

# **ABSTRACTS**

49<sup>th</sup> Annual Meeting and Symposium Hybrid and In-person February 21, 22, and 23, 2024

#### FORTY-NINTH ANNUAL MEETING AND SYMPOSIUM THE DESERT TORTOISE COUNCIL

Hybrid: in person and virtual by Zoom Wednesday, Thursday, and Friday, February 21–23, 2024

#### **ABSTRACTS OF PAPERS AND POSTERS**

(Abstracts arranged alphabetically by last name of first author) \*Speaker, if not the first author listed

#### Desert Tortoise protection and recovery: The slide towards extinction?

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The Center for Biological Diversity has worked for decades to support survival and recovery of the desert tortoise and its habitat in California, Nevada, Arizona, and Utah, using science-based advocacy, administrative processes, public information, and litigation. Natural and man-made threats to desert tortoise from development of new roads and routes, off-road vehicle use, mines, transmission lines, and large-scale renewable energy projects are increasing. Direct habitat loss, increasing habitat fragmentation and loss of connectivity impair the tortoise's ability to seek more suitable habitat in a changing climate. In addition, the spread of non-native plants and increased predation are pushing the desert tortoise farther from recovery and onto the slide towards extinction.

In California, the Center and others continues to pursue litigation challenging the BLM's West Mojave Plan for failure to comply with NEPA, FLPMA and the ESA, and continuing to allow off-road vehicles to run rampant in the West Mojave in critical habitat and elsewhere even as tortoise populations are crashing; we are currently awaiting a decision from the Court. The Center works to ensure BLM's implementation of the Desert Renewable Energy Conservation Plan (DRECP) provides critically needed conservation for tortoise and other species when new projects are proposed including new mineral exploration projects for both gold and lithium in the California desert. The Center is actively supporting the proposed Chuckwalla national monument proposal in Riverside and Imperial Counties that would increase protections for a substantial amount of desert tortoise habitat. We are also engaging in the proposed General Conservation Plan that FWS' has currently embarked on, for permitting take for desert tortoise on private lands in California. We are opposing proposed gold mining exploration in Imperial County in desert tortoise habitat. We also submitted comments on BLM's draft Strategic and Action plans for the Mojave desert tortoise.

In Utah, the Center as part of a strong conservation coalition continues our legal challenge to Interior's decision approving a new Northern Corridor 4-lane highway through the Red Cliffs National Conservation Area.

In Nevada, the Center fights against development in tortoise habitat including by: pushing back against the Clark County lands bill introduced again in 2022, but was derailed in the Senate, and it has not been re-introduced this year. It proposes to open up tens of thousands of acres of tortoise habitat to sprawling development from Las Vegas; advocating for Nevada to adopt a DRECP-like model for renewable energy that would steer development to lower conflict areas while protecting habitat and connectivity rather than the current approach driven by industry and new transmission such as Greenlink West which is impacting tortoise populations in Pahrump Valley, Amargosa Valley and elsewhere; and opposing new gold mining proposals near Beatty, in an essential climate connectivity corridor on the northern edge of the tortoise's range. The Center submitted a federal petition for listing the white-margined beards-tongue plant as threatened or endangered whose habitat is sympatric with the desert tortoise.

In the southeast, the Center challenged FWS' decision not to list the eastern gopher tortoise throughout its range and in the eastern portion of its range in early August 2023. In addition, the Center is fighting new policies in Florida that are intended to accelerate development in tortoise habitat.

#### Results of the second season of AI-assisted drone surveys in Washington County, Utah

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Resi has developed a method for conducting AI-assisted drone surveys for the Mojave desert tortoise (*Gopherus agassizii*) and for generating abundance and density population estimates using a novel implementation of distance sampling. The first full-scale implementation of the method was during the 2022 field season in Washington County, Utah. Paired drone and pedestrian surveys of Zone 6 of the Red Cliffs Desert Reserve were successful, but also resulted in several suggested improvements. These improvements, designed to maximize tortoise detectability for the drone method, were implemented in the 2023 season with good results.

In the 2023 season, drone surveys were performed in Zones 2-5 of Red Cliffs Desert Reserve in conjunction with scheduled pedestrian population monitoring surveys conducted by the Utah Division of Wildlife Resources. Rectangular transects were surveyed in Zones 2 and 3. A total of 4-5% of the surface area of these zones was photographed. Linear transects were surveyed in Zones 4 and 5. A total of 4% of the surface area of Zone 4 was photographed, and 12% of the much smaller Zone 5. The surveys resulted in the detection of 63 adult tortoises, 17 juveniles, and 2 carcasses. Mean adult tortoise density as estimated by distance sampling techniques varied from a low of 11 / km<sup>2</sup> in Zone 3 to a high of 46 / km<sup>2</sup> in Zone 2. Estimates produced by the drone surveys were similar to estimates derived from previous pedestrian surveys of these same areas.

The results of the 2023 field season suggest that the drone/AI survey method in its current form produces density and abundance estimates comparable to those produced by traditional pedestrian line distance sampling methods but at a much lower cost. The application of drones

and AI-assisted image search technology is ready to move out of the testing phase and be applied to concrete scientific and management problems throughout the range of the Mojave desert tortoise.

#### STUDENT PAPER

# Comparative genomic analysis of Sonoran and Mojave desert tortoises (*Gopherus morafkai* and *G. agassizii*) to identify the genetic basis of desert adaptations

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The *Gopherus* desert tortoises have adapted to unique desert environmental conditions in the Sonoran and Mojave deserts. In order to identify the genomic basis for species-specific adaptations, we used the reference genomes for *G. morafkai* and *G. agassizii* to calculate ratios of nonsynonymous to synonymous base pair substitutions to find genes under positive selection. There were 389 genes under positive selection and results indicate selection acting on traits related to environmental conditions. Genes associated with vision development were under selection particularly relating to low light conditions, possibly related to differences in above-ground circadian or seasonal behaviors. Genes associated with the immune system, the process of chromosomal segregation in mitosis and meiosis, as well as reproduction genes were also under selection, showing that these species are in the later stages of and actively in the process of reproductively isolating. Despite having few to no chromosomal rearrangements and low overall mutation rates, our results show these species have a high amount of adaptive divergence and may be a good model for understanding reproductive isolation.

#### Evidence of *Mycoplasma testudineum* and Testudinid herpesvirus infection since the early 1990s in the Mojave Desert

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Outbreaks of diseases contributed to federal listing of Agassiz's desert tortoise (*Gopherus agassizii*) as a threatened species in 1990. As part of an effort to understand health and diseases in desert tortoises, research was undertaken between 1992 and 1995 at sites representing three regions of the Mojave Desert, California: Desert Tortoise Research Natural Area (DTRNA), Ivanpah Valley, and near Goffs in Fenner Valley. The study was instrumental in the development of reference ranges for hematological and plasma biochemical variables, pivotal health parameters.

As part of the research, ill tortoises with *Mycoplasma agassizii* were identified, and clinical signs shown by the affected tortoises suggested the potential presence of other agents responsible for partially overlapping signs, including a new mycoplasma species (*M. testudineum*) and a herpesvirus. We retrospectively addressed these unanswered questions by analyzing archived samples of plasma collected between 1992 and 1995 during the four seasons at the three sites. Samples from all three sites were positive for *M. agassizii*. Sex did not affect ELISA values. However, site differences were significant, with higher A405 values at the DTRNA than those at Goffs or Ivanpah across all seasons. Within a site, A405 values were significantly higher in summer. Tortoises at DTRNA had elevated A405 across all seasons. In contrast, with minor exceptions, animals from Fenner Valley tested positive only in summer, while samples collected from Ivanpah had positive animals in both spring and summer. In contrast, relatively few animals tested positive for *M. testudineum*. Of the 16 positive results for *M. testudineum*, 10 were obtained from three tortoises at DTRNA (time point positives, n = 1, 3, and 6) and three tortoises in Fenner Valley (time point positives, n = 1, 1, and 4). No tortoises tested positive from Ivanpah. One positive sample of *Scutavirus testudinidalpha* (Testudinid herpesvirus) tested positive at DTRNA.

More evidence became available in later years. Between 2000 and 2002, ELISA tests for herpesvirus from the three sites revealed positive tests for the DTRNA and Ivanpah Valley but none at Fenner based on two samples. However, a necropsied tortoise from Goffs was positive for herpesvirus using the ELISA test in 2000. A 2012 report summarizing data collected using ELISA tests for herpesvirus throughout California deserts indicated examples of tortoises with positive serology and identified the herpesvirus as TeHV2.

With support from the Mojave National Preserve in 2018, we repeated testing at two of the sites, Ivanpah and Goffs/Fenner Valley, drawing on 43 adult tortoises. One tortoise had oral signs of herpesvirus and the same tortoise tested positive using the ELISA test for herpesvirus. Taken together, these retrospective data demonstrate the potential for all three pathogens to be present in the three wild populations, with *M. agassizii* the most common.

# Applications of near remote sensing imagery obtained with unmanned aerial vehicles on evaluating desert tortoise densities at local scales

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Mojave Desert tortoises (*Gopherus agassizii*) are patchily distributed throughout their range even in areas of suitable habitat, and due to this irregular pattern, we seek to examine influential factors on tortoise densities at local scales. At landscape scales imagery derived from satellites allows for the analysis of explanatory environmental covariates in relation to range wide tortoise densities. Recently, advancements in remote sensing using unmanned aerial vehicles (UAVs) have become a viable option for obtaining environmental data at finer scales. Imagery obtained using UAVs has the potential to bridge the gap between large-scale satellite imagery and small-scale species distribution and occupancy analyses. To evaluate the effectiveness of UAV imagery in local desert tortoise density analyses environmental covariates are derived from

imagery obtained with UAVs and input into models alongside desert tortoise mark-recapture data. Point process models (PPMs) are an effective tool for evaluating the influence of covariates on the density pattern of a set of points. Examining factors that shape tortoise densities at local scales will aide in our understanding of tortoise distributions and contribute to future studies on habitat quality in which the sole focus is not only range wide suitability but local suitability and density patterns as well. Exploiting new technologies and their applications such as UAVs and fine-scale remotely sensed imagery will provide new opportunities to further our knowledge on variation in the densities of endangered and threatened species like the desert tortoise.

#### **Mojave National Preserve Recovery Update**

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This update presents an overview of ongoing conservation initiatives and monitoring efforts undertaken for the desert tortoise (*Gopherus agassizii*) population within Mojave National Preserve (MOJA). Efforts focused on promoting the recovery of this iconic species, and various strategies were implemented to respond to emerging challenges.

