INDOOR-REARING AS A COMPONENT OF HEAD-STARTING THE MOJAVE DESERT TORTOISE (*GOPHERUS AGASSIZII*)

by

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(Under the Direction of Tracey D. Tuberville and Clinton T. Moore)

ABSTRACT

The Mojave Desert Tortoise population is declining and rearing neonate tortoises until they are more likely to survive (head-starting) is one strategy for population augmentation. In 2015 we began evaluating indoor-rearing to shorten the head-start period. We assigned 70 hatchlings to three treatment groups: indoor-reared (n=30), outdoor-reared (n=20), and directly released (n=20). Direct-release hatchlings were released in September 2015 and head-starts (outdoor- and indoor-reared) were captive for 7 months before release in April 2016. We evaluated size among groups before release and we monitored tortoises with radio-telemetry post-release until 27 October 2016. Indoor head-starts reached the size of 6-year-old wild tortoises by release but survival did not differ among treatment groups. Combined (all treatment groups) annual survival was 0.44 (95% CI, 0.34–0.58). Bird predation was the leading cause of mortality and tortoises had highest predicted survival (over 58 weeks) if they were >1.6 km from an active raven nest.

INDEX WORDS: Turtle, Desert tortoise, *Gopherus agassizii*, Head-starting, Conservation, Survivorship, Recruitment, Reintroduction, Reptile conservation

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Turtles are among the most threatened group of vertebrates (IUCN 2007). Habitat loss, harvest for human consumption and the pet trade, and road mortality (being hit by vehicles) are among the leading causes of decline among turtles globally (Gibbons et al. 2000, Gibbs and Shriver 2002). Other factors harming chelonians are increased desertification from global climate change (Lovich et al. 2014) and the spread of invasive species (Márquez et al. 2013, Drake et al. 2016). Chelonians have high mortality in their early life stages (eggs and hatchlings), but they usually have high survival as adults, and they reach reproductive maturity late in life, limiting their ability to respond to increased mortality of juveniles or adults (Congdon et al. 1994, Heppell et al. 1996, Heppell 1998).

Head-starting is a management tool that protects animals from predation during a vulnerable part of their life, before releasing them when they are larger, and presumably more likely to survive (Buhlmann et al. 2015, Burke 2015, Nagy et al. 2015). Turtles may be uniquely suited to head-starting because they have low survival in their early life stages, but high survivorship as adults (Tinkle et al. 1981, Congdon et al. 1994), and they require no parental care after hatching (Burke 2015). Population modeling suggests that head-starting can only be effective if it reduces juvenile mortality substantially; reducing egg and hatchling (first year) mortality alone is not likely to be successful in increasing population growth (Congdon et al. 1994). Also, increasing juvenile survival cannot effectively mitigate against factors that elevate adult mortality (Heppell et al. 1996). Furthermore, juvenile turtles' crypticity makes it difficult to evaluate the success of head-starting without intensive post-release monitoring (Reed et al. 2009). Thus, some have questioned the viability of head-starting (Heppell et al. 1996, Reed et al. 2009). However, head-starting may be useful in augmenting recruitment in areas where causes of increased adult and subadult mortality have been ameliorated if post-release monitoring shows appreciable increases in juvenile survival among head-starts (Buhlmann et al. 2015, Tuberville et al. 2015).

Desert tortoises are long-lived, large fossorial herbivores in the American southwest (Woodbury and Hardy 1948). The Mojave species of desert tortoise (*Gopherus agassizii*) has experienced precipitous declines across its range in the past several decades (Berry 1986, USFWS 1990). Causes of population decline include habitat loss (Berry 1986), spread of upper respiratory tract infection (Lenderle et al. 1997, Sandmeier et al. 2009), hyper-predation from ‘subsidized predators’ whose populations are increased through eating human-provided food (Boarman 2003, Esque et al. 2010), road mortality (Nafus et al. 2013, Peaden et al. 2015), and the spread of invasive grasses which are poorer quality food compared to native annual plants and which facilitate spread of wildfires (Drake et al. 2016).

Since the federal listing of the Mojave Desert Tortoise in 1990, there have been several management actions proposed in order to stabilize population decline, including restricting off-road driving on public lands, installing exclusion fencing along roadways, restoring degraded habitat, relocating individuals from areas slated for development, and head-starting (USFWS 1990, 2011). Although preserving habitat is a prerequisite in any effort to conserve desert tortoises, other manipulative techniques may be helpful as part of a multi-faceted management plan, including measures that increase recruitment (USFWS 1990, 2011). Because subadult and adult desert tortoises naturally have low risk of mortality, increasing the number of individuals that recruit into the subadult life stage through head-starting may be effective in increasing the growth rate of small populations (Reed et al. 2009), but post-release monitoring is essential to evaluate its efficacy.

Although head-starting may hold potential, its effectiveness in desert tortoises is little known. As head-start programs are already underway, it is important to assess the viability of this management practice, as conservation funds are limited and head-starting is resource intensive (Nagy et al. 2015*b,* Todd et al. 2015). The current method for head-starting desert tortoises involves: (1) obtaining eggs by radiographing wild females to detect calcified eggs, (2) bringing gravid females into predator-proof pens and allowing them to deposit their eggs in natural nests, (3) releasing the females and allowing the eggs to incubate naturally in the pens, and (4) rearing the hatchlings in outdoor predator-proof pens until they reach approximately 110 mm in midline carapace length (MCL; Nagy et al. 2015, Todd et al. 2015). Under natural conditions it may require 5–7 years to produce a predator resistant hatchling (>110 MCL); however preliminary results from supplemental feeding and artificial rain hold some promise in accelerating growth rates (Nagy et al. 2015*b*, Todd et al. 2015).

In addition to supplemental rain and food, rearing head-start hatchlings indoors and allowing them to stay active through the winter (rather than brumating in outdoor pens) has potential to extend the growing season, and thus reduce time in captivity (Buhlmann et al. 2015, Tuberville et al. 2015). This approach has proven successful in obtaining growth rates of approximately 70% (increase in MCL) during one over-wintering period in Gopher Tortoise (*Gopherus polyphemus*; Tuberville et al. 2015) and Blanding's Turtle (*Emydoidea blandingii*; Buhlmann et al. 2015) head-starts. High growth rates have also been observed in over-wintered captive desert tortoise hatchlings (kept active throughout the winter; Jackson et al. 1976);

however, tortoises in that study were fed an unsuitable diet of lettuce and tomatoes and they were not offered humid hide structures, thus leading to significant deformation (pyramiding) of the shell. Pyramiding of the shell has been shown to come from lack of humidity (Wiesner and Iben 2003). In our study, we provide head-starts with expert recommended (Jarchow et al. 2002) diet of readily available leafy greens and pre-mixed tortoise diet, and provide humid hide boxes to facilitate proper water balance and prevent shell pyramiding. Certainly there is the potential to increase growth through indoor-rearing, but monitoring other fitness outcomes such as physiological metrics (i.e., shell hardness and body condition) and post-release success will be key in evaluating indoor rearing in head-starting.

Evaluating body condition and shell hardness of head-started tortoises is important to insure that the action does not compromise tortoise health and viability (Tuberville et al. 2008, Nagy et al. 2011, 2015*b*). Shell hardness is important because calcification of the shell confers protection from predators (Kristan and Boarman 2003, Nagy et al. 2011, 2015*a*). Therefore, increasing shell hardness is one of the primary goals of head-starting. Body condition is important because it reflects tortoises' water balance and energy stores (Nagy et al. 2002, Loehr et al. 2007). Individuals with high body condition have large water reserves and are robust against seasonal water scarcity (Nagy et al. 2002, Loehr et al. 2007). Therefore, we monitor body condition among head-starts and compare to a free-ranging, directly released control group.

Selection of a proper release site is essential to the success of head-starting. Desert tortoises are most abundant in sandy alluvial valleys, 600–1200 m in elevation, where soils are friable for digging their extensive burrows, and where creosote (*Larrea tridentata*) is dominant (Woodbury and Hardy 1948, Luckenbach 1982, Nussear et al. 2009). Furthermore, at a finer scale, juvenile desert tortoises select for areas with large creosote bushes, fine soil grains, and abundant small mammal burrows in which to hide (Todd et al. 2016). In addition to meeting habitat requirements, release sites must be logistically accessible to facilitate monitoring, yet far enough from human activity to reduce predation pressure from subsidized predators such as ravens (*Corvus corax*) and coyotes (*Canis latrans*). Coyotes and ravens are considered 'subsidized,' because their numbers are artificially inflated in some areas by their consumption of human-produced food sources such as trash and agriculture (Boarman 2003, Esque et al. 2010). Ravens are drawn to powerlines and other raised structures, so selecting a release site that is far enough from these types of structures is important in mitigating predation risks (Kristan and Boarman 2003). After we initiated our experimental juvenile tortoise release, we noted a pair of ravens rearing young near our release plot. We used this opportunity to examine the effect of tortoises' proximity to an active raven nest on survival probability.

When animals are raised in captive conditions, there is often the concern that they will not know how to properly use cover and mediate activity to reduce predation and exposure risks. Head-started juvenile ploughshare tortoises (*Astrochelys yniphora*) used vegetative cover less often during the day than did their wild counterparts, but this difference in cover use did not appear to affect survivorship (Pedrono and Sarovy 2000). Monitoring cover use of head-started juveniles will be important to understand the effect that captive rearing may have on natural refuge-seeking behavior. Neonate tortoises are particularly vulnerable to predators, as their shells are soft, so their cryptic size and coloration are important in helping them avoid detection. An experimental study by Nafus et al. (2015) found that captive tortoises associated with rocks that were of similar size to their body rather than bare sand, in an apparent effort to blend in. Understanding whether head-starts achieve crypticity on the landscape—especially since they are unnaturally large for their age—could be important in understanding their predation risk. Here we monitor surface activity, cover use, and the number of 'tortoise-sized rocks' near head-start tortoises and our direct-release control group to investigate any effects of head-start rearing. We also used post-release surface activity level and cover use as predictors of individual fate to better understand how these behaviors could affect mortality risk.

Dispersal, or movement just after release, is a vulnerable time for head-start hatchlings, because they are at higher risk for predation and exposure while on the move (Hazard and Morafka 2002). Larger moves may allow animals to assess resource conditions and conspecific occupancy of their homes, but they also increase exposure and predation risk (Farnsworth et al. 2015). Temporarily penning animals in enclosures at the release site, or 'soft-release,' has shown to increase site fidelity in translocated turtles (Tuberville et al. 2005, Attum and Cutshall 2015), but pens have the potential to attract the attention of predators (Quinn 2016) and are sometimes logistically unfeasible to install (as is the case here). Several studies have monitored post-release movement of head-started tortoises. In a pilot study by Hazard and Morafka (2002), twelve head-started juvenile desert tortoises (ages 6–8 years) exhibited homing behavior immediately following their release; they moved toward their hatchery but eventually settled down close to their release sites (mean = 116 m) and all survived their first winter. A reintroduction study in Madagascar found relatively high site fidelity in head-started ploughshare tortoises (Pedrono and Sarovy 2000). Post release monitoring of 15 head-started Mojave Desert Tortoises found that most settled down for brumation within 150 meters of their release sites, with only three individuals settling down 200–600 m away (Nagy et al. 2015*a*). Here, we monitor post-release movement patterns of indoor and outdoor head-started juveniles compared to a direct-released control group to understand how rearing treatments may affect movement (and thus predation and exposure risks).

Ultimately, the goal of head-starting is to increase survival. However, because neonate tortoises are small and cryptic, they are notoriously difficult to study (Bjurlin and Bissonette 2004, Tuberville et al. 2015), so there are few estimates of juvenile desert tortoise survival. Because of their crypticity, evaluating success of desert tortoise head-starting with encounter surveys is unfeasible (Reed et al. 2009), so monitoring survival of head-starts through radio-telemetry is essential (Hazard et al. 2015, Nagy et al. 2015*a*, Tuberville et al. 2015). A study of 51 released head-started desert tortoises, age 2–15 y, found overall (all ages) survival of 79% percent for the first-year post-release, with smaller individuals (<110 mm in MCL) being more likely to die (Nagy et al. 2015*a*). An analysis of 16 outdoor-reared head-started tortoises (age 8–9 y) at the same site by Hazard et al. (2015) found that 7/16 (44%) of tortoises were predated by a single raven over six weeks, but that no head-starts over 84 mm and 125 g died. These head-start studies suggest that there is a threshold in size (whether 84 mm or 110 mm in MCL or some mass) at which head-starts become robust against predation and sizeable increases in survival are achieved (Hazard et al. 2015, Nagy et al. 2015).

Understanding natural baseline survival of neonate desert tortoises is important in drawing conclusions about any increase in survival afforded by head-starting. A field study by Bjurlin and Bissonette (2004) found that wild-hatched neonate desert tortoises had 84–91% survival from hatching to the onset of winter inactivity. This suggests that first-year survival may be much higher than previously thought; or that most first-year mortality occurs in spring (as fall survival was high); but they did not follow these hatchlings all year. Estimates of apparent annual survival (where missing animals were assumed dead) from previous cohorts of young tortoises (age 0–18 months) released by our research group ranged widely from year to year, and by release group (0.22–0.60; Todd et al. 2015). However, most mortality took place in spring and summer, with fall-released juveniles having 90-100% survival during initial dispersal (until brumation; Todd et al. 2015), supporting the finding of high fall survivorship by Bjurlin and Bissonette (2004).

Here, we use a comparative experimental approach to evaluate the efficacy of indoor rearing relative to outdoor-rearing and direct-release. We define three treatment groups: 1) head-started indoors for seven months, 2) head-started outdoors in predator-proof pens for seven months, and 2) directly released into the natural environment, which we considered as a treatment control. We monitor growth, body condition, and shell hardness index during the rearing period, and then monitor survival, activity, movement, and microhabitat use post-release with radio-telemetry. We compare growth and body condition among treatment groups during the head-start period, and evaluate any differences in movement, activity, and micro-habitat use post-release among tortoises from the 2015 cohort. We compared shell hardness in our indoor head-starts to shell hardness of older (3-4 y old) but similar-sized juveniles that had been reared in outdoor pens. We estimate survival of each treatment group, and use a predictive fate model to evaluate potential risk factors at the individual level.

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CHAPTER 2

COMPARING GROWTH AND PHYSIOLOGICAL CONDITION OF INDOOR-REARED, OUTDOOR-REARED, AND DIRECTLY RELEASED JUVENILE MOJAVE DESERT TORTOISES

INTRODUCTION

Head-starting seeks to increase the number of animals eventually recruited into the breeding population by rearing animals in protected conditions during their early life stages and releasing them into the natural environment at a larger size when they are presumably more likely to survive (Heppell et al. 1996, Burke 2015). Head-starting has been attempted with varying success with mammals (Sinn et al. 2014), birds (Cohn 2016), amphibians (Lannoo 2005), and reptiles (Jarvie et al. 2015, Tuberville et al. 2015). Turtles may be particularly suited to head-starting because they have low survivorship in the wild during their early life stages and high survivorship as adults under most natural conditions (Burke 2015). Head-starting has been attempted in several turtle species, including Blanding's Turtles (*Emydoidea blandingii*; Green 2015; Buhlmann et al. 2015), Gopher Tortoises (*Gopherus polyphemus*; Tuberville et al. 2015), Western Pond Turtles (*Actinemys marmorata*; Vander Haegen et al. 2009), and Kemp Ridley's Sea Turtles (*Lepidochelys kempii*; Caillouet Jr. et al. 2015). However, evaluating the efficacy of head-starting is difficult and modeling shows that head-starting cannot stabilize population growth rate under high levels of adult or subadult mortality (Heppell et al. 1996, Reed et al. 2009).

Mojave Desert Tortoises (*Gopherus agassizii*) are in decline throughout their range (Berry 1986, USFWS 1990). Habitat loss, road mortality, increased depredation from human subsidized predators (i.e., Common Ravens [*Corvus corax*] and Coyotes [*Canis latrans*]), spread of upper-respiratory tract disease, and habitat degradation from unnatural disturbance and invasive plants have all been identified as contributing causes (Berry 1986, Esque et al. 2010, Nafus et al. 2013).

Head-starting has been identified as a possible management action to help augment existing populations of Mojave Desert Tortoises (USFWS 2008, 2011), providing that the original causes of population decline have been mitigated. Several desert tortoise head-starting facilities have begun evaluating the efficacy of rearing hatchling tortoises in predator-proof outdoor pens before releasing them into the wild (Hazard and Morafka 2002, Nafus 2014, Nagy et al. 2015b). Estimates of the size threshold at which predation vulnerability of juvenile tortoises is reduced range from 84 mm (Hazard et al. 2010) to 120 mm in midline carapace length (MCL; B. Henen, Marine Corps Air Ground Combat Center Twentynine Palms, personal communication). Although supplemental feeding and artificial rain have the potential to increase growth in desert tortoises raised outdoors (Nafus 2014, Nagy et al. 2015*a*), outdoor rearing may take 5–9 years to produce a head-start of recommended size. Rearing tortoises indoors has the potential to decrease the time needed to raise tortoises to larger size by keeping juveniles active and growing during the winter months when growth would otherwise cease. No study has yet evaluated the effectiveness of indoor head-starting in desert tortoises.

Accelerating growth of captive tortoises by raising them indoors may have unknown consequences for the overall health and physiological condition of the animals. Thus, head-start studies should also monitor other physiological metrics such as body condition and shell hardness. Body condition (BC) is an estimate of an animal's body density and reflects its nutritional condition and water balance (Nagy et al. 2002, Loehr et al. 2007). The hardness of a turtle's shell plays a major role in its protection against predators (Nagy et al. 2011) and has been positively correlated with survivorship (Shine et al. 2001). In general, shell hardness increases with body size in juvenile desert tortoises (Nagy et al. 2011).

The goal of our study was to evaluate the feasibility of indoor rearing to reduce the time needed to head-start desert tortoises relative to outdoor rearing. We defined three treatment groups: indoor reared head-starts, outdoor reared head-starts, and directly released neonates (all from the 2015 cohort). We reared head-start treatment groups for seven months prior to release, and direct release neonates were placed in the natural environment shortly after hatching to serve as a control. We compared growth (mass and length) and body condition among the three treatment groups, and evaluated indoor-reared head-starts for shell hardness relative to similarly sized, but older (4-5 year-old), outdoor reared head-starts. We hypothesized that indoor-reared head-starts would grow faster and have higher body condition than either the direct-release or outdoor reared head-starts from the same cohort.

METHODS

Study site

The Mojave National Preserve is a 650,000 ha preserve in San Bernardino County, in the eastern Mojave Desert of southern California, managed by the National Park Service (NPS). All experiments and observations in this study were conducted in Ivanpah Valley in the northeastern part of the Mojave National Preserve. The primary habitat in Ivanpah Valley is “creosote bush scrub,” and is dominated by Creosote Bush (*Larrea tridentata*), White Bursage (*Ambrosia dumosa*), and low density *Yucca* (*Y. schidigera, and Y. brevifolia*; Turner et al. 1984, Todd et al. 2016). Although tortoises are commonly seen in Ivanpah Valley and habitat suitability is relatively high (Nussear et al., 2009), current tortoise densities are much lower than they were historically (77 – 85 tortoises per km^2 in 1977 to 1980; Turner et al. 1984). The area into which we released juvenile tortoises was a 0.7 km^2 plot centered 850 m from a powerline service road (plot center: N35° 19' 18.7" W115° 23' 58.3"). Other human disturbances within our study area include abandoned cattle grazing infrastructure (fencing and corrals), and a railroad grade approximately 4.5 km to the southeast.

Obtaining hatchlings

In May 2015 we captured female desert tortoises and brought them to the Ivanpah Desert Tortoise Research Facility (IDTRF) and radiographed them to determine whether they were gravid. Gravid females with at least three eggs were then transferred to predator-proof nesting pens at the IDTRF; all others were immediately returned to their capture sites. The nesting enclosure measured 30 x 30 m and was permanently subdivided with metal siding into 18 smaller pens (5 x 9 m). We constructed artificial burrows for each female tortoise to use as shelter and nesting sites. Burrows were at least 1 m in length and were constructed from 12-inch (310 mm) diameter cardboard Quik-Tube building forms® (Quikrete International, Atlanta, GA) that were cut in half longitudinally and buried at a 30 degree angle underground. We kept gravid females until they laid their eggs (within 30 d in most cases) and then returned them to their capture locations. As hatching approached (70 d into the estimated 90 d incubation period), we began monitoring pens for emerged hatchlings several times each day.

