mm, Wt.=610g, sample ABDSP006) and offspring AB6340 (MCL=70mm, Wt.=76g; sample ABDSP023) (probability=0.93). Male AB6357 was also identified as the father of offspring AB6341 (MCL=64mm, Wt.=64gm, sample ABDSP042) (probability=0.90). Adult male AB6313 (MCL=269mm, Wt.=4330g, sample ABDSP054) and adult female AB6349 (MCL=180mm, Wt.=1280g; sample ABDSP039) were identified as the parents of offspring AB6343 (MCL=60mm, Wt.=51g; sample ABDSP044) (probability=0.81); male AB6313 was also the most probable father of offspring AB6338 (MCL=68mm, Wt. 75g; sample ABDSP022) (probability=0.79), offspring AB6315 (MCL=54mm, Wt.=38g, sample ABDSP053) (probability=0.99), and offspring AB6356 (MCL=145mm, Wt.=670g; sample ABDSP057) (probability=0.66). Female AB6349 was not the most probable mother of the remaining offspring in the dataset. The third adult male (AB6336, MCL=279mm, Wt.=4282g; sample ABDSP037 fathered the following 4 offspring: AB6337 (MCL=89mm, Wt.=150g, sample ABDSP021), AB6311 (MCL=114, Wt.=241g; sample ABDSP041), AB6344 (MCL=105mm, Wt.=265g; sample ABDSP045), and AB6316 (MCL=83mm, Wt.=135g; sample ABDSP052) (all probabilities >0.99). The best maximum likelihood configuration of relatedness further identified 3 sets of 2-full-sibling pairs [AB6334 (MCL=84mm, Wt.=125g; sample ABDSP002) and AB6305 (MCL=114mm, Wt.=360g; sample ABDSP058) (probability=0.22), AB6360 (MCL=143mm, Wt.=610g; sample ABDSP006) and AB6340 (probability=0.73), and AB6333 (MCL=63mm, Wt.=70g; sample ABDSP020) and AB6330 (MCL=99mm, Wt.=228g; sample ABDSP033) (probability=0.56)]. It also identified 1 set of 3 full siblings [AB6337, AB6311, AB6344, and AB6313 (probability=0.54)]. There were also a number of half sibling pairs in the data.

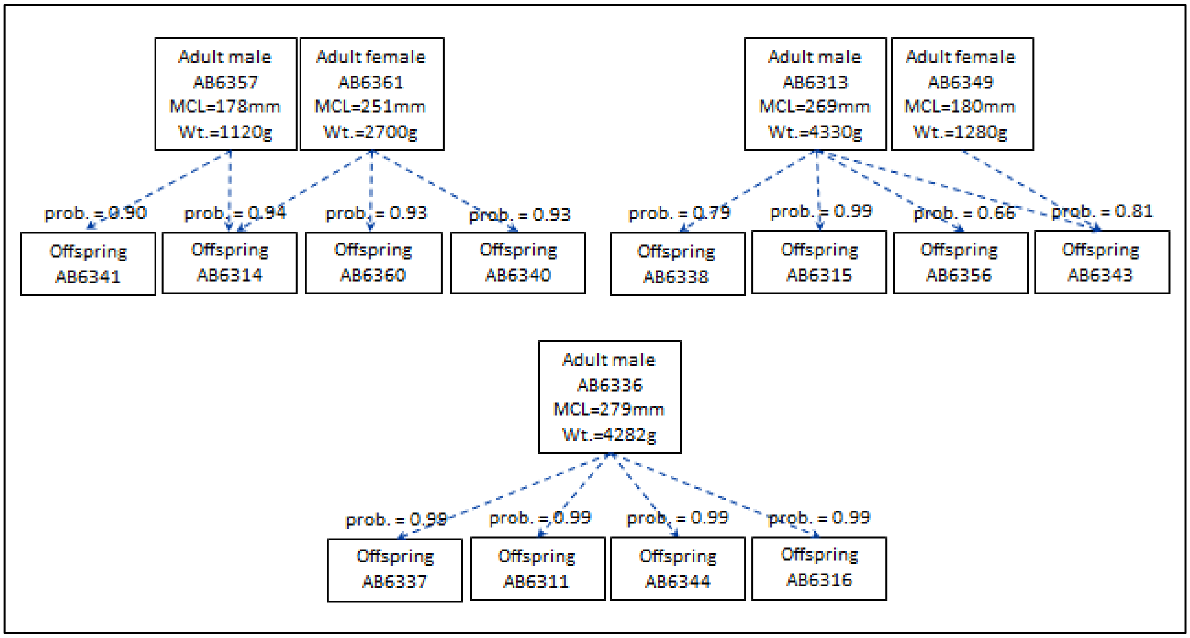


Figure 28. Desert tortoise pedigrees in Coyote Canyon Management Unit, Anza-Borrego Desert State Park, California, 2018. Based on best maximum likelihood configuration of relatedness for 31 tortoise samples (6 adult female blood samples, 5 adult male blood samples, & 19 blood and 1 tissue from tortoises with MCL<180mm). Animals not shown had either low or no probability of being related to the other individuals in the sample. Analyzed using Program Colony. See text for details.

Pinyon Ridge management unit -- The best maximum likelihood configuration of relatedness and parent-pair output from the 10 samples in this management unit identified a single offspring related to one of the sampled adults, with an additional 4 fathers and 3 mothers that were not part of the sample dataset. Adult female AB6332 (MCL=207mm, Wt.=1570g; sample ABDSP035) was consistent with being the mother of the animal that produced scat sample ABDSPScat052 (probability=0.68). This configuration also contained a single set of 3 full siblings that produced scat samples ABDSPScat023, Scat031, and Scat053 (probability=0.89) and a set of 2 full siblings (ABDSPScat035 &Scat36), although the probability of the latter was low (0.11). The remaining 2 scats had low probabilities (<0.09) of being full or half siblings.

Using all 10 samples (3 adults and 7 scats) combined while not accounting for sex or age structure further identified adult female AB6362 (MCL=263mm, Wt.=2750g; sample ABDSP007) as having a 0.48 probability of being a full sibling with 3 other animals identified by scat samples ABDSPScat023, Scat031, and Scat053. No other additional information about relatedness came out of these analyses.

Park-wide for full and/or half sibling groupings across sampling sites -- No evidence of intra-full sibling group fragmentation was found among the sampling sites across the park. There was also little evidence of intra-half sibling group fragmentation occurring among sites, with adult male AB6318 (MCL=252g, Wt.=3350g; sample ABDSP018) in the Pinyon Ridge management unit and adult male AB6355 (MCL=175mm, Wt.=1100g; sample ABDSP059) in the Coyote Canyon management unit having an estimated low probability (0.46) of being half siblings. Other half-sib groups with lower probabilities were equivocal.

Discussion

These genetic results are preliminary, and our analyses are ongoing. The appearance of low genetic diversity in ABDSP tortoises will be compared to other tortoise populations to ascertain whether this is relatively normal or lower than normal. Reasons for low genetic diversity are generally attributed to a small founder effect (i.e., low number of individuals that start a population) or a genetic bottleneck (i.e., a significant reduction in population size such that a low number of individuals reproductively contribute to subsequent generations). Given the information discussed in this report, plausible ways in which this system could be influenced by founder effects include: 1) a low number of individuals naturally colonized the ABDSP area sometime in the past, 2), some portion of the pet tortoises released into the park between 1958 and 1972 (or subsequent undocumented pets or offspring released) became naturalized and populated the park in the absence of natural colonization, and 3) the South family's newly hatched juvenile and other egg clutches possibly laid by their pet female tortoises may have survived and populated the area. Some combination of these scenarios is likely the case. All of these sources combined may still constitute a relatively small number of animals, containing low genetic diversity.

Genetic bottlenecks, on the other hand, can occur from a variety of pressures on existing populations, including overexploitation by humans. Although our analyses into bottlenecks continues, overexploitation of wildlife in the ABDSP area is believed to have occurred with the high numbers of miners, stage coach operations, explorers, and military operations in the region more than a century ago. In fact, Marshall South attributed his not seeing single bighorn during the first decade after his family's arrival in the park in the early 1930s to overexploitation by human travelers and residents prior to his family's arrival (South 1946).

Patterns of lower genetic diversity along species' range boundaries are common and expected when habitat is not contiguous (Richmond et al. 2013, Richmond et al. 2014). This is because extreme and shifting climatic conditions and increasing geographic isolation along these peripheral areas can contribute to relatively greater population fluctuations than in relatively more stable core areas. Because ABDSP extends southward like a peninsula from the larger desert tortoise range to the north (Nussear et al. 2009), it is not expected to receive gene flow from all directions like a core area would. A similar example is the low genetic diversity in the California gnatcatcher along the northern edge its range that may be partially due to smaller and more geographically isolated aggregations in northern latitudes (i.e., Ventura, Palos Verdes and Coyote Hills)(Vandergast et al. 2004). These authors also postulated that such populations along a species' range boundary may be important in allowing future range shifts in response to climate change. While this example presents a genetic signal along a species' leading (north) boundary as part of a northward range shift, demographic and genetic consequences of rapidly changing environmental conditions may also occur along species' trailing (southern) range boundaries. In support of this view, there is some preliminary evidence that desert tortoises may be exhibiting changes in reproductive phenology in response to extreme environmental conditions along their currently recognized southern range boundary north of Palm Springs, California (J. Lovich, pers. comm.), and it is reasonable to expect that such genetic and demographic signals may be pronounced in ABDSP due to it being situated farther south.

Our preliminary results also indicate that ABDSP's desert tortoises are *G. agassizii*, and show no evidence of hybridization, in support of Objective 1, H1. These results also indicate that genetic relatedness with reference populations to the north fit an isolation-by-distance pattern, indicating natural origins, consistent with Objective 1, H2. Additionally, there is evidence that gene flow has been influenced by the landscape (isolation-by-resistance), specifically with respect to lower genetic relatedness with reference populations across the Coachella Valley to the east of ABDSP, where prehistoric Lake Cahuilla divided these two regions during the late Pleistocene and Holocene (Norris and Norris 1961, Stokes et al. 1997). Covering up to 2,200 mi^2 (Norris and Norris 1961, Luttrell et al. 20007), Lake Cahuilla likely functioned as a barrier to tortoise dispersal and gene flow into the ABDSP region. Further refinement of these analyses may help improve our understanding of these processes.

