ROLE OF HEAD-STARTING TREATMENT AND CLIMATIC FACTORS ON POST-RELEASE SURVIVAL AND BEHAVIOR OF JUVENILE MOJAVE DESERT TORTOISES (*GOPHERUS AGASSIZII*)

by

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(Under the Direction of Tracey D. Tuberville and Clark S. Rushing)

ABSTRACT

Mojave Desert tortoise (*Gopherus agassizii*) populations have declined for decades, prompting the development of recovery tools. One conservation practice is head-starting, and continued optimization, including reducing rearing duration and considering tortoises' response to climate, will foster broader and more effective implementation. We released and radio-tracked 60 juvenile tortoises reared under two treatments: (1) rearing one year indoors, then one year outdoors ("Combo-reared"), and (2) rearing indoors only for one year ("Indoor-reared"). We quantified their behavior, movement, and first year survival. We found no difference in behavior or survival between treatments, but combo tortoises demonstrated higher site fidelity in their movements than indoor tortoises. Additionally, using long-term tracking data of 324 juvenile tortoises released between 2012-2020, we investigated the relative role of husbandry treatment and climate on long-term survival, focusing on drought. We found increasing drought severity decreased tortoise survival overall, and survival was not significantly different by head-starting treatment.

INDEX WORDS: Reptile, turtle, tortoise, desert tortoise, *Gopherus agassizii*, head-starting, population augmentation, climate change, survivorship, juvenile recruitment, Mojave, conservation, recovery

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Large-scale disturbances in natural environments have led to global biodiversity declines (Capdevila et al. 2021), especially in fragile and often overlooked desert ecosystems (Esque et al. 2021). While some disturbances can be natural (i.e. disease, hurricanes, etc.), human activity can escalate their impact on the ecosystem. For example, climate change is a major disturbance that has the potential to shift average temperatures and precipitation in deserts, and anthropogenic driven warming has contributed to ~46% of the 2000–2018 drought severity and ~19% of the 2021 drought severity in the southwestern U.S. (Williams et al. 2020, Williams et al. 2022). In an environment that already experiences harsh extremes, native desert species may not be able to adapt fast enough to anthropogenically-driven climate changes (Griffis-Kyle 2016, Riddell et al. 2019, Zhou et al. 2020). Deserts and shrublands ranked third out of fourteen biomes in the rate of temperature change during the most recent period of climate change (Loarie et al. 2009), indicating deserts are more likely to have a higher relative temperature increase as global climate change progresses. Developing recovery tools that support native species to mitigate threats to desert systems is a current conservation priority.

The Mojave Desert is located in the southwestern United States, and is a challenging environment for wildlife to occupy due in part to the extreme temperatures and scarce water sources. Since 2000, southwestern North America has also been experiencing a historic mega-drought, which is defined as a multi-decade event with periods of very high drought severity (Williams et al. 2020, Williams et al. 2022). An analysis of reconstructed tree rings and soil moisture levels revealed that the drought from 2000-2018 has been the driest period since the 15th century (Williams et al. 2020). Even in non-drought years, conditions can be extreme. Temperatures can range from a low of -11° C in the winter to a high of 42° C in the summer (Applied Climate Information System 2022). This region receives an average annual precipitation of 12.8 cm/yr with a range of 4.4-30.9 cm/yr, most of which falls in the cool season (Oct-April) (Hereford et al. 2006). The eastern and western Mojave deserts have different precipitation patterns, with a bi-seasonal pattern in the eastern portion and a winter dominant pattern in the west. In the Eastern Mojave Desert, approximately 66% of the total annual precipitation falls in the cool season, compared to 82% in the Western Mojave Desert (Hereford et al. 2006).

Over the last century, The Mojave has also experienced many changes, such as increased wildfires (Brooks 1999), aridification (Overpeck and Udall 2020), habitat loss due to development for housing (Morris et al. 1997), energy production (Parker et al. 2018), and tourism. Species such as the Vegas Valley leopard frog (*Rana* sp.) (Bradford et al. 2005, Hekkala et al. 2011), the Amargosa vole (*Microtus californicus scirpensis*) (Castle et al. 2020), multiple bird species (Iknayan and Beissinger 2018), and the Mojave Desert tortoise (*Gopherus agassizii*) (Allison and McLuckie 2018) have experienced population declines as a result of these changes. The Mojave Desert tortoise (hereafter 'desert tortoise' or 'tortoise') has been considered threatened under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service 1973) since 1990. Juvenile recruitment of desert tortoises has been declining (Allison and McLuckie 2018). Tortoises could face becoming endangered or extinct if juvenile recruitment continues to be low as the current adult population declines (Kristan III and Boarman 2003).

Desert tortoises are sensitive to changes in their environment, such as shifting climate, making them useful bioindicators for the integrity of the Mojave Desert (Berry and Medica 1995). They possess multiple traits that have allowed them to adapt to arid climates, including behavioral and physiological responses to environmental conditions. Long periods of inactivity, fasting, and retaining urine in their bladder help them maintain their body moisture until the next rainfall (McCoy et al. 2011). Desert tortoises also seek shelter in their burrows, which have relatively stable temperatures and humidity levels, to avoid the temperature fluctuations of the desert surface and reduce their water loss (Bulova 2002). However, these adaptions also lead to desert tortoises' body condition being strongly correlated to rainfall (McCoy et al. 2011). The herbaceous annuals on which desert tortoises depend for forage are also strongly affected by rainfall patterns (Hereford et al. 2006). Without sufficient rainfall in the cool season, winter annual plants will not germinate and be available in the spring when desert tortoises rely on them most (Beatley 1967). Desert tortoises can tolerate eating dry forage, but still rely on the opportunistic availability of free-standing drinking water from rain events, unlike many other desert animals that can obtain water from what they eat (Peterson 1996). Prolonged climate variation, especially recurrent droughts, has been demonstrated to reduce survivorship of desert tortoises even in otherwise protected areas (Lovich et al. 2014). Drought can affect tortoises both directly, causing dehydration, and indirectly, when there is no forage available or mesopredators "switch" to preying on tortoises

(Peterson 1994). In a climate change sensitivity model created by Barrows (2011), when terrain and soil variables were held constant and climate was altered on a gradient of increasing temperatures and decreasing precipitation, habitat for desert tortoises decreased by nearly 66% in the Mojave Desert. While many studies have examined the short-term effects of drought on desert tortoises (Peterson 1994, Duda et al. 1999, Longshore et al. 2003), few have been able to consider the long-term effects on a population.

A recovery tool that could mitigate tortoise declines is head-starting, the process of rearing juveniles of a species to an age or size at which they are less vulnerable and releasing them into the wild (U.S. Fish and Wildlife Service 2011, Burke 2015). Because reptiles often have high mortality in the early life stages, head-starting is one potential technique for speeding up the recovery of wild populations by increasing the number of juveniles that survive to less vulnerable life stages (Burke 2015). However, if the original cause of adult decline is not addressed, head-starting efforts will likely be in vain (Frazer 1992). Head-starting –especially when used in combination with other recovery tools – may help jumpstart populations. Head-starting is increasingly being used as part of the recovery efforts for many turtle species, including Blanding's turtles (*Emydoidea blandingii*) (Buhlmann et al. 2015, Thompson et al. 2020), diamondback terrapins (*Malaclemys terrapin*) (Herlands et al. 2004), western pond turtles (*Actinemys marmorata*) (Vander Haegen et al. 2009), and gopher tortoises (*Gopherus polyphemus*) (Tuberville et al. 2015). Head-starting practitioners have also continuously sought to improve the methods used to maximize turtles' post-release success. For example, Tetzlaff et al. (2019b) suggested that rearing duration was more important than providing enrichment (in the form of an enclosure incorporated with natural elements) for head-started eastern box turtles (*Terrapene carolina carolina*), likely due to the larger size of turtles reared for a longer period providing an advantage after release. They found that longer rearing durations came at no apparent cost to post-release behavior or survival, and concluded that rearing for several years to maximize head-start release size would be beneficial (Tetzlaff et al. 2019b).

Previous studies have evaluated various aspects of head-starting practices for desert tortoises, with early studies mainly focused on outdoor rearing in predator proof pens (Morafka et al. 1997). Nagy et al. (2015a) released and monitored 53 juvenile tortoises aged 2–15 years and found that post-release survivorship increased with increasing body size. They recommended that tortoises be held in captivity until reaching at least 100 mm midline carapace length (MCL) to reduce predation risk. However, tortoises usually take 6-8 years to reach this recommended release size in the wild or when reared outdoors because they grow very little during winter dormancy and summer estivation (Nagy et al. 2015a, Daly et al. 2018). Since 2011, the University of Georgia and the University of California, Davis have been evaluating head-starting as a recovery tool for desert tortoises in the Mojave National Preserve’s Ivanpah Valley, California, focusing on identifying the combination of husbandry and release conditions that result in greatest post-release survival (Todd et al. 2016, Daly et al. 2018, Daly et al. 2019, Tuberville et al. 2019, McGovern et al. 2020a, McGovern et al. 2020b, Candal 2021, McGovern et al. 2021). To shorten the time it takes for tortoises to grow to the releasable size recommended by Nagy et al. (2015a), Daly et al. (2019) tested the efficiency of indoor rearing, which involved keeping tortoises active and growing year-round, including when they would otherwise be dormant. They released and monitored 68 juvenile tortoises, of which 20 were released as hatchlings, 28 were indoor-reared for 7 months, and 20 were outdoor-reared for 7 months. They found that indoor-rearing produced tortoises much larger than those outdoor-reared, but post-release survival for the first six-months did not differ significantly among the groups (Daly et al. 2019), suggesting that 7 months was not enough time to produce tortoises that were large enough or had sufficient shell hardness to be predator resistant and be less vulnerable to exposure.

To reduce the risk of predation from common ravens (*Corvus corax*), Daly et al. (2019) recommended selecting release sites for head-started juveniles that are >1.6 km away from raven nesting structures and to avoid releasing tortoises in the spring when ravens are nesting and provisioning their young with prey such as desert tortoises. Selecting release sites that encourage site fidelity is also an important consideration for desert tortoises, as excessive movements away from the release site can be energetically costly and are the greatest cause of failure in reptile translocations (Germano and Bishop 2009). To encourage site fidelity, identifying release sites with suitable habitat for protection and forage should be a priority. Todd et al. (2016) demonstrated that juvenile tortoises selected habitats with abundant washes and high perennial plant density nearby, therefore selecting sites with these features for release would be beneficial. Another method of increasing site fidelity is to reduce tortoises' natural homing behavior. Hazard et al. (2015) found that choosing release sites at least 500 m away from the tortoises' rearing pens was a sufficient distance to reduce homing behavior and encourage juveniles to exhibit site fidelity in the release site.

A relatively new husbandry technique that has been successful is combo-rearing, which is a means of increasing tortoise size for one year indoors, and then allowing tortoises to gain wild behaviors and become accustomed to the natural habitat for a year outdoors. The combo-rearing method produced tortoises with significantly higher mass and body size and higher survival than same-aged outdoor-reared tortoises (McGovern et al. 2020a, McGovern et al. 2020b). Combo-reared tortoises also demonstrated high site fidelity and dispersed significantly less than outdoor-reared tortoises (McGovern et al. 2020a). However, because most of the growth that occurs during the year of indoor head-starting, it is not clear to what extent the high survival of combo-reared tortoises can be attributed simply to their larger size from their first year indoors, or to their exposure to natural environmental cues during their subsequent year outdoors.

Efforts to continue to maximize efficiency of head-starting, such as reducing rearing duration, will facilitate broader application of the technique. Furthermore, there are now sufficient monitoring data (radio-tracking from 2012-20222) to evaluate the relative role of climatic factors on post-release survival of head-started tortoises – an element that has not been considered in previous studies. My thesis includes two components. In Chapter 2, I compared two head-starting treatments – one year of indoor-rearing versus two years of combo-rearing (one year indoors to grow rapidly, one year outdoors to gain wild behaviors) – to determine whether similar behavioral and survival outcomes can be achieved with shorter head-starting duration. I compared the post-release movements, survival, and timing of settlement behaviors between the two treatments. In Chapter 3, I used multiple years of radio-tracking data from 324 juveniles reared under different head-starting treatments juveniles released from 2012-2020 to evaluate the relative role of head-starting treatment and climatic conditions on post-release survival. Collectively, the results of these two studies will inform head-starting programs of Mojave Desert tortoises on the most efficient rearing practices for rearing duration and the potential outcomes of releasing head-starts in certain climate conditions. This optimization of rearing practices will allow for greater application of head-starting and conservation of Mojave Desert tortoises.

LITERATURE CITED

Allison, L. J., and A. M. McLuckie. 2018. Population trends in Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **13**:433-452.

Applied Climate Information System. 2022. NOWData - NOAA Online Weather Data. Weather.gov.

Barrows, C. W. 2011. Sensitivity to climate change for two reptiles at the Mojave–Sonoran Desert interface. Journal of Arid Environments **75**:629-635.

Beatley, J. C. 1967. Survival of winter annuals in the northern Mojave Desert. Ecology **48**:745-750.

Berry, K. H., and P. A. Medica. 1995. Desert tortoises in the Mojave and Colorado deserts. Pages 135-137 *in* E. T. LaRoe, G. S. Farris, C. E. Puckett, P. D. Doran, and M. J. Mac, editors. Our living resources: A report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosystems. National Biological Service.

Bradford, D. F., J. R. Jaeger, and S. A. Shanahan. 2005. Distributional changes and populations status of amphibians in the Eastern Mojave Desert. Western North American Naturalist **65**:462-472.

Brooks, M. L. 1999. Does protection of desert tortoise habitat generate other ecological benefits in the Mojave Desert? Pages 68-73 *in* Wilderness Science in a Time of Change Conference. Us Dept Agr, Forest Serv Rocky Mt Forest & Range Exptl Stn, Missoula, Mt.

Buhlmann, K. A., S. L. Koch, B. O. Butler, T. D. Tuberville, V. J. Palermo, B. A. Bastarache, and Z. A. Cava. 2015. Reintroduction and Head-starting: Tools for

Blanding's Turtle (*Emydoidea blandingii*) Conservation. Herpetological Conservation and Biology **10**:436-454.

Bulova, S. J. 2002. How temperature, humidity, and burrow selection afect evaporative water loss in desert tortoises. Journal of Thermal Biology **27**:175–189.

Burke, R. L. 2015. Head-starting turtles: learning from experience. Herpetological Conservation and Biology **10**:299-308.

Candal, C. M. 2021. Pressure to perform: The role of stress Physiology in head-starting success for Mojave Desert tortoises. University of Georgia, Athens, GA.

Capdevila, P., N. Noviello, L. McRae, R. Freeman, and C. F. Clements. 2021. Global patterns of resilience decline in vertebrate populations. Ecology Letters.

Castle, S. T., P. Foley, D. L. Clifford, and J. Foley. 2020. A stochastic structured metapopulation model to assess recovery scenarios of patchily distributed endangered species: Case study for a Mojave Desert rodent. PLOS ONE **15**:e0237516.

Daly, J. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, and T. D. Tuberville. 2018. Comparing growth and body condition of indoor-reared, outdoor-reared, and direct-released juvenile Mojave Desert tortoises. Herpetological Conservation and Biology **13**:622-633.

Daly, J. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, and T. D. Tuberville. 2019. Survival and movements of head-started Mojave desert tortoises. Journal of Wildlife Management **83**:1700-1710.

Duda, J. J., A. J. Krzysik, and J. E. Freilich. 1999. Effects of drought on desert tortoise movement and activity. The Journal of Wildlife Management **63**:1181.

Esque, T. C., L. A. DeFalco, G. L. Tyree, K. K. Drake, and K. E. Nussear. 2021. Priority species lists to restore desert tortoise and pollinator habitats in Mojave Desert shrublands. Natural Areas Journal **41**:145-158.

Frazer, N. B. 1992. Sea turtle conservation and halfway technology. Conservation Biology **6**:179-184.

Germano, J. M., and P. J. Bishop. 2009. Suitability of amphibians and reptiles for translocation. Conservation Biology **23**:7-15.

Griffis-Kyle, K. L. 2016. Physiology and ecology to inform climate adaptation stratigies for desert amphibians. Herpetological Conservation and Biology **11**:563-582.

Hekkala, E. R., R. A. Saumure, J. R. Jaeger, H.-W. Herrmann, M. J. Sredl, D. F. Bradford, D. Drabeck, and M. J. Blum. 2011. Resurrecting an extinct species: archival DNA, taxonomy, and conservation of the Vegas Valley leopard frog. Conservation Genetics **12**:1379–1385.

Hereford, R., R. H. Webb, and C. I. Longpré. 2006. Precipitation history and ecosystem response to multidecadal precipitation variability in the Mojave Desert region, 1893–2001. Journal of Arid Environments **67**:13-34.

Herlands, R., R. Wood, J. Pritchard, H. Clapp, and N. Le Furge. 2004. Diamondback Terrapin (*Malaclemys terrapin*) head-starting project in southern New Jersey. Conservation and Ecology of Turtles of the Mid-Atlantic Region:13-21.

Iknayan, K. J., and S. R. Beissinger. 2018. Collapse of a desert bird community over the past century driven by climate change. Proceedings of the National Academy of Sciences **115**:8597–8602.

Kristan III, W. B., and W. I. Boarman. 2003. Spatial pattern of risk of common raven predation on desert tortoises. Ecology **84**:2432-2443.

Loarie, S. R., P. B. Duffy, H. Hamilton, G. P. Asner, C. B. Field, and D. D. Ackerly. 2009. The velocity of climate change. Nature **462**:1052-1055.

Longshore, K. M., J. R. Jaeger, and J. M. Sappington. 2003. Desert tortoise (*Gopherus agassizii*) survival at two eastern Mojave Desert sites: Death by short-term drought? Journal of Herpetology **37**:169–177.

Lovich, J. E., C. B. Yackulic, J. Freilich, M. Agha, M. Austin, K. P. Meyer, T. R. Arundel, J. Hansen, M. S. Vamstad, and S. A. Root. 2014. Climatic variation and tortoise survival: Has a desert species met its match? Biological Conservation **169**:214-224.

McCoy, E. D., R. D. Moore, H. R. Mushinsky, and S. C. Popa. 2011. Effects of rainfall and the potential influence of climate change on two congeneric tortoise species. Chelonian Conservation and Biology **10**:34-41.

McGovern, P. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, J. Hepinstall-Cymerman, J. A. Daly, and T. D. Tuberville. 2020a. Comparing husbandry techniques for optimal head-starting of the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **15**:626-641.

McGovern, P. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, J. Hepinstall-Cymerman, J. A. Daly, and T. D. Tuberville. 2020b. The effect of size on postrelease survival of head-started Mojave Desert tortoises. Journal of Fish and Wildlife Management **11**:494-506.

McGovern, P. A., J. M. Peaden, K. A. Buhlmann, B. D. Todd, and T. D. Tuberville. 2021. Comparing post-release cover and burrow use by differentially head-started Mojave desert tortoises in southern California. Conservation Evidence Journal **18**:37-43.

Morafka, D. J., K. H. Berry, and E. K. Spangenberg. 1997. Predator-proof field enclosures for enhancing hatchling success and survivorship of juvenile tortoises: a critical evaluation. Pages 147–165 *in* Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles: An International Conference Conservation. Wildlife Conservation Society Turtle Recovery Program and the New York Turtle and Tortoise Society, New York, New York, USA.

Morris, R. L., D. A. Devitt, A. M. Crities, G. Borden, and L. N. Allen. 1997. Ubanization and water conservation in Las Vegas Valley, Nevada. Journal of Water Resources Planning and Management **May/June**.

Nagy, K. A., L. S. Hillard, M. W. Tuma, and D. J. Morafka. 2015. Head-started desert tortoises (*Gopherus agassizii*): movements, survivorship and mortality causes following their release. Herpetological Conservation and Biology **10**:203-215.

Overpeck, J. T., and B. Udall. 2020. Climate change and the aridification of North America. Proceedings of the National Academy of Sciences of the United States of America **117**:11856-11858.

Parker, S. S., B. S. Cohen, and J. Moore. 2018. Impact of solar and wind development on conservation values in the Mojave Desert. PLOS ONE **13**:e0207678.

Peterson, C. C. 1994. Different rates and causes of high mortality in two populations of the threatened desert trotoise *Gopherus agassizii*. Biological Conservation **70**:101-108.

Peterson, C. C. 1996. Ecological energetics of the desert tortoise (*Gopherus agassizii*): Effects of rainfall and drought. Ecology **77**:1831-1844.

Riddell, E. A., K. J. Iknayan, B. O. Wolf, B. Sinervo, and S. R. Beissinger. 2019. Cooling requirements fueled the collapse of a desert bird community from climate change. Proceedings of the National Academy of Sciences **116**:21609-21615.

Tetzlaff, S. J., J. H. Sperry, B. A. Kingsbury, and B. A. DeGregorio. 2019. Captive-rearing duration may be more important than environmental enrichment for enhancing turtle head-starting success. Global Ecology and Conservation **20**.