Key recovery initiatives included the installation of a pivotal five-mile segment of tortoise fencing and the implementation of two tortoise crossing structures along Cima Road. These measures address habitat fragmentation and vehicular threats, ensuring safe passage and reducing mortality rates associated with road encounters. Additionally, the National Park Service has intensified its "Drive Like a Tortoise"<sup>TM</sup> public outreach campaign, employing speed enforcement, public events, and the installation of roadside signs to raise awareness and encourage responsible behavior around tortoise habitats.

Research endeavors play a crucial role in conservation, and MOJA continues its commitment to supporting head-starting research at the Ivanpah Desert Tortoise Research Facility. This initiative aims to enhance tortoise abundance in areas with depressed populations. Raven monitoring and control efforts continued in targeted areas. This update also details extensive monitoring activities, including over 400 hours dedicated to construction and roadwork tortoise monitoring, total sign surveys, and the identification of tortoises exhibiting Upper Respiratory Tract Disease (URTD) symptoms. MOJA and agency partners responded to major disasters like the York Fire, which consumed over 90,000 acres of desert habitat, and the Kelso train derailment, a major accident in a tortoise-occupied area. Looking ahead, planning will continue for additional tortoise fencing and crossings on Kelso-Cima and S. Kelbaker Roads. MOJA has reinforced its commitment to conservation and recovery of Mojave National Preserve's desert tortoise population and looks forward to continued success in the years ahead.

# The invertebrate *Galleria mellonella* differentiates virulence potential between species and strains of *Mycoplasma agassizii* and *Mycoplasma testudineum*

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Both Mycoplasma agassizii and Mycoplasma testudineum are etiological agents of upper respiratory tract disease in captive-raised and free-ranging Chelonian species. Both species have been isolated from clinically ill free-ranging and captive tortoises, but M. agassizii is associated with more severe clinical signs. While limited experimental infections have confirmed the pathogenicity and examined transmission dynamics, ethical considerations are a factor in continued research regarding host-pathogen interactions due to the federal protection status of many Chelonian species. Additionally, there is a lack of commercially available cell lines for temperature-restricted pathogens isolated from reptiles. To address these challenges, we established an alternative model of pathogenicity testing using the invertebrate Galleria mellonella. To assess the efficacy of this model we selected strains of M. agassizii and M. testudineum isolated from desert and gopher tortoises. A dose-response was run using low, medium, and high infection doses. Larval mortality, pupation, and emergence events were collected over 28 days. We found that, independent of strain, M. agassizii is more pathogenic in G. mellonella larva compared to M. testudineum. Interestingly, we also found that the pathogenicity of *M. agassizii* strain PS6 and 723 is highly dependent on dose when comparing differences within strains. Overall, G. mellonella is a tractable alternative model for assessing differences in virulence potential of M. agassizii and M. testudineum, and an important tool to understand how different species and strain impact clinical outcomes.

# Comparative genomics of *Mycoplasma agsasizii* isolates from wild *Gopherus* species, wild *Astrochelys radiata*, and captive *Manouria emys*

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Mycoplasmas have minimal genomes and most biosynthetic pathways are missing. The bacterial cell wall has been lost, leaving only a single bi-layer lipid membrane as the interface between bacterial and host cells. This close interface along with a reliance on hosts for nutrient scavenging inherently creates an intimate close contact between mycoplasmas and their hosts. While traditionally thought to be host specific, new and emerging mycoplasmas are increasingly being detected in novel hosts, and particularly in wildlife species. *Mycoplasma agassizii* was first identified in free-ranging desert tortoise populations in the 1990s and has subsequently been isolated from a wide range of hosts in the Testudinidae family. A long standing question, especially in Iight of captive release issues in desert tortoises, has been "was the pathogen introduced into wild populations". To better understand the evolutionary relationship between host and pathogen, we performed whole genome sequence analysis of isolates of *M. agassizii* from four free-ranging hosts with highly basal (*Gopherus* species, N=3) and recent (*Astrochelys radiata*, N=1) lineages.

Additionally, an isolate of *M. agassizii* from a clinically ill captive Burmese black mountain tortoise (*Manouria emys*), a sister clade of *Gopherus*, was included. Phylogenomics shows little divergence among the *M. agassizii* isolates, regardless of host. However, comparative system analysis identified variable gene arrangement and copy number of genes. Comparative genome analysis of *M. agassizii* obtained from wild animals in geographically disparate locations supports the hypothesis that *M. agassizii* was present in ancestral tortoise species and has co-evolved with *Testudines* hosts.

#### **Clark County Multiple Species Habitat Conservation Plan Update**

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The Clark County Desert Conservation Program (DCP) continues to administer the Multiple Species Habitat Conservation Plan (MSHCP) on behalf of the Cities, Clark County, and Nevada Department of Transportation as mitigation for an Endangered Species Act Section 10 incidental take permit for desert tortoise and 77 other species of plants and animals. The DCP has collected mitigation fees for 3,132 acres of take during the period of January through September 2023, leaving 42,736 acres of take authorization remaining under the current permit. The 2023-2025 Implementation Plan and Budget allocated up to \$16,530,362.00 for the funding of staff and projects starting in July 2023 to run through June 2025. Highlights of desert tortoise-related work conducted over the past year include: occupancy surveys on the Boulder City Conservation Easement (BCCE); completed year 9 of post-translocation monitoring of desert tortoises on the BCCE; post translocation of 51 desert tortoises onto the BCCE; post translocation monitoring in the Eldorado Valley; completed barrier fencing along Tule Springs National Monument; completed fence monitoring and road mortality surveys under the road warrior program; and continued work on public information and education through the Mojave Max program.

#### WINNER, ROBERT C. STEBBINS RESEARCH AWARD

### Anthropogenic uranium signatures in turtles, yortoises, and sea turtles from Nuclear Sites

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#### Anthropogenic uranium and plutonium radionuclides in 20<sup>th</sup> century desert tortoises

Cyler Conrad<sup>1,2</sup>, Jeremy Inglis<sup>3</sup>, Allison Price<sup>3</sup>, Allison Wende<sup>3</sup>, Matthew Sanborn<sup>3</sup>, Nilesh Mukundan<sup>4</sup>, Travis Tenner<sup>3</sup>, Kimberly Wurth<sup>3</sup>, Benjamin Naes<sup>3</sup>, Kristin H. Berry<sup>5</sup>, Katrina M. Derieg<sup>6</sup>, Margarete A. Walden<sup>7</sup>, Todd Esque<sup>8</sup>, Derek Hall<sup>9</sup>, Jeanette Perry<sup>9</sup>, Jeanne Fair<sup>10</sup>, Earl Middlebrook<sup>10</sup>, Shannon Gaukler<sup>11</sup>, Jenna Stanek<sup>11</sup>, Jessica Celmer<sup>11</sup>, Jeffrey Whicker<sup>11</sup>, Jamie L. Gerard<sup>11</sup>, Washington Tapia Aguilera<sup>12,13</sup>, James P. Gibbs<sup>14</sup>, and Blair Wolf<sup>15</sup> <sup>1</sup>Earth Systems Science Division, Risk and Environmental Assessment Group, Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA, 99352, USA; phone: 509-481-3626; email: cyler.n.conrad@pnnl.gov <sup>2</sup>Department of Anthropology, University of New Mexico, Albuquerque, NM, 87131, USA <sup>3</sup>Chemistry Division, Nuclear and Radiochemistry Group, Los Alamos National Laboratory, Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM, 87545, USA <sup>4</sup>Department of NanoEngineering and Chemical Engineering Program, University of California, San Diego, La Jolla, CA, 92093, USA <sup>5</sup>Western Ecological Research Center, Box Springs Field Station, Riverside, CA, 92518, USA <sup>6</sup>Natural History Museum of Utah, University of Utah, 301 Wakara Way, Salt Lake City, UT 84108, USA <sup>7</sup>Department of Natural Resources, University of Nevada–Reno, 1664 N. Virginia St., Reno, NV 89557, USA <sup>8</sup> Western Ecological Research Center, Boulder City Field Station, Boulder City, NV, 89005, USA <sup>9</sup>Nevada National Security Site, Mission Support and Test Services, LLC, 2621 Losee Road, North Las Vegas, Nevada 89030, USA <sup>10</sup>Bioscience Division, Genomics and Bioanalytics Group, Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM 87545, USA <sup>11</sup>Environmental Protection and Compliance Division, Environmental Stewardship Group, Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM 87545, USA <sup>12</sup>Galápagos Conservancy, 11150 Fairfax Blvd. #408, Fairfax, VA 22030, USA <sup>13</sup>University of Málaga, Campus Teatinos, Apdo 59.29080 Málaga, Spain <sup>14</sup>College of Environmental Science, State University of New York, 1 Forestry Dr., Syracuse, NY 13210, USA

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Between 1951-1962, 100 atmospheric nuclear weapons were tested at the Nevada Test Site (NTS; now Nevada National Security Site). Studies during and after this era of testing documented both the dispersion and composition of fallout matter and debris from these devices into portions of the western United States, especially in areas directly to the north and east of the NNSS. Various researchers during the 20<sup>th</sup> century hypothesized that it was possible fallout from atmospheric testing at the NTS would become incorporated into desert tortoise tissues, and preliminary counting of tortoise shell radioactivity in the 1980s supported this hypothesis. In 2023, we further tested and confirmed this hypothesis by identifying anthropogenic uranium (<sup>235</sup>U and <sup>236</sup>U) isotopes in a desert tortoise (meaning *Gopherus agassizii*) scute collected from southwestern Utah in 1959. Here, we report on new uranium and plutonium (<sup>239</sup>Pu and <sup>240</sup>Pu) isotopic results from a series of desert tortoises (all *Gopherus agassizii*) collected throughout southeastern Nevada, southwestern Utah, and northwestern Arizona. Our results confirm that fallout from the NTS was deposited in a wide region of the western United States, and although it occurs in minute concentrations, it is measurable and present within desert tortoise tissues several decades after atmospheric testing ended.

#### Using Areas of Critical Environmental Concern to conserve Desert Tortoises

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Under the Federal Land and Policy Management Act, the Bureau of Land Management (BLM) may designate Areas of Critical Environmental Concern (ACECs) to protect important resources and values on public lands during land use planning. A new proposed Public Lands Rule would provide tools for the BLM to improve the resilience of public lands in the face of a changing climate and increased development pressures, conserve important wildlife habitat and intact landscapes, and better recognize unique natural resources. ACECs would be emphasized in this new rule, to balance multiple uses that are extractive or destructive. We detail our efforts to nominate ACECs in Nevada and California in order to conserve Mojave desert tortoise (*Gopherus agassizii*) habitat and connectivity corridors in the face of ongoing utility-scale solar project build-out. The BLM designated our Ivanpah ACEC nomination in Ivanpah Valley, but also approved solar projects. We are currently nominating the proposed Cactus Springs ACEC in Indian Springs Valley, Nevada, to conserve a crucial linkage corridor for tortoises, as a conservation alternative to large-scale solar project applications in the area.