Pedigree tests revealed parent-offspring relationships in multiple sample sites in support of Objective 2, H2, and provided evidence of a naturally reproducing Mojave desert tortoise population in ABDSP. The presence of full and half-sibling groups provided further evidence of genetic and familial structure. Additionally, pedigree testing identified parent tortoises that were not included in our samples, specifically 5 missing fathers and 6 missing mothers in the Coyote Canyon management unit, and 4 missing fathers and 3 missing mothers in the Pinyon Ridge management unit. This is expected given that field sampling was not exhaustive. However, other plausible explanations include: 1) they were present in the system to successfully reproduce, but are now not present in the wild population due to mortality, poaching (USFWS 1994, Berry et al. 1996), or dispersal out of the sampling area, or 2) some of these parent tortoises (if full-parent pairs) are/were pet tortoises that produced offspring that were released into the park by pet owners this past decade. Any combination of these may also be possible.

The lack of evidence of geographically fragmented intra-full and intra-half sibling groups among sampling sites provided support for Objective 2, H.3. This finding indicates that sibling groups were geographically clustered within sampling sites rather than among sites, and thus was consistent with that expected of naturally dispersed siblings (vs. human-mediated introductions that may disperse individuals within sibling groups among sites in the park).

Analyses to address hypotheses under Objective 2 continue, and the results will be included as an addendum to this report. Additionally, in collaboration with Dr. Jeff Lovich of the U.S. Geological Survey's Southwest Biological Science Center, the authors are reanalyzing and refining our population assignment tests to include new reference population sites (Mesa near Palm Springs and Shavers Valley near the Salton Sea), which lie in between ABDSP and the 15 sites used in this current analysis. These new sites are within 20 miles of the park, and are anticipated to help us better understand the genetic origins of ABDSP's Mojave desert tortoises.

In summary, genetic evidence indicates that this population consists of wild, reproducing *G. agassizii* individuals. The origins of this population may be natural and/or human-mediated, and is likely to be both considering the history of the region.

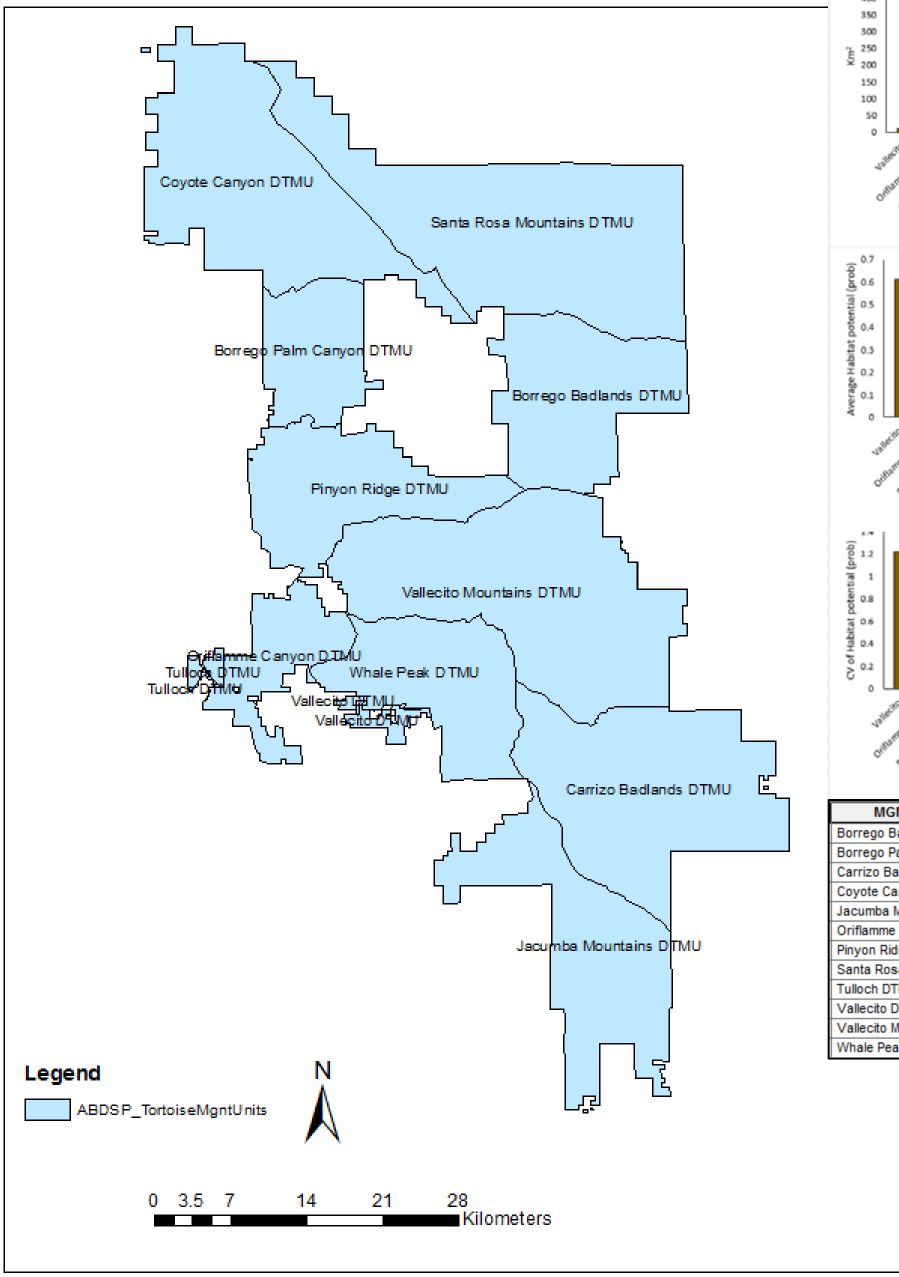
Framework for Population Monitoring

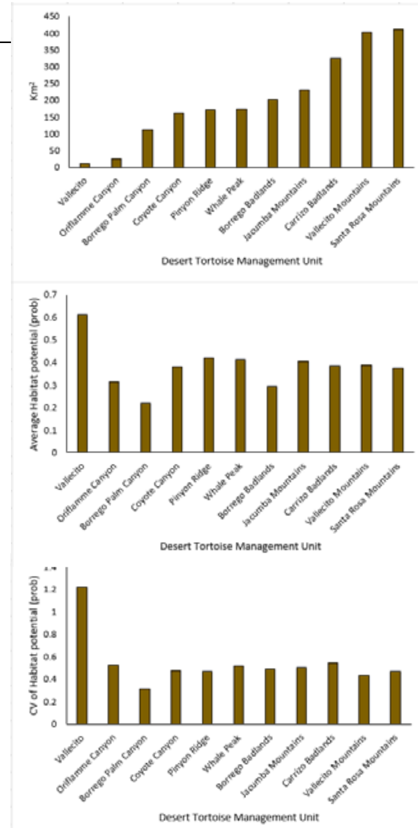
To help guide population monitoring, assess population status, and establish management goals and actions, the park was divided into 12 geographically distinct Desert Tortoise Management Units (DTMUs; Figure 29). These DTMU boundaries conform to existing Wildlife Management Units, and generally follow major mountain ridges, which provide natural breaks that keep core alluvial fan and adjoining slopes intact.

Anza-Borrego Desert State Volunteer Holly Valentine detecting a Mojave desert tortoise during the 2018 survey. Photo by ABDSP science volunteer Tim Valentine.

Variable baseline enviornmental conditions among DTMUs form the basis from which to establish DTMU-specific monitoring priorities and management and conservation measures. For instance, DTMUs vary in size and elevations (Figure 29). The average probability of habitat potential and coefficient of variation in habitat potential derived from Nussear et al.'s 2009 Maxent model also varies among DTMUs (Figure 29). Also, the relative frequencies of incidental tortoise sightings varied among these units (Figure 30).

The large size of ABDSP (1,000 mi^2, much of which is suitable habitat) presents challenges for wildlife monitoring programs. The level of survey effort in various habitat conditions and degrees of suitability can vary according to the specific question of interest, and questions regarding population trends versus understanding what factors govern annual rates of population growth and recruitment of young into a population that might be targeted for management actions can lead to different survey designs. Due to annual fluctuations in environmental and weather conditions across the park, annual surveys to assess population size, age structure, reproduction, survival, and cause-specific mortality would be helpful for developing effective data-driven management actions for desert tortoise conservation. Targeting known occupied sites for monitoring may prove useful in the park. However, stratified sampling (Cochran 1977) may be a more cost-effective approach. For example, the park can be divided into low, moderate, and high quality desert tortoise habitat according to Nussear et al.'s (2009) Maxent model (Figure 31), and monitoring could entail a relatively high level of survey effort in high quality areas, moderate surveying in moderate quality, and a relatively low level of surveying in low quality habitats. A number of alternative sampling allocation schemes within a stratified random sampling framework (such as proportional allocation of survey effort according to area of each





MGMT_NAME	MGMT_UNIT	ELE_RANGE
Borrego Badlands DTMU	622-H-N-04-a-100	0180-1280
Borrego Palm Canyon DTM	622-H-N-03-a-100	0640-6120
Carrizo Badlands DTMU	622-H-N-09-a-100	0220-3023
Coyote Canyon DTMU	622-H-N-02-a-100	0600-6160
Jacumba Mountains DTMU	622-H-N-10-a-100	0680-4520
Oriflamme Canyon DTMU	622-H-N-07-a-100	2160-5680
Pinyon Ridge DTMU	622-H-N-05-a-100	0680-4600
Santa Rosa Mountains DTM	622-H-N-01-a-100	0000-6040
Tulloch DTMU	622-H-N-00-a-100	4240-5480
Vallecito DTMU	622-H-N-08-a-100	1440-2400
Vallecito Mountains DTMU	622-H-N-06-a-100	0090-4480
Whale Peak DTMU	622-H-N-08-a-100	1000-5320

Figure 29. Desert Tortoise Management Units established in Anza-Borrego Desert State Park, California. Graphs and table depict size, average probability of habitat potential, coefficient of variation in habitat potential, and elevation range by DTMU. Habitat potential metrics derived from Nussear et al. (2009).