Thompson, D., G. Glowacki, D. Ludwig, R. Reklau, A. R. Kuhns, C. K. Golba, and R. King. 2020. Benefits of head-starting for Blanding's turtle size distributions and recruitment. Wildlife Society Bulletin **44**:57-67.

Todd, B. D., B. J. Halstead, L. P. Chiquoine, J. M. Peaden, K. A. Buhlmann, T. D. Tuberville, and M. G. Nafus. 2016. Habitat selection by juvenile Mojave Desert tortoises. The Journal of Wildlife Management **80**:720-728.

Tuberville, T. D., K. A. Buhlmann, R. Sollmann, M. G. Nafus, J. M. Peaden, J. A. Daly, and B. D. Todd. 2019. Effects of short-term, outdoor head-starting on growth and survival in the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **14**:171-184.

Tuberville, T. D., T. M. Norton, K. A. Buhlmann, and V. Greco. 2015. Head-starting as a management component for gopher tortoises (*Gopherus polyphemus*). Herpetological Conservation and Biology **10**:455-471.

U.S. Fish and Wildlife Service. 1973. Endangered Species Act of 1973.*in* United States of America, editor.

U.S. Fish and Wildlife Service. 2011. Revised recovery plan for the Mojave population of the desert tortoise (*Gopherus agassizii*).

Vander Haegen, W. M., S. L. Clark, K. M. Perillo, D. P. Anderson, and H. L. Allen. 2009. Survival and causes of mortality of head-started western pond turtles on Pierce National Wildlife Refuge, Washington. Journal of Wildlife Management **73**:1402-1406, 1405.

Williams, A. P., B. I. Cook, and J. E. Smerdon. 2022. Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. Nature Climate Change.

Williams, A. P., E. R. Cook, J. E. Smerdon, B. I. Cook, J. T. Abatzoglou, K. Bolles, S. H. Baek, A. M. Badger, and B. Livneh. 2020. Large contribution from anthropogenic warming to an emerging North American megadrought. Science **368**:314-+.

Zhou, H., Y. Chen, C. Zhu, Z. Li, G. Fang, Y. Li, and A. Fu. 2020. Climate change may accelerate the decline of desert riparian forest in the lower Tarim River, Northwestern China: Evidence from tree-rings of Populus euphratica. Ecological Indicators **111**.

CHAPTER 2

POST-RELEASE SURVIVAL AND BEHAVIOR OF HEAD-STARTED MOJAVE DESERT TORTOISES REARED WITH AND WITHOUT A SECOND YEAR OF OUTDOOR REARING

INTRODUCTION

The current rate of global biodiversity declines is leading to what scientists predict could be the world's sixth mass extinction event (Pievani 2014, McCallum 2015, Cowie et al. 2022). The previous five mass extinctions are thought to have been caused by natural global changes or disasters, but this sixth event will largely be due to anthropogenic influences accelerating the background rate of extinction by ~1000 times (Pimm et al. 2014). Despite the challenges to combating global change, conservation efforts can still mitigate some of the anthropogenic damage. Recovery tools must be developed and refined to be efficient in preserving the species that can be saved, and preventing more species from becoming endangered.

Turtles are a particularly threatened taxon, with over half of species listed as endangered (Lovich et al. 2018). Some traits, such as their long lifespan, delayed sexual maturity, and naturally low juvenile recruitment rates, make turtles vulnerable to decline when sudden changes occur in their environment because they cannot adapt quickly (Stanford et al. 2020, Berry et al. 2021). The Mojave Desert tortoise (*Gopherus agassizii*; hereafter "desert tortoise" or "tortoise") is one such turtle species that has experienced chronic declines and has been listed as threatened under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service 1973) since 1990. Desert tortoises are considered important keystone species because they construct burrows that are used by other species as refugia (Woodbury and Hardy 1948, Lovich 2011, Walde et al. 2014, Walde and Currylow 2015, Walde et al. 2016), they serve as bioindicators of ecosystem health (Berry and Medica 1995), and they are iconic species of the Mojave Desert (Kohn 2018). While tortoises have many traits allowing them to adapt to arid environments, including fasting, periods of inactivity, and seeking shelter in burrows, they are sensitive to changes in their environment. The added stressor of prolonged climate variation, especially the prolonged drought that the southwestern US has been experiencing since 2000 (Williams et al. 2020, Williams et al. 2022), reduces survivorship of desert tortoises even in otherwise protected areas (Lovich et al. 2014). Increased drought conditions and other threats, including wildfires (Brooks 1999), habitat destruction (Morris et al. 1997, Parker et al. 2018), and human-subsidized predators (Kristan III and Boarman 2003, Esque et al. 2010), have contributed to population declines of desert tortoises. From 2004 to 2014, abundance of adult desert tortoises declined in four out of five recovery units, and juveniles have declined in all recovery units since 2007 (Allison and McLuckie 2018). If these trends continue, desert tortoise populations will not recover.

To counter tortoise population declines, recovery tools are being investigated and refined for use at a larger scale (U.S. Fish and Wildlife Service 2011). One such tool, termed head-starting, is the practice of rearing juveniles to a size at which they are less vulnerable to mortality and then releasing them into the wild (U.S. Fish and Wildlife Service 2011, Burke 2015). When combined with other recovery tools, head-starting can help jumpstart conservation efforts to restore populations. For Mojave Desert tortoises, the recommended size at release is ~100 mm midline carapace length (MCL) to reduce mortality from exposure (such as dehydration) or predation (Nagy et al. 2015a). However, it can take up to 7 years for juveniles to reach that size in the wild or when reared outdoors (Daly et al. 2019). To speed up that process, a new husbandry approach called combo-rearing was developed that combines an indoor-rearing component for one year, during which tortoises can grow rapidly by being kept active year round, followed by outdoor-rearing for one year, during which tortoises are exposed to natural environmental cues (such as building burrows and initiating dormancy) in predator-proof pens. This husbandry approach was successful in producing healthy tortoises that attained the recommended release size within two years and that exhibited high survival rates after release (McGovern et al. 2020a, McGovern et al. 2020b, Candal 2021). It is unclear though, whether the tortoises' high survival rates are more influenced by their exposure to natural environmental cues during their year outdoors or to their larger size. Greater understanding of husbandry effects on tortoises' post-release success will help with continued optimization of the head-starting methods, thereby increasing efficiency and facilitating broader implementation as a recovery tool.

Here, we test whether the second year of combo-rearing during which tortoises are reared outdoors improves the release outcome relative to one year of indoor-rearing alone. Answering this question will help determine whether providing an outdoor experience before release should continue to be a priority for head-start programs. We compared the post-release settling behavior, movement, and first-year survival of combo-reared (one year indoors, one year outdoors) and indoor-reared (one year only) tortoises.

We hypothesize the following: 1) tortoises that have been exposed to natural environmental cues during outdoor-rearing (i.e., combo-reared) will settle into the environment more quickly post-release than tortoises that have only experienced indoor rearing, because they have performed settling behaviors before; 2) combo-reared tortoises will have reduced movements before and after settlement, because they will have performed exploratory movements before; and 3) after accounting for size, husbandry treatment will not affect tortoise survival post-release because both husbandry treatments produce healthy tortoises. From these hypotheses, we predict that: 1) tortoises in the combo-reared group will build their first burrows, initiate winter dormancy, and emerge in spring from dormancy earlier than those in the indoor-reared group; 2) the combo-reared group will have smaller home ranges, shorter dispersal distances, and shorter step-lengths than the indoor-reared group; and 3) that survival of head-started desert tortoises will not differ between treatments.

METHODS

2.1 Study site

The study was conducted in the Ivanpah Valley within the Mojave National Preserve (MNP) in San Bernardino County, California, USA. The area is within the Eastern Mojave recovery unit for the Mojave desert tortoise where the species has experienced the greatest decline in juvenile recruitment (U.S. Fish and Wildlife Service 2011, Allison and McLuckie 2018), making it an ideal place to study the effect of augmentation on the population. We conducted all head-starting operations at the Ivanpah Desert Tortoise Research Facility (IDTRF). The release site was approximately 15 km south of IDTRF (Fig. 2.1) and was dominated by Yucca Woodland habitat, with plant species such as creosote (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), yuccas (*Y. brevifolia*, *Y. shidigera*), pencil cholla (*Cylindropuntia ramosissima*), and littleleaf ratany (*Krameria erecta*) being the most abundant (Todd et al. 2016). The site also contained an abundance of small washes that animals often used as trails and small mammal burrows used as shelter sites by multiple wildlife species (Todd et al. 2016). Elevation ranged from 940 – 1075 m above sea level. From 2000-2022 the temperatures at the study site ranged from a long-term average high of 40.6 °C in the summer and an average low of -6 °C in the winter, and the annual precipitation ranged from 4.7 to 50.6 cm/yr. During our study period (2020 – 2021), the average annual precipitation was 5.4 cm/yr (Applied Climate Information System 2022).

2.2 Obtaining hatchlings

To obtain hatchlings, we captured adult desert tortoise females during late April – early May in 2018 and 2019, brought them to the IDTRF, and radiographed them to check for calcified eggs (Diagnostic Imaging Systems, Poskam, Colorado, USA; 60 kvp, 0.8 mAS, 74 cm focal length; Gibbons and Greene 1979). We immediately returned all non-gravid females to their capture location. We placed gravid females in individual 9m x 9m predator-proof nesting pens with pre-made burrows and natural vegetation (McGovern et al. 2020a, McGovern et al. 2020b). All gravid females were allowed to nest naturally and we gave them supplemental watering from a sprinkler system (Tuberville et al. 2019) and supplemental food. We radiographed females every 10 days to monitor for egg deposition, and when it was confirmed that they had nested, we returned them to the wild at their original capture location. We returned any females that had not deposited their eggs within 30 days to their last known burrow location, in accordance with our permits. Once eggs were deposited, they incubated naturally in the predator-proof pens. We began searching pens for emerging hatchlings around 80 days after the estimated nest date and we marked hatchlings with unique notch codes assigned by the U.S. Fish and Wildlife Service.

2.3 Experimental treatments

We compared two experimental rearing treatments in this study: combo-rearing and indoor-rearing. Combo-rearing involved rearing tortoises inside for one year, keeping tortoises active and eating year-round to achieve rapid growth, followed by one year of outdoor rearing to provide exposure to natural environmental cues while still being protected from predators. Indoor-rearing involved rearing tortoises indoors for one year only with the purpose of obtaining rapid growth. In September 2018, we selected 48 hatchlings for the combo-rearing treatment and in September 2019 we selected 48 hatchlings for the indoor-rearing treatment.

The indoor component for both rearing treatments began with assigning the selected tortoises to one of six large rearing tubs (8 hatchlings per tub). This was done in 2018 for the combo-reared group, and then again in 2019 for the indoor-reared group. To avoid any maternal effects bias, we split up tortoises from the same clutch so no siblings were placed together in a single tub (Nafus et al. 2015). Each tub consisted of a 190 L Rubbermaid stock tank (132 x 79 x 30.5 cm) equipped with natural substrate, humid hide boxes to promote normal shell growth and avoid pyramiding (Wiesner and Iben 2003), artificial plastic shelters, and a paper feeding plate. Lighting was provided using a 50 W ZooMed Repti Basking Spot Lamp bulb for daytime basking (37 °C), a ZooMed 50 W Infrared Basking Spot bulb for night-time heat (32 °C), and a 26 W Exo-Terra Reptile

UVB150 bulb (Rolf C. Hagen Corp., Mansfield, MA., U.S.A) for optimal calcium metabolism and vitamin D_3 conversion (McWilliams 2005), all suspended 30 cm above the tub and set with automatic timers to mimic a natural photoperiod. While tortoises were indoors, we provided food (a mixture of greens and moistened Mazuri® Tortoise Diet (Mazuri Exotic Animal Nutrition, St. Louis, MO); Daly et al. 2018) three times a week, supplemental calcium powder twice a week, and soaked tortoises once a week to maintain hydration. We recorded tortoise weight, midline carapace length (MCL), shell width, and shell height once per month during the indoor phase.

In the fall of 2019, we measured and weighed tortoises assigned to the combo-rearing treatment and placed them in outdoor pens. The pens were predator-proof, with pre-made burrows, natural terrain, and native vegetation. We provided them with supplemental food as they had received while indoors and water from a sprinkler system (Tuberville et al. 2019) once a week during the active season (~April – November). See McGovern et al. (2020a) for further husbandry details. Once combo tortoises had been moved outside, the tortoises selected for the indoor-rearing treatment were assigned to the indoor tubs.

By the time of release, tortoises assigned to the combo-reared treatment had spent one year indoors, then one year outdoors (2018-2020) and were two years old. Tortoises assigned to the indoor treatment had spent one year indoors only (2019-2020) and were one year old. Prior to release, we measured and weighed all tortoises to confirm they were fit to be released (i.e. around the recommended release size and healthy).

2.4 Release

Using ArcGIS (version 10.5), we identified a release site that met the following criteria: (1) 0.6 km^2 (300 m X 2000 m) rectangular area (McGovern 2019), (2) parallel to and ≥200 m from an access road to reduce tortoise interaction with the road, (3) and ≥1.6 km from any powerline to reduce raven predation (Daly et al. 2019). We divided the area into three blocks with a 400 m buffer between blocks to reduce the chance of tortoises settling in a different block than they were assigned. The use of blocks allowed for easier access to track tortoises post-release and reduced the amount of disturbance within the release area. Within each block, we created a grid of 20 release points 50 m apart throughout. We randomly selected 30 of the 48 tortoises from each treatment group to be released and for a total of 60 tortoises. Within each of the 3 blocks, we released 10 combo tortoises and 10 indoor tortoises, assigning individuals to separate release points, making sure to alternate treatment types assigned to each block (Fig. 2.1). Prior to the release day, we selected a release refugium within 10 m of each release point in the form of a kangaroo rat (*Dipodomys* spp.) burrow beneath a large perennial shrub and enlarged the burrow using a hand trowel to provide shelter to the released tortoise. We measured the selected tortoises and fitted them with an Advanced Telemetry Systems radio-transmitter (model R1670, 3.1 g or model R1680, 3.6 g) on the fifth vertebral scute with gel epoxy (Devcon 5-minute epoxy gel, ITW Engineered Polymers, County Clare, Ireland). On 1 October 2020 we released all tortoises.

2.5 Post-release monitoring

Using a 3-element Yagi antenna (AF Antronics, Inc., Urbana, IL) and a R1000 receiver (Communications Specialists, Inc., Orange, CA), we radio-tracked head-started tortoises 24-hours after release, twice a week for the first three weeks, then once per week until winter dormancy (fall 2020). After all tortoises ceased moving for the winter and entered winter dormancy in fall 2020, we reduced tracking to once every 10-14 days. In March 2021, when tortoises started emerging from winter dormancy, we returned to tracking weekly. We radio-tracked until December 2021, for a total of 15 months of monitoring. At each tracking event, we collected data on the tortoise's location to the nearest 3 m using a handheld GPS unit (Garmin GPSMAP 76CSx, Olathe, KS), cover usage (burrow/pallet, surface hidden, surface open), vegetation cover species, behavior, approximate distance from last tracking location, and time of day. When a mortality event occurred, we photographed the tortoise remains and attempted to determine cause (mammalian or avian predator, exposure).

2.6 Data Analysis

2.6.1 Settling behaviors

We characterized settling behavior using three metrics: timing of first burrow establishment, winter dormancy initiation, and spring emergence. Although we provided each tortoise with a burrow at release, we considered the milestone of a tortoise constructing its own first burrow as an early indication of the tortoise settling into its new environment. Burrows provide shelter from predators and temperature extremes, thus are likely to influence tortoise survival (Bulova 2002, McGovern et al. 2021, Tuberville et al. 2021). We also identified the date tortoises initiated winter dormancy once they remained in the same burrow for two weeks in the fall. Initiation of winter dormancy is another indication of settlement behavior as tortoises generally cease movement on the surface for several months, although they may emerge from their burrows to bask on warm winter days (Nagy and Medica 1986). Finally, we defined the spring emergence for each individual as the date on which the tortoise was documented to have moved at least one m from its winter dormancy burrow, even if only to bask and return to the same burrow. Spring emergence was an important behavior for gauging tortoises' sensitivity to changing environmental conditions. While in captivity, only combo tortoises had the opportunity to create their own burrows and be exposed to natural environmental cues, thus the timing of post-release settling behaviors might differ between combo and indoor tortoises. For each settling metric (i.e., response variable), we tested for a difference in the variance in days to a behavior between treatment groups using the Levene's test (Carroll and Schneider 1985). We used generalized linear models (GLM) to quantify how husbandry treatment affected settling behavior. Each response variable was measured in terms of days since release. Because each metric was constrained to be positive, real values, we used negative binomial models, with husbandry treatment as a categorical predictor variable, release MCL as a continuous predictor variable, and the number of days to each behavior as the response variables. When examining the number of days to dormancy initiation, we also included the number of days until their first burrow as an added continuous predictor to determine how the timing of the behaviors influenced the next behavior. Likewise, when examining the days to spring emergence we included the number of days until initiating dormancy as a continuous predictor. We constructed various candidate model sets for each behavior, including single parameter models, additive models, and one model with an interaction.

2.6.2 Movement

To evaluate home range sizes, we calculated the 95% and 50% Brownian bridge movement models (BBMM; Horne et al. 2007) for each tortoise using their locations during the full active season of 2021 (~April – November). We excluded data prior to their final settling metric (i.e., spring emergence) to avoid inflated home range estimates associated with exploratory behavior immediately following release. We included in the home range estimates one location associated with their first winter dormancy burrow from which they emerged in spring 2021, one location associated with their second winter dormancy burrow at the end of the 2021 active season (if applicable), and all the interim locations. We included the winter dormancy locations in the home range analysis due to the importance of dormancy burrows for tortoise survival and due to the extensive amount of time (~4.5 months) tortoises spend in them (Nagy and Medica 1986, Daly et al. 2019). We chose the BBMM approach because four individuals shifted the location of their home ranges mid-season, and the BBMM prevents home ranges from being overestimated when those large movements occur. The BBMM does this by assuming that locations are not independent and accounts for temporal autocorrelation, unlike the standard kernel density estimator (KDE), to create a home range based on the path an animal took while excluding the areas it likely did not use along the way(Horne et al. 2007). The BBMM has also been recommended for use in reptile studies using VHF tracking because it works well with tracking data and provides estimates with less error than KDE or minimum convex polygon approaches (Silva et al. 2020). Only individuals with >10 locations were used in this analysis, which excluded two combo tortoises that were predated within 15-49 days of their spring emergence. After calculating the 50%

and 95% BBMMs, we tested for a difference in the variances of home range sizes between treatments using the Levene's test (Carroll and Schneider 1985). We then compared home range size within 50% and 95% BBMMs using a gamma generalized linear model (GLM) with husbandry treatment and release MCL as predictor variables and home range size (in ha) as the response variables. Calculations and analyses were performed using the packages *adehabitatHR* and *adehabitatLR* (Calenge 2018) in Program R (R version 4.0.2, www.r-project.org). The candidate model set for each BBMM category included single parameter models, one additive model, and one model with an interaction.

We assessed long-term site fidelity of released head-starts by using ArcGIS and the tracking data to calculate Euclidian dispersal distances (straight-line distance between two points) for the following four dispersal periods: 1) pre-dormancy (release location to first dormancy location); 2) post-dormancy (first dormancy location to location at the end of the study); 3) total dispersal for tortoises that died or went missing (release location to last known alive location); and 4) total dispersal for the tortoises that survived (release location to location at end of study). When tortoises were found predated, we used their last known alive location to calculate their total dispersal instead of where they were found dead to avoid the potential bias due to predators carrying a tortoise to a farther distance. For each dispersal period, we used the Levene's test to assess the difference in variances between treatments. We used gamma GLMs (McGovern et al. 2020a) to compare the distances within each dispersal period, with the husbandry treatment and release MCL as the predictor variables and distance (m) as the response variables. For each dispersal period, we created a candidate model set with single parameter models and one additive model.

We also calculated daily movement using the average daily step-length for each tortoise for two time periods: four weeks post-release and eight weeks post-emergence in the spring. We chose these time periods because within approximately four weeks after release at least 40% of tortoises had initiated winter dormancy and within eight weeks following spring emergence the majority of tortoises (71.7%) had slowed their movements as part of summer estivation. We calculated daily average step length as distance (m) from the last observation divided by the number of days since last observation for every tracking event within both time periods. Therefore, every tortoise had a step-length estimated for each date within both time periods. We also created a binomial response for each step-length that assigned a value of 1 if the tortoise had moved on that date and 0 if not. Then we assigned the step-length estimates to the week after release or after emergence they occurred to assess the effect of time. We tested the variances of step-lengths between treatments using the Levene's test (Carroll and Schneider 1985). Within each time period, we compared the step-lengths of each treatment group using a zero-inflated gamma hurdle model. A zero-inflated gamma hurdle model uses a binomial model to quantify the probability of a tortoise moving (1 if tortoise moved, 0 if not) and a gamma model to compare the step-lengths of tortoises that did move. For both sub-models, we included husbandry treatment, body size, and week after event as predictor variables. For the binomial model, the response variable was whether a tortoise moved, and for the gamma model the response variable was the step-lengths (in meters). We created candidate model sets for each time period that included single parameter and additive models, and one model with an interaction.