We brought all emerged hatchlings inside and temporarily housed them by clutch until all hatchlings had emerged. We permanently marked each neonate by using nail clippers to notch the marginal scutes with a unique identification pattern (modified from Cagle 1939). Four hatchlings were removed from the study due to especially low body mass at hatching and/or developmental defects and are not considered further. We assigned healthy neonates to one of three treatment groups (described further below): 1) directly released (control group), 2) outdoor-reared, and 3) indoor-reared. Within each clutch, we randomly assigned individuals to treatment groups, attempting to divide each clutch as evenly as possible among treatment groups to avoid confounding clutch and treatment effects. As we made assignments, we kept a running tally of what proportion of each treatment group was populated. For any clutch not in a multiple of three, we randomly assigned the larger number of hatchlings to the treatment-group with fewest individuals in the tally. If there was a tie regarding which group was behind in the tally, we randomized which group got the larger number of hatchlings. When more than one hatchling from a clutch was assigned to a treatment, clutch-mates were distributed among replicate enclosures receiving the same treatment.

Experimental treatment groups

Indoor-reared

On 24 September 2015, approximately 21–46 d after hatching, we moved the indoor-reared head-start treatment group (hereafter indoor HS) to mesocosms inside the climate-controlled IDTRF. We set ambient temperature inside the IDTRF to a constant 24.4 °C. We used 50-gallon (189 L) Rubbermaid stock tanks (132 x 79 x 30.5 cm) filled with a layer of natural desert sand as substrate (Figure 2.1). We established six tanks, each of which housed five tortoises. We suspended Mini Combo Deep Dome Dual Lamp Fixtures (ZooMed Laboratories Inc., San Luis Obispo, California) over tanks and each held a 50 Watt ZooMed Repti Basking Spot Lamp bulb for daytime basking and a ZooMed 50 Watt Infrared Basking Spot bulb on the other side for night-time heat (Figure 2.1). Lights were connected to automatic timers designed to approximate natural photoperiod. The basking lights were timed to operate between 0600 and

1830 h, and the infrared lights were timed to operate between 1900 and 0530 h. The lights created basking spots of 37 °C during the day and 32 °C at night.

Each tub was outfitted with the three hides constructed from halved plastic pipe (11.5 cm in diameter and cut into 12-cm linear segments) and a paper feeding plate (Figure 2.1). Because inadequate humidity has been linked to 'pyramiding' of the shell as tortoises grow (Wiesner and Iben 2003), we provided a humid hide box in each mesocosm to promote smooth shell growth. We maintained humidity in the hides by cutting burrow-shaped entrance holes (one hole per hide) into the sides of lidded plastic tote boxes (Rubbermaid "Roughnecks"; 40 x 26 x 18 cm) and lined each tote box with approximately 7 cm of moist peat moss, which we re-moistened with a spray bottle every 3–4 d.

We fed the hatchlings ad libitum five times per week and soaked them weekly for 15 minutes in 1-2 cm of water, allowing them to drink. The diet was a mixture of five leafy greens supplemented with ZooMed Grassland Tortoise Diet (ZooMed Laboratories Inc., San Luis Obispo, California), a commercially available food pellet. The mixture consisted of, by mass, 50% leafy green mixture (see below), 25% food pellets, and 25% water (used to soften the pellets). At each feeding, the greens mixture consisted of equal amounts of five leafy greens readily available at grocery stores and selected to closely approximate the nutritional properties of the desert tortoise's natural forage. Collectively, they are high in calcium but low in protein (Jarchow et al. 2002). Our preferred choices of greens included dandelion (*Taraxacum officinale*), mustard greens (*Brassica juncea*), turnip greens (*Brassica rapa* var. *rapa*), collards (a cultivar of *Brassica oleracea*), and endive (*Cichorium endivia*). If one of the preferred choices was unavailable at the grocery store, kale (a cultivar of *Brassica oleracea*) or Swiss chard (*Beta vulgaris cicla*) was used as a substitute. Swiss chard contains oxalate and phytate which bind to calcium and render it physiologically unavailable, and kale contains goitrogens that inhibits iodine uptake, so their inclusion in the diet was minimized (B. Bastarache, Bristol County Agricultural School, personal communication). On 11 December 2015 we also began adding a light dusting of supplemental Rep-Cal Calcium with Vitamin D3 (Rep-Cal Research Labs, Los Gatos, California) to their food mixture twice per week.

Outdoor-reared

On 23 September 2015 we moved the 20 hatchlings in the outdoor-reared head-start treatment group (hereafter outdoor HS) to predator-proof, semi-natural pens. The 30 x 30 m rearing enclosure was constructed of chain-link (with buried chain-link to exclude digging mammals), and was covered with netting to exclude avian predators (Figure 2.2). This larger enclosure was subdivided into nine 10 x 10 m pens with sheet metal dividers. We divided the 20 outdoor-reared hatchlings among two of these pens (10 hatchlings per pen). The pens mimicked the natural environment of tortoises and contained natural vegetation, sandy substrate, rocks, dead woody structure, and "starter burrows" (Figure 2.2). The starter burrows were constructed from halved PVC pipe (13 cm diameter) buried in a 2–3 m trench at a 30% decline angle from the surface.

We provided artificial rain once a week during the active season (until late October) for 30-min with rotating garden sprinklers to allow hatchlings to drink and to stimulate growth of natural vegetation (Beatley 1974). We also provided supplemental food on watering days because watering stimulated hatchling exploration and feeding. The food mixture formula was the same as described above for the indoor-reared hatchlings. The amount fed was 5% of the total tortoise biomass in each pen, which was functionally ad libitum, but which minimized excess food to avoid attracting ants or rodents. We ceased supplemental watering and feeding during the fall and winter when these outdoor hatchlings normally became inactive outdoors.

Direct-release group

On 28 September 2015 we released 20 hatchings from our direct-release treatment group (hereafter DR) to the natural environment in Ivanpah Valley where their mothers had been captured. We used radio-telemetry to monitor these free-ranging hatchlings for post-release growth and survivorship (only growth and physiological condition reported here). We attached radio transmitters (BD-2, Holohil Systems Ltd., Ontario, Canada) with 7-month batteries to each tortoise on the fourth vertebral scute with 5-minute epoxy. We used a 3-element Yagi antenna (AF Antronics, Inc., Urbana, IL) and a R1000 receiver (Communications Specialists, Inc., Orange, CA) to locate each animal daily for the first 4 d following release, and then twice per week through the duration of the active season (until 12 November 2015). Throughout the winter, tracking frequency was reduced to once per week.

Morphometrics

We measured and weighed hatchlings immediately after emergence ("hatching" size) and then again prior to treatment group assignment on 22 September 2015 ("initial" size). We measured and weighed indoor head-starts approximately every 30 d until their release in late April 2016. We measured and weighed DR tortoises again during 11-15 March 2016 when we replaced their radio-transmitters. We measured indoor- and outdoor HS animals on 16 March 2016 to facilitate comparison among the three treatment groups. The indoor and outdoor HS animals were weighed and measured again prior to release in late April 2016.

We recorded mass with a digital gram scale to the nearest 0.01 g. We measured the following to the nearest 0.1 mm using vernier calipers: (1) midline carapace length (MCL, straight-line distance from the anterior edge of the nuchal scute to the inside of the natural notch in the supracaudal scute), (2) maximum shell height, and (3) maximum shell width on the bridge.

Physiological condition

Body condition

We calculated body condition (BC) for all surviving animals from all treatment groups based on measurements taken 5.8 months into the rearing period using the formula described by Loehr et al. (2004):

$$Body\ condition\ (\frac{g}{cm^3}) = \frac{mass\ (g)}{shell\ volume\ (cm^3)}$$

We computed shell volume (for the BC calculations) using the formula described by Loehr et al. (2004), where all input sizes are in mm and the product is in cm^3.

$$Shell\ volume\ (cm^3) = \frac{\pi \times MCL \times width \times height}{6000}$$

Shell hardness

We used a 4-inch (10.2 cm) tension-calibrated micrometer (L.S. Starrett Company, Athol, Massachusetts, USA; model: 3732XFL-4) as described by Nagy et al. (2011) to measure normal, uncompressed shell height (UCSH) at the center of the third vertebral scute (Figure 2.3). We then turned the micrometer spindle, compressing each tortoise's shell between the two measuring faces until the spindle ratchet slipped, and continued to slip continually for approximately 240 degrees. We then read the micrometer for a compressed shell height (CSH) reading. We calculated shell hardness index (SHI) as described by Nagy et al. (2011), where an index value of 100 corresponds to complete hardness:

$$Shell\ Hardness\ Index\ (SHI) = \frac{CSH}{UCSH} \times 100$$

We measured shell hardness of surviving indoor HS animals (n=29) just prior to release in April 2016. Of the 2015 cohort juveniles, we were only able to measure the indoor HS group for shell hardness. The outdoor HS and DR juveniles were too soft to safely measure and we were concerned that compressing these smaller tortoises could cause injury. Therefore, we compared the indoor HS 2015 cohort juveniles' shell hardness with shell hardness data taken in September 2015 from similar sized, but much older, outdoor-reared animals (2011–2012 cohorts) from other enclosures at the IDTRF (described in Todd et al. 2015).

Statistical methods

All statistical tests were performed in Program R (R Core Team 2014), with alpha set at 0.05. We presented data in text as means ± 1 standard error. To test for differences among treatment groups (2015 cohort) in MCL, BC, and mass, we used linear mixed effects models (LME; 'nlme' package, 'lme' function in R), with the metric of interest as the response variable and mothers' unique ID code as a random effect (non-numeric factor). Visual assessment of histograms of model residuals showed that model residuals generally approximated the normal distribution, with the exception of one outlier (a very small individual in the indoor-reared treatment group). We ran our analyses with and without this outlier, and outcomes were unchanged, therefore we felt comfortable proceeding with a LME approach on our full dataset (including the outlier). When significant differences were detected, we used Tukey's post-hoc comparisons ('glht' function in the 'multcomp' package) to further investigate treatment group differences.

We used ANCOVA ('lm' function) to test for treatment group (indoor HS 2015 cohort, outdoor HS 2011 cohort, and outdoor HS 2012 cohort) effects on shell hardness, and included MCL as a covariate. When treatments effects were detected, we performed Tukey's post-hoc multiple comparisons using the ‘glht’ function in the ‘multcomp’ package to further investigate treatment group differences.

RESULTS

In May 2015, we captured 31 female desert tortoises and radiographed them to determine whether they were gravid. Twenty-five of the 31 females were gravid with at least three eggs and were transferred to predator-proof nesting pens at the IDTRF. The other six females were returned to their capture sites. The 25 females collectively laid 123 eggs, from which 74 hatchlings successfully emerged (60.2% emergence success).

Sample size and survival

Of 70 neonates assigned to treatments, 20 were assigned to the DR group, 30 to the indoor HS group, and 20 to the outdoor HS group. We selected an unbalanced design—including more indoor HS animals—because previous cohorts included in our head-starting program included only releases of outdoor HS juveniles and DR neonates. Fifteen of the 20 (75%) DR juveniles survived from their release on 28 September 2015 until spring measurements in mid-March 2016; four were found dead and one went missing (unknown fate) before mid-March. All (20/20) outdoor HS tortoises survived until their release on 25 April 2016. All indoor HS tortoises (30/30) survived in captivity until mid-March, when data were taken for comparative purposes. However, on 08 April 2016 one indoor HS was found dead in its mesocosm; thus, survival for indoor HS group was 96.7% (29/30) through the head-start period.

Size and mass

Mean MCL at the start of the rearing period was 46.9 ± 0.2 mm (n=70) and MCL did not differ among treatment groups ($F_{2,67} = 1.765$, $p > 0.1$; Table 2.1). After 5.8 months (mid-March

2016) mean MCL differed significantly ($F_{2,67} = 249.3$, $p < 0.001$; Figure 2.4; Table 2.1) among treatment groups (DR: 48.8 ± 1.4 mm; indoor HS: 78.2 ± 1.0 mm; outdoor HS: 50.6 ± 1.2 mm). Indoor HS tortoises were larger (MCL) than either outdoor HS or DR juveniles ($p < 0.001$ in both cases), whereas outdoor HS and DR juveniles did not differ in MCL ($p = 0.543$).

Mean growth in size (% increase in MCL) over 5.8 months was significantly different ($F_{2,67} = 342.6$, $p < 0.001$; Table 2.1; Figure 2.4) among treatment groups (DR: 4.3 ± 2.7%; indoor HS: 70.7 ± 2.0%; outdoor HS: 8.3 ± 2.4%). As with size measured in March, growth in MCL was significantly greater among indoor HS tortoises than outdoor HS or DR tortoises ($p < 0.001$ in both cases). Indoor HS tortoises grew over 16 times faster in length than DR tortoises and over 8 times faster than outdoor HS tortoises. Outdoor HS and DR juveniles did not significantly differ in MCL growth ($p = 0.374$; Table 2.1; Figure 2.4). By head-start release in late April 2016, mean MCL was 87.2 ± 1.1 mm for indoor HS tortoises and 51.9 ± 0.6 mm for outdoor HS tortoises (Table 2.2).

Mean initial mass was 22.6 ± 0.3 g (n=70) and did not differ among treatment groups ($F_{2,67} = 0.573$, $p = 0.566$; Table 2.1). After 5.8 months (mid-March 2016), mean body mass differed significantly ($F_{2,67} = 197.3$, $p < 0.001$; Table 2.1; Figure 2.5) among treatment groups (DR: 24.5 ± 3.7 g; indoor HS: 94.7 ± 2.7 g; outdoor HS: 30.2 ± 3.3 g). Indoor HS tortoises were significantly heavier than DR juveniles and outdoor HS tortoises ($p < 0.001$ in both cases), whereas outdoor HS and DR juveniles did not differ in mass ($p = 0.402$).

After 5.8 months (mid-March 2016), mass increase was significantly different ($F_{2,67} = 277.3$, $p < 0.001$; Table 2.1; Figure 2.5) among treatment groups (DR: 8.8 ± 6.6%; indoor HS: 147.8 ± 5.1%; outdoor HS: 17.3 ± 6.0%). As with absolute mass measured in March, growth in mass was significantly greater among indoor HS juveniles than outdoor HS or DR juveniles

($p < 0.001$ in both cases). Indoor HS juveniles gained mass at over 16 times the rate of DR juveniles and over 8 times faster than outdoor HS juveniles. Outdoor HS juveniles and DR juveniles did not significantly differ in mass growth ($p = 0.326$; Table 2.1; Figure 2.5). By head-start release in late April 2016, mean mass was 122.6 ± 4.4 g for indoor HS tortoises and 30.9 ± 1.0 g for outdoor HS tortoises (Table 2.2).

Body condition

Body condition at the start of the rearing period was 1.08 ± 0.01 g/cm^3 (n=70) and did not differ among treatment groups ($F_{2,67} = 0.094$, $p = 0.91$; Table 2.1). After 5.8 months (mid-March 2016) mean BC differed significantly ($F_{2,67} = 6.498$, $p = 0.0032$; Table 2.1; Figure 2.6) by treatment group (DR: 1.08 ± .02 g/cm^3; indoor HS: 1.06 ± 0.01 g/cm^3; outdoor HS: 1.12 ± 0.01 g/cm^3). Indoor HS tortoises had lower BC than did outdoor HS tortoises, but DR juveniles were not different in BC from either indoor or outdoor HS animals ($p > 0.1$ in both cases) due to high variability in BC within the DR group. Analysis of subsequent BC just prior to release (late April) revealed that the significant difference BC between indoor and outdoor HS tortoises persisted ($F_{1,33} = 10.215$, $p = 0.0031$; Table 2.2).

Shell hardness

Size was a significant predictor of shell hardness ($p < 0.001$). For every 1.0 mm increase in MCL, shell hardness increased by 0.21 (Figure 2.7). Indoor HS tortoises (2015 cohort) had mean shell hardness index of 83.2 ± 0.6. Mean shell hardness index among older outdoor HS tortoises was 89.9 ± 0.6 for the 2011-cohort (n=26) and 87.1 ± 0.5 for 2012-cohort (n=38; Figure 2.7; Table 2.2). With size included as a covariate, indoor HS animals had significantly softer shells (lower shell hardness index) than did either 2011- or 2012-cohort outdoor HS animals ($F_{2,88} = 38.323$, $p < 0.001$ in both cases). In other words, an indoor HS tortoise was likely to have a softer shell than either a 2011- or 2012-cohort, outdoor HS tortoise of the same size. However, outdoor-reared 2011- and 2012-cohort HS tortoises did not differ from one another in shell hardness (p = 0.837; Figure 2.7; Table 2.2).

DISCUSSION

Indoor HS tortoises grew much faster than either DR or outdoor HS animals, but had lower body condition than outdoor HS neonates (2015 cohort) and softer shells than similar-sized, older outdoor HS juveniles (2012 and 2011 cohorts; 3-4 year-olds). Indoor HS tortoises reached the size (87 mm in MCL) of five- to six-year-old wild desert tortoises (Turner et al. 1987, Curtin et al. 2009). However, even with this rapid rate of growth, indoor HS tortoises did not reach the release-size threshold of 100 mm MCL recommended by Nagy et al. (2015*b*). However, indoor HS group did surpass the Hazard et al. (2015) recommended size of 84 mm in MCL. Growth in mass was also quite rapid among indoor HS tortoises; they grew nearly five times more in seven months than outdoor-reared neonates at Fort Irwin Air Force Base grew in one year (147% vs. 30%; Nagy et al. 1997).

Contrary to expectations, indoor HS tortoises had significantly lower body condition than did outdoor HS tortoises after 5.8 months of rearing and this difference persisted until time of release in late April 2016 (after 7 months of rearing). Water access did not drive this difference because a temporary failure in our water supply in April precluded watering of outdoor pens prior to the final determination of body condition. Furthermore, a study by Nagy et al. (2015*a*) comparing outdoor-reared head-starts with and without supplemental rain showed that body condition differed between the two groups only in drought years. Because both the indoor and outdoor HS groups in our study had weekly access to supplemental water throughout most of the head-starting period, it is unlikely that individuals from either group were dehydrated. It is possible that the rapid rate of growth among indoor-reared juveniles did not allow them to accumulate bone mass in proportion to their larger body size, making them lighter in proportion to their volume (Arendt and Wilson 2000). Perhaps indoor HS tortoises were less dense because they shunted their resources to new tissue instead of fat or their constant access to basking opportunities promoted growth and metabolic burning rather than fat storage and metabolic conservation (although we were not able to find literature supporting this hypothesis).

Hatchlings in the DR group had a high degree of variability in body condition and did not differ from either of the head-start treatment groups. Individual variation within the DR group could be due to very localized microhabitat differences among release sites and/or variation among individuals in their behavior or resource use. For example, variation in dispersal behavior may have driven the observed body condition variation, such that DR animals that moved greater distances post-release may have expended more water and energy reserves, resulting in lower body condition (although we did not test this hypothesis). In contrast, indoor and outdoor HS tortoises had limited potential to roam as widely, instead being confined to their enclosures during the study. Although there were substantial differences among individuals and treatment groups (indoor HS vs. outdoor HS) in body condition, no individuals in our study had alarmingly low body condition; none had body condition appreciably below previously reported values for clinically healthy tortoises. The highest (1.22 g/cm^3) and lowest (0.90 g/cm^3) values of body condition across all treatment groups were both from DR individuals, and they were similar to the range reported among adult Speckled Tortoises (*Homopus signatus signatus*) using the same body condition formula (0.910 – 1.128 g/cm^3; Leohr et al. 2004).