#### Heralds of the future? Mojave Maxine as a possible indicator of climate change

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The Desert tortoise (Gopherus agassizii) faces substantial threats from climate change within the California deserts. Tortoises in this region are experiencing escalating challenges of heightened frequency and severity of droughts, prolonged and hotter summers, and the depletion of indigenous vegetation pushed beyond its sustainable thresholds. These factors collectively result in a reduction of temporal windows of cooler conditions suitable for desert tortoise brumation and rest, necessitating prolonged periods of activity and wakefulness. This newly extended active phase imposes physiological stress on the tortoises, thereby contributing to heightened mortality rates. Since 2006, The Living Desert has conducted the Mojave Maxine winter emergence contest, an initiative designed to engage and foster behavioral changes among youth. The competition encourages young participants to advocate for a reduction in raven subsidies and the adoption of sustainable practices to coexist harmoniously with desert tortoises. Our data reveal a discernible correlation between increasing global temperatures and earlier emergence from winter brumation. Furthermore, we explore intricate associations between average local temperatures, annual rainfall patterns, early-season heat spikes, and the emergence patterns of these charismatic indicators of environmental change. Leveraging innovative datasets, such as those generated through this child-oriented guessing competition, may help assess the impacts of climate change and other environmental perturbations more broadly.

#### **STUDENT PAPER**

### Using line-distance sampling data to create spatial density analyses that allow for the exploration of covariates of desert tortoise density

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The Mojave Desert Tortoise Range-wide Monitoring Project, which employs line-distance sampling, was created to monitor the recovery of desert tortoises since 2001. Line distance sampling as a method of surveying is used to estimate the size and density of wildlife populations and can provide long-term, broad-scale reference points from which to estimate densities. For desert tortoises, these estimates are summarized and published by the US Fish and Wildlife Service (USFWS) at the stratum level (i.e., density estimates are limited to the scale of individual critical habitat units or other management units), but the strength of the line distance dataset lies in the broad extent that the surveys cover. We discuss a spatially explicit approach to understanding potential long-term desert tortoise population changes and demographic trends considering the influence of key environmental and anthropogenic stressors such as habitat loss and fragmentation from urbanization and off-highways vehicles, changes in vegetation, subsidized predators, and extended drought conditions. This approach provides further spatial and temporal context and insight to data collected through the longstanding range-wide monitoring efforts.

### Spatially explicit estimation of sex- and age class-specific densities of Mojave desert tortoises (*Gopherus agassizii*) within the Naval Air Weapons Station China Lake, California

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Obtaining reliable estimates of population density is critical to effective wildlife conservation and management. Densities of Mojave desert tortoises (*Gopherus agassizii*) traditionally have been estimated using line-distance sampling, but the burrowing behavior of tortoises poses issues for estimation with that design-based method. Spatial capture-recapture (SCR) models are flexible hierarchical models for estimating spatially explicit population density

and other demographic parameters from spatially and temporally replicated detection data; yet, few studies have explored the utility of SCR for estimating desert tortoise densities. We conducted a 4-day transect-based, search-area survey on a pilot demography plot within the restricted-access South Range of Naval Air Weapons Station China Lake (NAWSCL) in the western Mojave Desert to obtain tortoise detection data and applied SCR models to estimate spatially explicit sex- and age class-specific densities of desert tortoises. We obtained 99 detections of 24 individual tortoises (6 adult F, 4 adult M, 14 juveniles) during the 4-day survey. Explicitly accounting for the spatiotemporally varying survey effort, SCR model-averaged estimated mean tortoise density across the entire 2.53-km<sup>2</sup> parameter estimation area was 18.53 tortoises/km<sup>2</sup> (95% CI = 12.36-27.77; CV = 0.21). The estimated age class ratio was skewed towards juveniles (64% juveniles: 36% adults), whereas the adult sex ratio was female-biased (61% females: 39% males); corresponding densities were 11.86 juveniles/km2 (95% CI = 7.91–17.77), 4.08 adult females/km<sup>2</sup> (95% CI = 2.72-6.11), and 2.59 adult males/km<sup>2</sup> (95% CI = 1.73-3.89). Our study provides NAWSCL with preliminary local tortoise demographic estimates that can be used for long-term monitoring by establishing additional regional demographic monitoring sites. Additionally, we suggest further application of SCR models for estimating desert tortoise densities, as well as direct comparisons with traditional tortoise density estimation approaches, to update or improve density estimates and inform continued tortoise recovery.

# Understanding how Wile E. Coyote & friends outsmarted us all and the impacts predators have on Mojave desert tortoise recovery

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Predators in the Mojave and Colorado Deserts such as the American badger (Taxidea taxus), common raven (Corvus corax), bobcat (Lynx rufus), coyote (Canis latrans), golden eagle (Aquila chrysaetos), kit fox (Vulpes macrotis), mountain lion (Puma concolor), red-tailed hawk (Buteo jamaicensis), and others have a broad diet and can adapt to consume a variety of prey items depending on availability. Although these predators are critical to balancing the structure of desert ecosystems and influence the distribution, abundance, and diversity of their prey, they can have profound and negative impacts to sensitive and threatened species such as the Mojave desert tortoise (Gopherus agassizii). Landscape changes over the last 35 years have provided omnipresent human subsidies dramatically increasing the abundance of some commonly known tortoise predators (coyotes and common ravens). Additionally, the American badger, kit fox, and mountain lion have more recently emerged as predators that can exert severe effects to tortoise populations at a local level. While tortoises are likely opportunistically consumed by most predators at low levels over time and under variable environmental conditions, elevated adult tortoise mortality (as high as 43%) is most pronounced in years following drought throughout the listed range of the species. Resource managers and associated groups such as the Desert Tortoise Management Oversight Group (MOG) have endorsed recovery actions for tortoises that include predator monitoring and targeted control in some areas as well as concerted efforts to meaningfully reduce predator subsidies. Management efforts to reduce raven abundance and associated pressures on tortoises in California have largely been successful in recent years. Yet, actions and results for other predators are confounded by limited understanding of predator-prey dynamics, responses of predators to management actions, and rapid ecosystem and climate change. Here, we review the status of knowledge regarding predator impacts to Mojave desert tortoises, possible predator deterrent methods, and management options to improve species recovery initiatives.

# Personality, mternal effects, and outcomes in the wild for Mojave desert tortoises (Gopherus agassizii)

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Repeatable individual behavioral traits, or personalities, mediate the way that organisms interact with and respond to their environments. Such traits are known to predict survival in the wild, thus, in the context of conservation head-starting programs, it is critical to understand how they may be shaped by maternal effects and rearing regimens. As a first step toward understanding these dynamics, we assayed behavior in Mojave desert tortoise (Gopherus agassizii) mothers and offspring that were involved in a head-starting and translocation program. Tortoises were repeatedly tested to characterize exploratory tendencies and boldness. Mothers were also tested for response to predator cues. Radiotelemetry was used to track individual movement, habitat use, and survival in the wild. Both hatchlings and mothers exhibited significant repeatability in the majority of behavioral traits that were quantified. Some traits were correlated with each other, suggesting the possibility of a behavioral syndrome. Correlations between mothers and offspring were limited, but there were significant differences in behavioral traits across clutches. For mothers, we found significant associations between behavioral traits and time spent in burrows in the field. Field data for juveniles are still being collected and analyzed. Our results will be discussed in the context of desert tortoise conservation specifically and head-starting and translocation programs more generally.

#### Desert tortoise translocation of the Marine Corps Air Ground Combat Center (Combat Center) in 2023

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The 2023 annualized survival in the Combat Center's translocation was 92.1%, similar to the 88.2% for 2022. In 2023, Controls, Residents and Translocatees experienced 92.6, 88.2, and 95.5%, respectively, which were similar to 2022 values (82.6, 88.2 and 94.1%, respectively). Winter rainfall and annual plant production appeared heterogeneous in the Western Mojave Desert, with high annual plant production occurring sparsely across these translocation sites. This may have limited higher survival rates below that during earlier, wet years. Seventy percent (7 of 10) of mortalities were due to depredation of controls and residents. All of the depredations were due to coyotes and may have resulted from coyotes switching prey, if there was a paucity of

mammalian prey during 2023. One translocatee died of a vehicle strike. We could not determine the cause of death of one resident and one translocatee.

#### The California Department of Fish and Wildlife's Report on the Status of the Mojave Desert Tortoise (*Gopherus agassizii*)

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The California Fish and Game Commission was petitioned in 2020 by Defenders of Wildlife, the Desert Tortoise Council, and the Desert Tortoise Preserve Committee to uplist the Mojave Desert Tortoise from threatened to endangered under the California Endangered Species Act. In response, the California Department of Fish and Wildlife evaluated the best available scientific information on natural history, population trends, threats, and management efforts in order to make a recommendation to the Commission on whether the uplisting is warranted. Here, we go over the process for uplisting a species, present our findings, and discuss future steps the Department can take.

#### An Integrated Model Improves Inferences about Historical Patterns of Survival in the Mojave Desert Tortoise

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The Mojave desert tortoise (*Gopherus agassizii*) underwent precipitous population declines during the over the last six decades. During these declines, a small number of populations were monitored with mark-recapture methods through time, but inconsistent sampling and infrequent recoveries of dead, marked tortoises make it difficult to estimate the factors influencing survival. However, a large amount of data on tortoise telemetry has been collected in recent decades, providing an alternative and useful data source to augment and improve survival estimates. Here we describe an integrated, spatially explicit analysis of mark-recapture and known-fate telemetry data that we used to estimate spatial and temporal variation in survival of the Mojave desert tortoise. We compiled mark-recapture data from 2618 tortoises at 35 sites spanning 1977–2023 and telemetry data from 2861 tortoises from 24 sites spanning 1988–2022. We leveraged

information sharing from different data sources in an integrated analysis to produce robust estimates of survival and conditioned on the climate-related covariates. For the period 1977–2022, our integrated analysis revealed (1) higher survival of males than females, (2) no difference between survival of adults and juveniles, and (3) an effect of total precipitation from two prior winters (and active seasons) on survival. Temperature-related covariates were not supported by the analysis. A random site-by-year interaction effect explained considerable variance, suggesting that other drivers of survival remain unaccounted for. Our results demonstrate the strength of leveraging multiple data sources in describing historical patterns in demographic rates and identifying relationships between covariates with a lagged effect on survival. Our integrated analytical framework could be used to examine the effects of additional factors on tortoise survival and resulting population dynamics.