For the each of the model sets for the settling behavior and movement metrics, we selected the best-fitting model using the Akaike Information Criterion (AIC) method (Burnham and Anderson 2004). We performed all calculations and analyses for the model sets using the statistical package *lme4* (Bates et al. 2017) in Program R.

2.6.3 Survival

We used Kaplan-Meier survivorship curves to determine the overall survival rate over the 15-month monitoring period for all head-starts released and to compare the survival rates between treatment groups. Because some tortoises went missing during the study, we used right censoring to account for their unknown fates. We also used a Cox proportional hazard model (Cox 1972) to examine the effect of husbandry treatment and release MCL on the survival of head-starts. All calculations and analyses for the model sets were performed using the statistical package *survminer* (Kassambara et al. 2021) in Program R.

RESULTS

All of the following averages are reported as the mean ± 1 standard deviation. At the time of release (1 Oct 2020), the mean release MCL (hereafter “body size”) of combo tortoises (2018 cohort; n=30) was 110.8 ± 8.7 mm (range: 94 - 126.9 mm) and that of indoor tortoises (2019 cohort; n=30) was 102.6 ± 8.9 mm (range: 90.4 - 128.8 mm). Combo tortoises were significantly larger than indoor tortoises at release (p= 0.002).

3.1 Settling Behaviors

Tortoises took an average of 7.0 ± 4.4 d (range 1-19 d) post-release to build their first burrow, 27.4 ± 13.5 d (range 5-53 d) to initiate dormancy, and 191.8 ± 28.5 d (range 80-249 d) since release to emerge in the spring (Fig. 2.2; Table 2.1). These averages corresponded to the dates of 8 Oct 2020 for building a burrow, 28 Oct 2020 for initiating dormancy, and 10 April 2021 for spring emergence. The average dormancy duration was 164.4 days. The variances of settling behaviors were not significantly different between treatments (p>0.05 for all). The ranked AIC weights of the model sets for each behavior showed high model uncertainty (Table 2.2), and the null model was the top model for both days to first burrow and days to spring emergence. The top model for days to initiating dormancy included treatment type and days to building their first burrow. Indoor tortoises tended to initiate dormancy later than combo tortoises (β=0.3, 95% CI: 0.1, 0.5), and as the number of days to build a first burrow increased, the number of days until tortoises initiated dormancy tended to increase (β=0.02, 95% CI: -0.02, 0.1).

3.2 Movement

We estimated the 50% and 95% BBMM home ranges of 58 tortoises (30 indoor, 28 combo; Fig. 2.3). For the 50% BBMMs, combo tortoises' average home range size was 0.2 ± 0.1 (range 0 – 0.5) ha and indoor tortoises' was 0.4 ± 0.2 (range 0.1 – 0.9) ha. For the 95% BBMMs, combo tortoises had an average home range size of 2.8 ± 0.4 (range 2.2 – 4) ha and indoor tortoises had an average of 3.7 ± 1.5 (range 2.3 – 8) ha. On average, the indoor tortoises had larger home ranges than the combo tortoises (Fig. 2.3; Table 2.1). There was a significant difference in the variance of both 50% and 95% BBMM home ranges between treatments (p=0.04, p=0.01 respectively). The four individuals that shifted their home ranges mid-active season were all indoor tortoises. The top model for 50% BBMMs included treatment, with indoor tortoises tending to have larger home ranges (β= 0.5, 95% CI: 0.2, 0.9; Table 2.3). The top model for 95% BBMMs included the interaction of treatment and body size, where increasing body size was associated with decreasing home range size (β= -0.004, 95% CI: -0.02, 0.02), indoor tortoises tended to have larger home ranges (β= 1.7, 95% CI: -0.4, 3.9), and the interaction revealed a slightly greater negative effect of body size on home range for indoor tortoises compared to outdoor tortoises (β= -0.02, 95% CI: -0.05, 0.01; Table 2.3). There was high model uncertainty for both model sets (Table 2.3).

On average, indoor tortoises moved about twice the distance that combo tortoises did in all four dispersal categories (Fig. 2.4; Table 2.2). The farthest dispersal made by an indoor tortoise was 598.1 m from its release site, and the farthest a combo tortoise dispersed was 203.2 m from its release site. The variance was significantly different between treatments for the post-dormancy dispersal (p= 0.01) and for the total dispersal for the deceased and missing tortoises, with greater variation exhibited by indoor tortoises. The variance was not significantly different between treatments for the pre-dormancy dispersal or total dispersal for surviving tortoises (P>0.05 for both). The top model for all dispersal distances included treatment as the only predictor, with dispersal being greater for indoor tortoises than combo tortoises in all four distances (all β>0.3; Table 2.4).

We calculated the daily average step length for all 60 tortoises for four weeks post-release and eight weeks post-emergence to assess their daily movement. The average daily step-length of indoor tortoises 4-weeks post-release was 14.5 ± 13.4 (range 0.6 –

74) m, while that of combo tortoises was 12.6 ± 11.2 (range 0.5 – 95) m. Eight weeks post-emergence, the average daily step-length of indoor tortoises was 4.3 ± 4.9 (range: 0.1 – 26.3) m and of combo tortoises was 2.8 ± 2.3 (range 0.1 – 15.3) m. The variances in step-lengths were significantly different between treatments for both time periods ($P<0.05$). The best-fit models for comparing post-release step-lengths included the interaction of treatment and week for the binomial model (i.e., probability of movement; Table 2.5a), and the predictors of treatment and week without an interaction for the gamma GLM model (i.e., distance moved; Table 2.5b). After release, the probability of movement for all tortoises decreased over time ($\beta= -1.5$, 95% CI: -1.7, -1.2), and while indoor tortoises initially had a lower probability of movement than combo tortoises ($\beta= -2.2$, 95% CI: -3.3, -1.1), by the end of the four week period indoor tortoises had a greater probability of moving than combo tortoises ($\beta=0.9$, 95% CI: 0.6, 1.2; Fig. 2.5a). The daily step-lengths of tortoises after release decreased over time ($\beta=-0.1$, 95% CI: -0.2, -0.1), and indoor tortoises tended to have slightly larger daily step-lengths overall ($\beta=0.1$, 95% CI: -0.2,0.4; Fig. 2.5c).

The best fit models for post-emergence movement included the interaction of treatment and week for both the binomial and gamma GLM models (Table 2.6). After emergence, the general trend was that probability of movement decreased over time ($\beta= -0.1$, 95% CI: -0.2, -0.04), and indoor tortoises initially started with a lower probability of movement than combo tortoises ($\beta=-0.5$, 95% CI: -1.5,0.5). By the end of the eight-week period, indoor tortoises tended to have a higher probability of movement ($\beta=0.1$, 95% CI: 0.03, 0.2; Fig. 2.5b). The daily step-lengths of tortoises after emergence generally increased over time ($\beta=0.1$, 95% CI: 0.01,0.1), indoor tortoises initially had larger daily step-lengths than combo tortoises (β=0.6, 95% CI: 0.1,1.0), but at the end of the eight-week time period, combo tortoises had larger daily step-lengths than indoor tortoises (β= -0.2, 95% CI: -0.2, -0.1; Fig. 2.5d).

3.3 Survival

Of the 60 head-started tortoises, all survived through their first dormancy (~Nov 2020-April 2021) and 25 (41.7%) were known to have survived to their second dormancy and through the end of the study (Dec 2021). Thirty (50%) tortoises died (29 from predation, 1 possibly from exposure) and 5 (8.3%) tortoises went missing and were censored during the 2021 active season. By the end of the 15-month post-release monitoring period, overall survival was 47% (95% CI: 0.4, 0.6). When evaluating survival by treatment, combo tortoises had a survival rate of 42% (95% CI: 0.3, 0.7) and indoor tortoises had a survival rate of 51% (95% CI: 0.4, 0.7; Fig. 2.6). There was not a significant difference in the survival of tortoises by treatment (p=0.92) and there appeared to be a trend of tortoises with a larger release MCL being more likely to survive, but the trend was not statistically significant (p=0.07).

DISCUSSION

4.1 Overview

While combo tortoises tended to initiate dormancy earlier than indoor tortoises, there was not a meaningful difference in the timing of settling behaviors between treatments. Indoor tortoises demonstrated greater variability in most movement metrics than combo tortoises, suggesting that indoor tortoises are less predictable in their movements than combo tortoises. Indoor tortoise movement was still within the range of previously observed movements for desert tortoises (McGovern et al. 2020a). Despite the variability in movement between the two groups, survival did not differ between our treatments. The overall survival rate for tortoises in our study was lower than the first-year survival rate of other recent releases to our study site (McGovern et al. 2020a, Candal 2021). However, tortoises from those releases also experienced unusually high mortality in the active season of 2021.

4.2 Settlement

The timing of settling behaviors was not strongly influenced by husbandry treatment, except possibly in the initiation of dormancy. However, because all tortoises still initiated dormancy within the normal timeframe (~Oct-Nov), the difference between the treatment groups is not biologically meaningful. Therefore, our predictions that combo tortoises would exhibit settling behaviors earlier than indoor tortoises were not supported. During spring emergence, we also observed a number of tortoises, including tortoises from previous releases and not in this study, that emerged much later than normal, the latest being in early June. We speculate that the lack of late winter and early spring rains in 2021 kept some tortoises from emerging and becoming fully active again within the normal time period.

4.3 Movement

From our results, the effect of husbandry treatment on tortoise home range size appears to be weak, therefore our prediction that combo tortoises would have smaller home ranges than indoor tortoises was not supported. The prediction that combo tortoises would have higher site fidelity was supported, because combo tortoises tended to stay closer to their release sites than indoor tortoises, and indoor tortoises showed greater variability in most of the movement metrics than did combo tortoises. Although treatment type influenced the tortoises' dispersal and site fidelity, both treatment groups still demonstrated high site fidelity overall.

A common goal for turtle translocations or releases is having individuals demonstrate high site fidelity. Animals released as part of a head-starting or translocation program often have a period of exploratory movement before settling into the environment again (Rittenhouse et al. 2007, Russell et al. 2010, Hazard et al. 2015, Rueda et al. 2021, Smetzer et al. 2021). For turtles, this exploratory period is usually associated with increased surface activity, which in turn can leave individuals vulnerable to predation or other sources of mortality (Hazard and Morafka 2002, Rittenhouse et al. 2007). Dispersal away from the release site is also the leading cause of reptile translocation and release failure (Germano and Bishop 2009). The results of our study can help inform desert tortoise head-starting projects on the best ways to maximize site fidelity post-release.

4.3 Survival

While indoor tortoises tended to move more often and farther than combo tortoises, this difference in movement did not result in differences in survival between indoor and combo treatment groups. Body size did not have a statistically significant effect on survival, but there was a biologically meaningful trend suggesting body size is still an important factor. Other studies that did find an effect of body size on survival probability (Nagy et al. 2015a, McGovern et al. 2020b) saw an increase in survival probability until the recommended release size of ~100 mm MCL (Nagy et al. 2015a), when the probability started to plateau. There was likely not a statistically strong effect of body size detected in our analysis because all tortoises had an MCL of ≥80 mm and were close to that recommended release size.

The survival rate for both treatment groups (42% for combo tortoises, 51% for indoor tortoises) was lower than the rates of previous releases in the study site (96% for McGovern et al. 2020a, 79.3% for Candal 2021). We suspect that the differences in survival between studies stem from differences in climatic conditions tortoises experienced following release. At the time of release (October 2020), the Palmer Drought Severity Index (PDSI; measures relative severity of drought conditions over time, value of 0 is average conditions) was -1.9 indicating a period of drought was occurring. The PDSI continued to be negative for the rest of the study period (through Dec 2021) and reached a low of -3.9, which is the lowest PDSI value reported for the study site in the last 10 years (gridMET 2022). Drought conditions like these tend to reduce the amount of available forage and free-standing drinking water—both of which tortoises rely on (Peterson 1996). Drought has also been associated with reduced tortoise survival and movements (Peterson 1994, Duda et al. 1999, Longshore et al. 2003).

In addition, we observed an increase in tortoise predation from mesopredators, such as coyotes, relative to previous releases at the study site. When small mammal populations in the desert are low, which can happen during prolonged drought conditions (Beatley 1969), coyotes have been observed to use desert tortoises as a prey source more frequently, thus reducing local tortoise populations (Peterson 1994, Esque et al. 2010, Kelly et al. 2021). Also, when desert rodent populations are low, their burrows become less abundant and juvenile tortoises, which rely on small mammal burrows for refugia, can be impacted negatively (Todd et al. 2016). All of these factors likely contributed to the survival rate during the study being lower than past release years in the same study site (McGovern et al. 2020b, Candal 2021), with both husbandry treatment groups being equally affected. Further investigation into the effects of climate on head-started tortoises will give us valuable insight into post-release survival.

4.4 Enrichment from outdoor rearing

One major challenge of captive-rearing for reintroduction is ensuring animals are physically and behaviorally equipped for the wild. Animals reared in captivity may be unable to perform the natural, learned behaviors needed to survive in the wild if not given the opportunity to learn before being released (Einum and Fleming 2001, Mathews et al. 2005). One method employed by captive-rearing practitioners to equip animals with wild behaviors is to provide them with enrichment, such as natural habitat features in enclosures, anti-predator training, or foraging training (Reading et al. 2013, Tetzlaff et al. 2019a). In a literature review of captive-rearing and translocations, Tetzlaff et al. (2019a) found that animals released from captivity were more likely to survive if they had been given some type of enrichment before being released. In our study, the outdoor rearing component of combo-rearing could be considered enrichment because tortoises are provided with opportunities to build burrows, respond to weather conditions, forage on native plants, and experience winter dormancy before being released into the wild. From our study results, it appears that for the first year post-release, an outdoor-rearing component is not strictly necessary for tortoise survival, but it does provide valuable experiences to head-started desert tortoises that increases site fidelity and reduces variability in their movements. Future investigation into the influence of drought on tortoise movements would allow us to determine if the climate conditions in 2021 masked an effect of treatment on tortoise survival.

4.5 Management Implications

The essential first step in any conservation project is considering the specific goals, and head-starting programs are no exception. If the goal of a Mojave Desert tortoise head-starting program is to produce tortoises that will exhibit high probability of survival following release, our study suggests that indoor- or combo-rearing are likely to be equally successful, as there was no measurable influence of husbandry on survival. Indoor-rearing (without a subsequent year of outdoor rearing) is the most cost- and time-efficient means of producing tortoises and requires less infrastructure and resourses than combo-rearing. If, however, managers wish to release tortoises with more predictable home range sizes and higher site fidelity, our results demonstrate that combo-reared tortoises exhibit less among-individual variation in their movement and settling behavior. We also recommend that when severe drought conditions are occurring or predicted to occur, managers consider not releasing tortoises, to avoid the high rate of predation from mesocarnivores that we observed during the study. Ultimately, management decisions have to be made by weighing the benefits, risks, and costs of every rearing option.

LITERATURE CITED

Allison, L. J., and A. M. McLuckie. 2018. Population trends in Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **13**:433-452.

Applied Climate Information System. 2022. NOWData - NOAA Online Weather Data. Weather.gov.

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, G. Grothendieck, and P. Green. 2017. Package 'lme4'. CRAN.

Beatley, J. C. 1969. Dependence of desert rodents on winter annuals and precipitation. Ecology **50**:721-724.

Berry, K. H., L. J. Allison, A. M. McLuckie, M. Vaughn, and R. W. Murphy. 2021. *Gopherus agassizii*. The IUCN Red List of Threatened Species: e.T97246272A3150871.

Berry, K. H., and P. A. Medica. 1995. Desert tortoises in the Mojave and Colorado deserts. Pages 135-137 *in* E. T. LaRoe, G. S. Farris, C. E. Puckett, P. D. Doran, and M. J. Mac, editors. Our living resources: A report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosystems. National Biological Service.

Brooks, M. L. 1999. Does protection of desert tortoise habitat generate other ecological benefits in the Mojave Desert? Pages 68-73 *in* Wilderness Science in a Time of Change Conference. Us Dept Agr, Forest Serv Rocky Mt Forest & Range Exptl Stn, Missoula, Mt.

Bulova, S. J. 2002. How temperature, humidity, and burrow selection afect evaporative water loss in desert tortoises. Journal of Thermal Biology **27**:175–189.

Burke, R. L. 2015. Head-starting turtles: learning from experience. Herpetological Conservation and Biology **10**:299-308.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel Inference: Understanding AIC and BIC in model selection. Sociological Methods & Research **33**:261-304.

Calenge, C. 2018. Package ‘adehabitat’. CRAN.

Candal, C. M. 2021. Pressure to perform: The role of stress Physiology in head-starting success for Mojave Desert tortoises. University of Georgia, Athens, GA.

Carroll, R. J., and H. Schneider. 1985. A note on Levene's tests for equality of variances. Statistics & Probability Letters **3**:191-194.

Cowie, R. H., P. Bouchet, and B. Fontaine. 2022. The Sixth Mass Extinction: fact, fiction or speculation? Biological Reviews **97**:640-663.

Cox, D. R. 1972. Regression models and life-tables. Journal of the Royal Statistical Society: Series B (Methodological) **34**:187-202.

Daly, J. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, and T. D. Tuberville. 2019. Survival and movements of head-started Mojave desert tortoises. Journal of Wildlife Management **83**:1700-1710.

Duda, J. J., A. J. Krzysik, and J. E. Freilich. 1999. Effects of drought on desert tortoise movement and activity. The Journal of Wildlife Management **63**:1181.

Einum, S., and I. Fleming. 2001. Implications of stocking: Ecological interactions between wild and released Salmonids. Nordic Journal of Freshwater Research **75**:56-70.

Esque, T. C., K. E. Nussear, K. K. Drake, A. D. Walde, K. H. Berry, R. C. Averill-Murray, A. P. Woodman, W. I. Boarman, P. A. Medica, J. Mack, and J. S.

Heaton. 2010. Effects of subsidized predators, resource variability, and human population density on desert tortoise populations in the Mojave Desert, USA. Endangered Species Research **12**:167-177.

Germano, J. M., and P. J. Bishop. 2009. Suitability of amphibians and reptiles for translocation. Conservation Biology **23**:7-15.

Gibbons, J. W., and J. L. Greene. 1979. X-Ray photography: a technique to determine reproductive patterns of freshwater turtles. Herpetologica **35**:86-89.

gridMET. 2022. Drought - PDSI.*in* Climate Engine, editor. Google Earth Engine.

Hazard, L. C., and D. J. Morafka. 2002. Comparative dispersion of neonate and headstarted juvenile desert tortoises (*Gopherus agassizii*): A preliminary assessment of age effects. Chelonian Conservation and Biology **4**:406-409.

Hazard, L. C., D. J. Morafka, and S. Hillard. 2015. Post-release dispersal and predation of head-started juvenile desert tortoises (*Gopherus agassizii*): Effect of release site distance on homing behavior. Herpetological Conservation and Biology **10**:504-+.

Horne, J. S., Edward O. Garton, Stephen M. Krone, and J. S. Lewis. 2007. Analzing animal movements using Brownian bridges. Ecology **88**:2354–2363.

Kassambara, A., M. Kosinski, P. Biecek, and S. Fabian. 2021. Package ‘survminer’. CRAN.

Kelly, E. C., B. L. Cypher, and T. L. Westall. 2021. Predation on desert tortoises (*Gopherus agassizii*) by desert canids. Journal of Arid Environments **189**:4.

Kohn, H. 2018. The desert's canary: A narrative examination of the socio-political role of the threatened desert tortoise. Humboldt State University.

Kristan III, W. B., and W. I. Boarman. 2003. Spatial pattern of risk of common raven predation on desert tortoises. Ecology **84**:2432-2443.

Longshore, K. M., J. R. Jaeger, and J. M. Sappington. 2003. Desert tortoise (*Gopherus agassizii*) survival at two eastern Mojave Desert sites: Death by short-term drought? Journal of Herpetology **37**:169–177.

Lovich, J. E. 2011. *Gopherus agassizii* (Desert Tortoise) and *Crotalus ruber* (Red Diamond Rattlesnake). Burrow cooccupancy. . Herpetological Review **42**:421.

Lovich, J. E., J. R. Ennen, M. Agha, and J. W. Gibbons. 2018. Where have all the turtles gone, and why does it matter? BioScience **68**:771–781.

Lovich, J. E., C. B. Yackulic, J. Freilich, M. Agha, M. Austin, K. P. Meyer, T. R. Arundel, J. Hansen, M. S. Vamstad, and S. A. Root. 2014. Climatic variation and tortoise survival: Has a desert species met its match? Biological Conservation **169**:214-224.

Mathews, F., M. Orros, G. McLaren, M. Gelling, and R. Foster. 2005. Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation **121**:569–577.

McCallum, M. L. 2015. Vertebrate biodiversity losses point to a sixth mass extinction. Biodiversity and Conservation **24**:2497-2519.

McGovern, P. A. 2019. Changing the survival formula for the Mojave Desert tortoise (*Gopherus agassizii*) through head-starting. University of Georgia.