Shell development in turtles — especially increased hardness — is positively correlated with age and size and is thought to be important in conferring protection from predators (Nagy et al. 2011). Our indoor HS tortoises were harder than their much smaller DR and outdoor HS counterparts from the same cohort as only the indoor HS tortoises had shells hard enough to quantify shell hardness. However, the indoor HS tortoises had softer shells than similar sized (but older, 3–4 yr old) outdoor HS tortoises. Because tension calibrated micrometers differ among instruments in how much pressure they apply (Nagy et al. 2011), only limited conclusions can be drawn from comparisons of shell hardness among studies. Head-started tortoises in the Nagy et al. (2011) study with similar shell hardness to our indoor HS tortoises (SHI = 83) were approximately 1 year old and 40 mm in MCL. Nagy et al. (2011) found that outdoor reared HS tortoises approached full hardness at approximately 11.5 years old and 109 mm in MCL. Shell hardness may simply take more time to develop than the brief time we reared animals in captivity, even though growth can be accelerated through indoor rearing. In summary, after 7 months of rearing, indoor HS tortoises achieved sizes between the upper and lower size for release recommended in previous studies, but their shells were not as hard as similar-sized outdoor-reared tortoises from older cohorts.

Management implications

Indoor head-starting was successful in reducing the time required to produce head-started desert tortoises approaching the recommended size at release. However, we recommend that future studies evaluate indoor-rearing for a longer time period and/or incorporate a combination of indoor- and outdoor-husbandry (e.g., an entire year of indoor-rearing followed by a final year of outdoor-rearing) in an effort to increase shell hardness among head-starts while retaining the growth-enhancing benefit of indoor-rearing. Because our indoor HS tortoises had lower body condition than outdoor HS tortoises, we recommend that future head-start studies monitor body condition and evaluate the relationship between body condition and shell hardness, as body condition may increase as shells ossify. Ultimately, the success of indoor-rearing and head-starting in general can best be evaluated through post-release monitoring of survival and, eventually, reproduction.

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TABLES

Table 2.1. Summary statistics of growth metrics for juvenile Mojave Desert Tortoises (*Gopherus agassizii*) reared under three different husbandry treatments (direct-release, indoor head-started, outdoor head-started), including measurements taken shortly after hatching in Fall 2015 (‘initial’) and measurements taken in mid-March 2016 (‘final’, 5.8 months into the 7-month rearing period). Intervals are reported at 95% confidence. P-values (significant values in bold) correspond to one-way ANOVA for initial metrics and linear mixed effects models for all other metrics (with mothers’ identities as random effect). MCL= midline carapace length.

Metric	Treatment	*n*	Mean	SE	Lower CI	Upper CI	Min	Max	*p*-value
Initial MCL (mm)	Direct-release	20	46.5	0.5	45.4	47.2	42.7	49.1	0.079
	Indoor	30	45.8	0.4	44.8	46.4	40.5	48.5	
	Outdoor	20	46.8	0.5	45.5	47.3	42.9	50.2	
Initial mass (g)	Direct-release	20	22.6	0.7	21.0	23.8	17.7	27.2	0.523
	Indoor	30	22.3	0.7	20.5	23.2	15.5	26.7	
	Outdoor	20	23.1	0.7	21.0	24.0	16.1	27.8	
Initial body condition (g/cm^3)	Direct-release	20	1.08	0.02	1.05	1.11	0.98	1.19	0.778
	Indoor	30	1.07	0.02	1.04	1.10	0.85	1.22	
	Outdoor	20	1.07	0.02	1.03	1.10	0.96	1.18	
Final MCL (mm)	Direct-release	15	48.8	1.4	46.1	51.5	44.7	52.8	**< 0.0001**
	Indoor	30	78.2	1.0	76.2	80.1	54.6	87.1	
	Outdoor	20	50.6	1.2	48.3	53.0	45.4	55.4	
MCL growth (%)	Direct-release	15	4.3	2.7	1.4	9.6	0.4	7.5	**< 0.0001**
	Indoor	30	70.7	2.0	66.7	74.9	17.4	89.6	
	Outdoor	20	8.3	2.4	3.8	13.5	3.8	16.6	
Final mass (g)	Direct-release	15	24.5	3.7	16.8	31.6	17.2	30.4	**< 0.0001**
	Indoor	30	94.7	2.7	89.1	100.0	41.0	119.4	
	Outdoor	20	30.2	3.3	23.7	36.8	20.5	39.0	
Mass growth (%)	Direct-release	15	8.8	6.6	- 4.7	21.8	0.9	16.1	**< 0.0001**
	Indoor	30	147.8	5.1	138.7	159.1	33.9	209.7	
	Outdoor	20	17.3	6.0	7.9	31.9	7.0	34.4	
Final body condition (g/cm^3)	Direct-release	15	1.08	0.02	1.05	1.11	0.90	1.22	**0.0032**
	Indoor	30	1.06	0.01	1.04	1.08	0.97	1.15	
	Outdoor	20	1.12	0.01	1.09	1.14	1.03	1.21	

Table 2.2. Summary statistics of growth metrics from measurements of juvenile desert tortoises (*Gopherus agassizii*) taken prior to head-start release in late April 2016. Shell hardness data from indoor-reared head-starts (2015 cohort) are compared with data from outdoor-reared head-starts from 2012- and 2011-cohorts taken in September 2015. Intervals are reported at 95% confidence. P-values (significant values in bold) correspond to linear mixed effects model for follow-up analysis on body condition between indoor- and outdoor-reared head-starts (with mothers' identities as random effect) and ANCOVA on shell hardness data (with midline carapace length [MCL] as covariate).

Metric	Treatment	*n*	Mean	SE	Lower CI	Upper CI	Min	Max	*p*-value
	Comparison of 2015 indoor- and outdoor-reared head-starts								
Final MCL (mm)	Indoor	29	87.2	1.0	85.2	89.2	74	96.4	**< 0.0001**
	Outdoor	20	51.9	1.6	49.6	54.2	46.7	58.2	
Final mass (g)	Indoor	29	122.6	3.7	115.1	130.0	77.6	161.7	**< 0.0001**
	Outdoor	20	30.9	4.5	22.3	39.8	22.4	42.5	
Final body condition (g/cm^3)	Indoor	29	1.01	0.01	1.00	1.03	0.94	1.11	**0.0031**
	Outdoor	20	1.04	0.01	1.02	1.06	0.97	1.17	
	Comparison of 2015 indoor-reared group to outdoor-reared 2012 and 2011 cohorts								
Shell hardness index	Indoor 2015	28	83.2	0.6	81.0	83.3	75.4	91.4	**< 0.0001**
	Outdoor 2012	38	87.1	0.5	87.4	89.6	80.4	94.4	
	Outdoor 2011	26	89.9	0.6	87.8	90.1	85.1	95.4	

FIGURES

Figure 2.1. Husbandry setup for indoor-reared treatment group for Mojave Desert Tortoises (*Gopherus agassizii*) head-started for seven months at the Ivanpah Desert Tortoise Research Facility in Mojave National Preserve, California. Thirty juveniles that hatched in 2015 were distributed among six mesocosms containing natural sandy substrate, humid hide boxes and other artificial shelters, and white and infrared lights on automated timers for basking.

Figure 2.2. Head-starting pens at the Ivanpah Desert Tortoise Research Facility in the Mojave National Preserve, San Bernardino County, California that housed juvenile Mojave Desert Tortoises (*Gopherus agassizii*) from the outdoor-reared treatment group.

Figure 2.3. Juvenile Gopher Tortoise (*Gopherus polyphemus*) being measured for shell hardness with the micrometer method. Photo by Daniel Quinn.

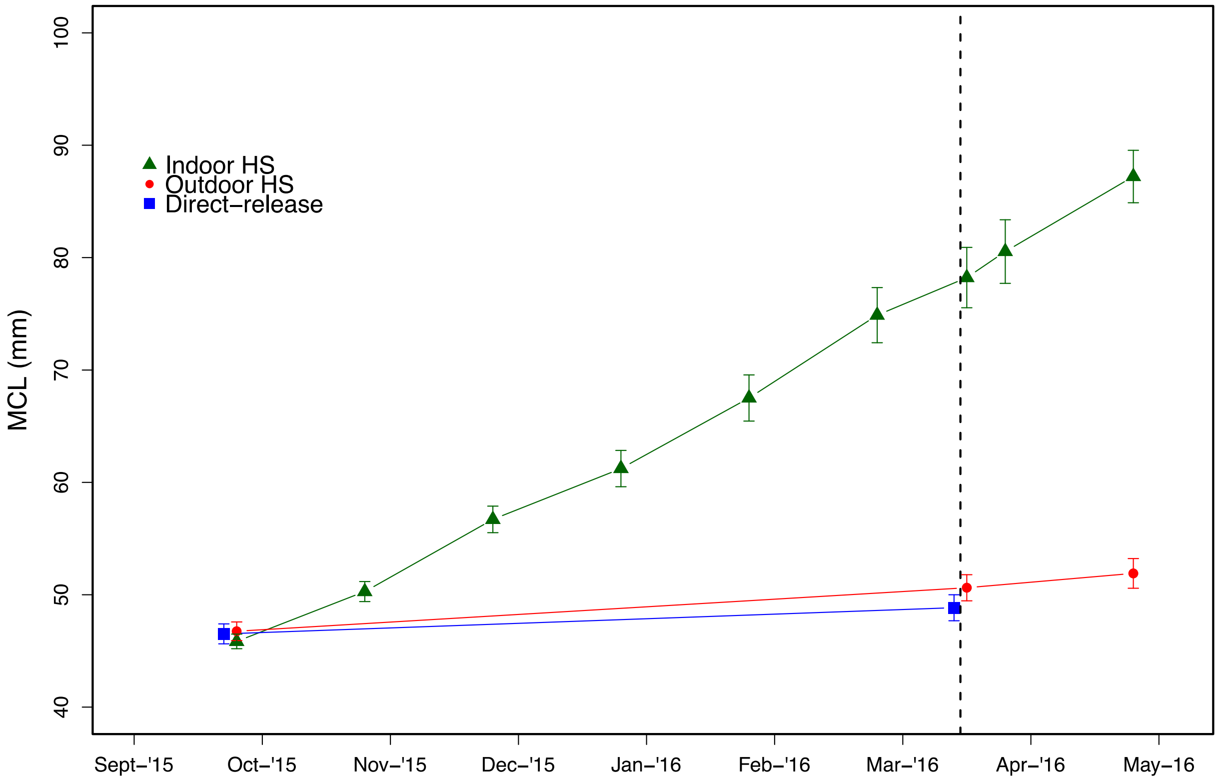

Figure 2.4. Means ± 95% confidence intervals for midline carapace length (MCL) of 2015 cohort juvenile Mojave Desert Tortoises (*Gopherus agassizii*) under three husbandry treatments. The dashed line indicates time when data were collected for comparative analyses among the three treatment groups.

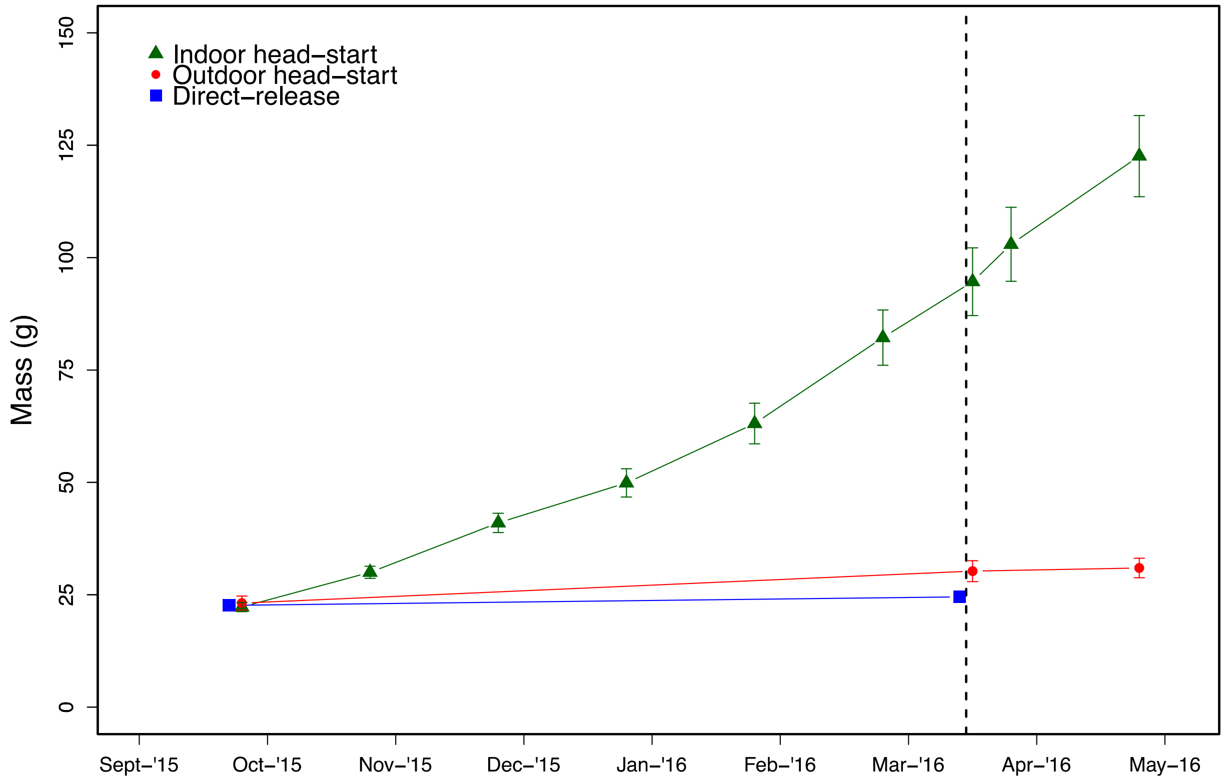

Figure 2.5. Means ± 95 confidence intervals for mass (g) of 2015 cohort juvenile Mojave Desert Tortoises (*Gopherus agassizii*) under three management treatments. The dashed line indicates time when data were collected for comparative analyses among the three treatment groups.

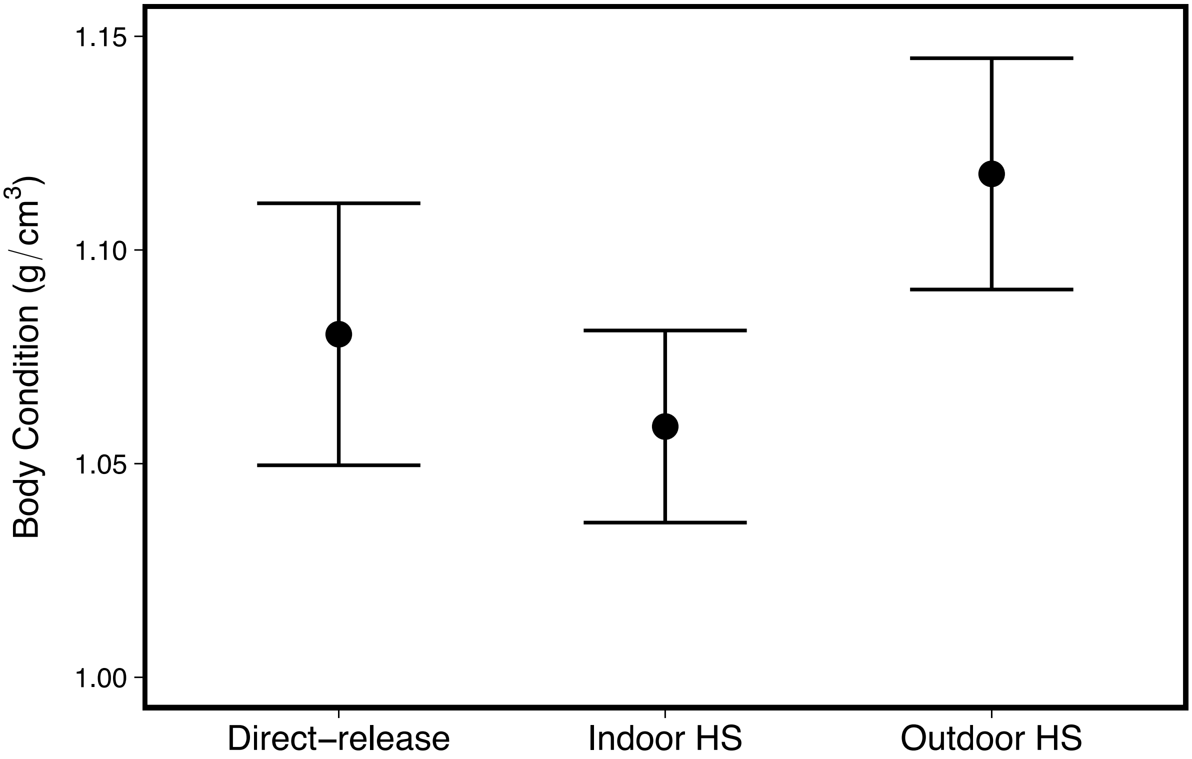

Figure 2.6. Body condition (g/cm3) of 2015 cohort juvenile Mojave Desert Tortoises (*Gopherus agassizii*) from three treatment groups: direct-release, indoor and outdoor head-started (HS) in March 2016. Error bars are 95 percent confidence intervals.

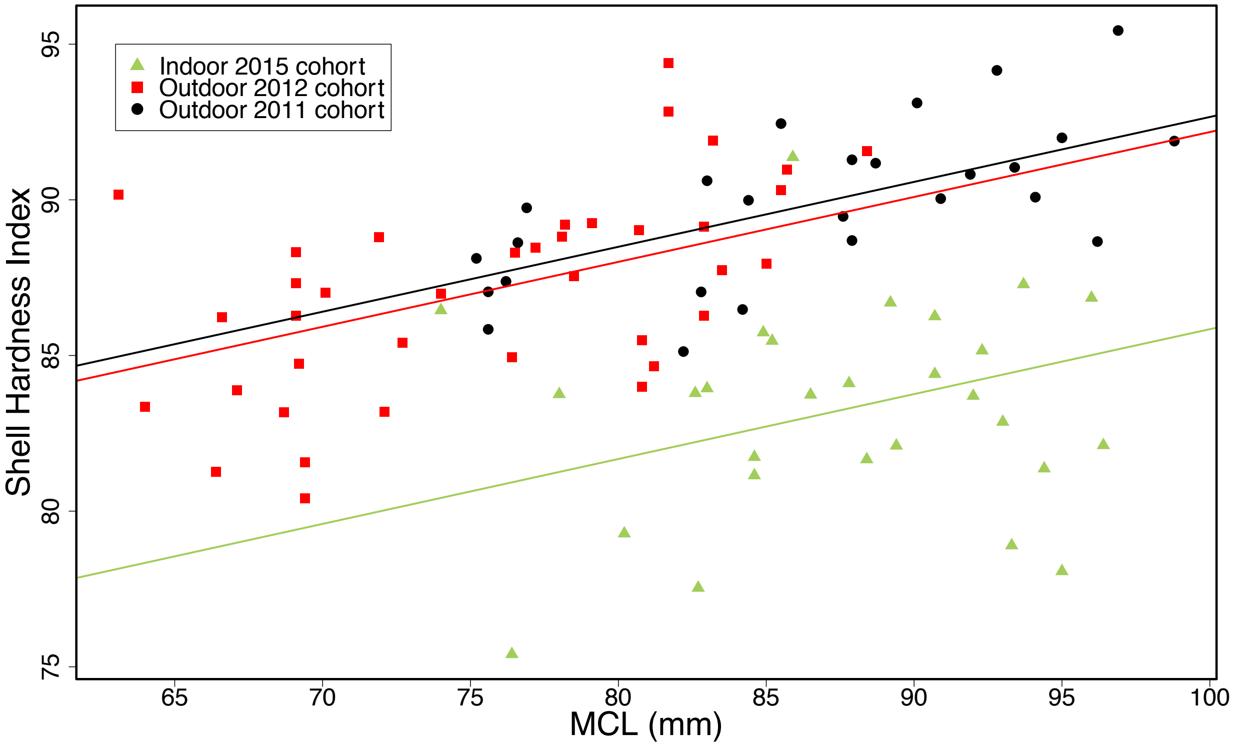

Figure 2.7. Shell hardness index versus midline carapace length (MCL) for three treatment groups of juvenile Mojave Desert Tortoises (*Gopherus agassizii*): 1) indoor-reared 2015-cohort juveniles [n=28; age=7.5 months], 2) outdoor-reared 2012-cohort juveniles [n=38, age=3 years], and 3) outdoor-reared 2011-cohort juveniles [n=26, age=4 years].