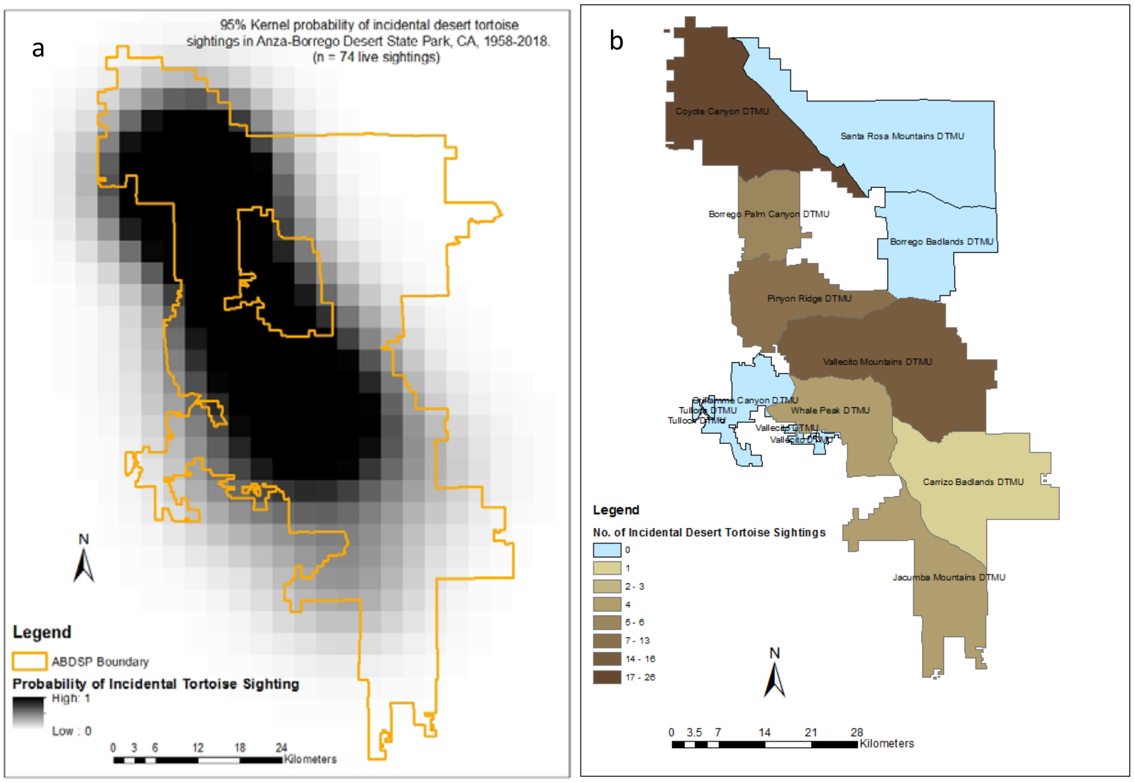


Figure 30. Estimated 95% kernel probability of incidental desert tortoise sightings (darker cells = higher probability of a sighting) (a), and frequency of incidental sightings in Desert Tortoise Management Units (b), Anza-Borrego Desert State Park, California, 1995-2018.

stratum) could be considered (Scheaffer et al. 1990). This could be duplicated in each DTMU. Optimal allocation, with sample sizes proportional to area (and variation) and inversely proportional to cost of conducting a survey, may ultimately improve sampling efficiency, reduce bias, and increase precision of population estimates.

Embedded within such a stratified random sampling framework would be field methods to detect tortoises and estimate targeted demographic rates in each sampling unit, including detection probability. For example, distance sampling has been used to monitor density of tortoises in the Mojave and Sonoran Deserts (Swann et al. 2002, Averill-Murray and Averill-Murray 2005, U.S. Fish and Wildlife Service 2016). Alternatively, occupancy sampling provides greater statistical power to detect annual declines in the proportion of area occupied by Sonoran Desert tortoises (Zylstra et al. 2010), and may prove beneficial in ABDSP, where vegetation cover is variable. Mark-recapture has been successfully applied to hundreds of species worldwide (Cormack 1964, Jolly 1964, Seber 1965), and does not require that animals be uniformly distributed, as is the case with distance sampling (Buckland et al. 1993). Experts in population estimation and sampling design should be consulted on methodologies to ensure statistical rigor and scientific validity.

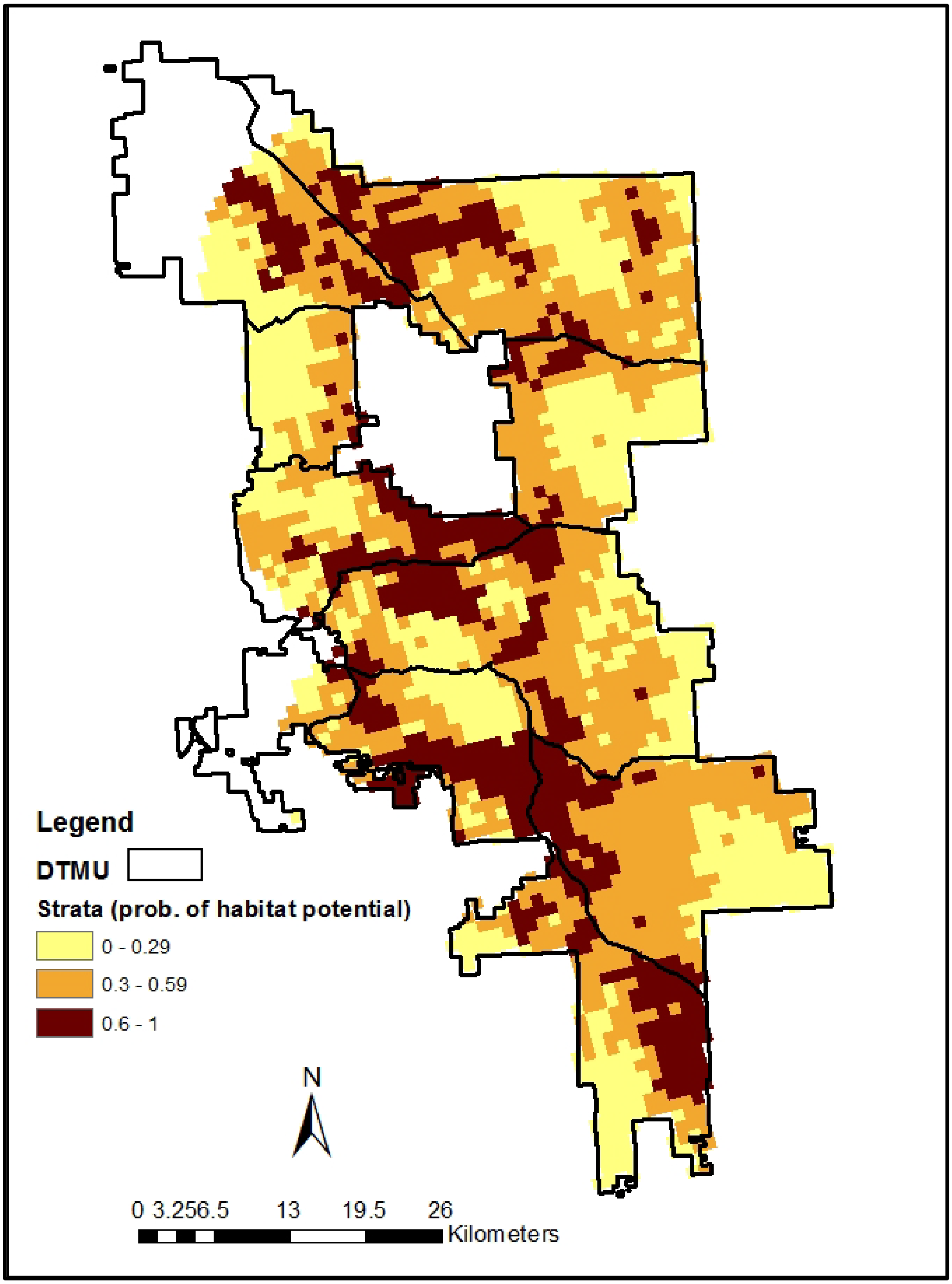


Figure 31. Three strata of probability of desert tortoise habitat potential: 1) low probability (0-0.29), 2) moderate (0.3-0.59), and 3) high (0.6-1.0) probability within 12 Desert Tortoise Management Units in Anza-Borrego Desert State Park, California. Habitat potential was derived from Nussear et al. (2009).

Baseline Estimates of Demography and Disease Prevalence

Baseline estimates were obtained from two separate surveys (March 2017 and 2018).

Presence (distribution of tortoise presence in ABDSP)

Presence and occupancy modeling can be used to describe the area occupied, site-specific probabilities of occupancy, factors driving occupancy, habitat selection, meta-population dynamics, and range shifts, all of which can help inform management (Machenzie et al. 2005). These methods rely on specific sampling designs that include spatial and temporal replication and associated data (Mackenzie and Royle 2005, Steenweg et al. 2018). At this time, formal "presence' or presence-absence" survey data were unavailable to estimate presence or occupancy throughout ABDSP. Given the hierarchical factors that ultimately influence the probability of incidental sighting records over the past 6 decades (see section about formalizing the incidental sightings database in this report), the existing incidental sighting records were not used to estimate occupancy. Despite limitations of these data, using them in a retrospective assessment of previous sightings over the past 60 years may elucidate a broad distributional pattern across the park. Future efforts should however evaluate the utility of these and new citizen-science data for estimating site occupancy in the park.