McGovern, P. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, J. Hepinstall-Cymerman, J. A. Daly, and T. D. Tuberville. 2020a. Comparing husbandry techniques for optimal head-starting of the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **15**:626-641.

McGovern, P. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, J. Hepinstall-Cymerman, J. A. Daly, and T. D. Tuberville. 2020b. The effect of size on postrelease survival of head-started Mojave Desert tortoises. Journal of Fish and Wildlife Management **11**:494-506.

McGovern, P. A., J. M. Peaden, K. A. Buhlmann, B. D. Todd, and T. D. Tuberville. 2021. Comparing post-release cover and burrow use by differentially head-started Mojave desert tortoises in southern California. Conservation Evidence Journal **18**:37-43.

McWilliams, D. A. 2005. Nutrition research on calcium homeostasis in lizards (with recommendations). International Zoo Yearbook **39**:69-77.

Morris, R. L., D. A. Devitt, A. M. Crities, G. Borden, and L. N. Allen. 1997. Ubanization and water conservation in Las Vegas Valley, Nevada. Journal of Water Resources Planning and Management **May/June**.

Nafus, M. G., B. D. Todd, K. A. Buhlmann, and T. D. Tuberville. 2015. Consequences of maternal effects on offspring size, growth and survival in the desert tortoise. Journal of Zoology **297**:108-114.

Nagy, K. A., L. S. Hillard, M. W. Tuma, and D. J. Morafka. 2015. Head-started desert tortoises (*Gopherus agassizii*): movements, survivorship and mortality causes following their release. Herpetological Conservation and Biology **10**:203-215.

Nagy, K. A., and P. A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. Herpetologica **42**:73-92.

Parker, S. S., B. S. Cohen, and J. Moore. 2018. Impact of solar and wind development on conservation values in the Mojave Desert. PLOS ONE **13**:e0207678.

Peterson, C. C. 1994. Different rates and causes of high mortality in two populations of the threatened desert trotoise *Gopherus agassizii*. Biological Conservation **70**:101-108.

Peterson, C. C. 1996. Ecological energetics of the desert tortoise (*Gopherus agassizii*): Effects of rainfall and drought. Ecology **77**:1831-1844.

Pievani, T. 2014. The sixth mass extinction: Anthropocene and the human impact on biodiversity. Rendiconti Lincei **25**:85-93.

Pimm, S. L., C. N. Jenkins, R. Abell, T. M. Brooks, J. L. Gittleman, L. N. Joppa, P. H. Raven, C. M. Roberts, and J. O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science **344**:987–997.

Reading, R. P., B. Miller, and D. Shepherdson. 2013. The value of enrichment to reintroduction success. Zoo Biology **32**:332–341.

Rittenhouse, C. D., J. J. Millspaugh, M. W. Hubbard, and S. L. Sheriff. 2007. Movements of translocated and resident three-toed box turtles. Journal of Herpetology **41**:115–121.

Rueda, C., J. Jiménez, M. J. Palacios, and A. Margalida. 2021. Exploratory and territorial behavior in a reintroduced population of Iberian lynx. Scientific Reports **11**.

Russell, J. C., A. J. C. McMorland, and J. W. B. MacKay. 2010. Exploratory behaviour of colonizing rats in novel environments. Animal Behaviour **79**:159-164.

Silva, I., M. Crane, B. M. Marshall, and C. T. Strine. 2020. Reptiles on the wrong track? Moving beyond traditional estimators with dynamic Brownian Bridge Movement Models. Movement Ecology **8**.

Smetzer, J. R., A. L. Greggor, K. L. Paxton, B. Masuda, and E. H. Paxton. 2021. Automated telemetry reveals post-reintroduction exploratory behavior and movement patterns of an endangered corvid, Alal (*Corvus hawaiiensis*) in Hawai'i, USA. Global Ecology and Conservation **26**:11.

Stanford, C. B., J. B. Iverson, A. G. J. Rhodin, P. P. v. Dijk, R. A. Mittermeier, G. Kuchling, K. H. Berry, A. Bertolero, K. A. Bjorndal, T. E. G. Blanck, K. A. Buhlmann, R. L. Burke, J. D. Congdon, T. Diagne, T. Edwards, C. C. Eisemberg, J. R. Ennen, G. Forero-Medina, M. Frankel, U. Fritz, N. Gallego-García, A. Georges, J. W. Gibbons, S. Gong, E. V. Goode, H. T. Shi, H. Hoang, M. D. Hofmeyr, B. D. Horne, R. Hudson, J. O. Juvik, R. A. Kiester, P. Koval, M. Le, P. V. Lindeman, J. E. Lovich, L. Luiselli, T. E. M. McCormack, G. A. Meyer, V. P. Páez, K. Platt, S. G. Platt, P. C. H. Pritchard, H. R. Quinn, W. M. Roosenburg, J. A. Seminoff, H. B. Shaffer, R. Spencer, J. U. V. Dyke, R. C. Vogt, and A. D. Walde. 2020. Turtles and tortoises are in trouble. Current Biology **30**:R721–R735.

Tetzlaff, S. J., J. H. Sperry, and B. A. DeGregorio. 2019. Effects of antipredator training, environmental enrichment, and soft release on wildlife translocations: A review and meta-analysis. Biological Conservation **236**:324–331.

Todd, B. D., B. J. Halstead, L. P. Chiquoine, J. M. Peaden, K. A. Buhlmann, T. D. Tuberville, and M. G. Nafus. 2016. Habitat selection by juvenile Mojave Desert tortoises. The Journal of Wildlife Management **80**:720-728.

Tuberville, T. D., K. A. Buhlmann, R. Sollmann, M. G. Nafus, J. M. Peaden, J. A. Daly, and B. D. Todd. 2019. Effects of short-term, outdoor head-starting on growth and survival in the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **14**:171-184.

Tuberville, T. D., D. P. Quinn, and K. A. Buhlmann. 2021. Movement and survival to winter dormancy of fall-released hatchling and headstarted yearling gopher tortoises. Journal of Herpetology **55**:88–94.

U.S. Fish and Wildlife Service. 1973. Endangered Species Act of 1973.*in* United States of America, editor.

U.S. Fish and Wildlife Service. 2011. Revised recovery plan for the Mojave population of the desert tortoise (*Gopherus agassizii*).

Walde, A. D., and A. Currylow. 2015. *Gopherus agassizii* (Mojave Desert tortoise) and *Coleonyx variegatus variegatus* (desert banded gecko). Spring burrow cohabitation. Herpetology Notes, **8**:501-502.

Walde, A. D., A. M. Walde, and A. P. woodman. 2016. *Gopherus agassizii* (Mojave Desert Tortoise). Burrow associate. Herpetological Review **47**:122.

Walde, A. M., A. D. Walde, and C. Jones. 2014. *Gopherus agassizii* (Mohave Desert Tortoise) and *Crotalus mitchellii* (Speckled Rattlesnake). Burrow Associate. . Herpetological Review **45**:688.

Wiesner, C. S., and C. Iben. 2003. Influence of environmental humidity and dietary protein on pyramidal growth of carapaces in African spurred tortoises (*Geochelone sulcata*). Journal of Animal Physiology and Animal Nutrition **87**:66-74.

Williams, A. P., B. I. Cook, and J. E. Smerdon. 2022. Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. Nature Climate Change.

Williams, A. P., E. R. Cook, J. E. Smerdon, B. I. Cook, J. T. Abatzoglou, K. Bolles, S. H. Baek, A. M. Badger, and B. Livneh. 2020. Large contribution from anthropogenic warming to an emerging North American megadrought. Science **368**:314-+.

Woodbury, A. M., and R. Hardy. 1948. Studies of the desert tortoise, *Gopherus agassizii*. Ecological Monographs **18**:145-200.

TABLES

Table 2.1. Metrics for all settling and movement analyses of head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) by husbandry treatment type: Combo-rearing (“Combo”) and Indoor-rearing (“Indoor”) on Mojave National Preserve, California, USA. Settling behaviors represent the number of days since release in October 2020 when each settling behavior (building first burrow, initiating dormancy, and spring emergence) was performed by tortoises. Home range size is the calculated Brownian Bridge Movement Model (BBMM) home range area in ha. Dispersal distances represent the Euclidian distance tortoises dispersed (m) pre-dormancy (release point to first dormancy point), post-dormancy (first dormancy point to last point), total dispersal (release point to last point) for tortoises that went missing or died, and total dispersal for tortoises that survived the study. Step-lengths represent the daily average distance moved (m) for four weeks post-release and eight-weeks post-emergence when tortoises moved. For all metrics, n=30 unless otherwise noted.

Metric	Treatment	Mean	SD	SE	Min	Max
Settling Behaviors						
Building Burrow	Indoor	7.4	4.7	0.9	1	19
	Combo	6.7	4.1	0.8	1	15
	All (n=60)	7.0	4.4	0.6	1	19
Dormancy Initiation	Indoor	31.5	14	2.6	8	53
	Combo	23.3	11.7	2.1	5	53
	All tortoises	27.4	13.5	1.7	5	53
Spring Emergence	Indoor	193.9	32.4	5.9	80	249
	Combo	189.6	24.3	4.4	151	249
	All (n=60)	191.8	28.5	3.7	80	249
Home Range Size						
50% BBMM area (ha)	Indoor	0.4	0.2	0.0	0.1	0.9
	Combo (n=28)	0.2	0.1	0.0	0	0.5
95% BBMM area (ha)	Indoor	2.8	0.4	0.3	2.2	4
	Combo (n=28)	3.7	1.5	0.1	2.3	8
Dispersal Distances						
Pre-dormancy dispersal (m)	Indoor	85.0	76.1	13.9	4.3	360.4
	Combo	59.5	40.0	7.3	10.1	158.1
Post-dormancy dispersal (m)	Indoor	106.1	126.9	23.2	4.5	546.4
	Combo	46.8	48.2	8.8	1.41	197.0
Total dispersal for missing and dead (m)	Indoor	154.1	161.4	41.7	8.9	598.1
	Combo	73.3	48.2	10.5	5.9	152.5
Total dispersal for surviving (m)	Indoor	156.2	101.7	26.3	42.1	387.7
	Combo	77.3	61.7	20.6	15.5	203.2
Step-lengths						
Step-length post-release (m)	Indoor	14.5	13.4	0.6	0.6	74
	Combo	12.6	11.2	0.5	0.5	95
Step-length post-emergence (m)	Indoor	4.3	4.9	0.2	0.1	26.3
	Combo	2.8	2.3	0.1	0.1	15.3

Table 2.2. AIC rankings for model set comparisons of the number of days post-release head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) a) built their first burrow, b) initiated dormancy, and c) emerged in the spring.

Response variables and associated, ranked candidate models	AICc	Δ AIC	AICc Weight	AIC Cum. Weight
a. Days to first burrow				
Null	**345.16**		**0.46**	**0.46**
Body Size	346.85	1.69	0.20	0.66
Treatment	347.00	1.84	0.18	0.85
Treatment + Body Size	348.04	2.88	0.11	0.96
Treatment * Body Size	349.93	4.77	0.04	1.00
b. Days to dormancy				
Treatment + Burrow	**472.33**		**0.34**	**0.34**
Treatment	472.98	0.65	0.25	0.59
Treatment * Burrow	473.80	1.47	0.16	0.76
Treatment+Body Size+Burrow	474.65	2.32	0.11	0.86
Treatment + Body Size	475.10	2.77	0.09	0.95
Null	476.84	4.52	0.04	0.98
Body Size	478.55	6.23	0.02	1.00
c. Days to emergence				
Null	**580.00**		**0.38**	**0.38**
Treatment + Dormancy	581.73	1.73	0.16	0.55
Treatment	581.89	1.89	0.15	0.70
Body Size	582.00	2.00	0.14	0.84
Treatment * Dormancy	583.55	3.55	0.06	0.90
Treatment+Dormancy+Body Size	584.09	4.09	0.05	0.95
Treatment + Body Size	584.13	4.13	0.05	1.00

Table 2.3. AIC rankings for model set comparisons of head-started juvenile Mojave desert tortoise (*Gopherus agassizii*) home range sizes of two categories: a) area of 50% Brownian bridge movement model (BBMM), and b) area of 95% BBMM. Top models are in bold.

Response variables and associated, ranked candidate models	AICc	Δ AIC	AICc Weight	AIC Cum. Weight
a. 50% BBMM home range area				
Treatment	**-43.26**		**0.41**	**0.41**
Treatment * Body Size	-43.17	0.09	0.39	0.80
Treatment + Body Size	-42.50	1.76	0.17	0.97
Body Size	-38.02	5.24	0.03	0.99
Null	-34.48	8.78	0.01	1.00
b. 95% BBMM home range area				
Treatment * Body Size	**152.81**		**0.43**	**0.43**
Treatment + Body Size	153.03	0.22	0.39	0.82
Body Size	155.91	3.09	0.09	0.91
Treatment	156.02	3.21	0.09	1.00
Null	167.56	14.75	0.00	1.00

Table 2.4. AIC rankings for model set comparisons of head-started juvenile Mojave desert tortoises' (*Gopherus agassizii*) Euclidian dispersal distances over four dispersal periods: a) pre-dormancy (from the release point to first dormancy point), b) post-dormancy (from the first dormancy point to end of the study), c) total dispersal for the deceased and missing tortoises (n=35) (from the release point to their last known alive point), and d) total dispersal for the surviving tortoises (n=25) (from the release point to the end of the study). All top models are in bold.

Response variables and associated, ranked candidate models	AICc	Δ AIC	AICc Weight	AIC Cum. Weight
a. Pre-dormancy dispersal				
Treatment	**629.65**		**0.61**	**0.61**
Body Size	631.89	2.24	0.20	0.81
Treatment + Body Size	632.02	2.38	0.19	1.00
b. Post-dormancy dispersal				
Treatment	**637.18**		**0.73**	**0.73**
Treatment + Body Size	639.48	2.30	0.23	0.96
Body Size	642.94	5.76	0.04	1.00
c. Total dispersal for deceased				
Treatment	**408.66**		**0.59**	**0.59**
Treatment + Body Size	409.51	0.85	0.38	0.97
Body Size	414.46	5.80	0.03	1.00
d. Total dispersal for surviving				
Treatment	**277.23**		**0.63**	**0.63**
Treatment + Body Size	279.41	2.18	0.21	0.84
Body Size	279.96	2.73	0.16	1.00

Table 2.5. AIC rankings for model set comparisons of head-started juvenile Mojave desert tortoises' (*Gopherus agassizii*) movement four weeks post-release: a) probability of movement, and b) the step-length in m. Top models are in bold.

Response variables and associated, ranked candidate models	AICc	Δ AIC	AICc Weight	AIC Cum. Weight
a. Probability of movement post-release				
Treatment * Week	**1590.54**		**1**	**1**
Treatment + Week + Body Size	1628.46	37.92	0	1
Treatment + Week	1628.56	38.02	0	1
Null	1896.70	306.17	0	1
Body Size	1897.41	306.88	0	1
Treatment	1898.85	308.31	0	1
b. Step-length post-release				
Treatment + Week	**6274.07**		**0.52**	**0.52**
Treatment * Week	6275.09	1.02	0.31	0.83
Treatment + Week + Body Size	6276.30	2.23	0.17	1
Null	6300.00	25.94	0	1
Treatment	6302.13	28.06	0	1
Body Size	6302.25	28.18	0	1

Table 2.6. AIC rankings for model set comparisons of head-started juvenile Mojave desert tortoises' (*Gopherus agassizii*) movement eight-weeks post-release: a) probability of movement, and b) the step-length in m. Top models are in bold.

Response variables and associated, ranked candidate models	AICc	Δ AIC	AICc Weight	AIC Cum. Weight
a. Probability of movement post-emergence				
Treatment * Week	**3200.79**		**0.82**	**0.82**
Treatment + Week	3205.94	5.15	0.06	0.88
Null	3206.05	5.26	0.06	0.94
Body Size	3208.21	7.42	0.02	0.96
Treatment + Week + Body Size	3208.25	7.46	0.02	0.98
Treatment	3208.26	7.47	0.02	1
b. Step-length post-emergence				
Treatment * Week	**5274.51**		**1**	**1**
Null	5323.20	48.70	0	1
Body Size	5325.46	50.95	0	1
Treatment	5325.48	50.98	0	1
Treatment + Week	5327.12	52.62	0	1
Treatment + Week + Body Size	5329.57	55.06	0	1

FIGURES

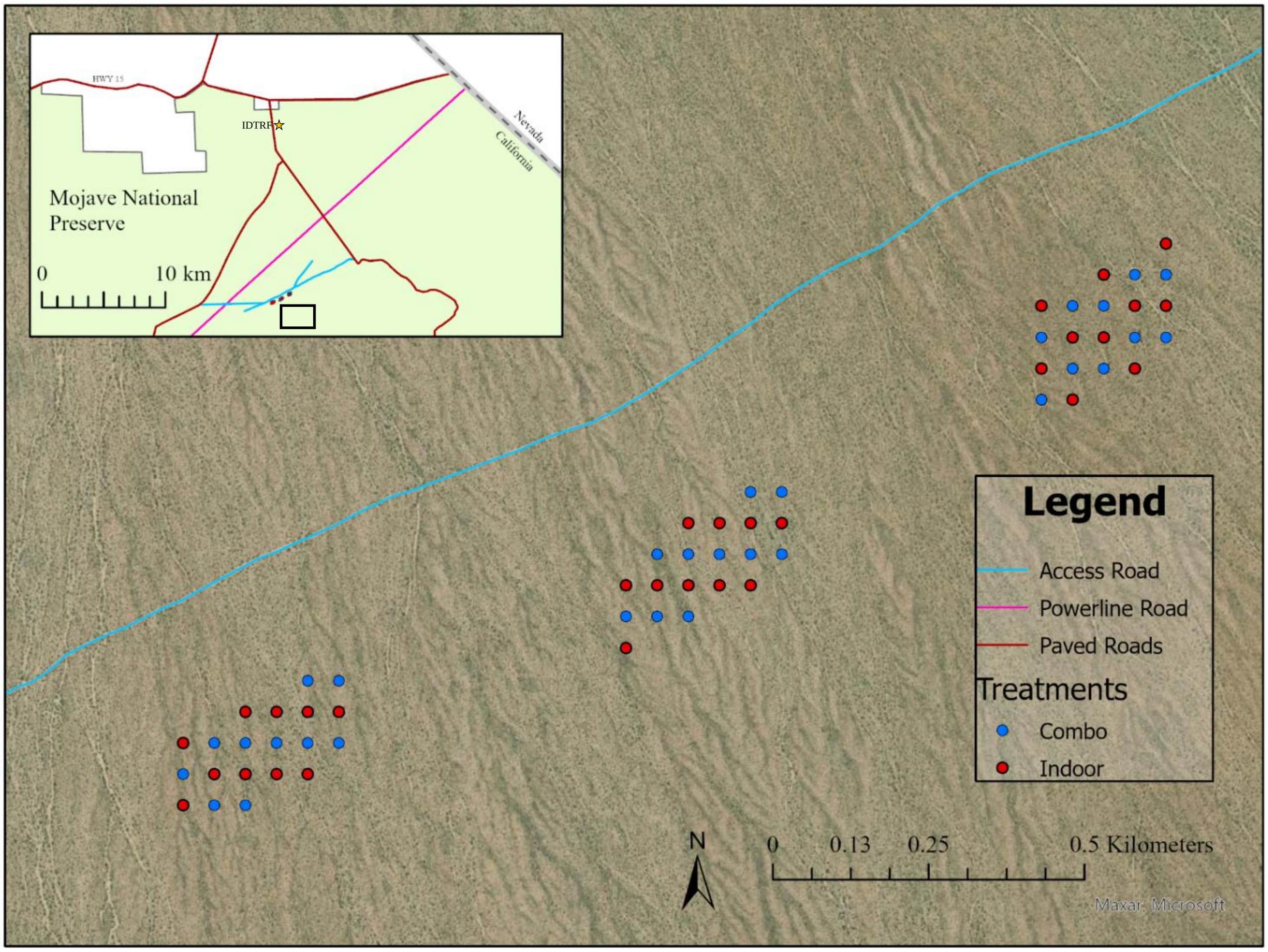

Fig. 2.1. Map of the release site and release assignments for head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) from two husbandry treatment groups: Combo-rearing ("Combo") and Indoor-rearing ("Indoor") in October 2020.