CHAPTER 3

POST-RELEASE MOVEMENT, MICROHABITAT USE, SURVIVORSHIP, AND MORTALITY RISK FACTORS AMONG INDOOR-REARED, OUTDOOR-REARED, AND DIRECTLY RELEASED JUVENILE MOJAVE DESERT TORTOISES (*GOPHERUS AGASSIZII*)

INTRODUCTION

Head-starting seeks to increase recruitment by rearing animals in protected conditions through early life stages before release into the natural environment at a body size at which survival is presumably enhanced (Heppell et al. 1996, Burke 2015). Turtles may be uniquely suited to head-starting because survivorship in the wild is low during early life stages and higher during adulthood under most natural conditions, and they require no parental care after hatching (Burke 2015). However, evaluating the efficacy of head-starting is often difficult and some have questioned its feasibility (Heppell et al. 1996). Certainly head-starting can only be a practical management tool if sources of adult mortality are addressed (Congdon et al. 1994, Tuberville et al. 2015). Reed et al. (2009) highlighted the difficulty in monitoring success of head-start releases, as detectability of young tortoises is low in post-release encounter surveys. Therefore, post-release monitoring via radio-telemetry is needed. Here, we use radio telemetry to monitor three treatment groups: indoor reared head-starts, outdoor reared head-starts, and a directly released control group. Few studies have evaluated post-release success of juvenile desert tortoises raised in predator proof pens (Hazard et al. 2015, Nagy et al. 2015*b*), but ours is the first to evaluate the feasibility of indoor head-starting (Chapter 2).

Dispersal, or movement just after release, is a vulnerable time for head-start hatchlings because they are at higher risk for predation and exposure while on the move (Pedrono and Sarovy 2000, Hazard and Morafka 2002). Larger movements may allow animals to assess resource conditions and conspecific occupancy of their homes, but they also increase exposure and predation risk (Farnsworth et al. 2015). Temporarily penning animals in enclosures at the release site, or 'soft-release,' has shown to increase site fidelity in translocated turtles (Tuberville et al. 2005, Attum and Cutshall 2015), but pens have the potential to attract the attention of predators (Quinn 2016) and are sometimes logistically unfeasible to install (as was the case in this study). Therefore, we monitored post-release movement patterns among head-starts and a direct-release control group to assess site movement and site fidelity.

Rearing animals indoors raises questions as to whether they will know how to use cover and modulate their activity to reduce mortality risks once released. Use of burrows, pallets (short burrows), and vegetative cover are important thermal and anti-predator refuges for desert tortoises (Woodbury and Hardy 1948). Here, we monitor use of cover and level of surface activity to investigate any differences in these behaviors among three treatment groups. Furthermore, juvenile desert tortoises have been shown to associate with soil substrate that is of similar body size (i.e., rocks), as opposed to fine sand, in an apparent effort to become more cryptic (Nafus et al. 2015). Yet, burrows are generally found in areas with finer soil grains (Todd et al. 2016) which are more friable. Therefore, understanding behaviors that affect crypticity (through comparison of animal size to surrounding soil grains) could be important in understanding predation risk, especially if head-starts are substantially larger than un-manipulated animals of the same age. Our study is the first to evaluate survivorship, movements, and microhabitat use by indoor-reared head-started desert tortoises through post-release monitoring.

METHODS

Study site

The Mojave National Preserve is a 650,000 ha preserve managed by the National Park Service and is located in the eastern Mojave Desert, San Bernardino County, California (Figure 3.1). Our study took place in Ivanpah Valley, at the northern edge of the Mojave National Preserve (Figure 3.1). The primary habitat in Ivanpah Valley is "creosote bush scrub," and is dominated by Creosote Bush (*Larrea tridentata*), White Bursage (*Ambrosia dumosa*), and low density *Yucca* (*Y. schidigera*, and *Y. brevifolia*; Turner et al. 1984). Although tortoises are commonly seen in Ivanpah Valley and habitat quality is relatively pristine (Nussear et al. 2009, Todd et al. 2016), tortoise densities are much lower than they were historically at the site (77–85 tortoises per km^2 in 1977 to 1980; Turner et al. 1984). Human disturbances within our study area include roads, powerline corridors, abandoned cattle grazing infrastructure (fencing and corrals), and railroad tracks.

Obtaining hatchlings

During May 2015, we captured radio-transmittered, but free-ranging adult female desert tortoises from Ivanpah Valley and radiographed them to determine if they were gravid. Gravid females were transferred to individual, outdoor, predator-proof nesting pens at the Ivanpah Desert Tortoise Research Facility (IDTRF). Gravid females were kept in nesting pens until they laid their eggs (up to 30 days), and then returned to their capture location. Eggs were left to incubate naturally in nests within the predator-proof enclosure. Over the period of hatchling emergence, each emerged hatchling was brought inside and temporarily housed by clutch. Four hatchlings were removed from the study due to especially low body mass (<15.5 g) at hatching and/or developmental issues (one had an eye deformity and two had very folded shells) and are not considered further.

Experimental treatment groups

In late September 2015 (within 21–46 days of hatching), we assigned healthy neonates to one of three treatment groups (described further below): 1) indoor-reared (n=30), 2) outdoor-reared (n=20), and 3) directly released (n=20; control group). We selected an unbalanced design—including more indoor-reared animals—because previous cohorts included in our head-start program included only releases of outdoor-reared and directly released neonates. Indoor-reared head-starts (hereafter 'indoor HS') were raised in the climate controlled IDTRF and housed in 50-gallon (189 liter) Rubbermaid polymer stock tanks with desert sand as substrate, humid hide boxes, and heat lamps (white during day and infra-red for night). Outdoor-reared head-starts (hereafter 'outdoor HS') were raised in semi-natural predator-resistant enclosures and were offered supplemental food and artificial rain via garden sprinklers once per week. Outdoor HS animals were allowed to undergo winter dormancy whereas indoor HS animals were kept active throughout the winter. Direct-release (hereafter DR) juveniles were released into the natural environment in September 2015, whereas indoor and outdoor HS juveniles were protected in captivity from September 2015 until April 2016 (seven months). Additional details regarding husbandry and handling of neonates can be found in Chapter 2.

Experimental releases

Using ArcGIS (version 10.2; ESRI, Redlands, CA), we delineated a 0.7 km^2 rectangular release plot (700 m x 1000 m) with the longer side parallel to the powerline service access road ("Powerline Road"; Figure 3.2). We chose this orientation to minimize variability in the distance from the powerline road across the release area (as we sighted ravens occasionally perching on these powerlines). We placed the center of the release plot (N35° 19' 18.7" W115° 23' 58.3") 850 m from the powerline road. We then used the “genregularpntsinpolys” (Generate Regular Points In Polygons) tool in Geospatial Modeling Environment (GME; version 0.7.3) to generate 70 regularly spaced points 100 meters apart (Figure 3.2). Elevation within the release plot was approximately 1000 m, and the dominant shrub species (Creosote Bush and White Bursage) were interspersed with Mojave Yucca, low density Joshua Trees, and various cacti.

We randomly assigned each of the hatchlings one of these “release centers,” for either DR release on 28 September 2015 or HS release (indoor and outdoor HS groups) on 25 April 2016. We placed a 20-m radius buffer around each release center within which we could preferentially select a point (release site) with large creosote bushes and at least several kangaroo rat (*Dipodomys* spp.) burrows for hatchling refugia (Todd et al. 2016) once in the field. Under our release protocol, the minimum possible distance between any two release sites was 60 m (Figure 3.2). We chose this spacing because it allowed us to begin the experiment with spatial independence between release points (Hurlbert 1984) while retaining a small enough study area to make regular radio-tracking logistically feasible.

On 28 September 2015, we released 20 hatchings from the DR treatment group at 20 of the pre-selected release sites described above. We released all hatchlings in the morning, between 0700 h and 0930 h, placing them inside kangaroo rat burrows under creosote bushes to avoid heat exposure. Seven months later (25 April 2016), we released 28 indoor HS tortoises and 20 outdoor HS in the same manner. After the spring release was already underway, we noted a mated pair of ravens rearing young in a nest on one of the powerline towers adjacent to the plot (approximately 500 m from the nearest released tortoise).

Post-release monitoring

Prior to release, we marked each tortoise by notching marginal scutes in unique patterns (modified from Cagle, 1939) and attached radio transmitters (BD-2, Holohil Systems Ltd., Ontario, Canada) to the fourth vertebral scutes with 5-minute epoxy. We used a 3-element Yagi antenna (AF Antronics, Inc., Urbana, IL) and a R1000 receiver (Communications Specialists, Inc., Orange, CA) to locate tortoises for monitoring post-release. We located each animal daily for the first four days following release during September 2015 (DR) and April 2016 (indoor and outdoor HS). After the initial week of dispersal, we tracked tortoises twice per week (every 3–4 days), a temporal resolution sufficient to capture mortality when tortoises were most active but not so frequent as to attract predators. We reduced tracking to once per week (every 5–9 days) depending on season and predation pressure.

Movement, surface activity, and microhabitat use

At each tracking event, we recorded tortoise location to the nearest ±3 m using a handheld GPS (Garmin model GPSMAP 76, Olathe, Kansas) and recorded attributes about the tortoise's activity and microhabitat use. When we observed tortoise behavioral response to the observer's approach (i.e., seeking cover or freezing in place), we recorded location and behavior when the tortoise was first sighted. Cover type was categorized as either shrub (identified to species), rock, pallet (short burrow, with length shorter than width), or burrow. Percent of tortoise covered is the percentage of the animal covered when looking down from directly above the tortoise. We categorized '% tortoise covered' in the percentage ranges: trace <1%, 1–10%, 10–25%, 25–50%, 50–75%, 75–95%, 95–100%. We categorized activity as walking, digging, foraging, resting, or not seen (in a burrow). We also counted the number of 'tortoise-sized-rocks' around the tortoises at each tracking. To enumerate, we placed a 1 m x 1 m quadrat around the tortoise (perpendicular to the tortoise' longitudinal axis; or squarely in front of the burrow or pallet if inactive) and counted the number of rocks with largest dimension that fell within 50–150% of the mean midline carapace length of the treatment group to which the tortoise belonged.

We subsequently converted % of tortoise covered to a binary variable for analyses, such that a tortoise was considered covered if more than 50% of its body was covered from above. Activity was also categorized to a binary variable: surface active (not in a burrow or pallet) or not surface active (at least part of its body inside a burrow or pallet). We used generalized linear models with mixed effects ("glmer" function in "lme4" package in R; R Core Team, 2014) to evaluate differences in % of tortoise covered, surface activity, and number of "tortoise-sized-rocks" among the three treatment groups from 25 April – 27 October 2016 (when all three treatment groups were in the field). In each model we included the random effect of individual to specify that repeated measures on individuals were not independent, avoiding pseudoreplication. Tukey's contrasts ("glht" function in "multcomp" package in R) were used to evaluate differences in means among the treatment groups. We used bootstrap re-sampling ("boot" package in R) to obtain treatment group mean estimates and 95% confidence intervals from at least 2000 successfully converged iterations of our models from re-sampled data.

To compare movement among the treatment groups we used the "movement.pathmetrics" function in GME to calculate distances moved between consecutive tracking events. For each animal, we calculated 1) mean distance moved per day during the first 4 d after release and 2) the mean distance moved between consecutive tracking events for the entire study. Both metrics of mean distance moved were 'natural log plus one' transformed (our data contained zeros) to improve normality and subsequently analyzed with one-way analysis of variance (ANOVA) followed by Tukey's tests. Means and confidence intervals were back-transformed for reporting.

We used the “point.distances” function in GME to calculate 1) each tortoise’s distance from release site after first 4 d of dispersal, and 2) its final distance from release site at the end of the movement study (by 27 October 2016). Two tortoises were censored (one went missing and one was found depredated) during their first 4 d of dispersal, and we removed these individuals from both analyses of distance from release site. Because post-release survival varied among individuals and thus affected number of days radio-tracked, we plotted distance from release site over time for individuals that survived the entire study to determine which individuals to include in our analysis of ‘final distance from release site.’ For these plots, we grouped individuals by release event: 1) DR and 2) HS release (combining both indoor and outdoor HS). Plots for both groups had a clear asymptote: a point in time at which individuals stopped moving farther from their release sites (Figure 3.3). If animals lived past that threshold date (29 October 2015 [31 d post-release] for DR and 18 August 2016 [115 d post-release] for HS), they were included in the analysis of final distance from release site. Both metrics of distance from release site were transformed to best improve normality: distance from release site after 4 d of dispersal was square-root transformed and final distance from release site was ‘natural log plus one’ transformed (the data contained zeros). Means and confidence intervals were back-transformed for reporting. Both metrics of distance from release site were analyzed with one-way analysis of variance (ANOVA) followed by Tukey’s tests.

Survivorship and mortality risk factors

Each time a tortoise was found dead, we carefully examined the area for signs of predators such as digging, tracks, or scat, and noted the location and condition of the carcass. Each carcass was photographed in situ and then retrieved for further analysis. We classified carcasses or chewed transmitters that were found adjacent to dug out burrows or that had clear tooth marks as “mammal predated.” We classified carcasses or transmitters that were found up in *Yucca* (*Y. schidigera or Y. brevifolia*) or directly beneath *Yucca* as “bird predated.” We classified remains without tooth marks or that were not directly in or under *Yucca* plants as predated by “unidentified predator.” Tortoises found intact and upside down (flipped on their carapace) or dead inside winter burrows were classified as “killed by exposure.” Individuals lost due to radio failure (battery failure or the unit fell off) were classified as “unknown fate.” We estimated survival for each treatment group using a Kaplan-Meier estimator using the “survival” package in program R. We right-censored data, meaning that we made no assumptions about the fate of missing animals.

Using only data from animals for which fate was known, we evaluated the effect of treatment group and individual level metrics (i.e., potential risk factors) on individual fate. We included treatment group, movement rate, surface activity, size at release, and average distance from a raven nest as potential predictors in a generalized linear model (GLM with binary response; alive = 1 and dead = 0). Movement rate was mean distance moved in meters per tracking event. Surface activity was characterized as the proportion of all observations for which the tortoise was surface active. Because of seasonal differences during which the HS (indoor and outdoor) and DR groups were tracked, we corrected each individual score of surface activity and mean distance moved by subtracting the treatment group mean score from the individual score. Therefore, the resulting individual metric indicated how far the individual deviated from its treatment group mean. Size at release was measured as midline carapace length (MCL in mm) and was similarly corrected by treatment group mean size. Mean distance from the raven nest was calculated for each individual using the “point.distances” function in GME. Over all tracking events where the tortoise was found alive, we averaged distances from the active raven nest.

We generated predictions and 95% confidence intervals (CIs) of survival based on distance from raven nest and proportion surface activity for each treatment group. To predict survival based on distance from the raven nest, we set individual scores (size, movement rate, and surface activity) to the treatment group mean for each animal, such that the group-corrected metric was zero. We then varied distance from raven nest from 400 – 1600 m and generated predictions every 10 m for each treatment group. To predict survival based on proportion of surface active observations, we set distance from the raven nest to 850 m (distance of the release plot center from the road) and set individual size and movement rate to treatment group mean scores (i.e., group-corrected score for each individual was zero). We varied proportion of surface active observations from zero to one and created 100 evenly spaced predictions along each curve (one curve for each treatment group).

RESULTS

All (20/20) outdoor HS tortoises survived in their head-start pens until their release on 25 April 2016. Twenty-nine of 30 indoor HS tortoises survived the rearing period. One indoor HS tortoise was found dead in its mesocosm due to natural causes (6.6 months into the indoor rearing period); thus, survival for indoor HS tortoises was 96.7% (29/30) through the HS period. One other indoor HS tortoise had a temporary skin condition—from which it later recovered—but it was excluded from the HS release and not considered further. Therefore, we released 68 tortoises into the natural environment: 20 DR, 20 outdoor HS, and 28 indoor HS.

Growth during the HS period varied greatly by treatment group. At the April 2016 HS release, midline carapace length (MCL ± SE) was 87.2 ± 1.0 mm for the indoor HS group, 51.9 ± 1.6 mm for the outdoor HS group, and 48.8 ± 1.4 mm for the direct-release group (Chapter 2).

Activity and microhabitat use

Tortoises in the three treatment groups were similar in their use of cover and levels of surface activity post-release, but tortoises differed in the number of tortoise-sized rocks around them. Sample sizes for these repeated measures analyses were n = 15 for the DR group (reflecting over-winter losses from this treatment group), n = 28 for indoor HS group, and n = 20 for outdoor HS group. Mean probability of being at >50% under cover upon observation was 0.85 (95 CI, 0.80–0.89) for DR, 0.86 (0.83–0.90) for indoor HS, and 0.87 (0.83–0.91) for outdoor HS, and treatment groups did not differ ($p > 0.10$; Figure 3.4). Mean probability of being surface active upon observation was 0.19 (0.15–0.24) for DR, 0.22 (0.18–0.27) for indoor HS, and 0.17 (0.13–0.21) for outdoor HS, and treatment groups did not differ ($p > 0.10$; Figure 3.5). On average, indoor HS tortoises had significantly fewer tortoise-sized rocks around them than either outdoor HS tortoises ($z = -7.26$, $p < 0.001$) or DR tortoises ($z = -6.86$, $p < 0.001$; Figure 3.6). Direct-release tortoises had >5x more tortoise-sized rocks around them than indoor HS tortoises (15.1 [12.2–18.1] rocks vs. 2.8 [2.2–3.37] rocks). Similarly, outdoor HS tortoises were surrounded by nearly 4x as many tortoise-sized rocks (10.5 [8.4–13.6] rocks) as indoor HS tortoises. The number of tortoise-sized rocks around DR and outdoor HS tortoises did not differ statistically ($z = 1.85$, $p = 0.151$; Figure 3.6).

Movement

Mean distance moved per day during initial 4 d of dispersal differed among treatment groups ($F_{2,65} = 3.79$, $p = 0.028$; Figure 3.7), and was 9.8 (95% CI, 5.9–15.9) m for the DR group, 8.0 (5.2–12.2) m for indoor HS tortoises, and 3.7 (2.0–6.4) m for outdoor HS tortoises (Table 3.1). During this initial dispersal period, DR tortoises moved significantly more than outdoor HS ones ($t = 2.59$, $p = 0.031$); however, DR and indoor HS tortoises did not differ ($t = 0.619$, $p = 0.810$) nor did indoor and outdoor HS tortoises ($t = 2.179$, $p = 0.082$; Figure 3.7).

During the first 4 d post-release, outdoor HS tortoises stayed significantly closer to their release sites than did indoor HS tortoises ($t = 2.63$, $p = 0.028$) or DR tortoises ($t = 3.31$, $p = 0.004$). Distance from release site by the end of the first 4 d was 41.6 (95% CI, 24.1–63.8) m for the DR group, 30.2 (17.7–46.0) m for the indoor HS group, and 8.4 (1.9–19.3) m for the outdoor HS group (Table 3.1; Figure 3.8). Direct-release juveniles dispersed nearly 5x farther than outdoor HS tortoises ($t = 3.31$, $p = 0.004$) and indoor HS dispersed 3.5x farther than outdoor HS ($t = 2.63$, $p = 0.028$; Figure 3.8). Direct-release and indoor HS tortoises did not differ in distance from release site after four days ($t = 0.950$, $p = 0.610$).

Mean distance moved per tracking event overall also differed by treatment group ($F_{2,65} = 14.96$, $p < 0.001$; Figure 3.9), with outdoor HS tortoises moving less than either indoor HS ($t = 5.46$, $p < 0.001$) or DR ($t = 3.19$, $p = 0.006$) tortoises. Mean distance moved per tracking event was 9.4 (6.8–13.1) m for DR tortoises, 14.5 (11.0–18.9) m for indoor HS tortoises, and 4.3 (3.0–6.2) m for outdoor HS tortoises (Table 3.1). Direct-release juveniles moved over 2x farther than outdoor HS juveniles between tracking events ($t = 3.19$, $p = 0.006$), and indoor HS tortoises moved over 3x farther than outdoor HS tortoises ($t = 5.46$, $p < 0.001$; Figure 3.9). Indoor HS and DR tortoises did not differ significantly in mean distance moved per tracking event ($t = 2.02$, $p = 0.116$).