Methods

Animal data from the 2018 genetic study conducted in 9 sites within the 5 selected DTMUs known to contain incidental sightings were used to portray the broad-scale distribution of tortoises in the park; these 5 DTMUs were Borrego Palm Canyon, Coyote Canyon, Jacumba Mountains, Pinyon Ridge, and Vallecito Mountains (Figure 29). Also, to elucidate broad distributional patterns, a 95% fixed kernel probability model was fit to the incidental sightings "presence-only" data from 1958-2018 (n = 74). This analysis has a number of assumptions due to the limitations of the incidental sightings data (see section in this report on formalizing the incidental sightings database).

Results

The 95% fixed kernel probability model revealed a relatively higher probability of desert tortoise sightings along a northwest-southeast gradient through the park over the past 6 decades (Figure 30a. This pattern was also apparent when examining the raw frequency of incidental sightings in DTMUs (Figure 30b). This species appears to be widespread across the park, as indicated by 7 out of the 12 DTMUs (58%) containing ≥1 incidental sighting over the past 6 decades (Figure 30b).

Live tortoises and/or recent tortoise sign (scat, burrow, and/or carcass) were observed in 4 of these DTMUs during that survey. Live tortoises and/or sign were not detected in the Borrego Palm Canyon DTMU during the 2018 survey, although 2 incidental sightings (with photographs) of 2 different adult tortoises (one was verified to be a male; J.A. Manning, pers. obs.) were reported in this DTMU in spring 2018. A third sighting of an adult female was reported on private lands immediately adjacent to this DTMU in the fall of 2018 (J.A. Manning, pers. obs.).

Accordingly, the minimum number of DTMUs known to be occupied by desert tortoises in 2018 was 5.

Sex ratio

Adult sex ratio (ASR, the proportion of females in the adult population) forms the foundation of a central concept in population and evolutionary biology, and influences mate choice, pair bonding, parental cooperation (Ancona et al. 2017, Schacht et al. 2017), and population viability. Environmental variation can underpin spatiotemporal shifts in ASRs within populations (Manning et al. 2015), and ASR can operate in concert with environmental variation to shape spatial distributions of polygyny thresholds (Manning and McLoughlin 2017). Thus, monitoring adult sex ratios can help elucidate possible changes taking place in a population or the environmental conditions that may drive population dynamics and viability.

Here, ASR was estimated in each sampling site surveyed within some of the selected DTMUs. Due to small sample sizes, data from 2017 and 2018 were pooled, which assumes adult survival is high, such that encountered adults were alive in both years (See Table 6 for details). The following are estimated ASRs for the 3 DTMUs with adequate sample sizes:

Coyote Canyon DTMU
New captures (2017 and 2018 combined)
Adult sex ratio (8f/14 adults with MCL >180mm) = 0.57

Pinyon Ridge DTMU
New captures from 2017 and 2018
Adult sex ratio (3f/4 adults with MCL >180mm) = 0.75

Vallecito Mountains DTMU
New captures from 2017 and 2018
Adult sex ratio (1f/3 adults with MCL >180mm) = 0.33

Comparing these ASRs to those from other studies across the tortoise's range may help elucidate the viability of these tortoise populations.

Reproduction

In 2018, the park documented mating in the wild (J.A. Manning, R. Woodard, and others, pers. obs. during genetics survey). This sighting of male desert tortoise AB6354 mating with female AB6339 in the Coyote Canyon DTMU was the first documented mating of Mojave desert tortoises in ABDSP. Tortoise egg shells were also discovered in a burrow located in the Vallecito Mountains DTMU, providing further evidence of breeding and egg clutches in at least two DTMUs in the park. The parent-offspring relationships found during pedigree construction provides evidence of successful reproduction has been occurring.

Table 6. Raw counts of live tortoises in desert tortoise management units surveyed in ABDSP in March 2018. Surveys were conducted in select areas within each management unit.

	2017				2018				
DTMU	Female	Male	Unk	ASR	Female	Male	Unk	ASR	Total
Coyote Canyon DTMU	2	1	6	0.33	6	5	19	0.42	37
Pinyon Ridge DTMU	0	0	1	-	3	1	0	0.25	5
Vallecito Mts. DTMU	0	1	1	-	2	1	0	0.33	5
Total	2	2	8		12	7	19		50

[1] Sex unknown due to absence of sex-specific morphological characteristics in non-adult tortoises (e.g., weight <1,000 grams).
[2] Coyote Canyon DTMU site grand total calculated as 9 captures in 2017 + 31 captures in 2018 - 3 recaptures of 2017 animals in 2018 = 37; no other DTMU contained recaptures in 2018.

Population size and viability

Population density is an important metric to estimate because it provides simple measure of tortoise population viability. According to the USFWS (1994), a viable population of desert tortoises must maintain an average minimum density of 10 adults per mi^2 (6 adults per km^2) in order to ensure that adults have ample opportunity to encounter likely mates. Survey methods for estimating population size are typically designed specific to a species and site. Methods, such as distance sampling (Buckland et al. 1993) and mark-recapture (Cormack 1964, Jolly 1964, Seber 1965) can account for different levels of survey effort and detection probability, and produce unbiased and comparable estimates of population size. Despite the importance of proper estimation of population size and density, the 2017 reconnaissance and 2018 genetic origins surveys were not intended for estimating density.

Methods

In the absence of statistically rigorous population estimates, raw counts were used here to provide insight into the minimum number of tortoises known alive in the park each year. While included in this report, the minimum number known alive is biased low from the true population size (given that detection of animals is always <100%, and thus not a standardized metric comparable among sites or years.

Although low numbers of tortoises detected during the 2017 initial reconnaissance survey (n = 12) did not allow estimation of population size, a subsequent resight survey in one small (<1 km^2) sampling site in the Coyote Canyon DTMU was conducted by Dr. Manning within 14 days, which allowed the use of the simple Chapman estimator with Poisson-corrected confidence intervals (CIs) to estimate population size (Ricker 1975, Seber 1973). Both, the initial reconnaissance survey and subsequent resight survey data included detections of individual tortoises from various size classes; the initial survey detected and uniquely marked 9 individual tortoises at the site, although the level of effort between the two sampling occasions differed.

To also help initiate discussion among park environmental scientists about how to best survey for and estimate tortoise densities into the future, I calculated two crude density estimates using the raw counts of living tortoises within 95% kernel areas estimated from tortoise or tortoise and tortoise sign point locations. To accomplish this, I first estimated adult density within a defined sampling site within its corresponding DTMU; here, I used a 95% fixed kernel analysis of adult tortoise capture locations to estimate the minimum geographic area occupied (e.g., Figure 32). Data used for this were the original capture sites for each individual live adult detected during the 2017 and 2018 surveys (e.g., an adult detected in both years was recorded once in this analysis). These kernel polygons are expected to be biased low due to using a single location from multiple individuals because tortoise home range sizes vary with sex, age, season, density, resource availability, and year (USFWS 1994). As such, the resulting densities are anticipated to be biased high. These analyses were performed using R package ‘adehabbitatHR’ (Calenge 2006) and visually portrayed in ArcGIS (ESRI, Redlands, CA). I used the *ad hoc* bivariate normal smoothing function, which avoids the drawbacks demonstrated with the least squares cross-validation function (i.e., high variability, tendency to undersmooth data, and multiple local minima; Horne and Garton 2009).

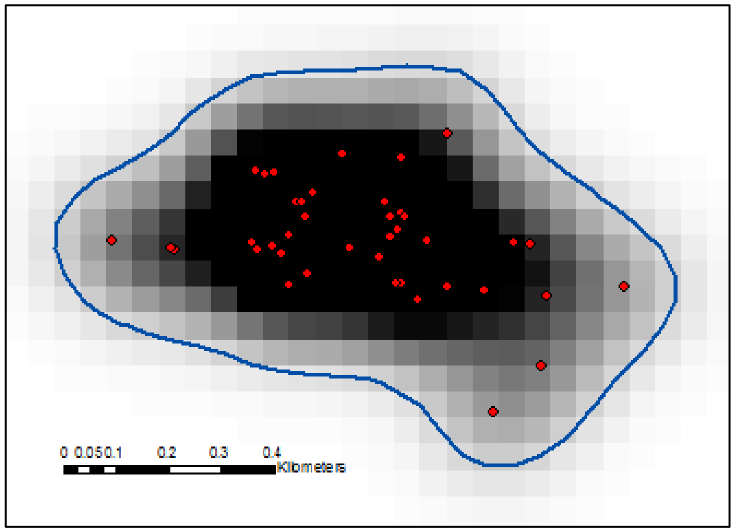


Figure 32. Estimated minimum geographic area occupied by desert tortoises (blue line = 0.5686 km^2) in a single sampling site within the Coyote Canyon Desert Tortoise Management Unit in Anza-Borrego Desert State Park, California 2017-2018. Area estimated as a 95% fixed kernel ad hoc bivariate normal function. Grid of light grey to black-colored cells portrays the probability density from all tortoises detected at the site during sampling (e.g., darker = higher probability that tortoises are found there). Data are single capture locations (red dots) from individual live tortoises (males, females, adults, and juveniles) captured during a 2017 initial reconnaissance survey or the 2018 genetic origins study (see this report for details). Three tortoises were recaptured in 2018; their initial locations from 2017 were used in this analysis.

Second, because small sample sizes precluded the estimation of adult density in all, but the one site within the Coyote Canyon DTMU, I calculated a measure of density in the remaining DTMUs where ≥1 tortoises were detected by dividing the raw count of adult tortoises (both sexes combined) by the 95% kernel minimal geographic area estimated at the corresponding site as a function of live animals, carcasses, scats, and burrows (Figure 33). This latter approach was intended to avoid biased low minimum geographic areas occupied and the corresponding biased high estimates of density that could arise from small samples detected from a possibly larger population of live animals. As pointed out above, these retrospective density estimates may be biased, and should be considered with caution.