### What's in a bite? Quantifying Mojave desert tortoise (*Gopherus agassizii*) foraging preference and consumed biomass

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The general foraging ecology of Mojave desert tortoises (Gopherus agassizii) has been well studied and tortoises have been found to be selective foragers based on individual preferences, nutrient requirements, mineral content, and the life stages of plants. Although bite count studies on tortoises have been conducted, the average biomass consumed in a tortoise bite has not been studied for adults or juveniles. To further quantify tortoise habitat use by evaluating tortoise forage preference, bite counts, and biomass consumed per species, we conducted a bite quantification study with 27 captive research juvenile tortoises from March – June 2022. As part of the study, we selected and grew seven Mojave desert forage species including six native forbs and one invasive nonnative Mediterranean grass (Bromus rubens). We then randomly but equidistantly placed clipped specimens of similar phenology and condition within specialized foraging enclosures and placed selected tortoises inside the enclosures to observe in 20-minute foraging trials. We found that tortoises consumed more California poppy (Eschscholzia californica) biomass (dry) per bite in the post-flowering life stage than any of other forage species. Biomass consumed per bite also varied with life stage. Tortoises also foraged on invasive B. rubens, but the overall biomass consumed was minimal in comparison with the native forbs. We found that foraging efficacy varies by species and changes as plants dry and senesce. Linking captive juvenile Mojave desert tortoise foraging behavior with our analyses can increase our understanding of tortoise dietary intake in relation to energy costs in their natural habitat and can be used to target specific habitats or forage species for restoration and conservation.

#### **STUDENT POSTER**

### Genome sequencing and annotation of the Texas Tortoise (*Gopherus berlandieri*) as a resource for population genetic studies

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The Texas tortoise (*Gopherus berlandieri*) is endemic to the thornscrub savannas and woodlands of south Texas and northern Mexico, but populations are declining due to pressures from habitat loss and may be susceptible to disease. The Texas tortoise is listed as threatened by the State of Texas. To identify and map the genetic diversity that is essential for the long-term survival and adaptation of this species, we generated a chromosomal-scale reference genome from a male individual from Cameron County, TX. The draft genome assembly for *G. berlandieri* has a scaffold N50 length of 137,584 kb and an L50 of 6. Genome annotation is currently underway using predicted annotation information from sister tortoises and transcriptome data from the Mojave Desert tortoise, *G. agassizii*. This reference genome will be framework for a population genomic analysis of 66 individuals from across south Texas to determine population genetic diversity, structure, historical effective population sizes as well as signatures of population-level and inter-specific positive selection. In addition, with the divergence between the *G. berlandieri* and desert *Gopherus* species lineages estimated at 10 million years ago, this will provide a more divergent genetic reference adapted to a non-desert environment, compared with *G. agassizii* and *G. morafkai*, which diverged about 5 million years ago.

#### **STUDENT PAPER**

#### Thermal ecology, activity patterns, and ecological niche modeling of *Gopherus* tortoises

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Almost 50% of modern tortoise species are endangered or already extinct. Niche modeling predicts extensive range contractions for many chelonian species due to climate change induced habitat loss. For example, tortoise habitat in arid environments is expected to increase in temperatures and prolonged drought reducing habitat availability. Desert-dwelling tortoises dig burrows or seek refuge in rock crevices to avoid the heat and potential risk of overheating. There are six tortoise species in North America and Mexico (genus *Gopherus*) and they differ in genetics,

distribution, morphology, body size, diet, habitat, and shelter site selection. Most *Gopherus* species have been assumed to have a similar physiology as *G. agassizii* despite differences in genetics and ecology and more recent evidence suggesting varying thermoregulatory strategies for different species. Therefore, understanding each species' specific habitat requirements and physiological properties are important to allow inferences about tortoises' potential to cope with temperature shifts and habitat alterations. Further, there is a lack of sufficient physiological data at an appropriate resolution of microhabitats to refine existing ecological niche models and improve predictions to guide conservation action for specific populations and species. Our study measures core physiological data, operative environmental temperatures, and movement and activity patterns of several species of *Gopherus* tortoises occupying differing habitats, ranging from desert habitat and arid grasslands to tropical deciduous forest habitat.

#### Mojave Desert Tortoise Week 2023: Making outreach count

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Establishing and sustaining an environmental education program is identified as a highpriority recovery action in the Mojave Desert Tortoise Recovery Plan. The goal of these planned events aspired to build public support for and involvement in Mojave Desert tortoise (Gopherus agassizii) recovery. The U.S. Fish and Wildlife Service (Service) organized and hosted a public outreach campaign to inform the public about desert tortoise conservation issues, aimed to change any misinformation of the Mojave desert tortoise, and raise awareness of how public actions may affect the species. The campaign called 'Desert Tortoise Week 2023' occurred within the first week of October; nevertheless, events occurred throughout the entire month. The education campaign was preceded by the hosting of hybrid (in-person and virtual) events and advertisement through social media. Events were hosted within multiple states and included a series of desert tortoise talks, guided tours, and presentations. The Service also implemented a social media campaign that challenged members of the public to engage in desert tortoise awareness activities including posting photos of desert tortoise habitat during recreational outings. The Service encouraged conservation partners to develop and host educational events to promote conservation and recovery actions for the desert tortoise. These partners developed educational virtual lesson plans, hosted webinars, recruited volunteers to remove invasive plants, and established scavenger hunts for the public to enjoy and learn more about the species. After Desert Tortoise Week 2023, our conservation partners reported public participation resulting in attendance of over 3,000 people at events and over 1.2 million social media impressions. Overall, Desert Tortoise Week 2023 was a success. The Service will continue to support and coordinate Desert Tortoise Week into the future.

# We aren't mucin around: The evolution of disparate mucin arrays in *Gopherus morafkai* and *G. agassizii*

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Characterizing genetic differences between sister species is important for understanding how related species respond to their local environment and can signal different management needs and concerns. Genetic differences can result in changes in a protein's function or changes to the conditions of when that protein is produced (expressed) in the organism. The Sonoran and Mojave desert tortoises (Gopherus morafkai and G. agassizii, respectively) live in different deserts with different amounts of rainfall, and exhibit different behaviors during the winter and timing of reproduction. Additionally, the Sonoran Desert tortoise has a comparatively lower disease incidence of *Mycoplasma agassizii*, the bacterium responsible for upper respiratory track disease (URTD). Using ten low coverage whole genome sequences for each species, we characterized the regions of 1% greatest genomic divergence between the two species. Genomic regions with high divergence included immune and mucosal genes. Further investigation uncovered that these species retain an unusual array of mucin (MUC) genes particularly those secreted in the lung and nasal mucosa, with many gene duplication events among and between the two lineages. These genes are important for osmoregulation and host defense against pathogens, particularly clearing bacterial infections. Differences in the function and number of mucin genes could indicate differential adaption to their respective habitats, where they have different amounts of rainfall and different rates of mycoplasmal infection and URTD. Additionally, we identified mutations in genes that are key for the circadian and circannual regulation of gene transcription. Such changes in the regulatory machinery could have potential ripple effects for the timing of biological functions with no change to the actual protein functions but could underlie differences in timing and patterning of brumation, mating, and laying between the species.

#### Using demographic study plots to inform range-wide xtatus for the Mojave desert tortoise

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Both the original 1994 and revised 2011 recovery plans for the Mojave population of the desert tortoise (*Gopherus agassizii*) stipulate that demonstration of a positive population growth trend over a 25-year period is a criterion for delisting (Recovery Criterion 1). In 1999, the federal and state land and resource manager's Desert Tortoise Management Oversight Group endorsed line distance sampling as the method that would be used on public lands to monitor desert tortoise populations. Line-distance sampling methods, which estimate imperfect detection rates and are repeatable and

comparable between years, have been used by USFWS to monitor Mojave desert tortoise populations in tortoise conservation areas range-wide since 2001, meeting Recovery Criterion 1a. While these methods have been effective for estimating densities and population trends of adult desert tortoises (>180 mm midline carapace length), they do not provide sufficient information needed to monitor and estimate vital rates, emphasizing a need for focused demographic studies to complement current range-wide monitoring, as outlined in Recovery Criterion 1b. In 2023, USFWS expanded the range-wide monitoring program for the desert tortoise by establishing and surveying two new 1 square kilometer demographic plots using capture-recapture methods in the Western Mojave Recovery Unit, with four more planned over the next two years as part of the Recovery and Sustainment Partnership Initiative. These sites were established as part of a range-wide effort to establish demographic study sites within each recovery unit to evaluate tortoise demography. Collectively, estimates from these sites can be compared with other established demographic sites throughout the Mojave Desert to improve estimates for tortoise vital rates so that fluctuations due to habitat disturbance, climate, and other confounding factors can be quantified and estimates of site persistence and potential re-colonization rates can be modeled.

#### Predator-prey dynamics in the Mojave Desert: An ecological Gordian Knot among coyotes, jackrabbits, and desert tortoises

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Identifying demographic and spatial relationships among predators and their prey is critical for disentangling the myriad of ecological, climatic, and anthropogenic effects on populations and for informing effective management at the population-level. When recovery of imperiled prey species is a primary objective, the intricacies of predator-prey dynamics are often obscured, leading to management that is typically focused on predator control, despite prey population growth and viability being governed by some combination of both bottom-up and top-down effects. Although multiple predator species have been implicated in the mortalities of threatened desert tortoises (Gopherus agassizzi), predation by coyotes (Canis latrans) remains one of the most frequently attributed causes of adult tortoise mortality throughout the Mojave Desert. The prevailing hypothesis is that coyotes exhibit 'prey switching', whereby increased coyote predation of desert tortoises occurs when abundance and availability of the preferred coyote prey base declines, particularly black-tailed jackrabbits (Lepus californicus), and/or anthropogenic resource subsidies increase, but evidentiary support for this remains limited. We present preliminary results from the first four years of an ongoing predator-prey dynamics study that is focused on the demographics and spatial ecology of coyotes and black-tailed jackrabbits in a desert tortoiseoccupied area of the Mojave Desert. Camera-trap-based population estimates indicated that coyotes inhabited the study area at consistently low densities that were uninfluenced by climatic conditions or the local jackrabbit population, and population-level coyote space use based on

radio-collar monitoring was negatively related to anthropogenic development. In contrast, the local black-tailed jackrabbit population exhibited fluctuations across time that were primarily influenced by seasonal variation in precipitation, despite predation being the leading cause of jackrabbit mortality, and population-level jackrabbit space use was positively associated with anthropogenic development. We discuss our preliminary findings in the context of contemporary predator-prey dynamics theory, coyote and jackrabbit ecology, and desert tortoise recovery.