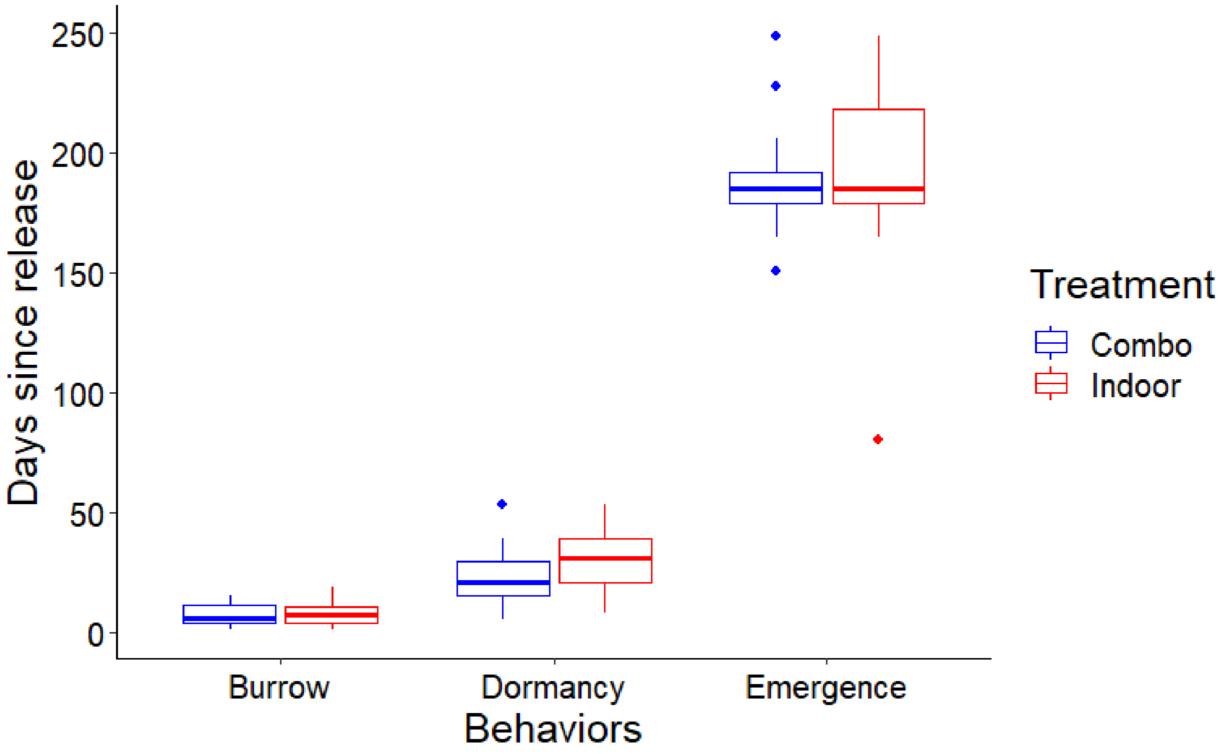

Fig. 2.2. Number of days since release in October 2020 when head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) performed each settling behavior (building first burrow, initiating dormancy, and spring emergence) by husbandry treatment type: Combo-rearing (“Combo”) and Indoor-rearing (“Indoor”). Boxplots show the mean number of days since release when each behavior was performed, the upper and lower quantiles, and outliers.

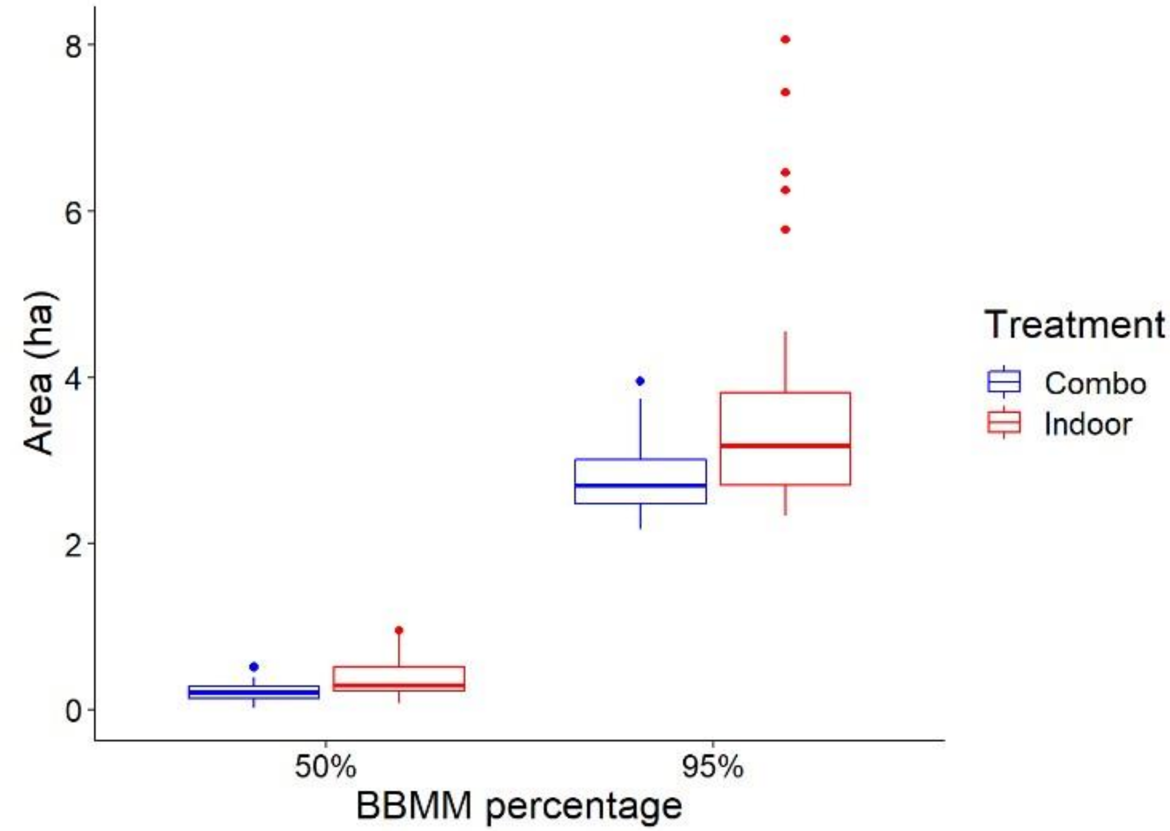

Fig. 2.3. Home range area (ha) for the 50% and 95% Brownian Bridge Movement models for head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) released in October 2020 by husbandry treatment type: Combo-rearing ("Combo") and Indoor-rearing ("Indoor"). Boxplots show the average home range size, upper and lower quantiles, and outliers.

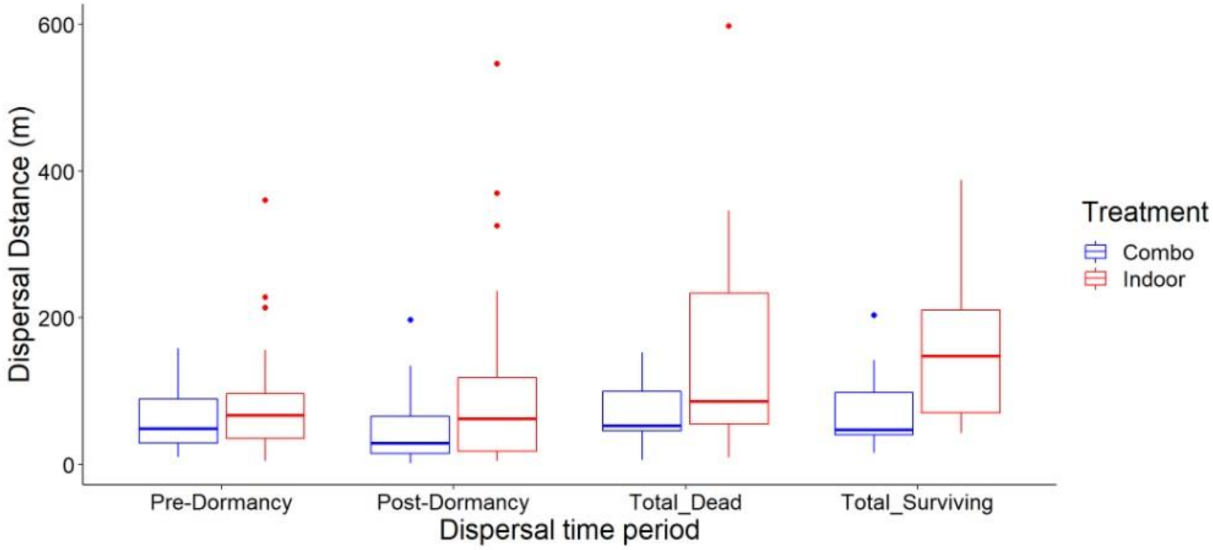

Fig. 2.4. Euclidian dispersal distances of head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) released in October 2020by husbandry treatment type: Combo-rearing ("Combo") and Indoor-rearing ("Indoor"). Pre-dormancy dispersal was calculated from the release point to first dormancy point (n=60), post-dormancy from the first dormancy point to the end of the study (n=60), total for the deceased and missing tortoises (n=35; Total_Dead) from the release point to their last known alive point, and total for the surviving tortoises (n=25; Total_Surviving) from the release point to the end of the study.

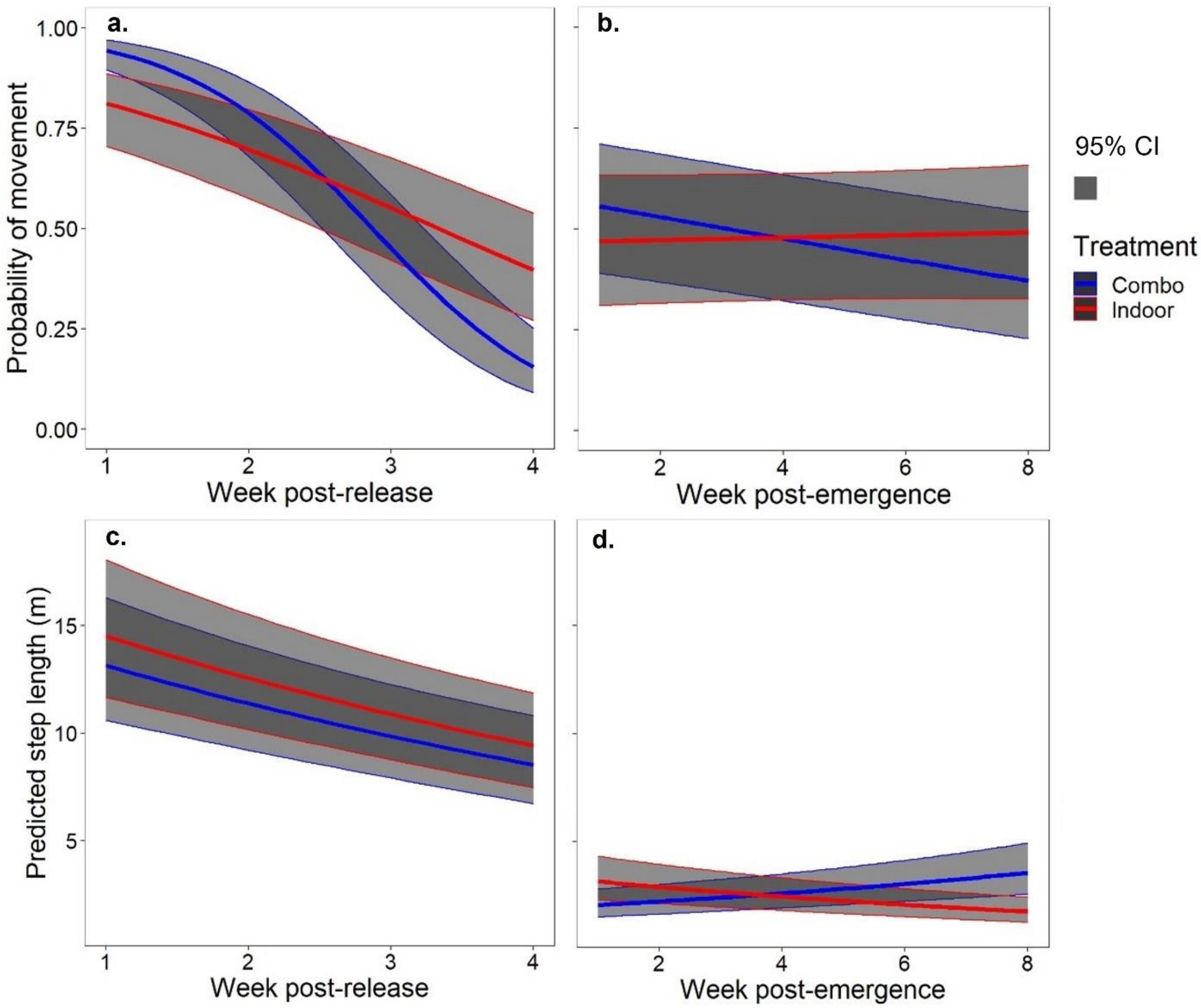

Fig. 2.5. Step-length predictions of head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) released in October 2020 by husbandry treatment type: Combo-rearing ("Combo") and Indoor-rearing ("Indoor"). Graphs show a) the probability of movement post-release, b) probability of movement post-emergence, c) predicted step-length (m) post-release, and d) predicted step-length (m) post-emergence. Shaded regions show 95% confidence intervals.

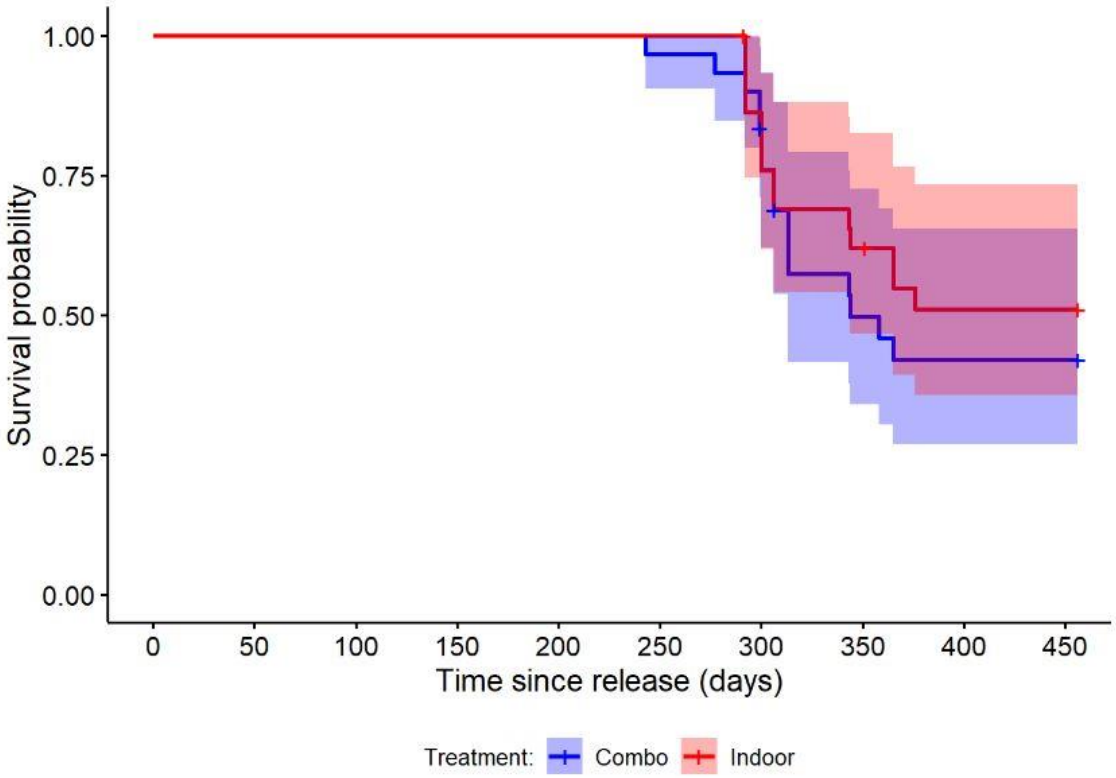

Fig. 2.6. Survival probability curves of head-started juvenile Mojave desert tortoises (*Gopherus agassizii*) over the 15 month study by husbandry treatment type: Combo-rearing ("Combo") and indoor-rearing ("Indoor") released in October 2020. Shaded regions show 95% confidence intervals. By the end of the 15 month post-release monitoring period, combo head-starts had a survival rate of 42% (95% CI: 0.3, 0.7) and indoor tortoises had a survival rate of 51% (95% CI: 0.4, 0.7).

CHAPTER 3

RELATIVE ROLE OF CLIMATIC CONDITIONS AND HUSBANDRY TREATMENT ON POST-RELEASE SURVIVAL OF HEAD-STARTED MOJAVE DESERT TORTOISES

INTRODUCTION

Some of the biggest threats to global biodiversity involve disturbances to the balance of natural systems, such as how climate change can affect organisms' ability to survive in their native habitats (Sheldon et al. 2011, Pievani 2014, Pimm et al. 2014). Climate change has the potential to shift species' distributions, shrink available habitat, change migration patterns, and affect species' behavior (Thomas et al. 2004, Deutsch et al. 2008, Barrows 2011, Foden et al. 2019). Thomas et al. (2004) predicted that by 2050, ~15–37% of the >1100 species in their study (including mammals, birds, herpetofauna, invertebrates, and plants) would be "committed to extinction" under a mid-range global warming scenario, which highlights the need for mitigation strategies and species conservation plans to be developed soon. Desert ecosystems are especially vulnerable to climate change, and were ranked third out of fourteen biomes in their rate of temperature change (Loarie et al. 2009), indicating deserts will likely have a higher relative temperature increase as global climate change progresses. Species in desert ecosystems are adapted to the naturally harsh conditions, but those species often cannot adapt quickly to rapid climate changes and are likely to be on the edge of decline if scarce resources disappear altogether (Griffis-Kyle 2016, Riddell et al. 2019, Zhou et al. 2020).

In the southwestern U.S., anthropogenically-driven climate warming has contributed to ~46% of the 2000–2018 drought severity and ~19% of the 2021 drought severity (Williams et al. 2020, Williams et al. 2022). This aridification of the southwest will likely persist as air temperatures continue to warm, because of the increased moisture demand by the atmosphere from the land surface and water bodies (Overpeck and Udall 2020). For the Mojave Desert, climate change often manifests as an increase in the frequency of extreme conditions, such as prolonged droughts interspersed with unusually wet years (Hereford et al. 2006). The Mojave Desert is currently experiencing a 22-year mega-drought, which is defined as a multi-decade event with periods of very high drought severity, that has likely been the driest period of time since 800 AD (Williams et al. 2020, Williams et al. 2022). These extreme conditions and fluctuations can increase vegetative growth in wet years but drastically decrease it during periods of drought (Hereford et al. 2006, McAuliffe and Hamerlynck 2010, Guida et al. 2014). These conditions also reduce the amount of available water in the landscape, which in turn can affect populations of other desert taxa. For example, native Mojave Desert species such as the Amargosa vole (*Microtus californicus scirpensis*) (Castle et al. 2020), the Vegas Valley leopard frog (*Rana* sp.) (Bradford et al. 2005), Townsend's big-eared bat (*Corynorhinus townsendii townsendii*) (Anderson et al. 2018), multiple bird species (Iknayan and Beissinger 2018), and the Mojave Desert tortoise (*Gopherus agassizii*) (Allison and McLuckie 2018) have all experienced population declines as a result of the increased climatic variation and other threats.

The Mojave Desert tortoise (hereafter "desert tortoise" or "tortoise") is a federally-threatened species under the U.S. Endangered Species Act (U.S. Fish and

Wildlife Service 1973) that has been declining since 1990. Desert tortoises are considered an umbrella species for studying and conserving Mojave Desert biodiversity because they are sensitive to changes in their environment (Berry 1997, Mouat et al. 1998). Their sensitivity allows them to serve as bioindicators of ecosystem health (Berry and Medica 1995), which benefits researchers attempting to assess the effects of various factors on desert species, including the effect of climatic conditions (McCoy et al. 2011, Nafus et al. 2017). Tortoises are especially vulnerable to climate change because they are ectotherms (Deutsch et al. 2008). Because of tortoises' important role in the desert and their declining population, conservation strategies are being developed to bolster their numbers, including head-starting (U.S. Fish and Wildlife Service 2011). Head-starting is the rearing of juveniles in captivity until they reach a size when they are less vulnerable to mortality by predation or exposure to the elements. When used in conjunction with strategies that protect adult populations and minimize the causes of overall population decline, head-starting can help jumpstart population growth for turtle species. Studies on improving desert tortoise head-starting have greatly increased our knowledge of the most efficient protocols (Hazard et al. 2015, Nagy et al. 2015a, Todd et al. 2015, Daly et al. 2019, Tuberville et al. 2019, McGovern et al. 2020a), but there are still many facets to uncover with this recovery tool.

One aspect of head-starting tortoises that has not been explored is the effect of climatic conditions on tortoise survival post-release. We synthesized a decade of radio-tracking data from 324 juvenile tortoises reared under different experimental head-starting treatments to determine the relative role of climatic factors, especially drought conditions, and treatment protocols on survival of released head-started desert tortoises in the Ivanpah Valley of the Mojave Desert. Specifically, we examined the effect of drought severity, air temperature, precipitation, experimental husbandry treatment type, release midline carapace length (MCL), and release weight on tortoise survival. Understanding how climatic factors affect the survival of head-started juvenile tortoises can give researchers greater insight into the ideal conditions for future releases and increase our understanding of how tortoise populations will respond to changes in climate.