Final distance from release site (13 months after DR release and 6 months after HS release) differed by treatment group ($F_{2,37} = 6.80$, $p = 0.003$; Figure 3.10). Mean final distance from release site was 164.8 (94.5–286.9) m for the DR group (n=16), 75.7 (41.5–137.3) m for the indoor HS group (n=14), and 32.2 (15.5–65.7) m for the outdoor HS group (n=10; Table 3.1). Direct-release tortoises moved >5x farther, on average, than did outdoor HS tortoises ($t = 3.66$, $p = 0.002$; Figure 3.10). However, indoor HS and DR groups did not differ statistically ($t = -1.934$, $p = 0.143$), nor did indoor HS and outdoor HS groups ($t = 1.856$, $p = 0.165$), in final distance from release site.

Survivorship and mortality risk factors

Of the 68 released tortoises, 27 (39.7%) were confirmed alive at the end of the study (6 November 2016). Thirty-seven of the 68 neonates released (55.9%) died with known fate, and bird predation was responsible for the largest proportion of mortality. Eighteen (26.5% of released tortoises) were bird predated, eight (11.8%) were mammal predated, seven (10.3%) were killed by unidentified predators, five (7.3%) were killed by exposure, and three (4.4%) were lost to radio failure (Figures 3.11, 3.12).

Combined (all treatment groups) annual survival (September 2015–2016) for the 70 tortoises was 0.44 (95% CI, 0.34–0.58). Combined survival for the entire study period (28 September 2015 – 6 November 2016, 58 weeks) was 0.43 (0.32–0.56). Survival was estimated at 0.37 (0.20–0.66) for the DR group (both annual and 58-week; Figure 3.13). Annual survival was 0.45 (0.30–0.67) and 58-week survival was 0.41 (0.27–0.64) for the indoor HS group (Figure 3.13). Finally, survival was estimated at 0.50 (0.32–0.77) for the outdoor HS group (both annual and 58-week; Figure 3.13). Overlapping 95% confidence intervals for survival estimates throughout the study indicate no significant difference in survival among treatment groups at any point in time (Figure 3.13).

Proportion of surface-active observations (relative to treatment group mean; $\beta = -6.808$, $z = -3.03$, $p = 0.002$) and distance from the raven nest ($\beta = 0.004$, $z = 2.56$, $p = 0.011$) were the only significant predictors of fate. Treatment group, size, and movement rate were not significant predictors of fate ($p > 0.05$ in each case). All surviving tortoises were surface-active on less than 30% of observations (Figure 3.14). Tortoises that were the least surface-active had the highest predicted survival probability (Figure 3.14). Tortoises that were farther from the raven nest were also more likely to survive. For every meter a tortoise was on average distant from the raven nest, its odds of surviving increased by 0.4%. At a larger scale, for every 100 m a tortoise was on average farther from the raven nest, its odds of surviving increased by 51.5% (Figure 3.15). None of the tortoises that were on-average farther than 1250 m from the raven nest died. Our modeled predictions of survival probability approached 1.0 at approximately 1600 m from the raven nest (Figure 3.15).

DISCUSSION

We saw no difference in survival among groups, but causes of mortality varied by treatment. For example, no indoor HS died from exposure. Of the five tortoises that were presumed killed by exposure (two DR and three outdoor HS) four were found toppled on their carapace and one (a DR) died inside its winter burrow. One could speculate that indoor HS tortoises' larger size might reduce their risk of toppling on their carapace. However, it could also be that the larger indoor HS tortoises were more successful in righting themselves once toppled. For a given species, water loss is negatively associated with size (Murphy et al. 2016), larger tortoises have greater water reserves (Nafus et al. 2015), and body size affects heating and cooling rates (Harris et al. 2015). Therefore, larger indoor HS tortoises would likely have more time to right themselves than smaller outdoor HS and DR tortoises before expiring from heat exposure and/or water loss, and we would expect them to generally be more robust against exposure mortality overall.

Predation by ravens was the largest source of mortality in our study, especially among the indoor head-start group. Previous cohorts of released juvenile tortoises at our site (age 0-18 months at release) had no confirmed incidences of raven predation during the first year after release (Todd et al. 2015). Raven populations are continuing to expand in the Mojave Desert due to human produced food subsidies (i.e., agriculture and garbage) and they are increasingly moving into natural areas such as our site (Boarman 2003, George 2015). A larger proportion of indoor HS tortoises were killed by ravens than the other treatment groups. Perhaps indoor HS tortoises were more easily sighted by ravens because of their larger size. We found fewer body-sized rocks around indoor HS tortoises than we found around tortoises in the other treatment groups, suggesting that they were less cryptic and more visible as ravens flew overhead searching for prey (Nafus et al. 2015).

Mammal predation accounted for a substantial proportion of mortality (11.8% of total released) and we believe that most of the ‘unidentified predator’ mortality (10.3% of total released) was by mammals because of the location and condition of carcasses (most were found buried in sand or on the surface with only part of their shells intact). We were unable to identify mammalian predators to species based on forensic evidence at the kill sites like Nagy et al. (2015*b*) did, and we cannot say with certainty that ‘mammal predated’ and ‘unidentified predator’ killed tortoises were not actually scavenged by mammals after dying from exposure or other causes.

Although we do not know which mammalian predators killed our tortoises, we should consider the mammalian tortoise predators at our site. At our site, we observed: Coyotes (known predator; *Canis latrans*), Kit Foxes (known predator; *Vulpes macrotis*), American Badgers (suspected predator; *Taxidea taxus*), and Ground Squirrels (suspected predator; *Spermophilus* spp.; Woodbury and Hardy 1948, Bjurlin and Bissonette 2004, Nagy et al. 2015*a*, 2015*b*). We did not observe any American Bobcats (*Lynx rufus*) at our site, although their range encompasses our research area. However, they are known to predate juvenile tortoises and are highly secretive (Nagy et al. 2015*b*), so predation by bobcats is possible even though we did not observe bobcats at our site. Nagy et al. (2015*b*) found that nearly 30% of released larger outdoor reared juveniles (111–140 mm in MCL) were killed by coyotes, but smaller juveniles (<110 mm MCL) suffered no confirmed coyote predation (although they were susceptible bird and bobcat predation). This heavy coyote predation came during the third year after release (2008), directly following a severe drought (2006–2007) when small mammal populations (coyotes' preferred prey base) were severely depleted (Nagy et al. 2015*b*). Because our study did not occur during a drought year it is more likely that mammal predated tortoises were killed by a variety of mammal predators instead of only coyotes.

We observed no differences among treatment groups in frequency of cover use or surface activity, suggesting that behavior was not driving the higher rates of raven predation of indoor HS tortoises. An initial study concern was whether indoor HS tortoises would be able to dig suitable refugia, as they were too large to fit in many of the unmodified small mammal burrows and the relatively thin layer of sand in their mesocosms did not provide them the opportunity to dig burrows while in captivity. However, from the first day post-release, we observed indoor HS tortoises digging to modify mammal burrows or constructing their own burrows. Tortoises from the other treatment groups mostly used small mammal burrows, although some were also observed digging their own. In short, tortoises from each treatment group used cover and burrow refugia similarly.

Although previous studies have found that size influences post-release survival of head-started juvenile desert tortoises (Hazard et al. 2015, Nagy et al. 2015*b*), our larger indoor HS tortoises did not exhibit greater survival than the smaller animals from the other two treatments. Likewise, individual size (corrected for by treatment group mean) was not a significant predictor of individual fate in our study. Nagy et al. (2015*b*) found that released outdoor-reared HS juveniles larger than 100 mm MCL were generally more robust against predation than smaller ones in general, and did not find any known raven-predated tortoises over 80 mm MCL. Analyses on data from released HS tortoises at the same site by Hazard et al. (2015) showed that juveniles (also outdoor-reared) over 84 mm were more likely to survive than those below that size threshold.

By these measures, we would have expected our indoor HS tortoises (87.2 mm mean MCL) to at least be robust to predation by ravens, if not entirely robust against predation more generally. However, outdoor-reared tortoises from these other studies (Hazard et al. 2015, Nagy et al. 2015*b*) were much older once they approached the size of our indoor HS tortoises, and likely had harder, more robust shells (Chapter 2). However, it is still unclear to what extent shell hardness (or lack thereof) drove our results. Of the 18 tortoises in our study that had confirmed raven predation, only three had holes pecked through the carapace. In most cases, the ravens removed the tortoise’s head, front limbs, and entrails, without puncturing the shell. This suggests that even if shell hardness was greater among our indoor HS tortoises, they would have still been susceptible to ravens.

It's unclear why outdoor HS tortoises exhibited such remarkably high site fidelity. After the four initial days of dispersal, they were on average <9 m from their release sites (compared to 30 m among indoor HS and 42 m among DR tortoises). After 185 days post release, outdoor HS tortoises were on average only 32 m from their release sites, over four times closer than outdoor-reared juvenile desert tortoises released by Hazard et al. (2015) after they had dispersed for only 20 days. Unlike tortoises in the DR and indoor HS treatment groups, outdoor HS tortoises had experience living in burrows. It may be that outdoor HS tortoises moved less because they readily found familiar refugia; however, we were not able to verify this as the mechanism driving differences in movement among treatment groups.

In contrast, the DR group (released September 2015) and the indoor HS group (released April 2016) both exhibited initial dispersal, moving rapidly from their release sites immediately following release, before finally settling down within 30 days in most cases. Direct-release tortoises exhibited significantly greater movement than outdoor HS tortoises in each metric we analyzed (mean move per day during initial four days post-release, distance from release after first four days post-release, mean move between consecutive tracking events overall, and final distance from release site). Similarly, directly released neonates (released in the fall, shortly after hatching) from previous cohorts released in Ivanpah Valley also moved farther than did released outdoor-reared tortoises (also from previous cohorts; K. A. Buhlmann, unpublished data). It's unclear to what extent season and weather may have affected movement of the DR and HS (indoor and outdoor) groups, as the DR group was released in the fall and HS groups were released the following spring. However, DR tortoises in our study settled farther from their release sites (165 m, by final measure), than did neonate (116 m) or juvenile (108 m) desert tortoises released (also in fall) by Hazard and Morafka (2002), but in both studies, tortoises stopped dispersing just over one month post-release. Furthermore, our DR tortoises seemed to disperse marginally farther than outdoor HS desert tortoises released in fall by Nagy et al. (2015*b*). They found that 12/15 individuals brumated within 150 m of their release sites and three brumated 200–600 m distant, but they did not report means or individual distances, making comparisons with our data difficult.

We considered that there could be an ontogenetic component as to why the DR hatchlings (age <1 mo. at release) moved more than the outdoor HS tortoises (age 7 mo. at release), such that newly hatched tortoises disperse from their nests after hatching. Although it's clear that marine and aquatic turtles undergo large movements (dispersal) immediately after hatching (Coleman et al. 2014, Congdon et al. 2015), it's unclear to what degree desert tortoises naturally disperse from their nests. Marine and aquatic turtle hatchlings must move from upland nests to their respective aquatic habitats, whereas tortoise burrows are ostensibly located in suitable juvenile tortoise habitat; thus, tortoises may not need to disperse far to find suitable resources. Pike (2006) found that largely un-manipulated neonate Gopher Tortoises (brought to the lab for less than 24 hours) did not exhibit high levels of movement in their first month, but moved more after emerging from brumation the following spring. This suggests that *Gopherus* hatchlings do not disperse immediately after emergence from nests like hatchlings of many marine and aquatic species.

Indoor HS tortoises dispersed qualitatively more than did outdoor HS tortoises, but they exhibited relatively high site fidelity. When comparing distance from release after the first four days of initial dispersal, indoor HS were significantly farther than outdoor HS. Although final distance from release site did not differ statistically between indoor HS and outdoor HS groups, indoor HS were on average twice as far from their release sites than outdoor HS, qualitatively reinforcing that dispersal was greater among indoor HS tortoises. However, compared to data reported by previous work on outdoor head-started desert tortoises (Hazard and Morafka, 2002; Hazard et al. 2015; Nagy et al. 2015*b*), both of our HS treatment groups (indoor and outdoor) exhibited high site fidelity. Furthermore, a release of five head-started Ploughshare tortoises claimed success because the juveniles stayed within 750 m of their release points (and because all five tortoises survived the 325-d study; Pedrono and Sarovy 2000). By this measure, all but one tortoise in our study (a DR tortoise that was 928 m from its release site) showed high site fidelity.

In investigating movement, we are ultimately concerned with how movement affects mortality risk. When comparing mean distance moved between consecutive tracking events overall, outdoor HS tortoises moved significantly less than did either indoor HS or DR tortoises, suggesting that outdoor HS tortoises might have lower predation risk. However, we found no differences in survival among the groups, and mean distance moved (corrected for by treatment group) was not a significant predictor of fate. Although it is reasonable to assume that greater movement comes with greater risks, distance to an active raven nest was a better predictor of fate in our study.

Although the primary goal of this study was to assess the viability of indoor head-starting, survival data from the largely unmanipulated DR group yielded important insights into first-year survival of wild hatchlings. The annual survival estimate of 0.37 among the DR group suggests that wild, unmanipulated neonates may have higher survival than has been historically assumed or estimated (except for recent head-start studies, we could find no field-based data on annual neonate survival in desert tortoises). This supports the assertion by Pike et al. (2008), that traditionally low estimates for juvenile reptile survival are unrealistic and mathematically untenable at producing recruitment to replacement level. A life-history study on Common Snapping Turtles (*Chelydra serpentina*) found that first year survival must be at least 0.47 for population stability. Because desert tortoises have approximately 5x fewer eggs on average per year than snapping turtles (5.6 eggs vs. 28 eggs; Turner et al. 1984, Congdon et al. 1994) first-year survival among desert tortoises likely need be even higher for population growth rate stability. Overall annual survival in our study (0.44) and especially survival among DR hatchlings (0.37) is not likely sustainable. However, recruitment among desert tortoises appears to be largely episodic, as interannual variability in rainfall drives variability in predation pressure (Morafka 1994) and reproduction (Turner et al. 1984). Without the substantial raven predation in our study, survival would likely have been higher and may have been adequate to support sustainable recruitment.

Furthermore, survival through the first fall was quite high among DR tortoises (80% through fall dispersal), and most mortality was experienced during the following spring. This supports findings from earlier work by our research group (Todd et al. 2015) and by Bjurlin and Bissonette (2004), which both found survival in dispersing neonates to be >80% during the fall (from emergence or direct-release in September until winter inactivity), suggesting that spring may be the most vulnerable period for young tortoises (although our findings are not conclusive in this regard). Fall releases of HS juveniles might be more successful than spring releases, as mortality risk appears be lower in the fall when dispersing head-starts would make their largest movements.

After controlling for potential covariates (movement rate, surface activity, size at release, and average distance from a raven nest), we found no effect of treatment group on survival probability. However, we did find some significant predictors of individual fate. Surface activity was a significant predictor of fate, but the relationship may be an artifact of the timing of mortality. For example, individuals succumbing to raven predation shortly after HS release would have been actively dispersing until death, thus providing a high proportion of surface-active observations. The design of our study (with two separate releases) made it unfeasible to model the temporal component of surface activity and risk.

Distance from the raven nest was a more useful predictor of fate, with tortoises farther from a raven nest having higher survival. While the siting of HS projects is constrained by where monitoring access is logistically feasible, managers also desire sites that minimize predation pressure on newly released juveniles. Thus, knowledge of how predation risk varies with distance from man-made structures that host avian predators—such as powerlines, or communication towers—is important to consider in the siting decision.

Although many ravens are predictably found at areas of high human activity (e.g., construction sites), nesting sites are more spatially distributed, making their location (and attendant predation risk) difficult to predict (Boarman 2003, Kristan and Boarman 2003). Although we knew that ravens occasionally perched on the powerlines near our release plot (500 m to the nearest edge), we did not anticipate the breeding activity that we discovered after we initiated our head-start release in April 2016. Previous research found that breeding ravens spend 75% of their foraging time within 600 m of their nests (Sherman 1993), suggesting that most of the tortoises in our study would experience relatively low predation risk from the breeding ravens (only five of 68 were within 600 m of the raven nest). In contrast, we found that tortoises were most likely to survive if they were on average at least 1.6 km from an active nest, suggesting that raven predation risk persists much farther from breeding sites than the 600 m previously suspected.

Management implications

Release site selection for reintroductions and population augmentation measures like head-starting must be made with careful assessment of anthropogenic risks. Head-starting is unlikely to be successful in areas where subsidized predators, or other systemic issues hampering recruitment are not properly managed. An ecosystem view must be applied to head-starting and reintroduction measures. Post-release survival of head-started desert tortoises is most likely to be greatest when they are released 1.6 km (or preferably farther) from raven nests or raised man-made structures that could attract ravens.

Our work also has implications for protocols of lethal raven control (i.e., shooting) under depredation permits for tortoise conservation. Current federal government protocols for lethal control of ravens target only “offending ravens” for removal if juvenile tortoise carcasses are found “within the presumed territories” of the ravens (USFWS 2008). Searching a brood-rearing raven’s entire 8.0 km^2 territory for tortoise carcasses is unrealistic (Boarman 2003). If ravens are nesting, the obvious first place to look for carcasses is beneath their nests. Because none of the 18 bird-killed tortoises in our study were found underneath the nest (or even within 400 m of the nest) our work suggests that identifying offending ravens can be extremely difficult (as also stated by Boarman et al. 2003). As ravens are opportunistic predators (Sherman 1993, Boarman 2003, George 2015), any raven should be considered a threat to juvenile desert tortoises if found foraging in desert tortoise habitat.

The timing of releases may be important in mitigating predation risk of released head-starts. The pair of ravens at our site began rearing their young just as our newly released HS (indoor and outdoor) tortoises began dispersing (late April–May). A fall HS release would insure that HS tortoise dispersal does not coincide with raven brood rearing (as raven nestlings fledge by mid summer), and it would also allow managers to identify areas of raven activity and nesting (to avoid) in the spring and summer, prior to HS release in the fall. Thus, fall may be a better time to release HS tortoises than spring.

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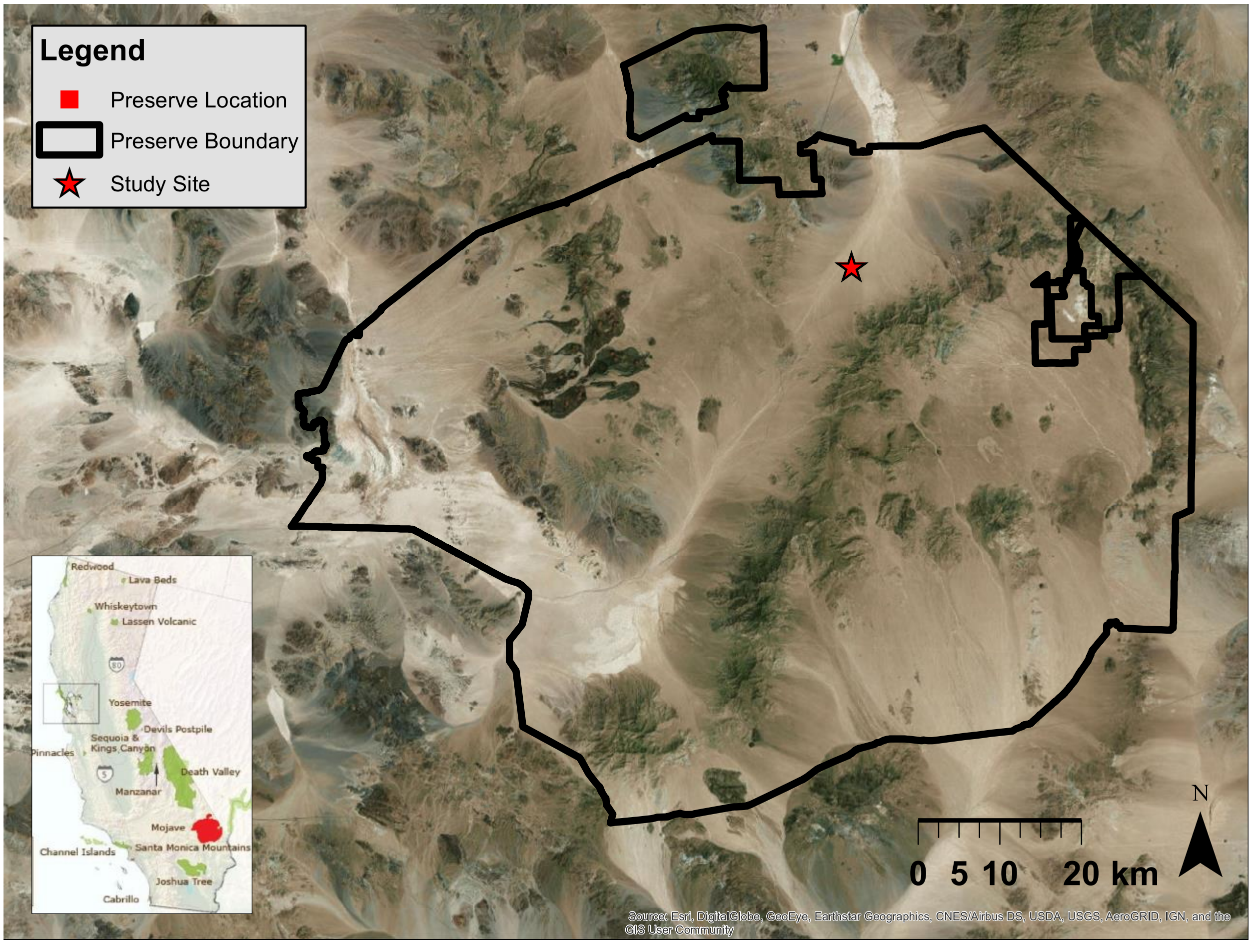
TABLES

Table 3.1 Post-release movement metrics from juvenile Mojave Desert Tortoises (*Gopherus agassizii*). Direct-released neonates were released in September 2015, whereas indoor- and outdoor-reared head-starts were kept in captivity for seven months prior to release in April 2016. We accepted significance at alpha = 0.05 and significant p values are in bold.