#### POSTER

#### Effects of Tropical Storm Hilary on desert tortoise (Gopherus agassizii) nest success

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Temperature underpins nearly all facets of reptile natural history and physiology, making climate change a growing threat. Altered climate regimes pose a particular threat to oviparous species given their reliance on maternal nest site selection for incubation conditions, which can shape offspring sex ratios and growth rates. Further, clutch phenology is influenced by regional patterns of precipitation and temperature. While research often focuses on the impacts of rising temperatures on populations, the intensity and unpredictability of meteorologic events as a result of climate destabilization may present additional stressors that threaten population growth. The Mojave Desert tortoise (Gopherus agassizii) has adapted to increasingly arid conditions and regional precipitation patterns of the Mojave Desert, characterized by winter dominant rainfall west of the 117<sup>th</sup> meridian and biseasonal precipitation to the east. In the western Mojave, desert tortoise eggs incubate during the summer when temperatures tend to be high and rainfall is low. Between August 19-21, 2023, the western Mojave experienced an unprecedented tropical storm, with 40 times the normal August precipitation falling in a 24-hour period. We monitored storm impacts on incubation temperature and nest outcomes. Using Hygrochron sensors placed near the middle egg of 17 nests, we recorded temperature and humidity at 90-minute intervals through the incubation period. Tropical Storm Hilary affected the third trimester of incubation for all nests. Increased soil moisture caused tortoise nest temperatures to rapidly drop, while nest humidity rapidly increased. Cool, moist conditions in late incubation slowed development and emergence timing, while also facilitating a suite of invertebrate nest predators resulting in lower-thanexpected hatching survival. Some eggs succumbed to late-stage embryonic death, while others succeeded in hatching but were vulnerable to invertebrate predators. These results highlight the challenges of more extreme and unpredictable climatic events for desert tortoise conservation and headstarting programs.

#### FEATURED SPEAKER

#### 15 Years In: Lessons being learned from assisted colonisation trials of Australia's rarest turtle

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Assisted colonisation – the introduction of a species outside its indigenous range for conservation purposes – has been suggested for several decades as a pragmatic response to climate change. But virtually no one has tried it. In this presentation I describe a collaboration between researchers and managers where assisted colonisation is being trialled to reduce the risk of extinction of a Critically Endangered freshwater turtle. The western swamp turtle is a long-lived reptile native to Perth and occupies seasonal wetlands on which it depends for food and reproduction. Much of its former habitat has been cleared, and the small fragments that remain are slowly drying due to declining rainfall and groundwater. Conservation translocations of juveniles raised in captivity have generally been successful, with more than 1000 individuals now released, but the long-term climatic suitability of translocation sites is a key concern. Consequently, based on biophysical modelling, multiple criteria analysis and empirical data, we have shifted our focus to evaluating release sites in cooler and wetter parts of south-west Australia. Beginning in 2016, juvenile turtles have been released into novel wetlands across a 350 km latitudinal gradient to study their growth, physiological performance, and survival. Now into our fourth trial, we have used each translocation experiment to answer increasingly complex questions, and to focus on the most promising wetlands for assisted colonisation south of the species known range. Our evidencebased approach is potentially a much-needed global example to break the apparent inertia between policy making and implementation of assisted colonisation initiatives.

#### Head-starting, survival and movement of Mojave desert tortoises at the Marine Corps Air Ground Combat Center

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Head-starting, in which eggs are hatched and juveniles raised in captivity before being returned to the wild, is one strategy for addressing population declines in turtles. Here, we report the survival and movement of 176 head-started Mojave desert tortoises (*Gopherus agassizi*) at the Marine Corps Air Ground Combat Center (MCAGCC) in Twentynine Palms, California. Juveniles

were hatched from eggs laid by wild, gravid females temporarily held in outdoor pens. Tortoises were released 5-13 years after hatching, in the spring and/or fall 2015 to 2019. Sex was determined for 69 individuals via endoscopy. We radio-tracked individuals following release, recording location and survival at least monthly. By December 2022, 56.3 percent of head-started tortoises were alive. Causes of death varied, with mammalian predators responsible for 42%. We used GLMs and GLMMs to explore drivers of survival and movement. Best performing models of survival included a significant effect of release group and lower survival with more surface activity and movement. The negative relationship between surface activity and survival persisted even if individuals that died within two months weren't included. With surface activity as the response, we found significant effects of release group and month, declines in activity with increasing time since release and a positive effect of size. Movement (average meters moved per day) varied with release group, and increased with age at release and distance to road. There was no evidence for homing behavior to the head-start facility. We found a consistent lack of sex effect on survival and movement. Overall, tortoises that were more active and moved more had higher mortality rates, supporting results from other studies. Ongoing work includes adding additional variables (e.g. landscape metrics and environmental variables) to models, and comparing survival and movement to tortoises that were not head-started. As such, conclusions may change by the meeting date.

### Inferring social contact networks in desert tortoises using fine scale GPS data - are they a solitary species, or is Bacon's law alive and well?

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Connectivity among individuals is important at several scales from linking populations together across landscapes, to social interactions within a population. The expansion of urban areas, and their associated infrastructure impacts habitat for many species, not only via direct losses, but also through habitat degradation, and fragmentation. Developed areas, and roads routes along rights of way provide additional impacts through habitat incursions from off highway vehicles, nesting or perching sites for subsidized predators, among others. Collectively these disturbances can impact the connectivity among animals, and may alter the genetic, demographic, and even the social structure of populations. We studied fine scale movements and population level connectivity of desert tortoises at 10 sites located in and around the Ivanpah Valley of Nevada and California which is an important area for range-wide connectivity of the species. We employed a system micro GPS dataloggers that record animal locations at hourly intervals, and collected more than 500,000 localities across the 10 sites and over several years. We use these data in a network modeling framework to quantify connection networks of animals within an among years to evaluate the relative connectedness within sites, and compare among sites with different levels of anthropogenic impacts. Further we identify key individuals that have elevated connectedness and the potential to provide genetic insights within the plots. Finally we quantify the microsite characteristics that are associated with tortoise contacts by analyzing the node locations of contacts among individuals. Collectively these analyses further our understanding of fine scale connectivity by providing information into the connectedness and the social hierarchies among animals within

these populations, and this will provide important ties in with genetic relatedness among these animals.

# Environment and diet influence fungal and bacterial gut microbiomes of Agassiz desert tortoise (*Gopherus agassizii*) in California

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Gopherus agassizii (Mojave Desert Tortoise) is widely considered a "keystone species" of the Mojave and Sonoran deserts for their role in native plant seed dispersal and for the habitat their burrows provide to myriad other species. Mojave Desert Tortoise populations are faced with challenges associated with habitat modification, human disturbance, and microbial pathogenesis. We evaluated fungal and bacterial microbiomes in wild and captive animals to obtain a more detailed picture of gut microbial diversity and composition in these tortoises, and how these factors relate to individual animal health. We concentrated on the influences of diet and environment on the Gopherus agassizii core fungal and bacterial microbiome as a starting point for obtaining valuable information to aid in future tortoise microbiome studies. We extracted DNA from desert tortoise fecal samples and paired soil samples collected from the environment. Fecal samples were collected opportunistically from animals who voided during routine health assessments, as paired with separate soil samples collected in tandem. We sequenced amplicons of the 16S rRNA gene and fungal ITS2 region using an Illumina MiSeq and compared the host microbiome to the soil communities in landscapes inhabited by these animals. We characterized microbial groups in hostassociated and environmental microbiomes and found they differed between sites or environmental sources and were ostensibly influenced by diet. The composition of fungal gut mycobiomes in wild animals were more similar to paired soil samples collected nearby than they were to the fungal gut mycobiomes in captive animals; however, this pattern was not detected in bacterial communities from these same animals. Although a small core microbiome was present, and shared across all tortoises, fungal consortia showed lower taxa richness than coexistent bacterial communities. Findings from this study will help provide valuable information about tortoise microbiomes and the ecological resilience of these tortoise populations.

#### **Desert Tortoise Council Activities - 2023**

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The Board of Directors (BOD) of the Desert Tortoise Council (Council) conducted an inperson business meeting at the Annual Symposium and held six online BOD meetings in 2023. In 2023, two BOD members stepped down due to other commitments and our BOD now consists of 15 members with five open Member-At-Large positions. Also in 2023, the Council added David Hedrick as a full-time Operations Manager whose duties include fundraising, assisting the BOD and Committee Chairs, implementing robust social media efforts, assisting with the Annual Symposium and training course implementation, and conducting outreach to the membership, the public, and other relevant organizations. The membership of the Council includes approximately 350 active members with over 3,100 contacts on our contact list. As of December 2023, the assets of the Council were \$233,801. Our Ecosystem Advisory Committee commented on 89 (97%) of the 92 notices received for projects or actions potentially affecting tortoises and their habitats in CA, NV, UT, and AZ and for interstate or national issues. The Council held the Introductory Course/Workshop with the virtual lecture in late October followed by two field workshops in early November. A total of 199 people completed the entire course and 17 people audited the lectures. Despite inclement weather and road closures, more than 200 people attended the 48<sup>th</sup> Annual Symposium in St. George, Utah. Ann McLuckie and Cameron Rognan conducted two field trips and SNEI, Inc. again sponsored the pre-conference mixer. The Symposium program included 59 oral presentations covering genomics, demography, connectivity of populations, population augmentation, and others. A high-level session on the issues related to Cannabis that are affecting tortoises and tortoise habitat was organized by Heidi Brannon and this important session is available on the Council's website. The David J. Morafka Memorial Research Award went to Daniel Guerra, a student studying thermal ecology in Texas Tortoises. The Linda J. Allison Memorial Grant, which was sponsored by contributions from Leeward Renewable Energy LLC was awarded to Jacqueline Dougherty and was presented by Chris Moore of Leeward Renewable Energy. The Glenn R. Stewart Travel Award was presented to Juila Joos. The Council's social media committee, which is composed of board members, the Operations Manager, and volunteers, greatly increased the Council's social media presence on various platforms in 2023. The Mexican Tortoise Conservation Committee, Agency Coordination Committee, and Advanced Training Course are in the process of being re-energized. Representatives from our Education and Outreach Committee attended The Wildlife Society Western Section conference to educate attendees about the mission of the Council, to sign up new members, and to sell SWAG as a fundraiser. The Fundraising Committee has also implemented fundraising events through Amazon and the social media platforms. The Council held "Drink Beer Save Tortoises" fundraising events in May and October at the Escape Craft Brewery in Redlands, California. The Fundraising Committee has scoped out other breweries throughout southern California for future "Drink Beer Save Tortoises" events and we intend to partner with other tortoise conservation groups on these events. The Council had a very busy 2023 and we look forward to an even busier 2024.