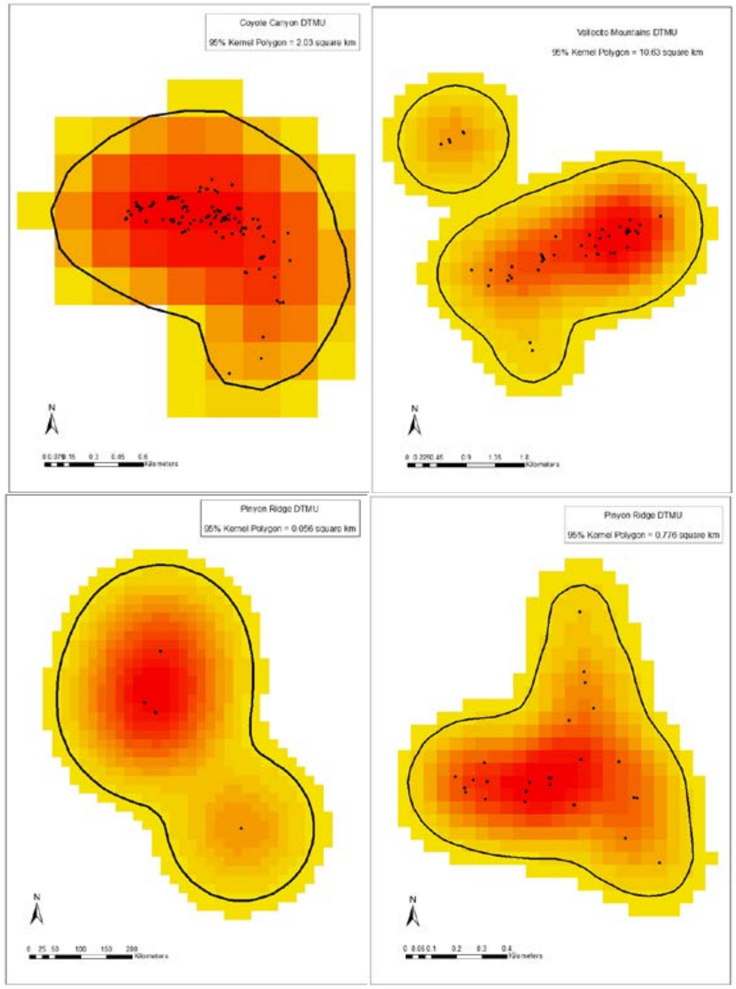


Figure 33. Estimated minimum geographic areas occupied by desert tortoises (black line) in a sampling site within each of three different Desert Tortoise Management Units (Coyote Canyon, Vallecito Mountains, and Pinyon Ridge) in Anza-Borrego Desert State Park, California 2017-2018. Area estimated as a 95% fixed kernel ad hoc bivariate normal function. Grid of yellow to red -colored cells portrays the probability density from all tortoises detected at the site during sampling (e.g., darker = higher probability that tortoises were found there). Data are single locations (black dots) of individual carcasses, scats, burrows , and live tortoises (males, females, adults, and juveniles) captured during the 2017 initial reconnaissance survey or the 2018 genetic origins study (see this report for details). Three tortoises were recaptured in 2018; their initial locations from 2017 were used in this analysis.

Results and discussion

The mark-resight survey resulted in 1 resight of a marked tortoise and 5 new unmarked tortoises (M=9 marked individuals on occasion 1, C=6 individuals on occasion 2, and R=1 resight of a previously marked individual during occasion 2), producing a simple Chapman-based population estimate N = 34 tortoises (Poisson-corrected CI = 10.4 to 60.9). However, a reduced sampling effort during the resight survey by a single observer (compared to the numerous observers during the first sampling occasion) likely lowered the detection probability and biased *N* high. Despite this, the estimated *N* indicates the presence of more than just a few individuals, but rather a population of tortoises in this vicinity.

In 2017, the minimum known number alive was 18 [a combination of 12 detected tortoises during that survey, 5 more unmarked individuals during the mark-resight survey, and an adult male tortoise that was also observed in an area not surveyed (Manning, J., unpubl data)].

In 2018, the minimum number known alive was 47 [12 uniquely marked individuals in 2017 and 36 uniquely marked individuals in 2018 minus 1 individual (AB6306) marked in 2017 that was later found dead in 2017]. These minimum numbers known to be alive are based on all size classes, and the 2018 number assumes that animals marked in 2017 that weren't resighted in 2018 (n = 8) survived without being detected in the March 2018 survey.

Adult densities varied among the three DTMUs where tortoises were located during the 2017 and 2018 surveys (Table 7). The estimated adult density in the site located within the Coyote Canyon DTMU was estimated at 26.38 adults/km^2; Table 7; Figure 32). This estimated density closely approximated and lies within the confidence interval of the independent mark-resight Chapman estimate of population abundance for the same area in 2017 (34, CI = 10.4-60.9), as detailed above. This correspondence suggests that despite the presumption that biased low 95% kernel areas and biased high estimates of density would emerge from using a single location from multiple individuals, the data available at that site may have been adequate for estimating density with these methods. As expected, however, the density based on the 95% kernel calculated from live tortoise and sign locations at this site was considerably lower (6.90/km^2; Table 7). However, both kernel-based density estimates and the Chapman estimate were above the minimum density to ensure population viability (USFWS 1994). Interestingly, this was the only DTMU where mating was observed. The estimated adult densities in the remaining 2 DTMUs where adult tortoises were detected during surveys were relatively low (Pinyon Ridge DTMU = 5.15/km^2 and Vallecito Mountains DTMU = 0.18/km^2; Table 7). These 95% kernels and associated densities should be interpreted with caution, as they likely represent areas smaller than tortoise home ranges, which can be 4-180 hectares (0.04-1.8 km^2) or considerably larger over a tortoise's lifetime (Boarman 2002c).

Table 7. Raw counts and densities of desert tortoises from standardized surveys in select, non-random sampling sites within Desert Tortoise Management Units in Anza-Borrego Desert State Park, California; 2017-2018 surveys combined.

	Raw Counts (N)				Adult Density (N/km^2)	
	Adults		Sub-adults			
DTMU	Male	Female	Sex Unknown	Total	Adult area	Total area*
Borrego Badlands DTMU						
Borrego Palm Cyn DTMU						
Carrizo Badlands DTMU						
Coyote Canyon DTMU	6	8	25	39	24.62 [1]	6.90**
Jacumba Mts DTMU						
Oriflamme Cyn DTMU						
Pinyon Ridge DTMU	1	3	1	5	NA	5.15
Santa Rosa Mts DTMU						
Tulloch DTMU						
Vallecito DTMU						
Vallecito Mts DTMU	2	1	1	4	NA	0.18
Whale Peak DTMU						

Note: Data are from non-randomly selected sites; empty cells reflect no survey.

* Estimated number of adult animals / area of 95% kernel polygon calculated from all live animals (any size and sex), carcasses, scats, and burrows at a site in the DTMU.

** Meets minimum density requirement for population viability (USFWS (1994); 6 adults per km^2 in order to ensure ample opportunity to encounter likely mates.

[1] Independent 2017 mark-resight Chapman estimate = 34, CI: 10.4-60.9 (Seber 1973, Ricker 1975; see text for details).

Disease assessment

Disease is a major threat to desert tortoises (USFWS 1994). Upper Respiratory Tract Disease (URTD) (*Mycoplasma spp.*) is widespread across the Mojave desert tortoise range. Thus, tortoise blood samples obtained from live tortoises captured during the 2018 genetic origins study were tested for the presence of URTD.

Methods

Disease assessments were conducted on animals captured in March 2018, and included blood analyses and an assessment of physical appearance for diagnostic symptoms. Blood samples were collected from the same 36 animals used for genetic testing. Blood serum or plasma were shipped to Dr. Mary Brown's lab at the University of Florida's Dept of Infectious Disease and Pathology to test ELISA serology for *Mycoplasma agassizii* (the most common test to detect exposure to *M. agassizii*, a cause of URTD in tortoises) and *Mycoplasma testudineum* (a test for a second species of mycoplasma that can be associated with URTD and eye infections).

Symptoms of URTD were assessed in each tortoise by biologists trained in symptom recognition. Blood collection for DNA analysis and disease assessment were collected by qualified personnel following the blood sampling protocols specified in the *Health Assessment Handbook*: https://www.fws.gov/Nevada/desert_tortoise/documents/reports/2016/may-2016-deserttortoise-health-eval-handbook.pdf. This USFWS approved manual references Hernandez-Divers et al. (2002). Digital photographs of live tortoise and each item of sign were also recorded.

Results

No evidence of URTD or other communicable diseases were detected in the ABDSP tortoises. However, the blood samples were collected earlier in the spring season than ideal for avoiding false negative presence of URTD (Roy Averill-Murray, pers. comm.). This is because it's more detectable after infected animals have been active later in the spring. As such, the presence of URTD can't be ruled out, although these results indicate that if present, it may not be prevalent in the ABDSP system. Reasons for this may be the geographic isolation of this population from the rest of the Mojave desert tortoise range. Given this finding, and the high rate of infection of this communicable disease, it is imperative that actions are taken to ensure that captive tortoises and those that have been held in captivity for even a short period of time not be released into (or back into) ABDSP. A public awareness campaign would be helpful to achieve this.

Threats

Various threats to the Mojave desert tortoise occur across its range (Boarman 2002a,b). Many threats, including habitat loss, disease, predation, and high levels of human access to tortoise habitat can have significant impacts not only to individual tortoises, but also on populations by cumulatively scaling up to reduce population growth and viability (Boarman 2002a,b). Because of the tortoise's K-selected natural history traits (see Introduction section of this report), which cause tortoise populations to recover slowly from population declines similar to that observed in elephants, whales, and rhinos, potential and realized sources of mortality are important to document and monitor. Boarman (2002a,b) specifically states:

> *"The loss of habitat, mortality from increased traffic, reduced quality of habitat altered by human presence and activity, fragmentation of populations, and the cumulative effects of other problems associated with humans (e.g., dogs, recreation, utility corridors, etc.) pose a significant and increasing problem for the viability of tortoise populations."*

High rates of predation can emerge in desert tortoise populations, causing significant population declines, and stochastic predation events can decimate cohorts and local populations (e.g., Medica et al. 2012). Predators include coyote, ravens, badgers, mountain lions, raptors, and various mesocarnivores (e.g., see http://tortoise-tracks.org/threats/predators). Of particular concern across the Mojave desert tortoise range are unnaturally high, human-subsidized numbers of ravens and coyotes (Boarman 2003, Todd et al. 2010).