We hypothesize that: 1) prolonged drought conditions cause increasing tortoise mortality over time due to direct and indirect effects of drought on tortoises such as bodily water loss and decreased amount of forage availability; 2) increased precipitation will positively affect survival probability because it is a scarce resource; 3) extreme high temperatures negatively affect survival of tortoises, which are ectotherms; 4) tortoises larger at release survive in the wild better than their smaller counterparts because they have greater defenses against predation and water loss; and 5) experimental treatment will not affect tortoise survival post-release, because all experimental treatments produce healthy tortoises. From these hypotheses, we predict that: 1) months with high drought severity will have decreased tortoise survival probability compared to months with low or average drought severity; 2) survival probability will be positively associated with monthly precipitation; 3) months with a higher number of days in which maximum temperatures exceed 35°C will have a reduced tortoise survival probability compared to months with fewer days that reach >35°C; 4) survival probability will be positively associated with release MCL and release weight; and 5) tortoise survival will be similar among treatments. Our overall objective was to explore the effects of climatic factors on tortoise survival and the potential for those factors to influence the outcome of recovery efforts.

METHODS

2.1 Study site

Head-starting operations and fieldwork were conducted in the Ivanpah Valley within the Mojave National Preserve, San Bernardino County, California, USA. The area is a part of the Ivanpah critical habitat unit and the Eastern Mojave Recovery Unit for Mojave Desert tortoises (U.S. Fish and Wildlife Service 2011). The habitat is primarily Yucca Woodland, with plant species such as creosote (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), yuccas (*Y. brevifolia, Y. shidigera*), pencil cholla (*Cylindropuntia ramosissima*), and littleleaf ratany (*Krameria erecta*) being the most abundant (Todd et al. 2016). The site also contained an abundance of small washes that animals often used as trails, and small mammal burrows used as shelter sites by multiple wildlife species (Todd et al. 2016). From 2000-2022 the temperatures at the study site ranged from an average high of 40.6 °C in the summer and an average low of -6 °C in the winter, and the annual precipitation ranged from 4.7 to 50.6 cm/yr. During our study period (2012-2022), the mean annual precipitation was 9.1 (range: 4.6 – 17.4) cm/yr (Applied Climate Information System 2022). The density of adult tortoises in the Ivanpah Valley has declined considerably from ~72 tortoises per km^2 in 1977 (Turner et al. 1984) to 2.8 tortoises per km2 in 2012 (Allison and McLuckie 2018). Observations of juveniles have also been decreasing since 2007 in all recovery units with the greatest declines occurring in the Eastern Mojave recovery unit (Allison and McLuckie 2018), making this site an ideal place to study the effect of population augmentation via head-starting. All captive husbandry operations occurred at the Ivanpah Desert Tortoise Research Facility (IDTRF), approximately 15 km north of the release sites.

2.2 Obtaining hatchlings

Beginning in April 2011, we captured wild female desert tortoises and fitted them with VHF radio transmitters. We tracked females monthly using a 3-element Yagi antenna (AF Antronics, Inc., Urbana, IL) and a R1000 receiver (Communications Specialists, Inc., Orange, CA). From 2011-2021 (excluding 2014) we collected females during late April-early May, brought them to the IDTRF, and radiographed them to check for calcified eggs (Diagnostic Imaging Systems, Poskam, Colorado, USA; 60 kvp, 0.8 mAS, 74 cm focal length; Gibbons and Greene 1979). We placed all gravid females in outdoor nesting pens at IDTRF and released all non-gravid females at their original capture location. All pens were 9m x 9m, predator-proof, and included pre-made burrows and natural vegetation(McGovern et al. 2020a, McGovern et al. 2020b). Females were given supplemental food and supplemental watering from a sprinkler system (Tuberville et al. 2019) and were allowed to nest naturally. Once females were placed in pens, we radiographed females weekly to monitor for egg deposition. When it was confirmed that they had nested, we returned them to the wild at their original capture location. In accordance with permit, no females were kept longer than 30 days unless specifically approved by permitting agencies. After the eggs had incubated naturally for ~80 days, we searched for emerging hatchlings in the pens. Each hatchling was then given a unique shell notch code assigned by the U.S. Fish and Wildlife Service.

2.3 Experimental treatments

The juvenile tortoises included in this study include all juveniles produced in captivity and released into the wild, after which we monitored their fate via radio-telemetry. Husbandry methods varied over the study period as we tested and optimized different rearing techniques. The experimental head-starting treatments included: direct release of hatchlings, outdoor-rearing for up to two years, outdoor-rearing for at least six years before release (hereafter “six+”), indoor-rearing, and combo-rearing (Table 3.1).

Directly-released individuals were protected from predators and exposure as eggs, and then released into the wild shortly after hatching in captivity in the fall (Table 3.1). Individuals that were directly released allowed researchers to evaluate the behavior and survival of hatchling tortoises in the wild, because finding wild hatchlings is difficult due to their small size and evasiveness. Directly-released hatchlings also served as a control for comparing movement and survival of tortoises head-started in captivity for short durations (Daly et al. 2019, Tuberville et al. 2019).

Individuals in the outdoor-rearing group spent between seven months and two years in captivity in outdoor pens similar to the nesting pens used for females (Daly et al. 2018, Tuberville et al. 2019; Table 3.1). They were given supplemental water every 1-2 weeks during the active season (~April – November) and, starting with the 2015 cohort, supplemental food (made up of a mixture of greens and moistened Mazuri® Tortoise Diet (Mazuri Exotic Animal Nutrition, St. Louis, MO); Daly et al. 2018). Pens had pre-made burrows and space for tortoises to build their own burrows as well. Outdoor rearing allowed these tortoises to experience environmental cues, such as weather conditions and natural forage, and to exhibit natural behaviors, such as constructing their own burrows and undergoing winter dormancy. Tortoises in the “six+” treatment were held in the same outdoor pens with supplemental food and water for at least six years Table 3.1), allowing them to reach a larger body size than the younger outdoor-reared tortoises.

Indoor-rearing involved rearing tortoises in large rearing tubs inside for seven months to one year (Daly et al. 2018; Ch 2 of this thesis; Table 3.1). Tubs were equipped with natural substrate, artificial plastic shelters, and humid hide boxes to promote normal shell growth and avoid pyramiding (Wiesner and Iben 2003). Lighting was provided using a 50 W ZooMed Repti Basking Spot Lamp bulb for daytime basking (37 °C), a ZooMed 50 W Infrared Basking Spot bulb for night-time heat (32 °C), and a 26 W Exo-Terra Reptile UVB150 bulb (Rolf C. Hagen Corp., Mansfield, MA., U.S.A) for optimal calcium metabolism and vitamin D_3 conversion (McWilliams 2005), all set with automatic timers to mimic a natural photoperiod. Researchers fed indoor-reared tortoises three times a week, provided calcium powder two times a week, and soaked tortoises once a week to maintain hydration. Tortoises were kept active through the winter and therefore grew faster than tortoises undergoing dormancy outdoors at the time, thereby decreasing the time for tortoises to reach the recommended release size of ~100 mm MCL (Nagy et al. 2015a)

Combo-rearing combined an indoor-rearing component for one year, during which tortoises could grow rapidly by being kept active year-round, followed by outdoor-rearing for one year, during which tortoises were exposed to natural environmental cues in the predator-proof pens (McGovern et al. 2020a, Candal 2021; Table 3.1). Combo-reared tortoises attained sizes similar to the “six+” treatment with just two years of head-starting and were larger in size than their same age siblings assigned to the outdoor treatment. See McGovern et al. (2020a) and (Daly et al. 2018) for further husbandry details on outdoor-, indoor-, and combo-rearing.

2.4 Releases

We performed releases of head-started tortoises every year from 2012-2020 except in 2014 and 2017 (Table 3.1). Before each release, we weighed all tortoises and measured their shell height, width, and midline carapace length (MCL). We also fitted each tortoise with radio transmitters either from Holohil Systems Ltd. (Ontario, Canada; model BD-2) or Advanced Telemetry Systems (MN, U.S.A; models R1670, 3.1 g or R1680, 3.6 g) attached to the fourth or fifth vertebral scute with gel epoxy. Release locations varied over the study, but all sites were within 11 km of each other and ~8-15 km south of IDTRF. We assigned tortoises to individual release points, which were prepped with a release refugium in the form of a kangaroo rat (*Dipodomys* spp.) burrow beneath a large perennial shrub that we enlarged using a hand trowel to provide shelter to the released tortoise. The release points were often grouped into blocks, with 20-30 individual release points each at least 30 m apart. The use of blocks allowed us easier access to track tortoises post-release and reduced the amount of disturbance within the release area.

From 2012 to 2016, the time of year releases occurred varied, with some in the spring and some in the fall. The tortoises in the 2016 release experienced unusually low survival rates (44%) due to predation from a single pair of ravens during their spring release (Daly et al. 2019). This occurrence prompted us to change our release protocol to releasing in the fall only, to avoid the spring raven nesting period, and to use release locations that were ≥1.6 km away from any natural or man-made structure that could be used by ravens for nesting. All releases occurring after 2016 followed these two criteria.

2.5 Post-release monitoring

We monitored all tortoises using 3-element Yagi antenna (AF Antronics, Inc., Urbana, IL) and a R1000 receiver (Communications Specialists, Inc., Orange, CA). Our tracking frequency varied throughout the years of the study, but all tortoises were tracked at least once a month. Generally, tracking during winter dormancy (November to March) could be reduced until the time of spring emergence. In later years (2018-2020), tortoises were tracked 24 hours after release, twice a week for the first three weeks, then once per week until winter dormancy (mid-November) when tracking was reduced to once every 10-14 days. At each tracking event, we collected data on the tortoise's location to the nearest 3 m using a handheld GPS unit (Garmin GPSMAP 76CSx, Olathe, KS), cover usage (burrow/pallet, surface hidden, surface open), vegetation cover type, behavior, approximate distance from last tracking location, and the time of day. When radio transmitter batteries were close to dying, we would replace them and perform a health check on tortoises, including taking body measurements and touching up the notch codes markings for easier identification. Starting in fall 2020, a portion of tortoises that had been tracked were recaptured and then released without a new transmitter and removed from the telemetry study to reduce tracking demands. All other surviving tortoises were radio-tracked through June 2022.

2.6 Data Analysis

We compiled the following information on juvenile tortoises that had been tracked at any point from Sept 2012 – June 2022: experimental husbandry treatment, release weight, release MCL, release date, and their fate at each tracking event (alive, dead, not found). We created encounter histories for each tortoise with one "occasion" for each month, starting in Sept 2012 and ending June 2022 (total of 118 months) using binary code where 10 meant the tortoise was alive, 11 meant it died in that month, and 00 meant it was not observed in that month and therefore censored. Tortoises whose final fate was unknown (went missing or we released without a transmitter) were censored. We created survival models using the package *RMark* (Laake 2022) in Program R (R version 4.0.2, www.r-project.org), which serves as an alternative interface for MARK software (version 9.0, http://www.phidot.org/software/mark/downloads/).

We also compiled climatic data over the study period in the form of monthly average Palmer Drought Severity Index (PDSI), monthly precipitation in mm, and the number of days per month having a max temperature >35° C, which we obtained from ClimateEngine.com using the gridMET database. The PDSI is a measure of the relative severity of drought conditions over time that incorporates soil moisture, air temperature, and recent weather conditions, with a value of 0 indicating average conditions, positive values indicating wet conditions, and negative values indicating drought conditions. We choose to consider monthly precipitation in addition to PDSI, as tortoise survival could alternatively or also be influenced by more immediate precipitation patterns and my goal was to determine which variable better represented tortoises' response to drought. Considering both precipitation and PDSI was also beneficial because monthly precipitation can reflect the short-term effects of drought while the PDSI reflects the relatively long-term effect of drought on the ecosystem. The effect of air temperature was measured as the number of days within each month that the maximum temperature was

>35°C, based on the tortoise handling requirements of the U.S. Fish and Wildlife, because tortoises tend to start overheating at that temperature (U.S. Fish and Wildlife Service 1991, The Desert Tortoise Council 1994 (Revised 1999))

Survival models included the following individual-level predictor variables: experimental husbandry treatment, release weight, and release MCL as individual-specific predictor variables. In addition, we included the time-specific predictor variables of monthly PDSI, monthly precipitation in mm, and the number of days per month with a max temperature >35° C. To account for the difference in release protocols before and after 2016, we created a dummy variable to distinguish animals released under each protocol (with post-2016 releases occurring in Fall only and ≥1.6 km from potential raven nesting structures), and incorporated it into each model as a covariate. We built six additive models each with one of the predictor variables and the protocol dummy variable. Given that the focus of our study was testing specific hypotheses about individual factors influencing tortoise survival, we focused on effect sizes and their corresponding uncertainty within each model rather than selecting among alternative models. RMark also cannot currently run mixed effects models, therefore we could not include any variables as a random effect (Rotella et al. 2004, Fromberger et al. 2020).

RESULTS

We released and radio-tracked a total of 324 head-started tortoises from 2012-2022 (Table 3.1). The longest-tracked head-started tortoise was still alive at the end of the study, having been tracked for 105 months. Among the tortoises (113 pre-2016 and 210 post-2016 under the current release protocols), 42 were directly released, 58 were indoor-reared, 92 were outdoor-reared for up to two years, 54 were reared outdoors for six+

years, and 78 were combo-reared. Of the 324 total tortoises, 158 died, 56 went missing, 29 were released from the study after 1-3 years of being tracked, and 81 were still alive and being tracked as of June 2022. Six+ tortoises had the largest average MCL at release, followed by combo, indoor, outdoor, and direct release tortoises (Fig. 3.1).

3.1 Individual-specific variables

Over the whole study period, six+ tortoises had the highest estimated monthly survival rate (0.989), followed by combo (0.979), outdoor (0.975), direct release (0.968), and finally indoor (0.967) (Fig. 3.2; Table 3.2). However, the 95% confidence intervals for all treatment types were not significantly different from each other, indicating that survival rates did not differ among the captive husbandry treatments. The models for both release MCL (Table 3.3) and release weight (Table 3.4) showed a positive effect on survival, with survival increasing as both size metrics increased (Fig. 3.3 & 3.4). The 95% confidence intervals for the MCL and weight variables overlapped zero, suggesting their effect was statistically insignificant. However, the confidence intervals were also very close to overlapping zero, so there still could have been a meaningful biological effect of MCL and weight that was not detected by our models.

3.2 Time-specific variables

A graph of the yearly average survivals of tortoises with known fates can be seen in Fig. 3.5. There was a positive relationship between PDSI and survival of all tortoises, indicating tortoise survival was higher during non-drought conditions (positive PDSI values) than during drought conditions (negative PDSI values; Table 3.5). Monthly precipitation also had a positive trend on tortoise survival, indicating that tortoise survival increased with increased monthly precipitation (Table 3.6). However, because the 95% confidence interval for the precipitation estimate overlapped zero, the effect was not statistically significant. When evaluating the effect of the number of days in each month with a temperature >35° C, there was negative relationship to tortoise survival, indicating that tortoise survival decreased in months that had a higher number of days >35° C (Table 3.6).

The effect of the change in protocols post-2016 had a positive trend on tortoise survival in all 6 models, and was statistically significant in all models except the one that considered treatment alone. However, in the treatment model the 95% confidence intervals for the protocol variable were close to not overlapping.

DISCUSSION

4.1 Overview

All head-started Mojave Desert tortoises in our study survived about as well as each other. Surprisingly, tortoise release weight and MCL did not have a strong effect on survival, but both still had a positive trend, indicating larger tortoises survived better than smaller tortoises. Tortoise survival decreased as drought severity grew more intense (negative PDSI values), and increasing monthly precipitation had a weak positive effect on survival. In addition, tortoise survival decreased when the number of days in which maximum temperatures exceed >35°C per month increased. Tortoises also tended to survive better under the post-2016 release protocols than under the previous protocols, indicating that continuing to release head-starts in fall (rather than spring) and at distances ≥1.6 km away from raven nesting structures is warranted.

4.2 Effects of treatment and size

From our results on the effect of treatment, we can infer that any captive husbandry type is able to produce tortoises that can survive in the wild well, given they are healthy at release. This finding supports our hypothesis that experimental husbandry treatment would not affect survival post-release. Previous studies have found that tortoises with larger MCL have higher survival after release likely because they are less vulnerable to predation (Nagy et al. 2015a, McGovern et al. 2020b), and our results support that finding and our hypothesis on the effect of size despite the weak effects in the current study.

4.3 Climatic effects on turtle populations

Population declines of turtles often have multiple contributing causes, and climate change is usually one of those causes (Lovich et al. 2018). Climate change can have both direct and indirect effects on turtles, including altered sex ratios and clutch sizes, decreased suitable habitat, and reduced food availability (Chessman 2011, McCoy et al. 2011, Hedrick et al. 2018). Turtles have mechanisms for acclimating to or avoiding unfavorable weather conditions such as aestivation during a drought (Nagy and Medica 1986), regulating body temperature with basking, or delaying reproduction (Lovich et al. 2015), but if the unfavorable conditions are prolonged, population declines can occur (Chessman 2011). Greater variation and more frequent extremes in climate, like what has been observed in the Mojave Desert, could have a greater effect on sensitive species than effects associated with consistently higher temperatures or lower precipitation (Chessman 2011, Garcés-Restrepo et al. 2019). Deserts are already extreme environments, and studies like ours will help determine to what extent increased climatic variation will affect the sensitive species, such as desert tortoises, that live there.

4.4 Effect of drought on desert tortoises

Our results suggest that the effect of drought is more clearly seen when using long-term measures of drought, such as the PDSI, than when using short-term measures, such as monthly precipitation. Our hypothesis that drought conditions would decrease tortoise survival were supported because increasing PDSI value (non-drought conditions) had a positive effect on survival. Increasing monthly precipitation did not appear to have a strong effect on survival, likely because the effect of precipitation in the desert seems to have a delay in impacting the system as a whole. The spring and winter annual plant production, which sustains many species such as small-mammals (Beatley 1969) and tortoises, depends on the cool season rains (Oct-April) in the Mojave Desert (Beatley 1967, 1974, Hereford et al. 2006). When cool season rains are reduced or do not occur, the effects are seen months later when the amount of forage is reduced, long-lived perennial plants such as *Ambrosia* spp. (which serve as cover for many animals) experience mortality events (McAuliffe and Hamerlynck 2010), and herbivorous species have population reductions (Beatley 1969, Duda et al. 1999, Longshore et al. 2003) during the spring and summer. The PDSI likely does a better job of capturing this phenomenon than monthly precipitation because it can incorporate soil moisture, recent temperatures, and recent climate conditions into the index value (Rhee and Carbone 2007).

The effect of air temperature on tortoise survival is likely due to the risk of tortoises overheating as their critical thermal maximum (body temperature at which they start to enter hyperthermia) is between 39° C and 44° C (The Desert Tortoise Council 1994 (Revised 1999)). The negative relationship between temperature and survival could also be due to the risk of predation during moderately warm months, when tortoises are more surface active than during winter dormancy and summer estivation.

The effect of drought on tortoises can also be observed in the difference in climate between the Mojave Desert and another nearby desert with a similar tortoise species – the Sonoran Desert. Until 2011, Sonoran desert tortoises (*Gopherus morafkai*) and Mojave desert tortoises were thought to be the same species (Murphy et al. 2011) because of their similar ecology. The Sonoran Desert tends to have more reliable and greater (by about 85 mm on average) summer precipitation than the Mojave Desert (Germano 1994), which benefits the Sonoran desert tortoise. Possibly because of this greater availability of water, Sonoran desert tortoises are able to forego reproduction in years that are unfavorable (Campbell et al. 2015), whereas Mojave desert tortoises have developed a “bet-hedging” strategy, where they lay a clutch of eggs every year despite the conditions because the risk of a continued drought next year is high anyway (Lovich et al. 2015). In a similar study to ours, Zylstra et al. (2013) evaluated climatic effects on the survival of Sonoran desert tortoises using the PDSI, and found a negative relationship between increasing drought conditions and survival at a scale similar what we found for Mojave desert tortoises. While it seems both tortoise species’ survival rates are similar when it comes to responding to drought, because the Mojave is more arid than the Sonoran to start, the continued aridification occurring in the southwest means Mojave desert tortoises will continue to be at a disadvantage (Overpeck and Udall 2020).

4.5 Impact and future directions

Understanding the status of such an important Mojave Desert species as the desert tortoise can help researchers gauge what is going on in the rest of the ecosystem (Mouat et al. 1998). This is especially true with the ongoing threat of climate change, as desert tortoises have been one of the first species to have its response to changes be investigated (McCoy et al. 2011, Sieg et al. 2015, Barrows et al. 2016, Mitchell et al. 2021). There is still much to examine about the effect of climatic factors and drought on the ecosystem of the Mojave Desert, and our study could be considered a first stepping stone to continued study into tortoises' status during different climatic events. Future research could focus on the interface of drought and urbanization or habitat loss, as development in the Mojave Desert for energy production and housing continues to expand (Morris et al. 1997, Parker et al. 2018, Todd et al. 2021). At our study site specifically, continued tracking of head-started tortoises will give us great insight into how climate is affecting tortoises and the Mojave as a whole. Our model could also be improved as more individual tortoises are tracked, thus increasing the sample sizes of each treatment group. Our study contributes to population recovery efforts by providing a better understanding of how climatic conditions affect head-started desert tortoises, specifically that drought conditions can lower tortoise survival. Therefore, practitioners should consider avoiding releasing tortoises during times of high drought severity to reduce tortoise mortality as much as possible.