#### Juvenile Mojave desert tortoises shift night microhabitats during summer heatwaves

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Behavioral plasticity can buffer animals against physiological impacts of climate change, but research is needed to understand the limits and consequences of behavioral responses to warming. Increased night activity by diurnal animals is a potential response to warming thought to require considerable behavioral flexibility. We hypothesized that juvenile Mojave desert tortoises (Gopherus agassizii), diurnal ectotherms that typically rely on burrows to conserve limited energy and water reserves during summer, respond to rising burrow temperatures by shifting from using burrows at night to overnighting at the surface where cooler temperatures reduce metabolic expenditure. This strategy could be energetically adaptive but may increase predation risk. Using time-lapse cameras at burrows and iButton temperature loggers affixed to juveniles and placed at burrow and surface microhabitats during a summer heatwave, we found that individuals often emerge from burrows on evenings when their temperatures approach the voluntary maximum of well-fed, hydrated laboratory animals (~34°C). Tortoises remained inactive at the surface before returning to burrows after sunrise. By shifting to surface microhabitats at night, tortoises lowered mean nighttime body temperatures by up to ~12°C and reduced energy expenditure during the heatwave. Future research will examine how this behavioral adjustment to temperature influences predation risk and water loss.

#### POSTER

#### A sequential bottom-up approach to restore desert tortoise (*Gopherus agassizii*) in the Orocopia mountains of Southern California.

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North American deserts are widely diverse not only between areas but within ecoregions. The Coachella Valley and surrounding areas in the Sonoran Desert show rainfall patterns more in line with some of the driest parts of the Mojave Desert. Factors like these create unique site conditions for implementing traditional restoration approaches. To improve desert tortoise habitat in the Orocopia Mountains of southern California, The Living Desert Zoo and Gardens has implemented a sequential restoration approach. This area was selected because it connects populations in the northern part of the Chuckwalla bench with known populations to the north in Joshua Tree National Park. Our approach consists of improving micro catchment hydrology and water retention with grade control structures. Subsequently, key drought tortoise forage (*Opuntia basilaris*), was outplanted across such improved areas. Finally, known native annuals will be seeded to enhance the seed bank and ensure higher forage availability. We have tested (ongoing research) approaches to minimize seed predation as part of these efforts. Our preliminary results

indicate that combining grade control structures with topsoil cover minimizes predation. We expect these structures to also function as "mulch" during wetter seasons, enhance the survival of native species, and create sites for seed dispersal. Given the uncertainty that climate change will bring to restoration efforts across all ecosystems, we hypothesize that this bottom-up approach, starting with hydrology, will provide significant climate adaptation advantages to our restoration sites.

#### FEATURED SPEAKER

#### Interpreting laboratory results and protecting the health of wild and captive chelonians while preparing radiated tortoises (*Astrochelys radiata*) for rewilding

Bonnie L Raphael<sup>1</sup>, Marv B Brown<sup>2</sup>, Tsanta Fiderana Rakotonanaharv<sup>1</sup>, Aiona Nieto Claudin<sup>3</sup>, Jamie Palmer<sup>3</sup>, Sean Perry<sup>4</sup>, Susan L. Bartlet<sup>5</sup>, Paul P Calle<sup>5</sup>, Kevin Toregrossa<sup>5</sup>, Sarah O'Brien<sup>3</sup>, Kathleen Apakupakul<sup>3</sup>, Alexandra M Brune<sup>2</sup>, Stephen Nelson<sup>6</sup>, Richard Vetter<sup>1</sup>, Matthew Evans<sup>7</sup> Jessica Mitia Koloina Andrianantenaina<sup>8</sup>, Rakotobe Tindra Navalona<sup>8</sup>, Joanne Larry Rambeloson<sup>8</sup>, Mirana Maelys Tolojanahary<sup>8</sup>, Carrela Anjaramamitiana Rasolondraibe<sup>1</sup>, Tony Dieudonne Ralivaniaina<sup>1</sup>, Fidy Rasambainarivo<sup>9</sup>, Santatra Randrianarisoa<sup>9</sup>, and Sharon L Deem<sup>3</sup> <sup>1</sup>Turtle Survival Alliance, Madagascar, Paraguay, USA, Charleston, SC 29406 USA, <sup>2</sup>Department of Infectious Diseases & Immunology, University of Florida, College of Veterinary Medicine, Gainesville, FL 32608 USA <sup>3</sup>Institute for Conservation Medicine, Saint Louis Zoo, Saint Louis, MO 63110 USA <sup>4</sup>Mississippi Aquarium Gulfport, MS 39501, USA. <sup>5</sup>Zoological Health Program, Department of Herpetology, Wildlife Conservation Society, Bronx, NY 10464 USA <sup>6</sup>Zoo Knoxville, Herpetology Department, Knoxville, TN 37914USA <sup>7</sup>Reptile Department, Smithsonian Institute, National Zoo, Washington, DC USA <sup>8</sup>Mention Enseignement de Sciences et de Medecine Veterinaires, Faculté de Medecine, Université d'Antananarivo

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The Turtle Survival Alliance (TSA) is a conservation organization with a mission to protect and restore wild populations of tortoises and freshwater turtles through science-based conservation, global leadership and local stewardship. TSA is committed to zero turtle extinctions in the 21<sup>st</sup> century and a planet where turtles thrive in the wild and are respected and protected by all humans. The Radiated tortoise (Astrochelys radiata) is endemic to southern Madagascar, inhabiting xeric spiny forests, sandy coastal scrub and coastal rocky outcroppings. Males average 40 cm in length and females 36 cm. The largest specimen currently known is a 24 kg female. Eggs are laid in clutches of 1-5 eggs 1-3 times per year to synchronize with hatching after up to 10 months at the onset of rainy seasons. It was once one of the most abundant tortoises on earth with an estimated population of 12 million individuals and has been reduced by 80% over the past 20 years. Illegal wildlife trade is the primary driver of the species decline. The TSA began a systematic reintroduction process of previously confiscated Radiated tortoises 2019. Periodic sampling of some captive and wild animals for the purpose of establishing normal health parameters had taken place since 1998, with enhanced sampling and molecular techniques in a more focused manner in 2016. In 2020, 2022 and 2023, teams of health professionals and biologists performed intensive sampling of statistically significant numbers of tortoises as well as individual

handling and microchipping 6,000 tortoises in two TSA facilities in Madagascar which were holding a total of 24,000 tortoises. 3,000 tortoises were cleared for release based on testing. Molecular testing has included *Mycoplasma sp*, herpesvirus, ranavirus, adenovirus and chelonian intranuclear coccidia. In November 2022, *Mycoplasma agassizii* was documented in recently confiscated tortoises in quarantine (n=26/40) and in tortoises (n=4/149) held under human care for several years. In spring of 2023 free ranging Radiata (n=275) underwent sampling at 5 novel sites. Antibody testing via ELISA revealed exposure to both *M. agassizii* and *M. testudineum* in wild and captive animals. Positive cultures and genomic sequencing have been obtained.

#### PLENARY SPEAKER

#### Earth beyond six of nine planetary boundaries

Katherine Richardson<sup>1</sup>, Will Steffen<sup>2†</sup>, Wolfgand Lucht<sup>3,4</sup>, Jørgen Bendtsen<sup>1</sup>, Sarah E. Cornell<sup>5</sup>, Jonathan F. Donges<sup>3,5</sup>, Markus Drüke<sup>3</sup>, Ingo Fetzer<sup>5,6</sup>, Govindasamy Bala<sup>7</sup>, Werner von Bloh<sup>3</sup>, Georg Feulner<sup>3</sup>, Stephanie Fiedler<sup>8</sup>, Dieter Gerten<sup>3,4</sup>, Tom Gleeson<sup>9,10</sup>, Matthias Hofmann<sup>3</sup>, Willem Huiskamp<sup>3</sup>, Matti Kummu<sup>11</sup>, Chinchu Mohan<sup>8,12,13</sup>, David Nogués-Bravo<sup>1</sup>, Stefan Petri<sup>3</sup>, Miina Porkka<sup>11</sup>, Stefan Rahmstorf<sup>3,14</sup>, Sibyll Schaphoff<sup>3</sup>, Kirsten Thonicke<sup>3</sup>, Arne Tobian<sup>3,5</sup>, Vili Virkki<sup>11</sup>, Lan Wang-Erlandsson<sup>3,5,6</sup>, Lisa Weber<sup>8</sup>, Johan Rockstrüm<sup>3,5,15</sup> <sup>1</sup>Globe Institute, Faculty of Health, University of Copenhagen, Copenhagen, Denmark <sup>2</sup>Australian National University, Canberra, Australia <sup>3</sup>Potsdam Institute for Climate Impact Research, Potsdam, Germany <sup>4</sup>Department of Geography, Humboldt-Universität zu Berlin, Berlin, Germany <sup>5</sup>Stockholm Resilience Centre, Stockholm University, Stockholm, Sweden <sup>6</sup>Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden <sup>7</sup>Centre for Atmospheric & Oceanic Sciences, Indian Institute of Science, Bangalore, Karnataka-560012, India <sup>8</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel & Faculty for Mathematics & Natural Sciences, Christian-Albrechts-University Kiel, Kiel, Germany <sup>9</sup>Department of Civil Engineering, University of Victoria, Victoria, British Columbia, Canada <sup>10</sup>School of Earth & Ocean Sciences, University of Victoria, Victoria, British Columbia, Canada <sup>11</sup>Water & Development Research Group, Aalto University, Espoo, Finland <sup>12</sup>Global Institute for Water Security, University of Saskatchewan, Saskatoon, Saskatchewan, Canada <sup>13</sup>Waterplan (UC S21), San Francisco, California, USA <sup>14</sup>Institute of Physics & Astronomy, University of Potsdam, Potsdam, Germany <sup>15</sup>Institute for Environmental Science & Geography, University of Potsdam, Potsdam, Germany

This planetary boundaries framework update finds that six of the nine boundaries are transgressed, suggesting that Earth is now well outside of the safe operating space for humanity. Ocean acidification is close to being breached, while aerosol loading regionally exceeds the boundary. Stratospheric ozone levels have slightly recovered. The transgression level has increased for all boundaries earlier identified as overstepped. As primary production drives Earth system biosphere functions, human appropriation of net primary production is proposed as a control variable for functional biosphere integrity. This boundary is also transgressed. Earth system modeling of different levels of the transgression of the climate and land system change boundaries illustrates that these anthropogenic impacts on Earth system must be considered in a systemic context. Title and abstract from Richardson, K., et al. 2023. Earth beyond six of nine planetary boundaries. Sci. Adv. 9, eadh2458.

#### POSTER

# Cool air at night, tortoise delight: Validating iButtons for characterizing nighttime microhabitat use in juvenile desert tortoises (*Gopherus agassizii*) during a heatwave

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Faced with climatic extremes, desert species may be at increased risk of depleting water and energy reserves in summer, thus, understanding their thermal ecology and microhabitat use is more crucial than ever to predict responses to climate change. Like many species, desert tortoises (*Gopherus agassizii*) utilize burrows as refugia from high daytime surface temperatures to avoid overheating and conserve energy and water. However, nighttime temperatures are lower at the surface than in burrows, particularly in smaller, less ventilated burrows. This raises the question of whether juvenile tortoises will shift from using burrows at night to overnighting at the surface to reduce energy expenditure in hotter seasons. Cameras can address this question, but they are limited by tortoise movements among burrows and do not provide key information on temperature. In August of 2023, we observed juvenile tortoise activity in the wild using time-lapse cameras and simultaneously recorded tortoise and environmental temperatures using temperature loggers (iButtons) affixed to juveniles and positioned in burrow and surface microhabitats. We will use camera data to assess whether temperature data from tortoises and burrow and surface environments can be used to reliably estimate nighttime microhabitat use and potential activity shifts in response to climate change.