During the 2017 and 2018 surveys, ravens were observed carrying toilet paper and trash to a raven nest in the Coyote Canyon DTMU (Figure 34). One juvenile-sized tortoise carcass was found with classic puncture marks from a common raven's beak (USFWS 2008; Figure 35). Boarman (2002c, 2003) and others discuss ravens with regards to desert tortoise predation, and actions to control ravens across the tortoise's range have received much attention by resource agencies (USFWS 2008). Methods to reduce ravens include the removal and control of human refuge that attracts these birds and coyotes. Wildlife-proof trashcans are critical in remote campgrounds, and working with local dumps and dump stations to ensure that dumpster lids are in place and closed at all times will help reduce raven congregations that spill over miles into desert tortoise habitat from these food subsidies.

Figure 34. Raven carrying toilet paper over occupied tortoise habitat, ABDSP, 2018.

Figure 35. Carcass 1-1 (genetics sample 024) with missing portion of plastron indicative of raven predation, Anza-Borrego Desert State Park, California. March 2017. Photo by J.A. Manning.

There was also evidence that canines chewed on adult tortoises in the Coyote Canyon DTMU (Figures 36 and 37). These may have been coyotes, but domestic dogs are also known to chew on tortoises. Although park regulations require dogs to be on leash and remain on designated roads, field surveyors reported seeing unleashed dogs in the vicinity of tortoises. Additionally, feeding coyotes can subsidize their local populations, is prohibited in the park and the Borrego Springs area, and enforcement should continue (California Department of Fish and Wildlife and DPR regulations).

Roads in desert tortoise habitat can cause a variety of impacts to tortoises, including mortality and illegal collecting (poaching; Berry et al. 1996). Vehicular activities in desert

Figure 36. Damaged gular horn on adult male tortoise AB6329 in Coyote Canyon DTMU, Anza-Borrego Desert State Park, California, 2018. Damage is indicative of chewing (canine teeth) by coyote (*Canis latrans*) or domestic dog (*C. familiaris*). Person in background of photo is J.A. Manning.

Figure 37. Damaged gular horn and carapace on adult female tortoise AB6332 in Pinyon Ridge DTMU, Anza-Borrego Desert State Park, California, 2018. Damage is indicative of chewing (canine teeth) by coyote (*Canis latrans*) or domestic dog (*Canis familiaris*). Photo by J.A. Manning.

Figure 38. Heat map of variable levels of vehicle and foot use in the Coyote Canyon Desert Tortoise Management Unit. (Data acquired Nov 6, 2018 via Strava: https://www.strava.com/heatmap#12.83/-116.50218/33.40484/hot/all).

tortoise habitat is considered a primary threat to tortoises across their range (Boarman 2002a,b, and others). ABDSP contains variable levels of vehicle use in tortoise habitat. For example, a heat map of vehicle use in the Coyote Canyon DTMU (Data via Strava: https://www.strava.com/heatmap#12.83/-116.50218/33.40484/hot/all) reveals variable frequencies of vehicle activity along the designated dirt roads in that DTMU (Figure 38). The potential of extracting quantitative use statistics from Strava could prove useful for future investigations into wildlife-vehicle interactions. Additionally, adult and juvenile-size tortoises were commonly observed on designated dirt roads during surveys and incidental sightings (Figure 39), with some tracks of breeding adult tortoises observed being intersected by tracks of unauthorized vehicles in wilderness dry washes in occupied breeding habitat (Figure 40). Additionally, the mortality of a juvenile size tortoise (AB6306: wt = 68g, MCL = 62mm) was documented in 2017, showing symptoms characteristic of being crushed (Figure 41). In line with this observation, the Initial Study and Checklist for the Coyote Canyon Public Use Plan (CDPR Oct 2 1995), stated: *"as a federal and state threatened species, … tortoises in Coyote Canyon are entitled to full protection under the law. Potential impacts include being run over by vehicles or capture and removed by visitors"* (CDPR 1995:16). Although the specific cause of being crushed was unknown, it was located on a dirt road where vehicular and equestrian activities are common. Because 98% of baby tortoises perish before reaching adulthood due to being a K-strategist (MacArthur and Wilson 1967, Wilbur and Morin 1988, Turner et al. 1987), even a low number of baby tortoises lost to tortoise-vehicle collisions can significantly impact population viability.

Poaching tortoises for a cultural observance is not uncommon in other areas (USFWS 1994, Berry et al. 1996). Poaching has not been reported in ABDSP, although the park acknowledges the potential for capture and removal by visitors (CDPR 1995:16). According to Berry et al. (1996), nearly 8% of tortoise burrows found in their study area showed evidence of being excavated by humans and the number increased closer to dirt roads. Their findings indicate that poaching tends to occur closer to roads and that the presence of roads may facilitate it. Another human-related source of desert tortoise mortality in some areas is gunshot deaths (Berry et al 2008). Although this has not been observed in ABDSP, it has been identified as an important cause of mortality in Red Rock Canyon State Park's desert tortoises (Berry et al. 2008).

Figure 39. Desert tortoise tracks crossing a designated dirt road in Anza-Borrego Desert State Park, California, 2018. Photo by Jeff Manning.

Figure 40. Tracks from vehicles that traveled in a wilderness dry wash closed to vehicles, which intersected adult-sized desert tortoise tracks in Anza-Borrego Desert State Park, California. 2017. Photo by J.A. Manning.

Figure 41. Juvenile tortoise AB6306 (MCL=62mm, Wt.=68g) was delivered to ABDSP Headquarters by a visitor after being crushed by an unknown source. Anza-Borrego Desert State Park, California, 2017. Photo by J.A. Manning.

Long periods of drought can also have significant impacts to desert tortoises (Duda et al. 1999, Berry et al. 2002b), and may interact with the factors above to reduce population viability. Consequently, monitoring of tortoise demographic rates and research on how the above mortality factors interact with the park's tortoise population in the presence of changing climatic conditions may help in establishing short and long-term tortoise conservation actions.

Conservation Actions

Land managers across the tortoise's range are engaged in habitat conservation measures that include restoration and management to help recover the species (Abella and Berry 2016). The DPR implements land acquisition, resource, facility, and visitor use management strategies that foster long-term sustainability of natural animal populations and the processes that influence the dynamics of animal populations (DPR DOM Section 0311.2 General Animal Management Policy; see Boxes 1 and 2 in the Introduction). In line with this, the Initial Study and Checklist for the Coyote Canyon Public Use Plan (CDPR Oct 2 1995), states:

> *"The park is outside the recognized distribution of the tortoise, but the species is found in the mountains east of Salton Sea, forty miles from Coyote Canyon. Most records for the park are from Sheep Canyon in Coyote Canyon and include nine records from 1973 to 1993. An observation during April 1993 of newly hatched tortoises is thought to represent captive individuals released into the park, or perhaps a remnant natural population. In any event, the tortoises in Coyote Canyon are entitled to full protection under the law."* (CDPR 1995:16).

As such, ABDSP has been engaged in a variety of actions that provide direct or indirect conservation benefits to desert tortoises. These actions provide benefits to desert tortoises, and thus constitute recovery actions as defined under the federal Endangered Species Act.

State of California - The Resources Agency — Arnold Schwarzenegger, Governor
DEPARTMENT OF PARKS AND RECREATION — Ruth Coleman, Director

Resolution 15-2005
adopted by the
CALIFORNIA STATE PARK AND RECREATION COMMISSION
at its regular meeting in San Diego, California
February 11, 2005

Anza-Borrego Desert State Park®
Anza-Borrego Desert State Wilderness Expansions

WHEREAS, the units of the State Park System are classified by the State Park and Recreation Commission into a category specified in Public Resources Code (PRC) Section 5019.50;

WHEREAS, one of these classifications is "State Wilderness," which consists of those areas of relatively undeveloped, state-owned or leased land, which have retained its primeval character and influence or have been substantially restored to a near-natural appearance; where the earth and its community of life are untrammeled by man and where man himself is a visitor; and

WHEREAS, a State Wilderness (per PRC 5019.68) has outstanding opportunities for solitude or a primitive and unconfined type of recreation; and

WHEREAS, a State Wilderness must be of sufficient size to make practicable its characteristics, or is of sufficient size as to make practicable its preservation and use in an unimpaired condition;

WHEREAS, the Anza-Borrego Desert State Park® General Plan has proposed that the Anza-Borrego Desert State Wilderness be expanded by expanding existing wilderness management areas and adding 1 new wilderness management area (as delineated in the General Plan document, and further delineated in *Attachment A: Naming and Classification of Various Parcels Within Anza-Borrego Desert State Park®*), to provide for the recognition and protection of the unique wilderness environment; and

WHEREAS, the proposed expansions comprise thousands of acres that meet the definition of State Wilderness; and

WHEREAS, the designation and preservation of this area will protect the wilderness qualities for future generations;

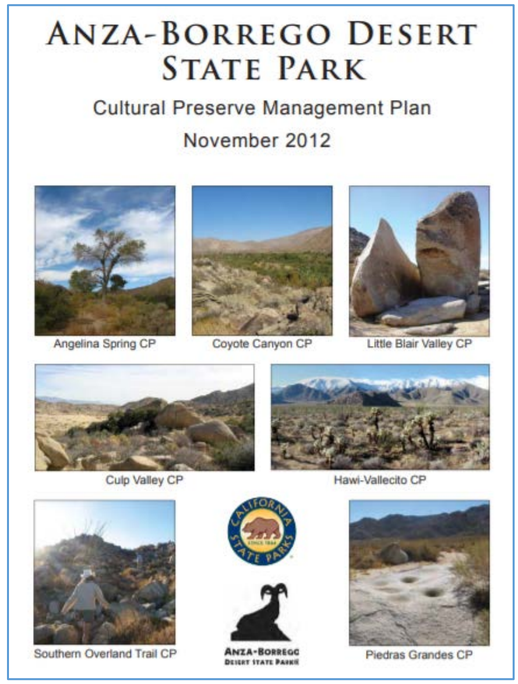
NOW THEREFORE, BE IT RESOLVED: pursuant to Section 5019.50 of the Public Resources Code, that the California State Park and Recreation Commission hereby classifies the portions of Anza-Borrego Desert State Park® as a State Wilderness as described in *Attachment A: Naming and Classification of Various Parcels Within Anza-Borrego Desert State Park®* and in the *Anza-Borrego Desert State® Park General Plan*.