Metric	Treatment	*n*	Mean	Lower CI	Upper CI	Min	Max	*F*	*p*	Tukey's HSD
Mean move during first 4 days of dispersal (m)	Direct-release (A)	20	9.8	5.9	15.9	0	60.2	3.79	**0.028**	A-C*
	Indoor (B)	28	8.0	5.2	12.2	0	57.0			
	Outdoor (C)	20	3.7	2.0	6.4	0	21.6			
Distance from release site after 4 days of dispersal (m)	Direct-release (A)	19	41.6	24.1	63.8	0	235.6	6.03	**0.004**	A-C**, B-C*
	Indoor (B)	27	30.2	17.7	46.0	0	219.5			
	Outdoor (C)	20	8.4	1.9	19.3	0	50.7			
Mean move overall (m)	Direct-release (A)	20	9.4	6.8	13.1	2.1	74.4	14.96	**<0.001**	A-C**, B-C***
	Indoor (B)	28	14.5	11.0	18.9	6.5	37.9			
	Outdoor (C)	20	4.3	3.0	6.2	0	14.3			
Final distance from release site (m)	Direct-release (A)	16	164.8	94.5	286.9	17.8	928.3	6.80	**0.003**	A-C**
	Indoor (B)	14	75.7	41.5	137.3	12.2	603.2			
	Outdoor (C)	10	32.2	15.5	65.7	10.2	237.2			

Significance: $p < 0.001$ ‘ *** ‘; $p < 0.01$ ‘**’; $p < 0.05$ ‘*’

FIGURES



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 3.1. Location of study site for Mojave Desert Tortoise (*Gopherus agassizii*) head-start experiment in the Ivanpah Valley of the Mojave National Preserve (MNP), California, USA. Inset map of California shows the location of the MNP in red and a red star marks the location of our study site within MNP.

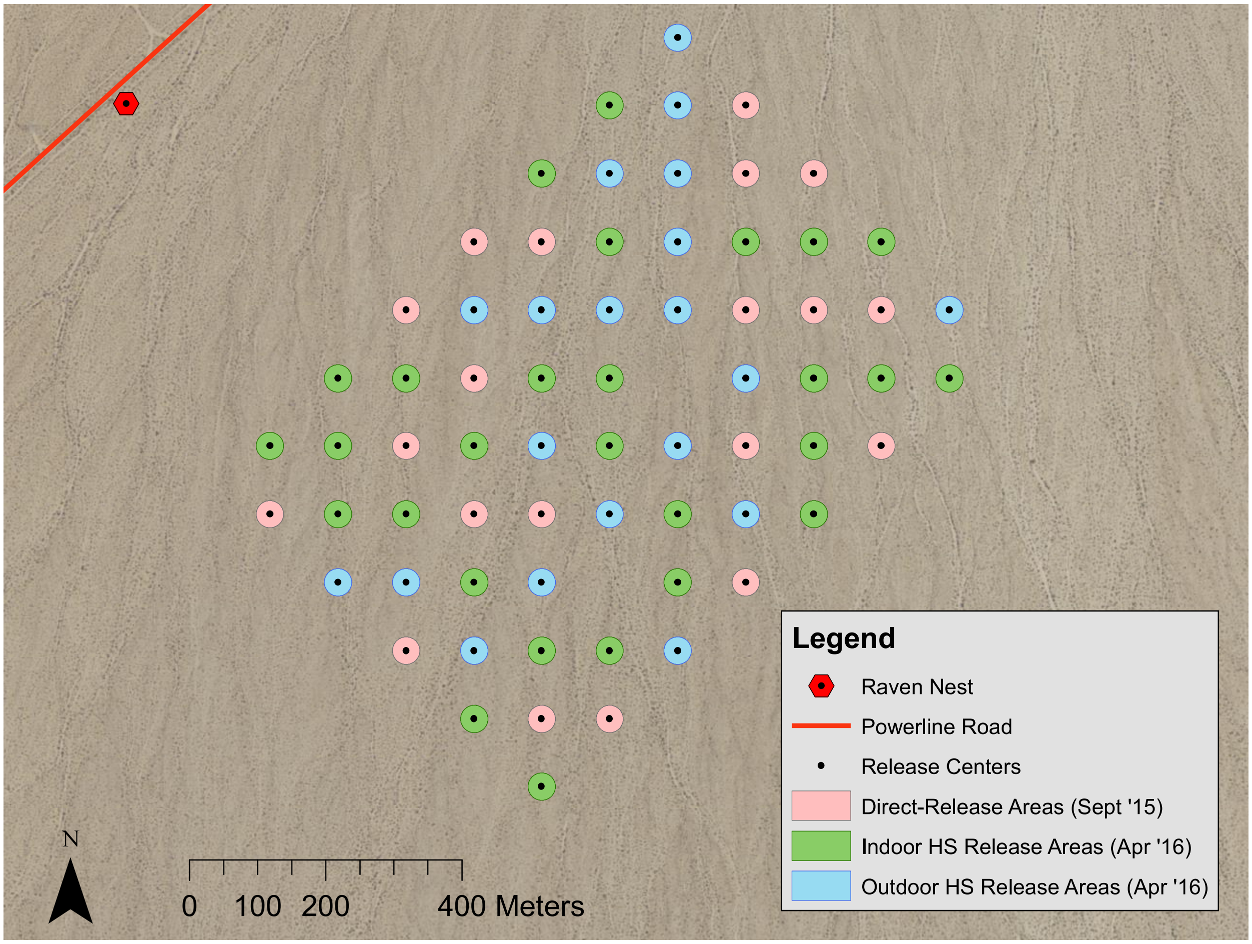


Figure 3. 2. Map of release site with 68 release centers for juvenile Mojave Desert Tortoises (*Gopherus agassizii*) in the Mojave National Preserve, California. Sixty-eight tortoises were released in a 700 m x 1000 m plot. Twenty tortoises were directly released (DR) on 28 September 2015. Seven months later (25 April 2016) 28 indoor head-started (HS) and 20 outdoor (HS) tortoises were also released. All DR and HS tortoises were from the 2015 hatchling cohort.

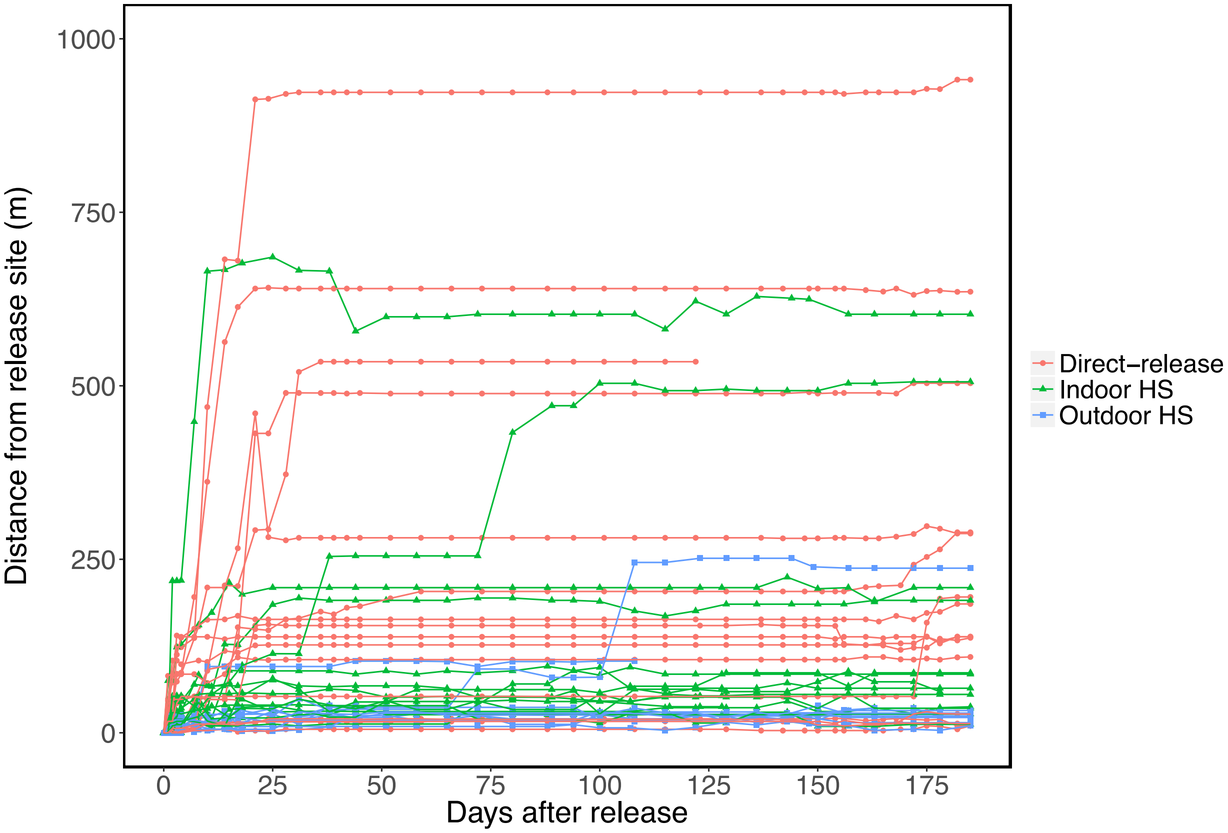


Figure 3.3. Distances moved from release centers during the first 185 days following release for juvenile Mojave Desert Tortoises (*Gopherus agassizii*) in the eastern Mojave Desert as part of a head-start (HS) experiment. Although we released a total of 68 tortoises, those (28) that died early in the study (before they ceased moving farther from their release sites) were not included in this plot. These data are from 40 tortoises in three treatment groups: direct release (n=16), indoor HS (n=14), and outdoor HS (n=10). Direct-release juveniles were released on 28 September 2015. Indoor HS and outdoor HS tortoises were released seven months later on 25 April 2016.

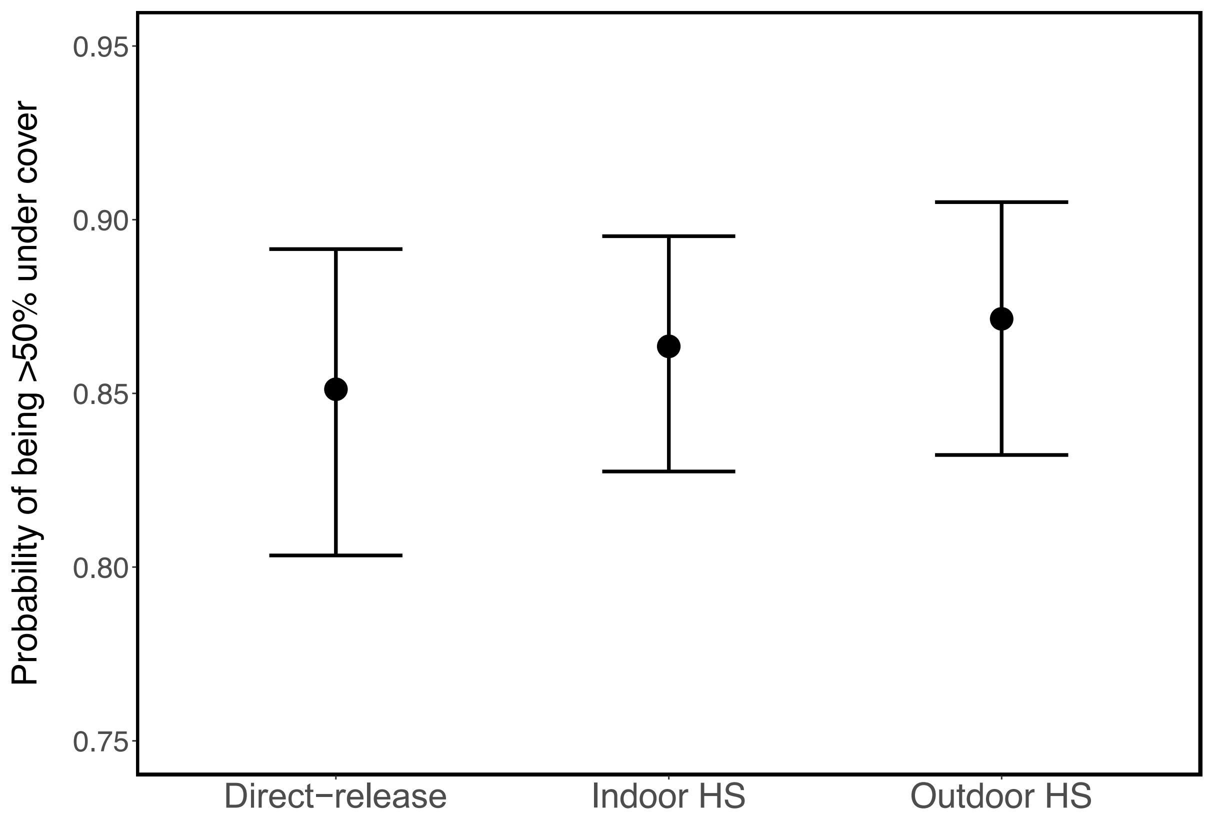


Figure 3.4. Estimated probability of being >50% under cover upon observation for juvenile Mojave Desert Tortoises (*Gopherus agassizii*). Data are from three experimental head-start (HS) treatment groups: directly released, indoor HS, and outdoor HS. Error bars are 95% confidence intervals.

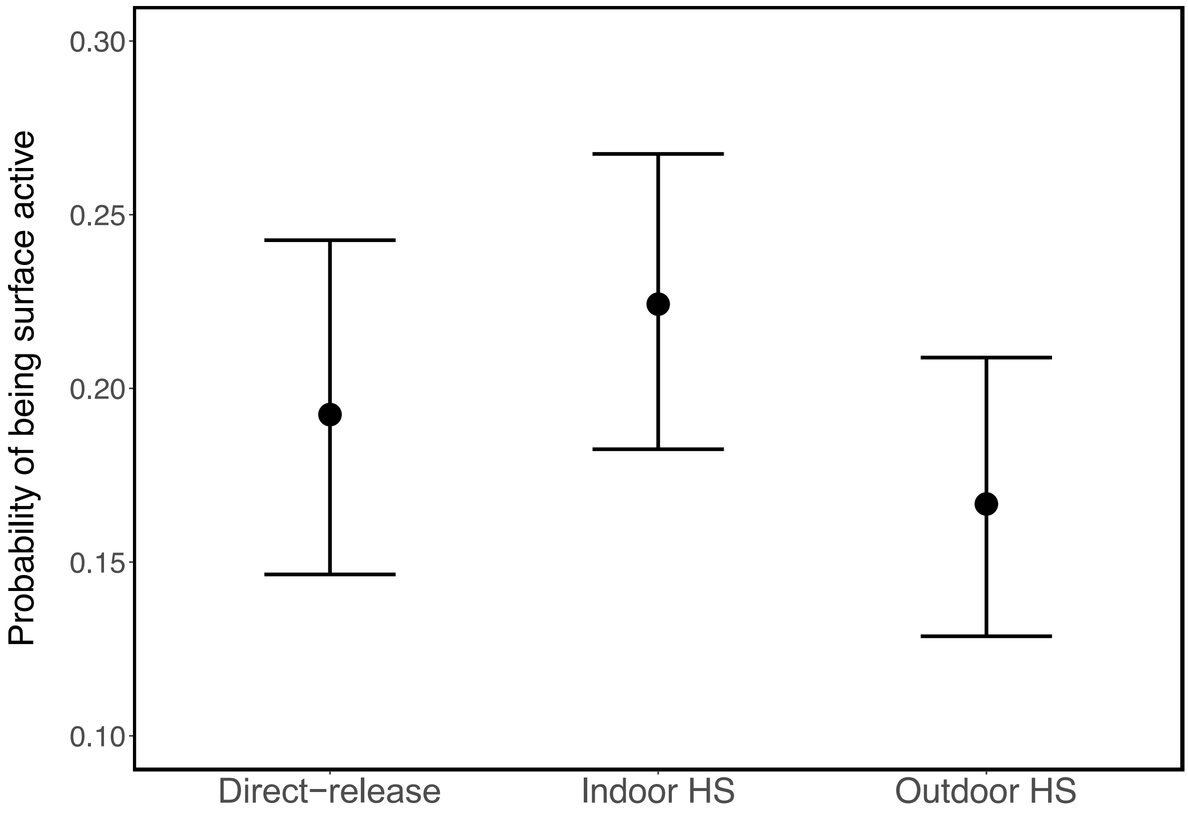


Figure 3.5. Estimated probability of being surface active upon observation for juvenile Mojave Desert Tortoises (*Gopherus agassizii*). Data are from three experimental head-start (HS) treatment groups: directly released, indoor HS, and outdoor HS. Tortoises were considered surface active if no part of their body was inside a burrow or pallet (shallow depression). Error bars are 95% confidence intervals.

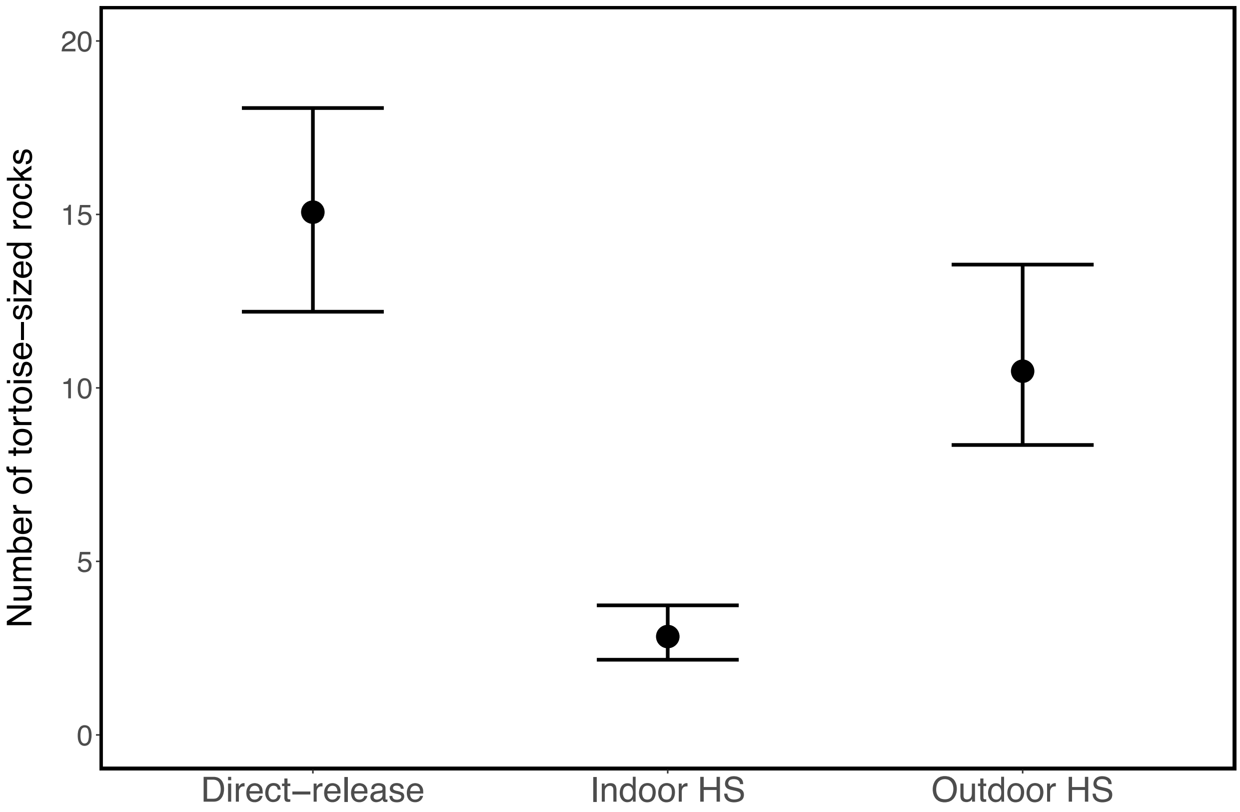


Figure 3.6. Mean number of “tortoise-sized” rocks within 1 m x 1 m plots surrounding juvenile Mojave Desert Tortoises (*Gopherus agassizii*) from three experimental treatment groups: directly released, indoor head-started and outdoor head-started (HS). Rocks were considered tortoise-sized if their largest dimension was within 50-150% of the mean treatment group size (midline carapace length by treatment group). Error bars are 95% confidence intervals.