# Behavioral change programming to reduce human provided food subsidies for rRavens in the desert tortoise's range

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Raven populations in the Southwestern United States benefit from the food, water, and shade we provide to them due to uncovered trash, excessive watering of the land, and buildings. The subsidies are substantial enough that raven populations have increased by up to 1,700% in the California Deserts, where the birds are the greatest threat to the survival of the desert tortoise (*Gopherus agassizii*), causing up to 50% of annual tortoise deaths. To address these subsidies that humans provide, The Living Desert conducted a behavioral change program in Hi-Desert communities that encouraged restaurants and food-related businesses to cover their dumpsters to prevent ravens from consuming trash. The campaign recently expanded to include 40 businesses

in Desert Hot Springs. Due to our interventions, restaurant dumpster closures increased by 12% shortly after our interventions and, 3 months after our outreach, sustained a 6% increase. Research assessing the efficacy of pro-environmental behavior change efforts document that it is common to see a large initial increase in the desired behavior shortly after an intervention; yet this increase often diminishes over time. Allcott & Rogers (2014) refer to this occurrence as "action and backsliding." However, repeated interventions can diminish the cyclical repetition of the "action and backsliding" over time, with behavior change becoming more persistent and sustained with continued interventions. Conducting repeated interventions allows for repeated feedback on each restaurant's dumpster closure rate, comparative feedback with how the restaurant is performing in relation to other local restaurants, and motivation as businesses are persistently evaluated, all of which have been found to contribute to long-term behavior change. These latest findings about The Living Desert's behavioral change program indicate the importance of repeated and ongoing efforts and periodic reinforcement to decrease the human provided subsidies available to ravens across the entire range of the desert tortoise.

#### **STUDENT PAPER**

# Effects off fixed and tracking monofacial photovoltaic solar arrays on the operative temperatures available to a 4 kg Mojave desert tortoise

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Solar energy development coincides with habitat for many sensitive species, including the Mojave desert tortoise (Gopherus agassizii, tortoise). While utility-scale solar energy reduces net carbon emissions and should ultimately benefit climate-sensitive species, peer-reviewed literature suggests that air temperatures (1 m above ground) 11 m inside and 100 m adjacent to utility scale photovoltaic solar arrays (array) incur significant warming effects between 11:00 pm and 9:00 am and correspond with significantly lower creosote (Larrea tridentata) leaf xylem water up to 400 m away from an array. To understand whether such air temperature effects translate into operative temperature (T<sub>e</sub>) effects and thus behavioral or physiological effects for large ectothermic animals, we added thermal inertia to hourly temperature anomalies collected using iButtons inserted inside copper biophysical T<sub>e</sub> models. T<sub>e</sub> models were positioned in the sunniest (T<sub>sun</sub>) and shadiest (T<sub>shade</sub>) microhabitats available at >10 m inside (array), 100 m adjacent to (adjacent), and >2 km away from (undisturbed control) a fixed and tracking monofacial array, located in the eastern Riverside County Development Focus Area, California. Preliminary spectral density estimates for the series of differences between the mean maximum spring array  $T_{sun}$  and control  $T_{sun}$ , suggest that the mean maximum spring T<sub>sun</sub> available to a 4 kg tortoise inside an array is consistently lower than the mean maximum spring T<sub>sun</sub> control during daylight hours. Conversely, the mean maximum spring array T<sub>shade</sub> (shaded by photovoltaic panels) is consistently warmer than the mean maximum spring control T<sub>shade</sub> during several daylight hours. Yet, whether a lower array T<sub>sun</sub> offsets a higher array T<sub>shade</sub> is unknown and should be the focus of future investigations of tortoise ecology inside and adjacent to arrays. These preliminary findings and others presented suggest that the thermal

environment inside unvegetated, monofacial arrays is significantly altered from natural conditions, underscoring the need for further investigation.

#### **STUDENT PAPER**

### Vegetation cover, topography, and low-traffic roads influence habitat use and movement of Sonoran desert tortoises

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Wildfire, the invasion of non-native grasses, human development, and linear barriers like roads are replacing habitat and reducing landscape connectivity for the Sonoran desert tortoise (Gopherus morafkai). These processes are largely driven by anthropogenic activities and are increasing in severity throughout the Sonoran Desert. Studies examining how natural and anthropogenic landscape features influence G. morafkai's habitat use and movement may inform management plans specific to the species. To examine how tortoises select habitat and respond to roads, we recorded movements from 17 adult tortoises using GPS dataloggers over two summers. We used these movements in integrated step-selection analyses (iSSA) to quantify fine-scale habitat selection by G. morafkai and to examine how their movement behavior is influenced by roads. We calculated relative selection strength (RSS) to estimate how selection (or avoidance) varies in response to natural landscape characteristics and roads. We found that tortoises were approximately three times more likely to select areas with the mean vegetation coverage on the landscape than areas of bare earth or the highest vegetation coverage. Tortoises generally selected to move through rugged but not extremely rugged terrain and areas close to incised washes. Tortoises also avoided areas close to low-traffic roads and increased their speed when approaching or crossing them. Our findings suggest that management actions that aim to prevent activities that remove or significantly increase vegetation cover (e.g., off-roading or invasion of non-native grasses, respectively) and reduce the impacts of roads (e.g., restoring unused roads) may help preserve tortoise movement through the landscape and thus enhance landscape connectivity for the species.

#### STUDENT PAPER

#### Distribution of Mycoplasma spp. across the Texas range of Gopherus berlandieri

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Texas tortoises are experiencing anthropogenically mediated decline due to development driven habitat loss (e.g., industry) and collection of wild animals. These disturbances not only fragment the habitat of Texas tortoises, but they may also affect immune function leading to increased susceptibility to disease. Upper respiratory tract disease (URTD) has been detected in closely related desert tortoises and may cause morbidity and mortality in infected individuals. However, data on the presence of URTD-causing pathogens is limited in Texas tortoises, hindering proper management actions. Our objectives are to 1) test for the presence of two URTD-causing pathogens (Mycoplasma agassizii and Mycoplasma testudineum) across the U.S.A. range of Texas tortoises and 2) assess whether tortoises are more susceptible to these URTD-causing pathogens based on biological characteristics and anthropogenic stressors. To ensure our efforts are as widely representative as possible, we established a network of biologists and community scientists to aid in survey efforts. We have collected plasma samples from Texas tortoises throughout their range in the U.S.A. and tested for the presence of Mycoplasma agassizii and Mycoplasma testudineum with enzyme linked immunosorbent assays (ELISA). We will collect geographic variables, such as distances to regions of development and distance to roads, biological data (age, sex, size, observed clinical URTD signs) to understand if certain anthropogenic stressors are associated with Mycoplasma spp. presence. Thus far, 3/65 tortoises have tested positive for M. testudineum, and 2/65 have tested positive for *M. agassizii*. An additional 18/65 tested suspect for *M. testudineum*, 4/65 tested suspect for *M. agassizii*, and 8/65 tested as suspect for both. This knowledge will aid in relocation efforts of Texas tortoises in proximity to development.

#### The advantage of size in desert tortoise head-starting and the challenge of extreme weather

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The appeal of desert tortoise head-starting rests in the increase in survival that accrues as tortoises grow in captivity, presumably improving their odds of survival after release. We released head-started Mojave Desert Tortoises spanning 70–145 mm midline carapace length in the Mojave National Preserve in lower Ivanpah Valley in San Bernardino County, California in

Fall 2018 (n=78), Fall 2019 (n=72), and Fall 2020 (n=60) and monitored their fates using radiotelemetry. In 2019, annual survival averaged 83% and size was positively correlated with survival. In 2020, annual survival increased to 93% and the positive relationship between size and survival was modest. In 2021, annual survival fell to 60% and there was no relationship between size and survival. Of the 85 known tortoise mortalities, 77 were killed by mammals (91%) and 62 of the 85 mortalities (73%) occurred in 2021. The region was in 'moist' to 'neutral' drought categories until an exceptionally dry winter and subsequent hot year pushed the area into 'extreme drought' for most of 2021. Others have attributed elevated predation rates on adult tortoises during droughts to coyotes when preferred prey like jackrabbits become scarce; indeed, the number of sightings of jackrabbits relative to covotes reported to iNaturalist plummeted in 2021 in this region. We likewise suspect that drought-induced prey switching by coyotes and other mammal predators explains the increased mortality of head-started tortoises by predators that occurred in 2021 irrespective of tortoise size. Considering the challenge of extreme weather like that in 2021, releasing larger head-started tortoises will likely benefit populations by improving survival in non-drought years, ensuring that, with more tortoises alive on the landscape before droughts, more may remain in the aftermath of such bottlenecks.

#### The Northern Corridor Highway and the Washington County Habitat Conservation Plan – two roads to nowhere

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In January 2021, the U.S. Department of the Interior (Interior) issued two decisions with grave consequences for the Upper Virgin River Recovery Unit of the Mojave Desert Tortoise (*Gopherus agassizii*): a right-of-way (ROW) grant to the Utah Department of Transportation (UDOT) to construct a four-lane high speed highway (the Northern Corridor highway) through Mojave desert tortoise critical habitat, the Red Cliffs National Conservation Area and Washington County Desert Tortoise Reserve; and an incidental take permit increasing "take" of tortoise under the Washington County Habitat Conservation Plan. The Northern Corridor highway would be sited directly through the most important high-density cluster of desert tortoises in the entire Upper Virgin River Recovery Unit.

Advocates for the West, representing Conserve Southwest Utah and other conservation organizations challenged these decisions in federal court. In November 2023, the U.S. District Court for the District of Columbia sided with plaintiffs, and remanded back to Interior the Northern Corridor Highway ROW, the incidental take permit, and associated land use plans and other decisions. The court ordered Interior to issue new decisions by November 2024.

Interior has recently re-started the decision-making process for the ROW and incidental take permit, and a supplemental draft environmental impact statement is scheduled for publication in April 2024. UDOT continues to push for the Northern Corridor highway ROW, and Washington County, Utah's application for an incidental take permit remains on the table. And Advocates for the West, Conserve Southwest Utah and our allies continue to pushback against these decisions.

Tucci will discuss the Northern Corridor highway and Washington County habitat conservation plan and their collective impacts on the desert tortoise, the lawsuit and results, and

how injecting sound science into the agency decision making process can impact the outcome of Interior's decision-making process.