Attest: This Resolution was duly adopted by the California State Park and Recreation Commission on February 11, 2005, at its duly noticed public meeting in San Diego, California.

By: ORIGINAL SIGNED BY Date: 2-11-05
Louis Nastro
Assistant to the Commission
For Ruth Coleman, Director
California State Parks
Secretary to the Commission

Designated State Wilderness and Cultural Preserves

The state legislature established the California Wilderness Preservation System in 1974 (Trumby 1984). State wilderness areas are recognized as areas where the earth and its community of life are untrammeled by man and where man himself is a visitor who does not remain (California Public Resources Code 5019.68). It is further defined to mean an area of relatively undeveloped state-owned or leased land which has retained its primeval character and influence or has been substantially restored to a near-natural appearance, without permanent improvements or human habitation, other than semi-improved campgrounds, or structures



which existed at the time of classification of the area as state wilderness. The state legislature established an 87,000-acre wilderness area in the Santa Rosa Mountains of ABDSP in 1974 (Dawson and Thornkike 2002), and subsequent wilderness designations across nearly 2/3 of ABDSP resulted in roughly 400,000 acres of designated state wilderness in 12 separate areas of the park ranging in size from the now 106,000-acre Santa Rosa Mountains State Wilderness to the 5,200-acre Desert Oasis State Wilderness. In total, the state wilderness area in ABDSP constitutes 87% of that designated in California, and much of it supports suitable desert tortoise habitat (Figure 11). As such, the wilderness areas in ABDSP surely benefit the park's desert tortoise population. In addition, seven State Cultural Preserves were designated in various portions of the park. These DPR Cultural Preserves are an internal unit classification within state park units consisting of distinct nonmarine areas of outstanding cultural interest established within the boundaries of other state park system units for the purpose of protecting such features as sites, buildings, or zones which represent significant places or events in the flow of human experience in California. As such, these provide additional land and habitat protections that certainly benefit natural biological processes that tortoises depend on.

Land acquisitions

The CDPR and the Anza-Borrego Desert State Park Foundation have worked together on numerous land acquisitions along the park borders, which has resulted in thousands of acres of desert habitats being protected that benefit tortoises in the park. Borderland acquisitions also function as geographic buffers to existing occupied tortoise habitat in the park.

Livestock removal

Cattle and feral horses roamed parts of ABDSP, particularly Coyote Canyon, where their space use overlapped that of areas known to be occupied by desert tortoises (CDPR 1995). As part of the park's efforts to restore the natural condition and animal community in this region of the park, the free-ranging cattle were removed from Coyote Canyon in 1984, and the small population of feral horses (N=29) were removed in 2003. Subsequent habitat monitoring to assess benefits to desert tortoises was not conducted, but improvements to forage, water, and soil disturbance in nesting and burrow areas likely occurred in this important area.

Caution road signs

In 2017, ABDSP installed interim caution signs in the Coyote Canyon DTMU to inform motorists of the presence of desert tortoises. In the summer of 2018, formal roadside caution signs were created; where and when to erect these signs is currently a topic of discussion.

Interpretative signage

Temporary interpretative signs were installed in the Coyote Canyon DTMU in 2017 and 2018; these signs continue to be developed as new scientific information about the park's tortoise population emerges.

Habitat management

The presence of Sahara mustard was recorded during the 2017 and 2018 tortoise surveys. This information was used to direct Sahara mustard removal efforts. A mustard removal effort in targeted areas of the Coyote Canyon DTMU was conducted by 8 park employees for over 4 days in the spring of 2018 to improve desert tortoise habitat.

Actions taken to protect against predation

Wildlife proof trash cans were installed in the Coyote Canyon DTMU in 2018.

Acknowledgements

The California Department of Parks and Recreation funded much of this work, and additional funding by the Desert Tortoise Council (Palmdale, California) was awarded to J.A. Manning on January 15, 2018 to investigate genetic origins. This work would not have been possible without the field biologists and volunteers who contributed their time and expertise, especially Rachel Woodard. *The 2017 survey crew*: Rachel Woodard, Corey Mitchell, Shannon Hoss, Max Maurer, Chris Bedwell, Kemp Anderson, Kelly Herbinson, and Corey Chan. *The 2018 survey crew*: Permitted individuals were Rachel Woodard, Shannon Hoss, Chris Bedwell, Crystal Bedwell, Charles Peterson, Kemp Anderson, Andrea Currylow, Corey Chan, Danna Hinderle, Corey Mitchell, Peter Woodman, and Christopher Fabry. Trainees and volunteers under Rachel Woodard were Lara Law, Bonny Ahern, Craig Bansmer, Paul Flores, Susan Seville, Ben DeLancey, Samantha Burrell, and Sarah Teed. State Park volunteers/employees were Diana Fong (Ocotillo Wells State Recreation Area), Tim Valentine, Holly Valentine (SD Co. SAR), John Lenberg (ABDSP), Regina Reiter (ABDSP), Jennifer Howland (DPR CDD), Mike Ortiz (DPR CDD), Lisa Gonzales-Kramer (DPR CDD), Conrad Gonzales-Kramer, Loraine Beland (ABDSP), Steve Beland (ABDSP), Lora Funk (ABDSP), and Denise Harter (SD Co. Tracking Team). I thank L. Louise Jee (retired DPR CDD Research Analyst) for the countless hours discussing incidental sighting records, personal observations, and park history, Dennis Stephen (DPR CDD Park Interpretive Specialist) for providing critical input during the development and placement of interpretive and caution road signs, and the DPR CDD Natural Resources Program employees who endured long hours removing Sahara Mustard, raking out illegally established off-road tracks, and erecting closure signs to protect tortoises and their habitat. The CDD maintenance crew installed wildlife-proof (raven-proof) trash cans. I thank Diana Lindsay for her helpful comments, sharing articles, and invaluable assistance in being an intermediary with the Marshall South family regarding their pet tortoises. I also thank the 3 anonymous CDPR reviewers who provided insightful edits and suggestions that helped improve the report. I especially thank Roy Averill-Murray (USFWS), Kim Field (USFWS), Daniel Marquez (USFWS), and Rebecca Jones (CDFW) for providing critical input and thoughtful suggestions; this program would not have succeeded without these contributions.

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Appendix A. Cowan, E. 1972. Haven for tortoises and pupfish. Desert Magazine 35:31-33. Source: https://archive.org/details/Desert-Magazine-1972-03.

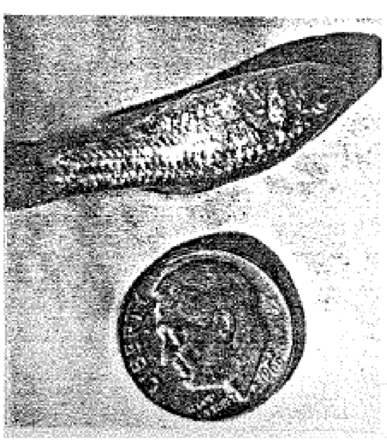
Haven for Tortoises and Pupfish

by Ernie Cowan

VISITORS TO Anza-Borrego Desert State Park will be surprised to learn that there is something new under the sun there. We have enjoyed several weekend outings as a result of a new experiment by park rangers to keep two species of animals one step ahead of the grim reaper.

In a program unique to California State Parks, rangers are trying to reintroduce two kinds of animals now extinct within the nearly half-million acre park in eastern San Diego County.

The animals are the desert tortoise and the desert pupfish. Nearly 80 tortoises have been collected by rangers and 65 have been released in a remote area of the Pinyon Mountains. A few pupfish were put in a new home all their own last year and now number several thousand.



Park Supervisor Jack Hesemeyer and naturalist Ernie Brown are responsible for this interesting idea designed to replenish the desert park with some of the animals that were native there in the past.

The idea of replanting tortoises began about two years ago when someone brought their pet tortoise to park headquarters in Borrego Springs. The owners felt sorry for their pet living in the city, so decided to return it to its natural home.

Brown and some of the other park rangers found other people in the city had desert tortoises as pets, so a few more were collected and it was decided to release them in a suitable area with hopes they would breed.

A San Diego newspaper ran a story about the tortoises and as a result 77 were collected and brought to the park, including 12 we collected in our own area. A special wire enclosure has been built behind park headquarters where new arrivals are kept until they can be released with a number of others. Visitors are welcome to come and see these docile reptiles in a natural setting.

Park Naturalist Ernie Brown (above) examines a desert tortoise. A female desert pupfish (left) compared in size to a dime.

Naturalist Brown feels there are probably "thousands" of tortoises in backyards of California cities. The tortoise is a harmless animal and makes a good pet. This fact has contribulted to the decline of this animal since they have been picked up and carted off by the thousands.

California has recognized this problem and has made it illegal to possess a desert tortoise.

Over the past 18 months, park rangers have released three groups of tortoises in a remote area of upper Fish Creek. The area is accessible only on foot, but it's well worth the several miles of walking to see how these new park residents are doing. Check with park rangers for specific directions.

We have hiked into the area several times in the past few months and have found seven live tortoises and three that have not survived. It is not known why the three died, but one we found had eight eggs inside its shell.

Before being released, each tortoise was engraved with its own identification number. They were weighed, measured and

continued next page

Appendix A (continued).

all information was carefully recorded.

As yet no detailed survey has been made of the fledgling herd, but naturalist Brown plans a survey this spring when the tortoises are most active. In the meantime anyone who finds a tortoise with a number on its back is asked to leave it alone and report the number and location to rangers.

If you are not the active type, there is still plenty for you to see in the park at the special pupfish pond built at the mouth of Borrego Palm Canyon.

Here supervisor Hesemeyer arranged for an honor camp crew to build a natural-looking shallow pond. Rocks were placed around the pond, water plants were introduced, and after the water "cured" for a time, 50 of the tiny pupfish were moved into their new plastic-lined home.