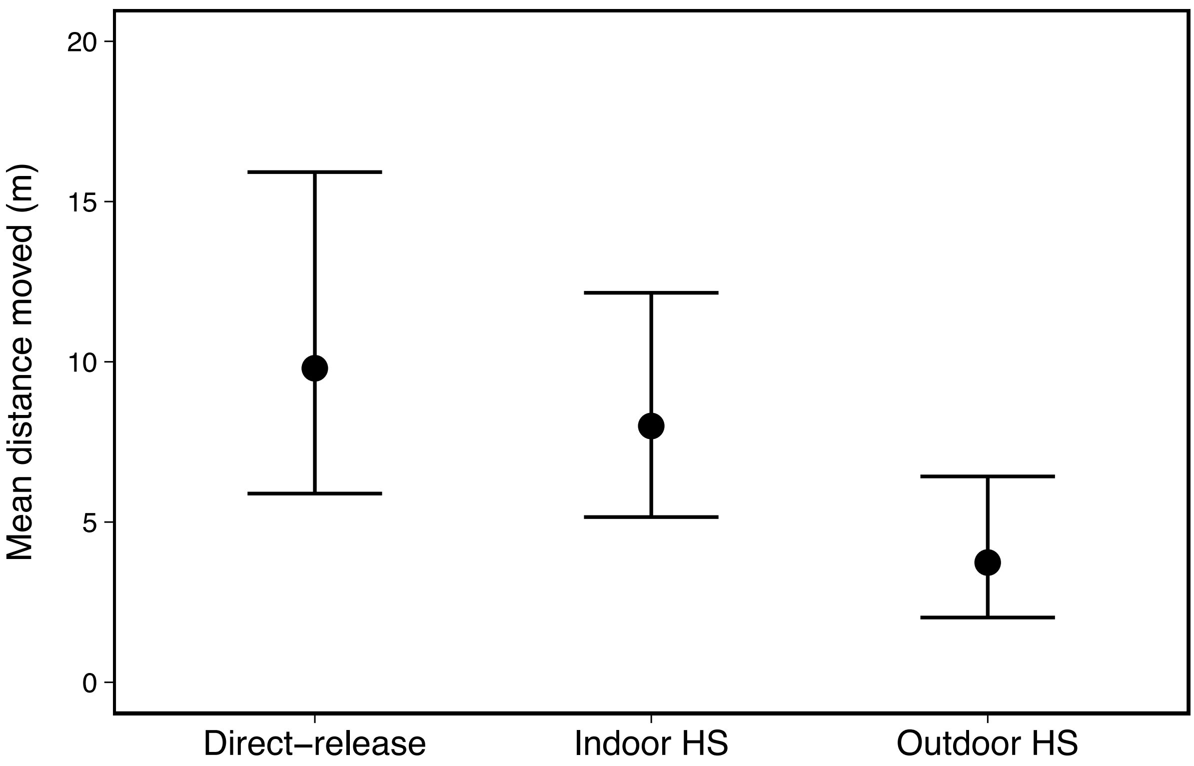


Figure 3.7. Mean distance moved per day by juvenile Mojave Desert Tortoises (*Gopherus agassizii*) during the first four days after release into the natural environment. Direct-release (n=20) juveniles were released on 28 September 2015. Indoor head-started (HS; n=28) and outdoor HS (n=20) tortoises were released seven months later on 25 April 2016. We calculated mean distance moved per day for each individual and conducted ANOVA and Tukey's tests by treatment group on individual mean scores. Error bars are 95% confidence intervals. Although confidence intervals overlap, direct-release tortoises moved significantly more than outdoor HS tortoises ($t = 2.59$, $p = 0.031$).

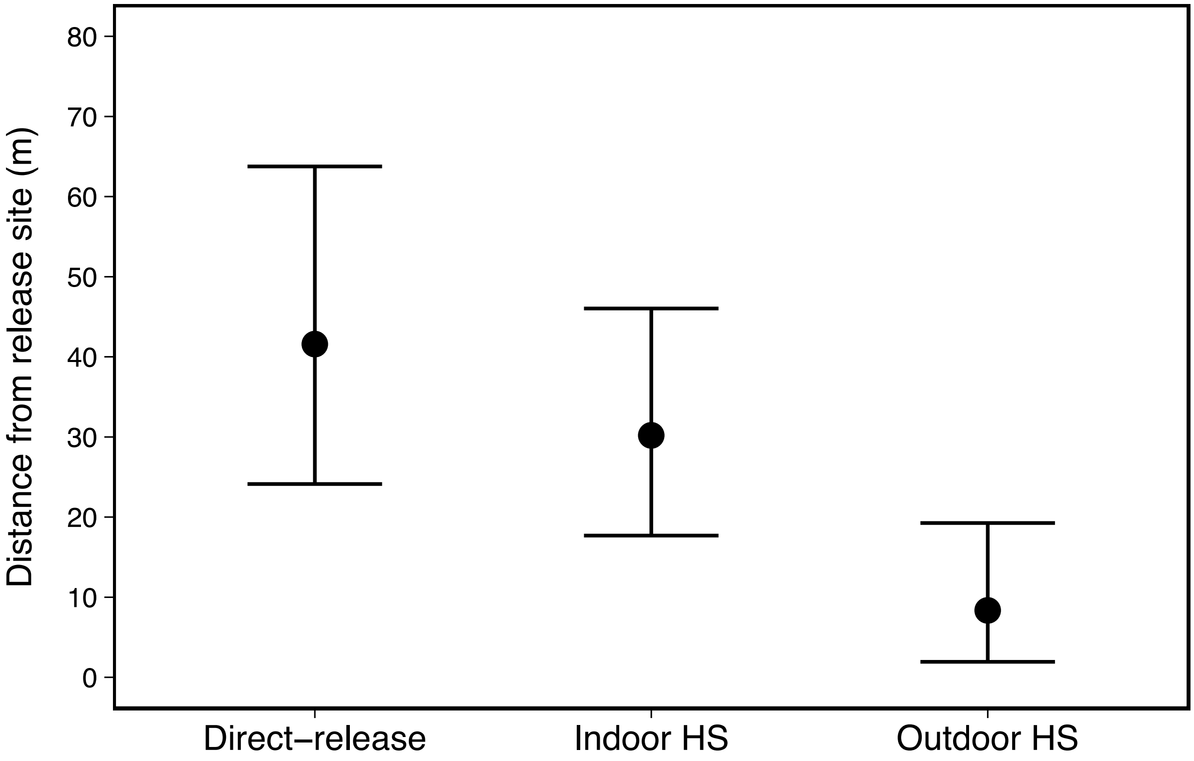


Figure 3.8. Mean distance from release site after four days of initial dispersal by juvenile Mojave Desert Tortoises (*Gopherus agassizii*) from three treatment groups. Direct-release (n=20) juveniles were released on 28 September 2015. Indoor head-started (HS; n=28) and outdoor HS (n=20) tortoises were released seven months later on 25 April 2016. Error bars are 95% confidence intervals.

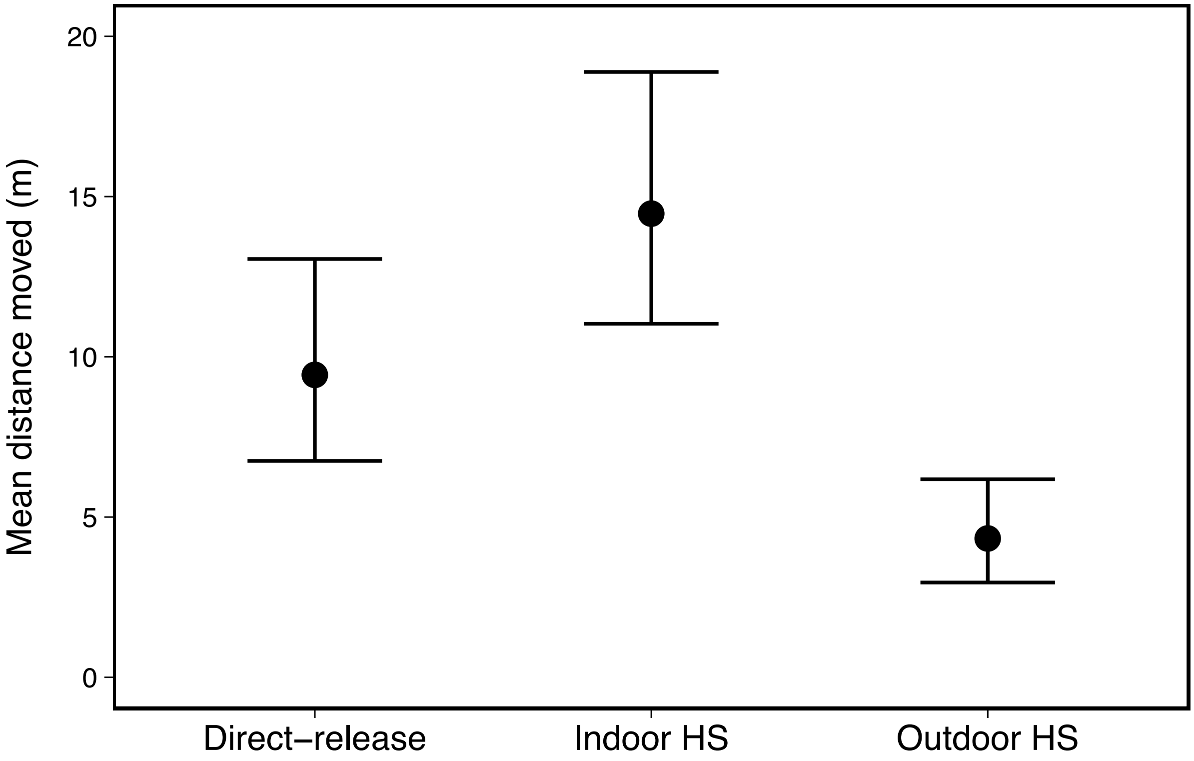


Figure 3.9. Mean distance moved between consecutive tracking events by juvenile Mojave Desert Tortoises (*Gopherus agassizii*). Direct-release (n=20) juveniles were released on 28 September 2015. Indoor head-started (HS; n=28) and outdoor HS (n=20) tortoises were released seven months later on 25 April 2016. We calculated means for each individual from their release until 27 October 2016, and conducted ANOVA and Tukey's tests by treatment group on these individual mean scores. Error bars are 95% confidence intervals.

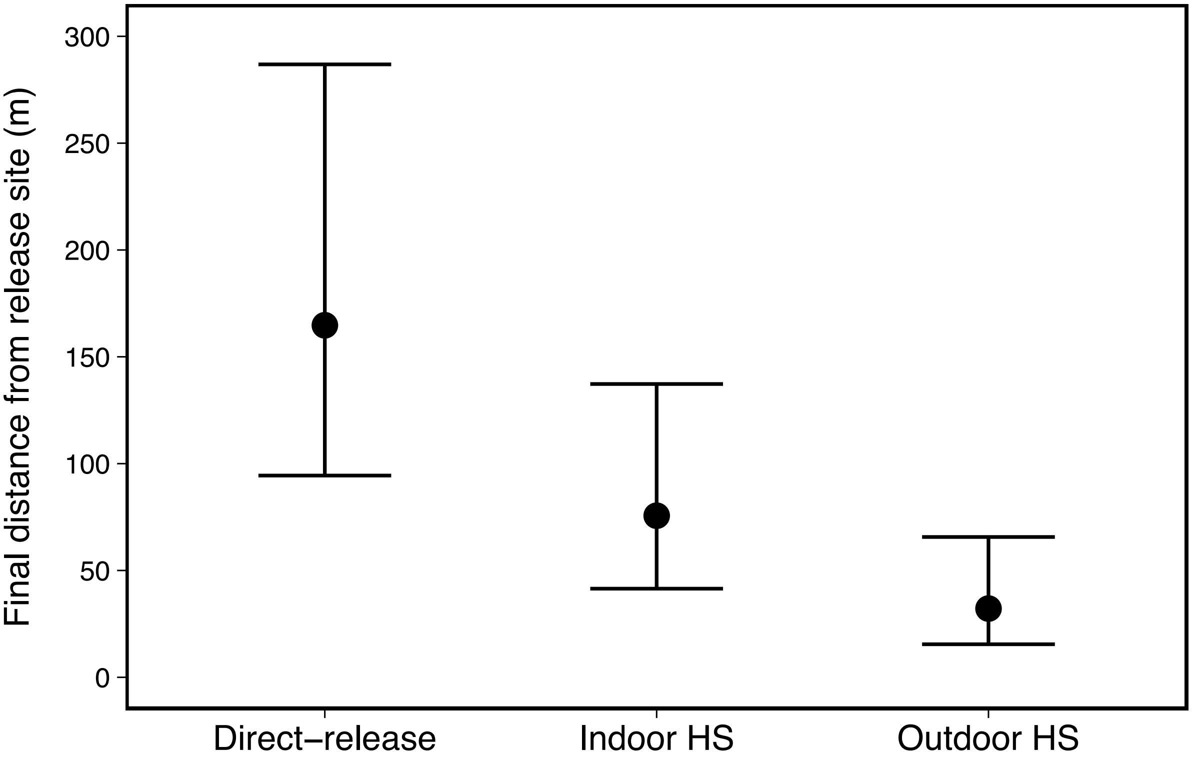


Figure 3.10. Final distance from release site from juvenile Mojave Desert Tortoises (*Gopherus agassizii*) in three experimental treatment groups. Data are presented as treatment group means with 95% confidence intervals. Although we released a total of 68 tortoises, those (28) that died early in the study (before they ceased moving farther from their release sites) were not included in this plot. These data are from 40 tortoises in three treatment groups: direct release (n=16), indoor HS (n=14), and outdoor HS (n=10). Direct-release juveniles were released on 28 September 2015. Indoor HS and outdoor HS tortoises were released seven months later on 25 April 2016.

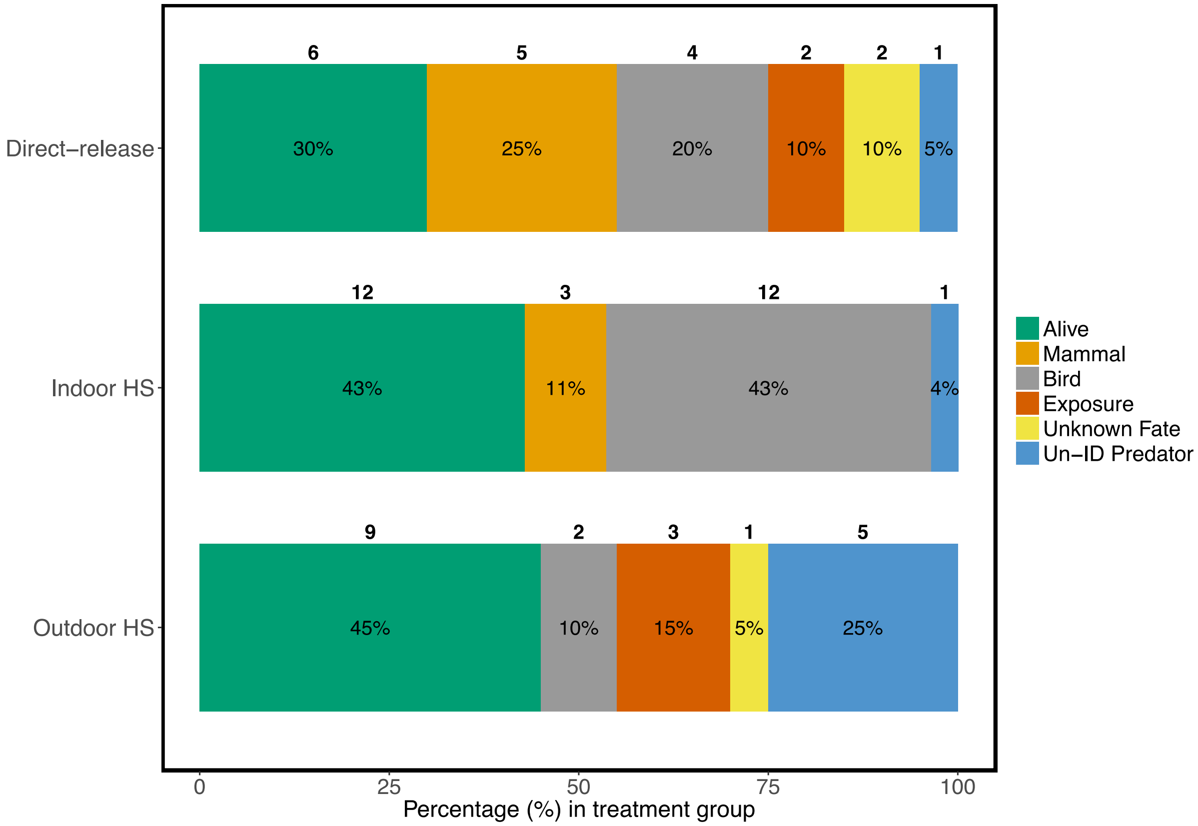


Figure 3.11. Fates of 68 juvenile Mojave Desert Tortoises (*Gopherus agassizii*) released into the eastern Mojave Desert. Directly released neonates (control group, n=20) were released in September 2015 whereas indoor head-started (HS; n=28) and outdoor HS (n=20) juveniles were released in April 2016, after seven months of head-starting. Bold numbers above bar segments are the number of individuals with each fate. Percentages are percentage of animals with each fate by treatment group rounded to the nearest percent.