LITERATURE CITED

Allison, L. J., and A. M. McLuckie. 2018. Population trends in Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **13**:433-452.

Anderson, A. P., J. E. Light, O. M. Takano, and M. L. Morrison. 2018. Population structure of the Townsend’s big-eared bat (*Corynorhinus townsendii townsendii*) in California. Journal of Mammalogy **99**:646–658.

Applied Climate Information System. 2022. NOWData - NOAA Online Weather Data. Weather.gov.

Barrows, C. W. 2011. Sensitivity to climate change for two reptiles at the Mojave–Sonoran Desert interface. Journal of Arid Environments **75**:629-635.

Barrows, C. W., B. T. Henen, and A. E. Karl. 2016. Identifying climate refugia: A framework to inform conservation strategies for Agassiz's desert tortoise in a warmer future. Chelonian Conservation and Biology **15**:2-11.

Beatley, J. C. 1967. Survival of winter annuals in the northern Mojave Desert. Ecology **48**:745-750.

Beatley, J. C. 1969. Dependence of desert rodents on winter annuals and precipitation. Ecology **50**:721-724.

Beatley, J. C. 1974. Phenological events and their environmental triggers in Mojave Desert ecosystems. Ecology **55**:856-863.

Berry, K. H. 1997. The desert tortoise recovery plan: An ambitious effort to conserve biodiversity in the Mojave and Colorado deserts of the United States. Pages 43G-440 *in* Conservation, Restoration, and Management ofTortoises and Turtles-An International Conference. New York Tunle and Tonoise Society.

Berry, K. H., and P. A. Medica. 1995. Desert tortoises in the Mojave and Colorado deserts. Pages 135-137 *in* E. T. LaRoe, G. S. Farris, C. E. Puckett, P. D. Doran, and M. J. Mac, editors. Our living resources: A report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosystems. National Biological Service.

Bradford, D. F., J. R. Jaeger, and S. A. Shanahan. 2005. Distributional changes and populations status of amphibians in the Eastern Mojave Desert. Western North American Naturalist **65**:462-472.

Campbell, S. P., R. J. Steidl, and E. R. Zylstra. 2015. Recruitment of desert tortoises (*Gopherus agassizii* and *G. morafkai*): A synthesis of reproduction and first-year survival. Herpetological Conservation and Biology **10**:583-591.

Castle, S. T., P. Foley, D. L. Clifford, and J. Foley. 2020. A stochastic structured metapopulation model to assess recovery scenarios of patchily distributed endangered species: Case study for a Mojave Desert rodent. PLOS ONE **15**:e0237516.

Chessman, B. C. 2011. Declines of freshwater turtles associated with climatic drying in Australia's Murray-Darling Basin. Wildlife Research **38**:664-671.

Daly, J. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, and T. D. Tuberville. 2018. Comparing growth and body condition of indoor-reared, outdoor-reared, and direct-released juvenile Mojave Desert tortoises. Herpetological Conservation and Biology **13**:622-633.

Daly, J. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, and T. D. Tuberville. 2019. Survival and movements of head-started Mojave desert tortoises. Journal of Wildlife Management **83**:1700-1710.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences **105**:6668-6672.

Duda, J. J., A. J. Krzysik, and J. E. Freilich. 1999. Effects of drought on desert tortoise movement and activity. The Journal of Wildlife Management **63**:1181.

Foden, W. B., B. E. Young, H. R. Akçakaya, R. A. Garcia, A. A. Hoffmann, B. A. Stein, C. D. Thomas, C. J. Wheatley, D. Bickford, J. A. Carr, D. G. Hole, T. G. Martin, M. Pacifici, J. W. Pearce-Higgins, P. J. Platts, P. Visconti, J. E. M. Watson, and B. Huntley. 2019. Climate change vulnerability assessment of species. WIREs Climate Change **10**:e551.

Fromberger, M. A., A. J. Campomizzi, Z. M. Lebrun-Southcott, A. L. Pintaric, N. M. Macdonald, and E. Nol. 2020. Factors affecting Bobolink nest survival across grassland types. Avian Conservation and Ecology **15**.

Garcés-Restrepo, M. F., J. L. Carr, and A. Giraldo. 2019. Long-term variation in survival of a neotropical freshwater turtle: Habitat and climatic influences. Diversity **11**:97.

Germano, D. J. 1994. Growth and age at maturity of North American tortoises in relation to regional climates. Canadian Journal of Zoology.

Gibbons, J. W., and J. L. Greene. 1979. X-Ray photography: a technique to determine reproductive patterns of freshwater turtles. Herpetologica **35**:86-89.

gridMET. 2022. Drought - PDSI.*in* Climate Engine, editor. Google Earth Engine.

Griffis-Kyle, K. L. 2016. Physiology and ecology to inform climate adaptation stratigies for desert amphibians. Herpetological Conservation and Biology **11**:563-582.

Guida, R. J., S. R. Abella, W. J. Smith, H. Stephen, and C. L. Roberts. 2014. Climatic change and desert vegetation distribution: Assessing thirty years of change in southern Nevada's Mojave Desert. The Professional Geographer **66**:311-322.

Hazard, L. C., D. J. Morafka, and S. Hillard. 2015. Post-release dispersal and predation of head-started juvenile desert tortoises (*Gopherus agassizii*): Effect of release site distance on homing behavior. Herpetological Conservation and Biology **10**:504-+.

Hedrick, A. R., H. M. Klondaris, L. C. Corichi, M. J. Dreslik, and J. B. Iverson. 2018. The effects of climate on annual variation in reproductive output in Snapping Turtles (*Chelydra serpentina*). Canadian Journal of Zoology **96**:221-228.

Hereford, R., R. H. Webb, and C. I. Longpré. 2006. Precipitation history and ecosystem response to multidecadal precipitation variability in the Mojave Desert region, 1893–2001. Journal of Arid Environments **67**:13-34.

Iknayan, K. J., and S. R. Beissinger. 2018. Collapse of a desert bird community over the past century driven by climate change. Proceedings of the National Academy of Sciences **115**:8597–8602.

Laake, J. 2022. Package ‘RMark’.

Loarie, S. R., P. B. Duffy, H. Hamilton, G. P. Asner, C. B. Field, and D. D. Ackerly. 2009. The velocity of climate change. Nature **462**:1052-1055.

Longshore, K. M., J. R. Jaeger, and J. M. Sappington. 2003. Desert tortoise (*Gopherus agassizii*) survival at two eastern Mojave Desert sites: Death by short-term drought? Journal of Herpetology **37**:169–177.

Lovich, J. E., J. R. Ennen, M. Agha, and J. W. Gibbons. 2018. Where have all the turtles gone, and why does it matter? BioScience **68**:771–781.

Lovich, J. E., J. R. Ennen, C. B. Yackulic, K. Meyer-Wilkins, M. Agha, C. Loughran, C. Bjurlin, M. Austin, and S. Madrak. 2015. Not putting all their eggs in one basket: bet-hedging despite extraordinary annual reproductive output of desert tortoises. Biological Journal of the Linnean Society **115**:399-410.

McAuliffe, J. R., and E. P. Hamerlynck. 2010. Perennial plant mortality in the Sonoran and Mojave deserts in response to severe, multi-year drought. Journal of Arid Environments **74**:885-896.

McCoy, E. D., R. D. Moore, H. R. Mushinsky, and S. C. Popa. 2011. Effects of rainfall and the potential influence of climate change on two congeneric tortoise species. Chelonian Conservation and Biology **10**:34-41.

McGovern, P. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, J. Hepinstall-Cymerman, J. A. Daly, and T. D. Tuberville. 2020a. Comparing husbandry techniques for optimal head-starting of the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **15**:626-641.

McGovern, P. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, J. Hepinstall-Cymerman, J. A. Daly, and T. D. Tuberville. 2020b. The effect of size on postrelease survival of head-started Mojave Desert tortoises. Journal of Fish and Wildlife Management **11**:494-506.

McWilliams, D. A. 2005. Nutrition research on calcium homeostasis in lizards (with recommendations). International Zoo Yearbook **39**:69-77.

Mitchell, C. I., D. A. Friend, L. T. Phillips, E. A. Hunter, J. E. Lovich, M. Agha, S. R. Puffer, K. L. Cummings, P. A. Medica, T. C. Esque, K. E. Nussear, and K. T. Shoemaker. 2021. 'Unscrambling' the drivers of egg production in Agassiz's desert tortoise: Climate and individual attributes predict reproductive output. Endangered Species Research **44**:217-230.

Morris, R. L., D. A. Devitt, A. M. Crities, G. Borden, and L. N. Allen. 1997. Ubanization and water conservation in Las Vegas Valley, Nevada. Journal of Water Resources Planning and Management **May/June**.

Mouat, D., A. R. Kiester, and J. Baker. 1998. Analysis and Assessment of Impacts on Biodiversity: A Framework for Environmental Management on DoD Lands within the California Mojave Desert: A Research Plan. U.S. EPA NHEERL, U.S.DA. Forest Service.

Murphy, R., K. Berry, T. Edwards, A. Leviton, A. Lathrop, and J. D. Riedle. 2011. The dazed and confused identity of Agassiz’s land tortoise, Gopherus agassizii (Testudines: Testudinidae) with the description of a new species and its consequences for conservation. ZooKeys **113**:39-71.

Nafus, M. G., T. D. Tuberville, K. A. Buhlmann, and B. D. Todd. 2017. Precipitation quantity and timing affect native plant production and growth of a key herbivore, the desert tortoise, in the Mojave Desert. Climate Change Responses **4**.

Nagy, K. A., L. S. Hillard, M. W. Tuma, and D. J. Morafka. 2015. Head-started desert tortoises (*Gopherus agassizii*): movements, survivorship and mortality causes following their release. Herpetological Conservation and Biology **10**:203-215.

Nagy, K. A., and P. A. Medica. 1986. Physiological ecology of desert tortoises in southern Nevada. Herpetologica **42**:73-92.

Overpeck, J. T., and B. Udall. 2020. Climate change and the aridification of North America. Proceedings of the National Academy of Sciences of the United States of America **117**:11856-11858.

Parker, S. S., B. S. Cohen, and J. Moore. 2018. Impact of solar and wind development on conservation values in the Mojave Desert. PLOS ONE **13**:e0207678.

Pievani, T. 2014. The sixth mass extinction: Anthropocene and the human impact on biodiversity. Rendiconti Lincei **25**:85-93.

Pimm, S. L., C. N. Jenkins, R. Abell, T. M. Brooks, J. L. Gittleman, L. N. Joppa, P. H. Raven, C. M. Roberts, and J. O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science **344**:987–997.

Rhee, J., and G. J. Carbone. 2007. A comparison of weekly monitoring methods of the Palmer Drought Index. Journal of Climate **20**:6033-6044.

Riddell, E. A., K. J. Iknayan, B. O. Wolf, B. Sinervo, and S. R. Beissinger. 2019. Cooling requirements fueled the collapse of a desert bird community from climate change. Proceedings of the National Academy of Sciences **116**:21609-21615.

Rotella, J. J., S. J. Dinsmore, and T. L. Shaffer. 2004. Modeling nest–survival data: a comparison of recently developed methods that can be implemented in MARK and SAS. Animal Biodiversity and Conservation **27.1**:187–205.

Sheldon, K. S., S. Yang, and J. J. Tewksbury. 2011. Climate change and community disassembly: impacts of warming on tropical and temperate montane community structure. Ecology Letters **14**:1191–1200.

Sieg, A. E., M. M. Gambone, B. P. Wallace, S. Clusella-Trullas, J. R. Spotila, and H. W. Avery. 2015. Mojave desert tortoise (*Gopherus agassizii*) thermal ecology and reproductive success along a rainfall cline. Integr Zool **10**:282-294.

The Desert Tortoise Council. 1994 (Revised 1999). Guidlines for handling desert tortoises during construction projects.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. De Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. Van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. Townsend Peterson, O. L. Phillips, and S. E. Williams. 2004. Extinction risk from climate change. Nature **427**:145-148.

Todd, B. D., B. J. Halstead, L. P. Chiquoine, J. M. Peaden, K. A. Buhlmann, T. D. Tuberville, and M. G. Nafus. 2016. Habitat selection by juvenile Mojave Desert tortoises. The Journal of Wildlife Management **80**:720-728.

Todd, B. D., T. D. Tuberville, and K. A. Buhlmann. 2015. Innovative mitigation of solar energy impacts on desert tortoises: Head-starting and habitat selection of juvenile desert tortoises.

Todd, B. D., T. D. Tuberville, and K. A. Buhlmann. 2021. Mitigating impacts of solar energy development on desert tortoises. California Energy Commission.

Tuberville, T. D., K. A. Buhlmann, R. Sollmann, M. G. Nafus, J. M. Peaden, J. A. Daly, and B. D. Todd. 2019. Effects of short-term, outdoor head-starting on growth and survival in the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **14**:171-184.

Turner, F. B., P. A. Medica, and C. L. Lyon. 1984. Reproduction and survival of the desert tortoise (*Scaptochelys agassizii*) in Ivanpah Valley, California. Copeia **4**:811-820.

U.S. Fish and Wildlife Service. 1973. Endangered Species Act of 1973.*in* United States of America, editor.

U.S. Fish and Wildlife Service. 1991. Interim techniques handbook for collecting and analyzing data on desert tortoise populations and habitats.

U.S. Fish and Wildlife Service. 2011. Revised recovery plan for the Mojave population of the desert tortoise (*Gopherus agassizii*).

Wiesner, C. S., and C. Iben. 2003. Influence of environmental humidity and dietary protein on pyramidal growth of carapaces in African spurred tortoises (*Geochelone sulcata*). Journal of Animal Physiology and Animal Nutrition **87**:66-74.

Williams, A. P., B. I. Cook, and J. E. Smerdon. 2022. Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. Nature Climate Change.

Williams, A. P., E. R. Cook, J. E. Smerdon, B. I. Cook, J. T. Abatzoglou, K. Bolles, S. H. Baek, A. M. Badger, and B. Livneh. 2020. Large contribution from anthropogenic warming to an emerging North American megadrought. Science **368**:314-+.

Zhou, H., Y. Chen, C. Zhu, Z. Li, G. Fang, Y. Li, and A. Fu. 2020. Climate change may accelerate the decline of desert riparian forest in the lower Tarim River, Northwestern China: Evidence from tree-rings of Populus euphratica. Ecological Indicators **111**.

Zylstra, E. R., R. J. Steidl, C. A. Jones, and R. C. Averill-Murray. 2013. Spatial and temporal variation in survival of a rare reptile: a 22-year study of Sonoran desert tortoises. Oecologia **173**:107-116.

TABLES

Table 3.1. Tortoises released in the Ivanpah Valley in Mojave National Preserve, California, USA by release date, cohort, and experimental husbandry treatment type. The number of tortoises alive, missing or released, and dead by the end of the study in June 2022 are listed as well.

Release Date	Cohort	Age at release (months)	Treatment	No. of animals	No. alive	No. missing or released	No. dead
Fall 2012	2011	12	Outdoor	6	0	3	3
Fall 2012	2012	0	Direct release	12	0	1	11
Spring 2013	2011	18	Outdoor	6	0	4	2
Spring 2013	2012	6	Outdoor	12	0	4	8
Fall 2013	2013	0	Direct release	10	1	6	3
Fall 2015	2015	0	Direct release	20	1	7	12
Spring 2016	2015	7	Indoor	28	5	4	19
Spring 2016	2015	7	Outdoor	20	1	6	13
Fall 2018	2016	24	Combo	24	7	7	10
Fall 2018	2016	24	Outdoor	24	6	3	15
Fall 2018	2011	84	Six+	15	3	6	6
Fall 2018	2012	72	Six+	15	6	6	3
Fall 2019	2017	24	Outdoor	24	8	10	6
Fall 2019	2017	24	Combo	24	7	6	11
Fall 2019	2012	84	Six+	6	4	1	1
Fall 2019	2013	72	Six+	18	6	7	5
Fall 2020	2018	24	Combo	30	11	3	16
Fall 2020	2019	12	Indoor	30	15	1	14
			Totals:	324	81	85	158

Table 3.2. Parameter estimates and 95% confidence intervals from the modeling of head-started juvenile tortoise survival as a function of experimental treatment from 2012-2022. The protocol variable incorporates the effect of the two protocols used in the study before and after 2016. The reference level is the direct release treatment.

Variable	Estimate	SE	95% CI
Intercept	3.419	0.203	3.021, 3.818
Combo	0.028	0.351	-0.660, 0.717
Indoor	-0.220	0.282	-0.773, 0.333
Outdoor	0.072	0.278	-0.473, 0.617
Six+	0.717	0.403	-0.072, 1.508
Protocol	0.437	0.232	-0.017, 0.891

Table 3.3. Parameter estimates and 95% confidence intervals from the modeling of head-started juvenile tortoise survival as a function of midline carapace length (MCL) in mm at release from 2012-2022. The protocol variable incorporates the effect of the two protocols used in the study before and after 2016.

Variable	Estimate	SE	95% CI
Intercept	3.328	0.291	2.758, 3.899
MCL	0.001	0.004	-0.007, 0.009
Protocol	0.600	0.274	0.063, 1.138

Table 3.4. Parameter estimates and 95% confidence intervals from the modeling of head-started juvenile tortoise survival as a function of tortoise weight (in grams) at release from 2012-2022. The protocol variable incorporates the effect of the two protocols used in the study before and after 2016.

Variable	Estimate	SE	95% CI
Intercept	3.361	0.130	3.104, 3.617
Weight	0.0004	0.001	-0.001, 0.002
Protocol	0.563	0.239	0.093, 1.032

Table 3.5. Parameter estimates and 95% confidence intervals from the modeling of head-started juvenile tortoise survival as a function of the Palmer Drought Severity Index (PDSI) value from 2012-2022. The PDSI is a measure of drought severity with negative values representing drought conditions, a value of 0 representing average conditions, and positive values representing wet conditions. The protocol variable incorporates the effect of the two protocols used in the study before and after 2016.

Variable	Estimate	SE	95% CI
Intercept	3.549	0.143	3.268, 3.830
PDSI	0.125	0.051	0.025, 0.226
Protocol	0.733	0.168	0.403, 1.063

Table 3.6. Parameter estimates and 95% confidence intervals from the modeling of head-started juvenile tortoise survival as a function of the monthly precipitation in cm from 2012-2022. The protocol variable incorporates the effect of the two protocols used in the study before and after 2016.

Variable	Estimate	SE	95% CI
Intercept	3.359	0.138	3.087, 3.631
Precipitation	0.002	0.005	-0.008, 0.012
Protocol	0.652	0.164	0.329, 0.974

Table 3.7. Parameter estimates and 95% confidence intervals from the modeling of head-started juvenile tortoise survival as a function of the monthly precipitation in cm from 2012-2022. The protocol variable incorporates the effect of the two protocols used in the study before and after 2016.

Variable	Estimate	SE	95% CI
Intercept	3.839	0.148	3.548, 4.130
# of days >35°C	-0.050	0.006	-0.064, -0.036
Protocol	0.627	0.164	0.304, 0.949

FIGURES

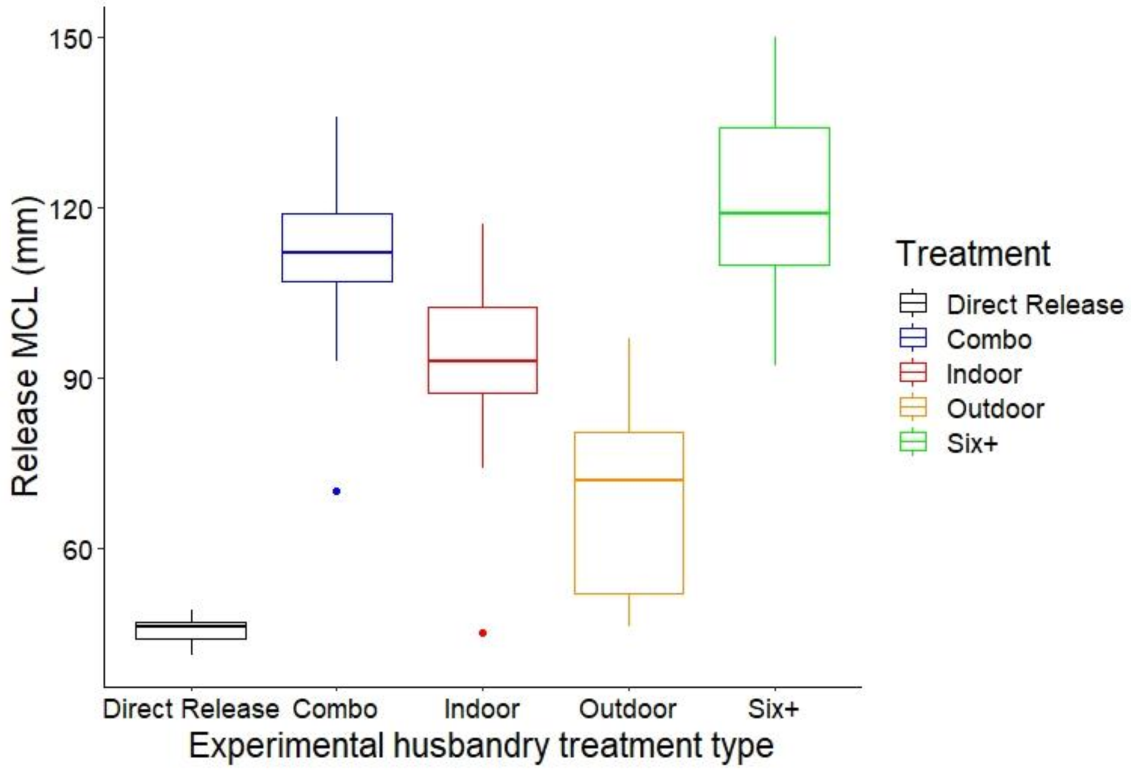

Fig. 3.1. Mean midline carapace length (MCL) (mm) of head-started Mojave desert tortoises (*Gopherus agassizii*) at time of release into Mojave National Preserve, San Bernandino County, CA. Boxplots show the average release MCL, upper and lower quantiles, and outliers.