Now visitors can sit beside the pond and watch for the colorful iridescent-blue male to pop to the surface, or the silvery brown female to swim into view. There is also an aquarium at park headquarters with pupfish for visitors to see.

The pond has also provided an added bonus. It has become a favorite watering place for many of the desert birds and smaller animals. The nature photographer has a field day there in the early morning hours of summer. Tracks of a fox, coyote, bobcat or ringtail cat can often be found in the damp sand.

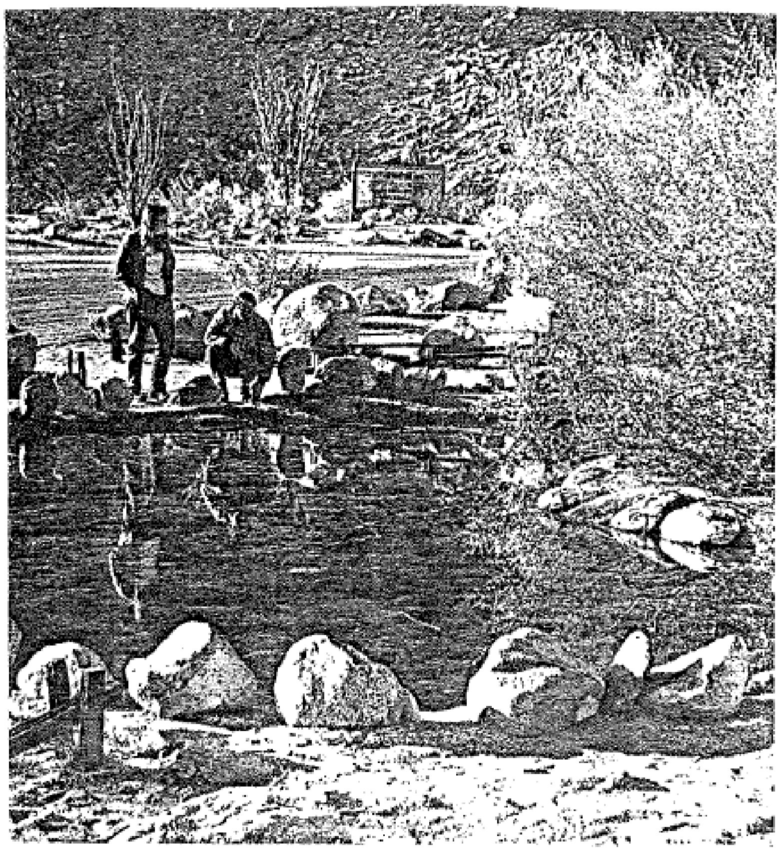
In addition to supporting fish, the pond near ranger headquarters is a watering place for desert animals. Visitors look for tracks.

The pupfish in the park pond were caught in the Salton Sea. Brown says the Salton Sea is their major population stronghold now. The only other known location of this particular species of pupfish in San Sebastian Marsh, east of the park boundary in Imperial County.

The pupfish is scientifically important because of its unique ability to survive under a wide range of living conditions. This tiny little fish of one and one-half inches is capable of living in water from near freezing to 108 degrees and in nearly pure water to water with salinity twice that of the ocean.

These fish face a threat, however, since

Appendix A (continued).



increasing salinity of the Salton Sea could kill all living things there in another five to ten years, according to some leading scientists.

Brown says the pupfish was once found in Fish Creek when it was running, Carrizo Marsh and other all-year water sources in the park. Man is the biggest enemy of the pupfish, however. Water pollution and the lowering of water tables threaten these fish with oblivion. A detailed census has not been taken as yet, but Brown estimates at least 20,000 now thrive in the pond.

This spring it is hoped some of the tiny fish can be transplanted in places such as Yaqui Well, Sentenac Canyon, Mountain Palm Spring and Carrizo Marsh, reversing the process of extinction that has already taken its toll. By spreading the fish around, it will be that much more difficult for them to disappear in case of disaster.

Perhaps the most important thing about these efforts of park rangers is what they are providing for the visitor. Our family has taken the time to seek out these new residents of the park and as a result we have learned a little more about nature and some of its wonders.

The tortoise and the pupfish also show us again the contrast of this amazing arid land. In an environment of little water, a tiny fish survives that may hold the answer to scientific questions of lasting import. At the same time a sluggish reptile, unchanged for eons, fights a new enemy for survival. With the help of the state parks he may win! □

Appendix B. San Diego, California Evening Tribune, December 17, 1971. Source: https://nl.newsbank.com/nl-search/we/Archives/?p_product=HA-CAET&p_action=keyword&p_theme=histpaper#coverageMap.

65 COLLECTED, RELEASED NEAR BORREGO SPRINGS

Race is on to save near-extinct desert tortoise

Dec 17, 1971 Evening Tribune Dispatch

BORREGO SPRINGS — The plodding desert tortoise, now extinct here, may be making a comeback as a result of efforts by Anza-Borrego Desert State Park rangers.

Close track is being kept of 65 tortoises released in the past 18 months in a remote area of the Vallecito Mountains.

Since their release, some have been found dead, but there is evidence the others are adapting well and will adjust to their new habitat.

Park naturalist Ernie Brown said he has been unable to make a detailed study so far, but he is encouraged by early findings.

"I plan to make a walking survey of the area in the spring," Brown said. "That is when the tortoise is most active."

Brown came up with the idea of re-establishing the tortoise locally after several were brought to the park.

"People were keeping them as pets in the city and they don't do well there at all," Brown said. "After we got the first ones, other people began to hear about the program, so more came in."

Sixty-seven tortoises were brought to the park by interested persons. Brown said some students made projects of collecting the tortoises from city backyards. All but two have been released.

In the summer of 1970, the first batch was released. The second group was released this spring, and the last group was planted in September.

Each tortoise was weighed, measured and a small identifying number etched in its shell before release.

Brown considered several areas before deciding on a valley area in the Vallecito Mountains.

"This area is remote enough so the tortoises are protected from the casual visitor, and it is a suitable environment for them," Brown said.

Brown said the tortoises disappeared in the first place because "people picked them up for pets, took them home, and most have died or just escaped and are forgotten."

Last week a group hiked into the area where the tortoises were released to check on them before they begin hibernating.

Brown said evidence shows many of the tortoises are doing well.

"There is plenty of sign of activity," he said. "They have been doing a lot of digging, and they are expanding their range."

One dead female containing eight eggs was found. It was impossible to tell if the eggs were fertile.

"We are hoping spring will bring with it some new tortoises," Brown said. "That would really indicate they are taking hold."

If the experiment is a success, rangers hope to expand their efforts, releasing new additions in other suitable park areas.

"Then we have to start an education program that would teach people to look, enjoy and leave the tortoise alone," Brown said.

Appendix C. The Blade: Toledo, Ohio, December 20, 1971 (page 13). Source: https://news.google.com/newspapers?nid=8_tS2Vw13FcC&dat=19711220&printsec=frontpage&hl=en.

THE BLADE: TOLEDO, OHIO, MONDAY, DECEMBER 20, 1971

Desert Tortoise Shows Gain In Its Race Against Extinction

65 Released In California Area Leaving Many Signs Of Activity

BORREGO SPRINGS, Calif. (UPI) — A race to save the desert tortoise from extinction may be paying off.

Over the last year and half 65 tortoises have been released in a remote area of the Vallecito Mountains in southern California's Anza-Borrego Desert State park.

"There is plenty of signs of activity," according to naturalist Ernie Brown. "They have been doing a lot of digging, and they are expanding their range."

The first batch of the tortoises brought to the park by interested persons was released in the summer of 1970. The second was freed this spring and the latest in September.

Before then, rangers were alarmed that tourists were taking tortoises home with them and out of their natural environment many of them died. A publicity campaign seems to have ended the turtlenaping.

The rangers weigh and measure each tortoise, etching an identifying number into its shell before releasing it.

A valley in the Vallecito Mountains was selected because "it's a suitable environment for them and the tortoises are protect from the casual visitor," Mr. Brown said.

One Delivery Stops Another

PAMLICO, S.C. (UPI) — The Rev. Sam Truitt was delivering his Sunday sermon when someone motioned to him from the front door of the church and gave the Baptist minister a message.

"If anybody feels like com-

Appendix D. Some Mojave desert tortoises in Anza-Borrego Desert State Park, 2017-2018. Photos by J.A. Manning.


Unmarked tortoise, ABDSP, 2018.


Unmarked tortoise, ABDSP, 2018.



Male tortoise AB6313 eating wildflowers & hosting male chin glands, ABDSP, 2018.


Unmarked tortoise, ABDSP, 2018.


Unmarked tortoise, ABDSP, 2017.

Appendix E. Rules and regulations pertaining to desert tortoises in California. Source: http://www.swparc.org/resources/desert-tortoise/

Rules and Regulations pertaining to desert tortoises in California: The Mojave desert tortoise is the only native tortoise species that resides in California. California law has prohibited removal of desert tortoises from the wild since 1972. The desert tortoise has been listed as Threatened by the California Department of Fish and Wildlife (Department) since 1989 and by the United States Fish and Wildlife Service since 1990.

Lawfully obtained desert tortoises may be privately adopted, subject to specific rules. Per California Code of Regulations, Title 14, Section 674, desert tortoises may be possessed only under the authority of a permit issued by the Department.

Per Fish and Game Code 5000, it is unlawful to sell, purchase, harm, take, or transport any tortoise (*Gopherus*spp.) or parts thereof, or to shoot any projectile at a tortoise. Per Fish and Game Code 5001and the abovementioned regulation, desert tortoises legally acquired and possessed prior to March 7, 1973, may continue to be possessed, but documentation, marking, and a special permit are required. Possession of desert tortoises is illegal except under the authority of a permit issued by the Department, and transfer of captive desert tortoises is not permitted without prior Department approval. Per California Code of Regulations, Title 14, Section 40, an individual shall not release into the wild any desert tortoises previously held in captivity without prior written approval from the Department. Per California Code of Regulations, Title 14, Section 43, it is illegal to captive breed desert tortoises without a permit from the Department.