#### Use of aerial drones to locate Bolsón Tortoise populations in the Bolsón de Mapimi, Mexico

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The Turtle Conservancy (TC) and Habitat para la Biodiversidad (HABIO) recently purchased Ranchos San Ignacio and Guimbalete in the Bolsón de Mapimi Biosphere Reserve in Durango and Coahuila (respectively), Mexico for the purpose of conserving lands for the Critically Endangered bolsón tortoise (Gopherus flavomarginatus). Resi Solutions partnered with the TC and HABIO to search for bolsón tortoise populations on Rancho San Ignacio using aerial drones in conjunction with computer vision software that searches the aerial drone imagery for burrows and tortoises. In May 2023 we initiated aerial drone flight missions at Rancho San Ignacio. Our goal was to cover large areas to identify highly visible, larger burrows and to follow up with pedestrian inspections. The AI system identified 195 bolsón tortoise burrows and 31 bolsón tortoises in the resulting imagery, including a large, densely populated colony in a flight area near the western border of the ranch where 109 burrows and 4 tortoises were detected. In November 2023, we performed pedestrian checks of the burrows in this colony and recorded a total of 135 tortoise burrows. Most (94%) burrows were assessed as being actively occupied by tortoises. We confirmed that 10 (9%) of the burrows identified by the AI were false positives and 11 (10%) were double counted. The AI also missed 50 tortoise burrows that were encountered opportunistically during the pedestrian checks, representing about 37% of the confirmed tortoise burrows in the colony. The pedestrian verification of the burrows allowed us to observe tortoise behaviors, collect detailed information about tortoise burrows and threats to bolsón tortoises, and to identify smaller or obscured burrows that were not detected from the air. We recommend that aerial drone surveys be used in conjunction with pedestrian surveys to maximize finding tortoise burrows and to collect important ecological data.

#### Tracking movement and breeding of Mojave desert tortoises using genetic pedigrees

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Genetic tools are increasingly being used to supplement telemetry and behavioral studies to understand fine scale demographic and movement patterns of endangered species. We developed a panel of 105 microsatellite loci for the Mojave desert tortoise (Gopherus agassizii) to enhance the specificity and reliability of genotyping using high-throughput sequencing. We genotyped 318 adults and 155 juveniles and subadults (mean MCL = 111 mm, range 44-179; hereafter, subadult) captured in the Ivanpah Valley connectivity study area between 2011 and 2023. While most adults have been tracked using telemetry, most subadults were too small for transmitters and genetic data may be particularly useful for tracking parentage and movement of these individuals. Using all genotyped tortoises, we inferred the genetic pedigree using maximum likelihood methods. Subadults were more likely than adults to have a parent sampled on the landscape (54% of all sampled subadults had at least one sampled parent in the pedigree compared to 9% of adults). Similarly, subadults had more siblings sampled on the landscape than adults (29% vs. 12.5%). Still, these numbers suggest that there are a high proportion of unsampled parents, which may exist outside of current study plots. Only 23% of all sampled adult males and 33% of sampled adult females were identified as parents of the sampled offspring. Most sires were associated with only 1 dam; however, one sire in McCullough Pass had 4 dams. McCullough Pass also has the highest estimated density of tortoises and smallest home range sizes among study plots, and among the highest proportion of subadults assigned to sampled parents. Relatives were usually found close together, with an average distance of 600 m (max 9.5 km) between initial capture points of parent-offspring and full-sibling pairs, and distance was positively correlated with MCL. The mean distance between half-sibling pairs was 2 km (max 18 km). These results mirror our telemetry results which found that most territories were relatively small (median 37 HA) and long-distance movements were infrequent. Results confirm that genetic pedigrees are informative for inferring movement and the extent of genetic neighborhoods. Family relationships are being compared with social interaction networks and resource and habitat quality assessments to better understand the factors underlying reproductive success. Finally, ongoing field surveys are targeting regions between long-term study plots to fill gaps in the pedigree.

### Faster somatic growth may contribute to climate change resilience for a terrestrial vertebrate ectotherm

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Mean body size in ectotherms is generally predicted to decrease with increased temperature, a prediction termed the "temperature-size rule," although contrary patterns of body size and temperature in both inter- and intra-specific systems have been reported. In long-lived, large-bodied terrestrial vertebrate ectotherms, age-at-maturity and female body size can have significant effects on fecundity. In addition, size-specific predation and resource limitations can affect mean body size in a population, all of which can lead to concerns for population viability under different climate change scenarios. We performed hierarchical Bayesian analyses on legacy mark-recapture datasets in order to model the influence of environmental conditions (*e.g.*, temperature, precipitation) on growth rates, size-at-maturity, and mean body size for the Mojave desert tortoise and the gopher tortoise. Understanding how these vital rates respond to environmental gradients can help inform predictions of population persistence under climate change scenarios.

#### Building a Bolson Tortoise (Gopherus flavomarginatus) Recovery Program in the US

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The IUCN Red List's "Critically Endangered" Bolson tortoise (Gopherus flavomarginatus) is endemic to the Chihuahuan Desert, the northern tip of which extends into southern New Mexico in the US. Starting in the fall of 2006 with 30 adult/sub-adult and 7 hatchling Bolson tortoises, biologists with the Turner Endangered Species Fund (TESF) spearhead a project that includes establishing robust captive breeding and head-starting programs on media mogul Ted Turner's southern NM ranches that encompass suitable habitat. Recent progress toward TESF's ultimate goal of establishing free-living, minimally managed Bolson tortoise populations in the US imparts optimism that these tortoises can also serve as the foundation for a US-based Bolson tortoise recovery program. To date, TESF's project has produced over 700 juvenile tortoises ranging in age from 0-17 years old. Regular, long-term monitoring of marked individuals makes it possible to determine parameters such as typical growth rates, survivorship, genetics, kinship, predation pressures, etc. Moreover, two of the oldest (currently 15 and 16, respectively) and largest juvenile female tortoises showed signs in 2023 of potential egg production in 2024; when these females produce eggs, they will represent the first documented cases of known age and size at first egg production. Furthermore, in 2021 and 2022 we experimentally released 101 juvenile Bolson tortoises in a remote location on one of the ranches. Survivorship thus far is >95%, and movement and "settling" patterns of juvenile tortoises in their new northern Chihuahuan desert home are

beginning to emerge. Thus far,  $\sim$ 88% of the juvenile tortoises remain within  $\sim$ 1,200 m (0.75 mi) of their release location.

# Canid deterrents: Are tools used to deter gray wolves (*Canis lupus*) from depredating livestock applicable in Mojave desert tortoise (*Gopherus agassizii*) recovery efforts?

#### Elizabeth Willy

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Gray wolves (*Canis lupus*) began to recolonize southern Oregon in 2011. Livestock production is important to the local economy, but also creates an attractant for wolves. Because gray wolves are federally protected under the Endangered Species Act in the western two-thirds of Oregon, only nonlethal tools to deter wolves from depredating livestock are allowed in this area. Many non-lethal tools were implemented in the last 12 years, with varied levels of success. The non-lethal tools used include, but are not limited to, human presence, turbo fladry, noise makers, and drones—which may also have utility in deterring other canids such, as covotes. These deterrent tools may be applicable to improved management strategies for coyotes and other mammal predators within targeted areas of the Mojave and Colorado Deserts, aiding Mojave desert tortoise (Gopherus agassizii) recovery initiatives in highly valued habitats. This presentation aims to provide information for continued discussion, innovation, and development of non-lethal tools and management practices needed to reduce or eliminate depredation sensitive wildlife within of desert ecosystems.

# U.S. Fish and Wildlife Service Desert Tortoise Recovery Updates: An ongoing need for integrated recovery actions and future collaboration

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The Desert Tortoise Recovery Program collaborated with numerous partners on projects in 2023, contributing to recovery of the desert tortoise through many direct and indirect avenues. Throughout our work on desert tortoise recovery, it remains clear that continued collaboration among federal, state, private, and public partners is integral to the recovery of the species across the range. However, future recovery efforts not only need to focus on collaboration, but also integrated and adaptive management actions to achieve success. Specific recovery updates include:

- 1) We continued our efforts to conduct range-wide surveys of desert tortoises and began establishing new demographic plots to improve our understanding of vital rates.
- 2) We continued further development and implementation of a series of integrated raven management tools to reduce raven densities in tortoise habitat.
- 3) We coordinated with federal and non-federal partners on a variety of projects to promote recovery, including enhancement of desert tortoise habitat, ongoing translocation studies,

expansion of road fencing and connecting culverts, head starting, and drone survey research.

- 4) We collaborated with partners on several large-scale planning initiatives, including the Mojave Desert Sentinel Landscape designation, the Department of Defense Recovery and Sustainment Partnership Initiative, development of the revised BLM Solar Programmatic Environmental Impact Statement, the BLM's new Strategic Plan for Mojave Desert Tortoise and its associated Action Plan, and development of the Fish and Wildlife Service's General Conservation Plan for Desert Tortoise in California.
- 5) We coordinated efforts with partners to provide feedback and guidance on maintaining vegetation and improving compatibility with wildlife at utility-scale solar facilities. Additionally, we provided input on numerous renewable energy projects throughout the range.
- 6) We contributed to several outreach events for desert tortoises, including Earth Day, National Public Lands Day, and the fifth annual Desert Tortoise Week, during which we organized several events across California, Nevada, Utah, and Arizona.

We published three papers with collaborators on topics including range-wide occupancy trends, travel management planning, and spatial density modeling. Additionally, we produced guidance on husbandry practices for wild tortoises in captivity, tortoise fence turnarounds, shade structures, and gate guard.

**Bolson Tortoise Conservation in the United States** 

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We (USFWS) finalized a Safe Harbor Agreement on the Armendaris Ranch, for the Bolson tortoise (*Gopherus flavomarginatus*) in 2023 as a first step toward conservation of the species in the United States. The Armendaris Ranch is a 344,955 acre (139,598 hectare) private ranch in central New Mexico. This newly created United States population has 121 free-ranging animals and additional releases, to bolster numbers, are planned. The wild population in the tri-state area of Mapimí, Mexico is thought to be at less than 2,500 individuals. This 50-year Safe Harbor Agreement provides a net conservation benefit to both the Mexican and New Mexican population.

With finalization of the Safe Harbor Agreement, the New Mexico Ecological Services Field Office is assuming recovery responsibility for efforts in the United States. We will continue to work with partners in Mexico and the United States to help assure conservation in both countries. We are considering the possibility of additional tortoise release locations on protected lands in suitable habitat. Habitat modeling efforts indicate that suitable habitat may exist on Sevilleta and Bosque del Apache National Wildlife Refuge's. We are interested in finding other potential sites to locate conservation populations that have large areas of suitable habitat, protection from human mortality or collection, and lack of conflict with human activities or other species.