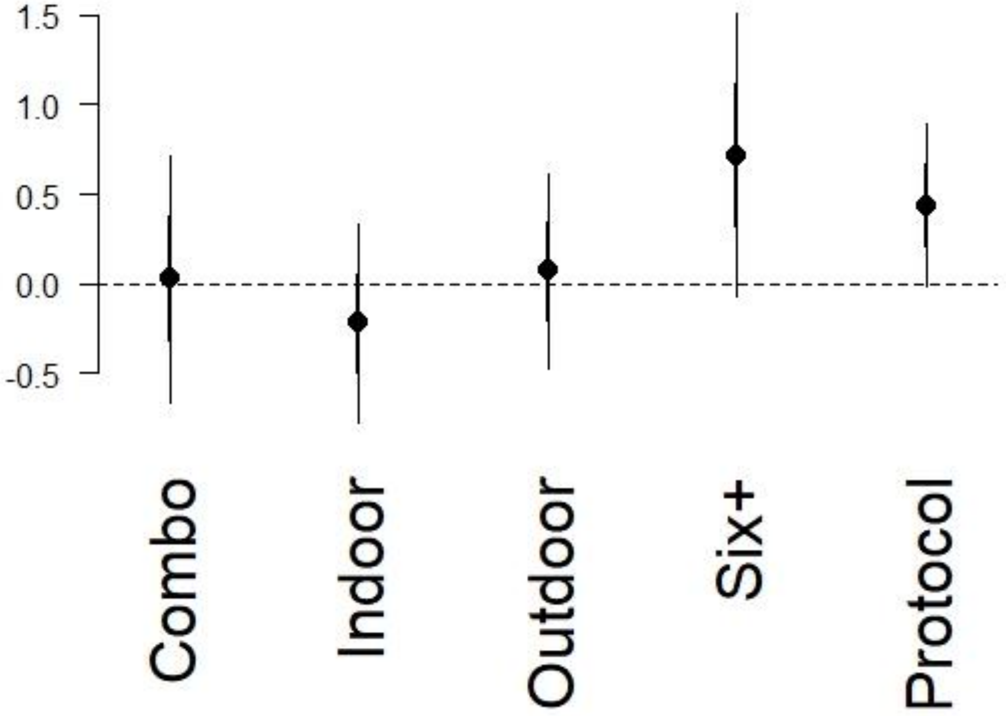

Fig. 3.2. Plot of the coefficients from the modeling of head-started juvenile tortoise survival as a function of experimental treatment from 2012-2022 and the effect of protocol type (pre-2016 vs. post-2016 protocols). The reference level is the direct release treatment. The thicker lines indicate ±1 standard deviation and the thinner lines indicate ±2 standard deviations from the estimates.

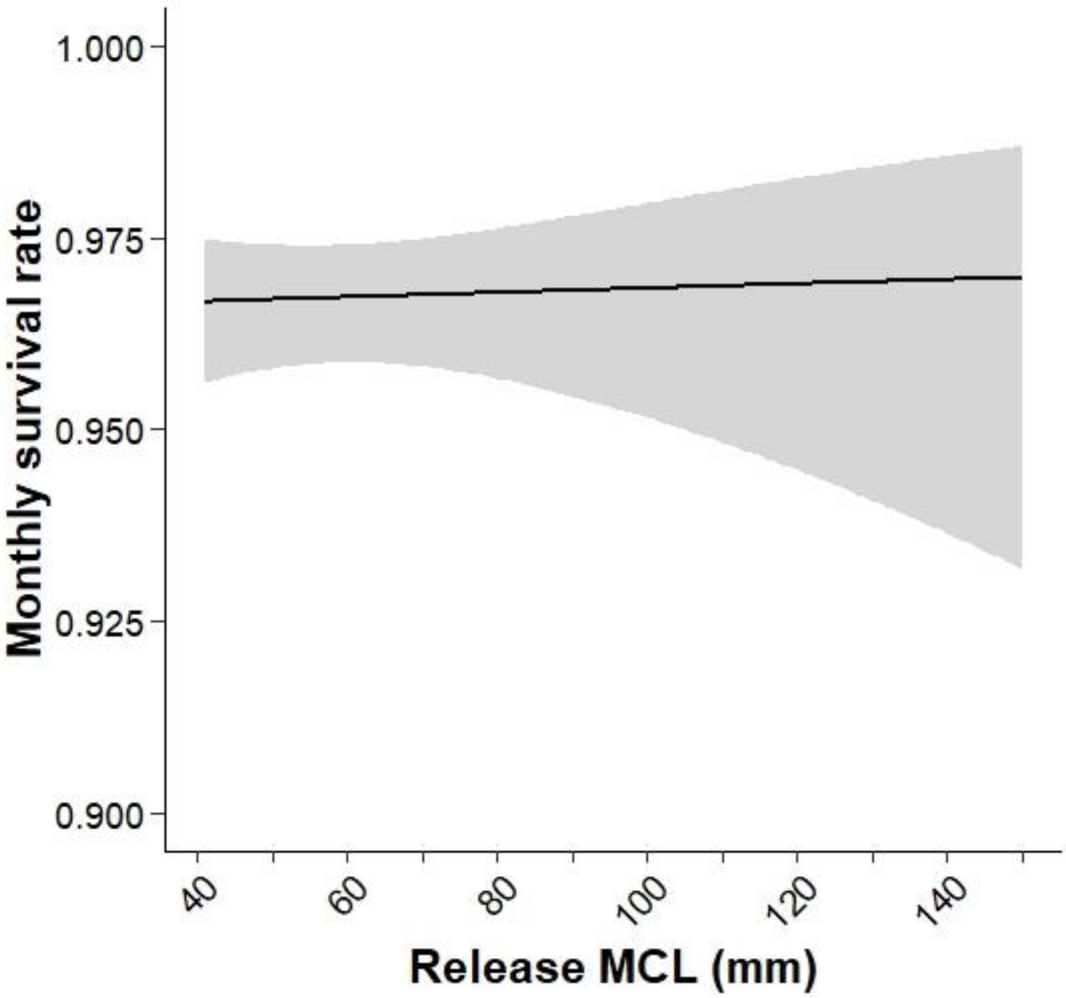

Fig. 3.3. Predicted survival rate of head-started Mojave desert tortoises (*Gopherus agassizii*) by midline carapace length (MCL) in mm at release. We tracked 324 tortoises over the course of the study from Sept 2012 – June 2022. Shaded areas indicate 95% confidence intervals.

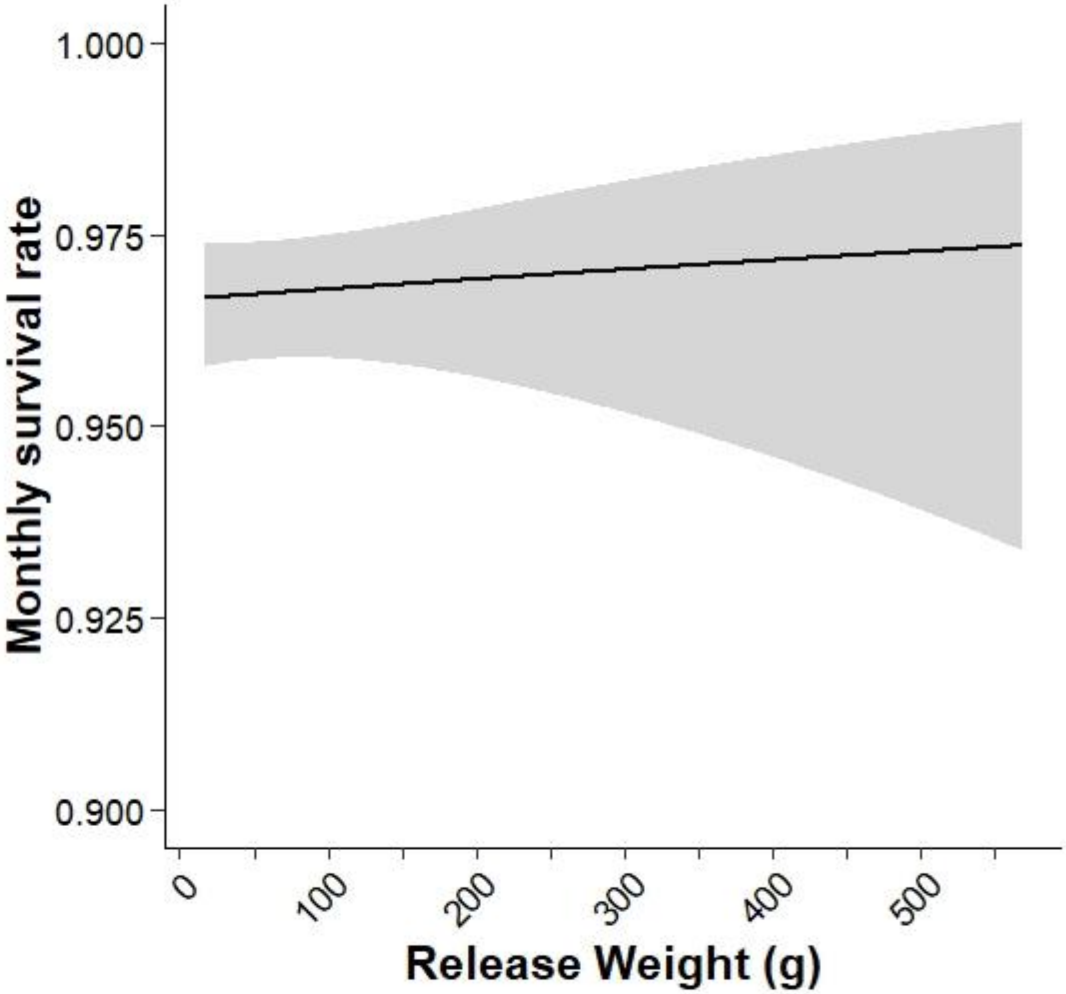

Fig. 3.4. Predicted survival rate of head-started Mojave desert tortoises (*Gopherus agassizii*) by weight in g at release. We tracked 324 tortoises over the course of the study from Sept 2012 – June 2022. Shaded areas indicate 95% confidence intervals.

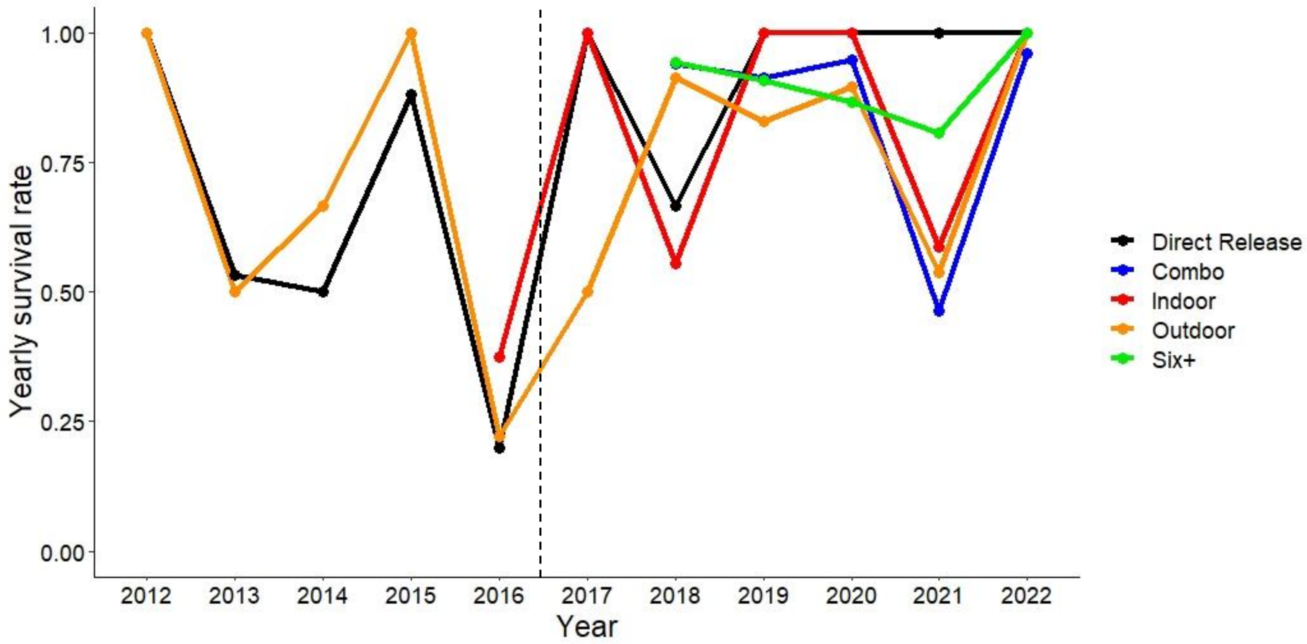

Fig. 3.5. Yearly raw survival rates of head-started Mojave desert tortoises (*Gopherus agassizii*) with known fate by experimental husbandry treatment. The dashed line indicates when our release protocols changed (post-2016) to releasing only in the fall and at a site >1.6 km away from raven nesting structures. Tortoises with unknown fates were excluded from this graph. Survival rates in 2012 reflect survival from September to December in that year, and survival rates in 2022 reflect survival from January to June in that year.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Head-starting as a conservation strategy has been criticized as being costly (Reed et al. 2009), a “half-way” technology because it does not solve the original problem of adult decline (Frazer 1992), and of having a low success rate (Dodd and Seigel 1991). However much of this criticism has been given without allowing enough time to fully evaluate the effects of head-starting in a long-lived species (Pritchard 1981, Allen 1990). More recently, head-starting programs have been shown to be beneficial tools in combination with other conservation efforts that increase subadult and adult survival for long-lived species (Heppell et al. 1996, Burke 2015, Shaver and Caillouet 2015, Spencer et al. 2017). Head-starting of desert tortoises for population recovery has been evaluated and improved upon for almost 30 years. Outdoor-rearing of tortoise was first explored by Morafka et al. (1997), and successive developments in husbandry practices, such as supplemental food and water (Nagy et al. 2015b, Tuberville et al. 2019), improved tortoise health and growth in captivity. Indoor-rearing was then explored as a method of speeding up the rearing process by keeping tortoises active and growing through the winter dormancy period (Daly et al. 2019). To build on the momentum of indoor-rearing, McGovern et al. (2020a) tested the efficacy of combo-rearing, which is the rearing of tortoises inside for one year to facilitate rapid growth, and then one year of outdoor-rearing to provide tortoise with the opportunity to experience environmental cues. The combo-rearing method was successful at producing tortoises that were healthy, significantly larger than outdoor-reared tortoises of the same age, and survived well post-release. However, it is unclear the extent to which this success can be attributed to either the tortoises' size, other factors associated with head-starting experience, or environmental conditions tortoises experience following release. These previous studies created the foundation of continued head-starting improvement that this thesis examined. We explored the improvement of Mojave Desert tortoise head-starting through evaluating the second year of combo-rearing and the relative role of climatic factors on survival of tortoises post-release.

In Chapter 2, we compared two head-starting treatments – one year of indoor-rearing versus two years of combo-rearing (one year indoors to grow rapidly, one year outdoors to gain wild behaviors) – to determine whether similar behavioral and survival outcomes can be achieved without the second year outdoors. We found that rearing head-starts solely indoors resulted in tortoises with higher variability in movement when compared to combo-rearing, therefore combo-reared tortoises had higher site fidelity. This variability did not result in a difference in the first-year survival, however. There was also no meaningful difference in the settling behaviors (building first burrow, initiating winter dormancy, spring emergence) between the two husbandry treatments. From these results, we concluded that either indoor- or combo-rearing would be successful methods of head-starting tortoises, but if researchers want to increase the site fidelity of released tortoises, combo-rearing would be the better choice.

In Chapter 3, we evaluated the effect of climatic conditions, especially conditions of drought, and experimental treatment on tortoise survival post release. We used multiple years of radio-tracking data from 324 juveniles reared under different head-starting treatments released from 2012-2020. We found that the increasing severity of drought decreased tortoises' probability of survival significantly, regardless of husbandry treatment type. Tortoise survival also decreased with an increased number of days in which maximum temperatures exceed >35°C per month. Increasing precipitation had a positive, but weak, effect on tortoise survival. Over the study period, the estimated survival probability of tortoises in each experimental treatment were similar, indicating head-started tortoises survived well post-release and head-starting in general can be a successful conservation tool.

Together, the two components of this thesis help inform future head-starting operations, and provides a stepping stone to further optimization of the technique. Further climatic modeling would be especially useful as the Mojave desert likely faces continued drought conditions in the near future (Overpeck and Udall 2020, Williams et al. 2022). Head-starting will continue to be a helpful tool along with other conservation efforts to restore populations of the important Mojave Desert tortoise.

LITERATURE CITED

Allen, C. H. 1990. Give “headstarting” a chance. Marine Turtle Newsletter **51**:12-16.

Burke, R. L. 2015. Head-starting turtles: learning from experience. Herpetological Conservation and Biology **10**:299-308.

Daly, J. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, and T. D. Tuberville. 2019. Survival and movements of head-started Mojave desert tortoises. Journal of Wildlife Management **83**:1700-1710.

Dodd, C. K., Jr, and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica **47**:336-350.

Frazer, N. B. 1992. Sea turtle conservation and halfway technology. Conservation Biology **6**:179-184.

Heppell, S. S., L. B. Crowder, and D. T. Crouse. 1996. Models to evaluate headstarting as a management tool for long-lived turtles. Ecological Applications **6**:556-565.

McGovern, P. A., K. A. Buhlmann, B. D. Todd, C. T. Moore, J. M. Peaden, J. Hepinstall-Cymerman, J. A. Daly, and T. D. Tuberville. 2020. Comparing husbandry techniques for optimal head-starting of the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **15**:626-641.

Morafka, D. J., K. H. Berry, and E. K. Spangenberg. 1997. Predator-proof field enclosures for enhancing hatchling success and survivorship of juvenile tortoises: a critical evaluation. Pages 147–165 *in* Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles: An International Conference

Conservation. Wildlife Conservation Society Turtle Recovery Program and the New York Turtle and Tortoise Society, New York, New York, USA.

Nagy, K. A., S. Hillard, S. Dickson, and D. J. Morafka. 2015. Effects of artificial rain on survivorship, body condition, and growth of head-started desert tortoises (*Gopherus agassizii*) released to the open desert. Herpetological Conservation and Biology **10**:535-549.

Overpeck, J. T., and B. Udall. 2020. Climate change and the aridification of North America. Proceedings of the National Academy of Sciences of the United States of America **117**:11856-11858.

Pritchard, P. C. H. 1981. Criteria for scientific evaluaticn of head-starting. Pages 3-4 Marine Turtle Newsletter.

Reed, J. M., N. Fefferman, and R. C. Averill-Murray. 2009. Vital rate sensitivity analysis as a tool for assessing management actions for the desert tortoise. Biological Conservation **142**:2710-2717.

Shaver, D. J., and C. W. Caillouet. 2015. Reintroduction of Kemp's ridley (*Lepidochelys kempii*) sea turtle to Padre Island National Seashore, Texas and its connection to head-starting. Herpetological Conservation and Biology **10**:378-435.

Spencer, R. J., J. U. Dyke, and M. B. Thompson. 2017. Critically evaluating best management practices for preventing freshwater turtle extinctions. Conservation Biology **31**:1340-1349.

Tuberville, T. D., K. A. Buhlmann, R. Sollmann, M. G. Nafus, J. M. Peaden, J. A. Daly, and B. D. Todd. 2019. Effects of short-term, outdoor head-starting on growth and survival in the Mojave Desert tortoise (*Gopherus agassizii*). Herpetological Conservation and Biology **14**:171-184.

Williams, A. P., B. I. Cook, and J. E. Smerdon. 2022. Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. Nature Climate Change.