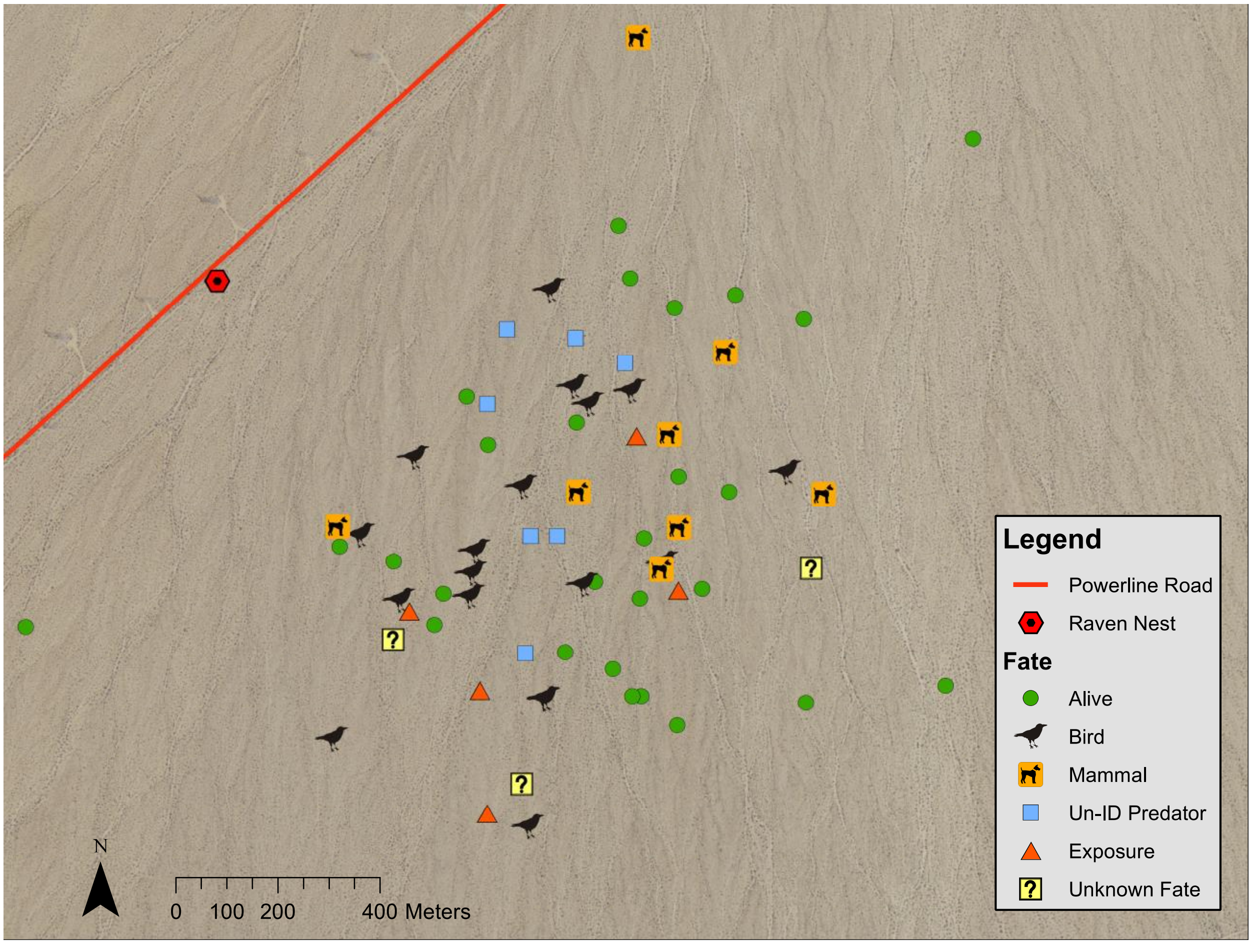
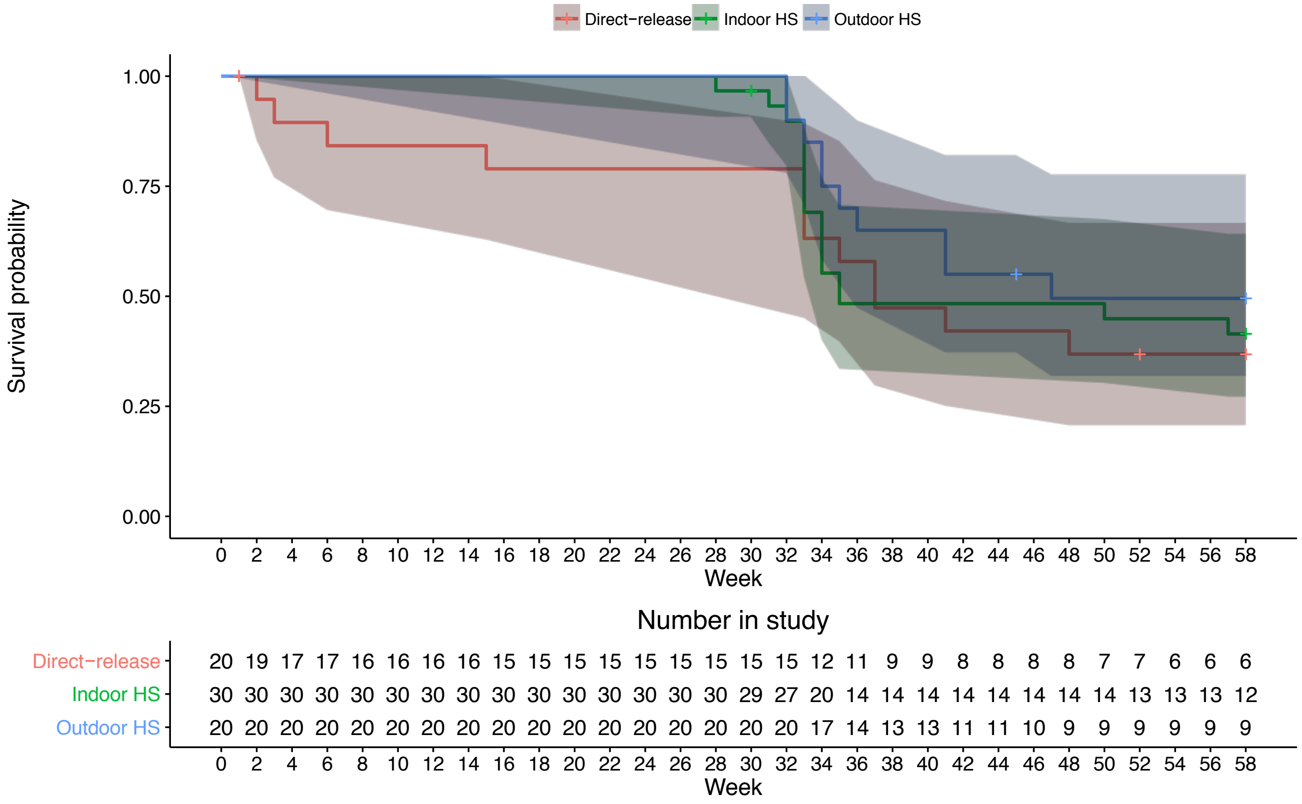


Figure 3.12. Study area showing last known live locations and fate for 68 released juvenile Mojave Desert Tortoises (*Gopherus agassizii*) in the eastern Mojave Desert. Ravens occasionally perched on the powerlines near our site throughout the study. However, we first noticed signs of activity (mated pair rearing young) at the raven nest on the day we released 48 of our 68 tortoises (25 April 2016).



Week	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58
Direct−release	20	19	17	17	16	16	16	16	15	15	15	15	15	15	15	15	15	12	11	9	9	8	8	8	8	7	7	6	6	6
Indoor HS	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	29	27	20	14	14	14	14	14	14	14	14	13	13	13	12
Outdoor HS	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	17	14	13	13	11	11	10	9	9	9	9	9	9

Figure 3.13. Kaplan-Meier survival curves for 70 Mojave Desert Tortoises (*Gopherus agassizii*) in three treatment groups: directly released (n=20), indoor head-started (HS; n=30), and outdoor HS (n=20). Estimates are shown from 28 September 2015 until 6 November 2016 (58 weeks). Direct-release tortoises were released into the natural environment at week zero (28 September 2015) whereas indoor and outdoor HS tortoises were released at week 30 (25 April 2016). Data were right-censored, in that no assumptions were made about the fate of lost or missing animals. Shaded bands are 95% confidence intervals.

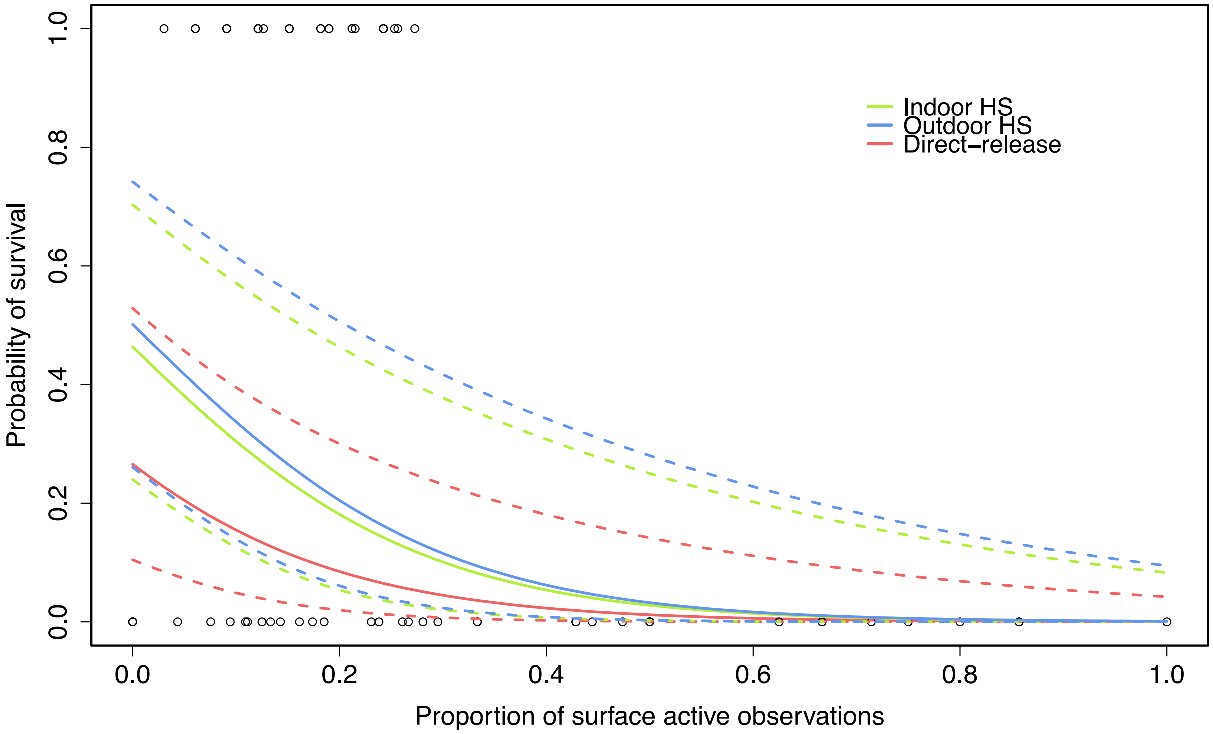


Figure 3.14. Observed survival fates (open circles) and predicted survival probabilities based on surface activity for Mojave Desert Tortoises (*Gopherus agassizii*) in three treatment groups: directly released, indoor head-started (HS), and outdoor HS. We predicted survival probability for each treatment group by setting distance to raven nest to 850 m and size and mean distance moved per tracking event to the treatment group mean for each individual. We then varied proportion of surface active observations from 0–1 and generated predictions of survival probability along that curve. Solid lines are predicted model estimates and dashed lines are upper and lower limits of 95% confidence intervals.

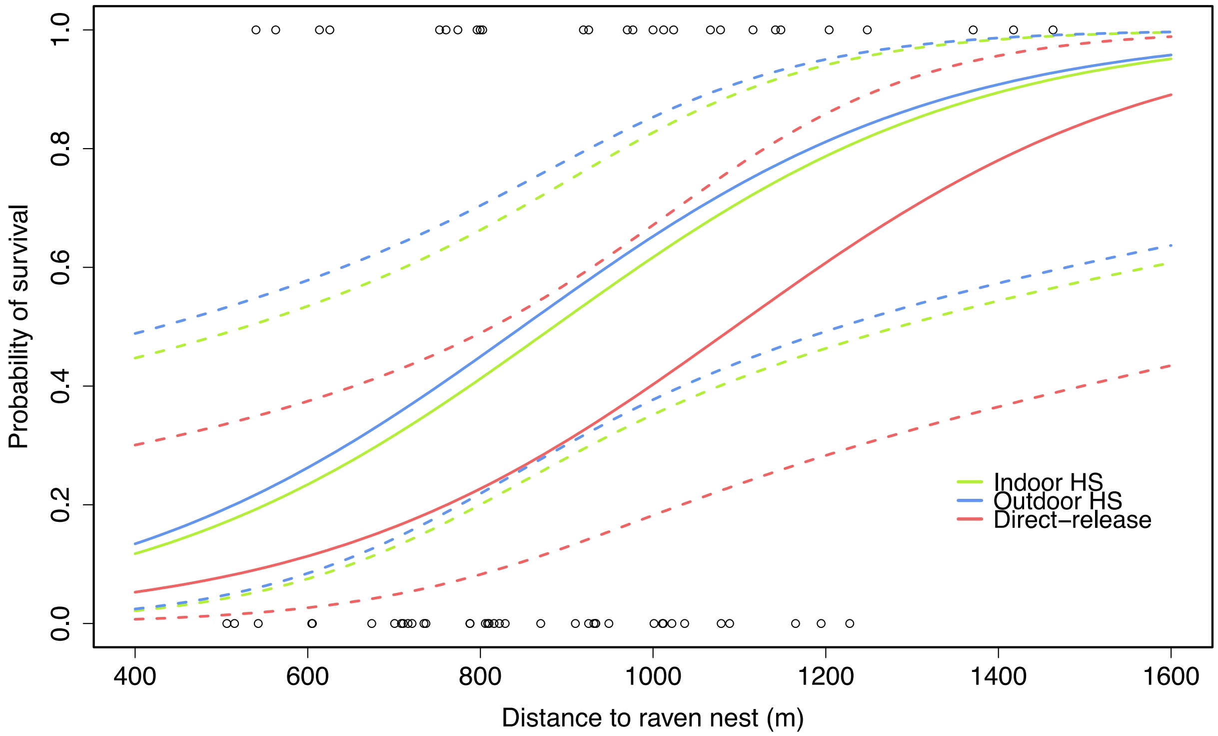


Figure 3.15. Observed survival fates (open circles) and predicted survival probabilities based on distance from a raven nest for juvenile Mojave Desert Tortoises (*Gopherus agassizii*) in three treatment groups: directly released, indoor head-started (HS), and outdoor HS. We predicted survival probability for each treatment group by setting distance moved per tracking event, size, and proportion of surface active observations to the treatment group mean for each individual. We then varied distance from the raven nest from 400–1600 m and generated predictions of survival probability along that curve. Solid lines are predicted model estimates and dashed lines are upper and lower limits of 95% confidence intervals.

CHAPTER 4

CONCLUSIONS

Indoor head-starting in Mojave Desert Tortoises was successful at increasing growth relative to outdoor-rearing and direct-release, but ultimately there was no difference in post-release survival among the treatment groups. Indoor head-starts reached the size of 5-6 y old wild tortoises (87 mm in carapace length; Turner et al. 1987, Curtin et al. 2009) by the time of release (April 2016). However, they had lower body condition than outdoor-reared tortoises of the same age, and softer shells than older outdoor-reared tortoises of the same size. However, they appeared clinically healthy and were alert and active. Nagy et al. (2011) found that shells among outdoor-reared head-starts were not fully developed (reached 98% shell hardness) until they reached 110 mm in midline carapace length or approximately 11 y of age. It is likely that shell hardness simply takes more time to develop than our seven-month indoor-rearing period. Perhaps a hybrid of indoor and outdoor-rearing (i.e., rearing tortoises indoors for some time before a final year of outdoor-rearing) could be useful in exploiting the growth enhancement of indoor rearing yet give tortoises time for their shells to harden.

Ultimately, post-release survival among the treatment groups did not differ, and all treatment groups experienced substantial raven predation. Annual survival overall (all treatment groups combined) was 0.44 (95% CI, 0.34–0.58), which is similar to estimates for annual survival of young tortoises (ages 0–18 months) released previously at the same site (Todd et al. 2015). The two previous peer-reviewed studies on survivorship of released outdoor-reared desert tortoises (Hazard et al. 2015, Nagy et al. 2015) found that larger head-starts were more likely to survive, and all raven predated head-starts were <80 mm in MCL in both studies. Therefore, we were surprised that indoor head-started tortoises were qualitatively more susceptible to predation by ravens than the smaller tortoises in our direct-release and outdoor head-started treatment groups (43% of released indoor head-starts were raven predated, as opposed to 20% of direct-release tortoises, and 10% of outdoor-reared head-starts).

We did not detect any differences among treatment groups in cover use or surface activity—behaviors that could influence predation risk. There was some initial concern that indoor head-starts may be too naive to use cover, dig burrows (they were too large to fit into most kangaroo rat burrows without modification) or modulate their surface activity to minimize risks. However, indoor head-starts were immediately successful in finding refugia; some dug their own burrows and others widened small mammal burrows to fit their width. Indoor head-starts may have been simply more visible to ravens. We found that indoor head-starts were surrounded by fewer 'tortoise-sized' rocks (rocks that were within 50-150% of their treatment group's mean size) than were direct-release or outdoor head-started tortoises, suggesting that they were less cryptic (Nafus et al. 2015). This difference was not likely driven by tortoise behavior, but was simply due to the fact that most rocks in our study site were small, and were closer in size to the smaller tortoises in the direct-release and outdoor-reared treatment groups.

Movement level differed by treatment group, with direct-release tortoises dispersing the farthest, and outdoor head-starts dispersing the least, but most tortoises in each treatment group settled within 300 m from their release site in the first month after their release. Only one tortoise settled over 700 m from its release site (a direct-release tortoise that settled 928 m distant). Except for tortoises in our outdoor head-start group (which moved exceptionally little), dispersal among our study animals was relatively similar to dispersal reported by other tortoise head-start studies (Pedrono and Sarovy 2000, Hazard and Morafka 2002, Hazard et al. 2015, Nagy et al. 2015). Although it is reasonable to assume that movement comes with risk, movement (mean distance moved between consecutive tracking events) was not a significant predictor of individual fate in our study.

Although we did not originally design this study as a raven focused experiment, ravens became an important part of our study when a pair of ravens began rearing chicks 500 m from the nearest edge of our release site. Raven predation became the leading cause of death among our study animals. However, none of the 18 bird-predated tortoise carcasses were found within 400 m of the raven nest, suggesting that identifying “offending ravens” for targeted removal (i.e., shooting) by searching under or near nests (USFWS 2008) is likely to be unfruitful. It is likely that many brood-rearing ravens kill juvenile tortoises but do not bring their carcasses to their nests, but rather eat them at perches closer to the kill sites. Thus, tortoise-killing ravens may be falsely classified as non-offending under current government guidelines and not targeted for removal.

Raven nests are quite spatially dispersed (Kristan and Boarman 2003), so the location of the active nest very close to our site should be considered a stochastic event, but predation by ravens on juvenile desert tortoises is common (George 2015, Hazard et al. 2015). Therefore, subsequent desert tortoise head-start efforts should avoid areas of potential raven activity as much as possible when selecting release sites. When evaluating average distance from the raven nest as a mortality risk factor, we found that tortoises were most likely to survive if they were at least 1.6 km from the raven nest. This indicates that brood-rearing ravens can have sizeable impacts on tortoise survival farther from their nests than previous research suggests (Sherman 1993).

Future directions and management implications

Future desert tortoise head-start research should consider evaluating a hybrid of indoor- and outdoor-rearing (e.g., rearing tortoises indoors for some time before a final year of outdoor-rearing). This may take advantage of the growth enhancing benefit of indoor-rearing, yet give head-starts more time for their shells to ossify and allow them to acclimate to the outdoors before their release. Future head-start studies should evaluate shell hardness and body condition, in addition to growth, to insure that head-starts are physiologically robust.

Our work has important implications for lethal control of ravens for conservation of desert tortoises and other sensitive species upon which ravens prey. Managers are authorized to kill ravens only if they are considered "offending ravens" (USFWS 2008). In the context of desert tortoise conservation, ravens are considered "offending" if observers find carcasses of juvenile tortoises "within their presumed territories" (USFWS 2008). As Boarman (2003) pointed out, it is unrealistic to find all juvenile tortoise carcasses within the 8 km^2 territory of a brood-rearing raven. Because none of the bird-predated tortoises were found within 400 m of the raven nest, searching under or near the nest would have been unsuccessful in detecting these predated juveniles. All ravens foraging in desert tortoise habitat should be considered a threat to juvenile tortoises.

Analysis of potential mortality risk factors found that tortoises were most likely to survive if they were on average at least 1.6 km from an active raven nest. Therefore, head-starts should be released at least 1.6 km (or preferably farther) from raised man-made structures that could attract ravens. The pair of ravens at our site began rearing their young just as our newly released head-starts (indoor- and outdoor-reared) were dispersing in late April and May. Releasing head-starts in the fall would insure that head-start dispersal does not coincide with raven brood-rearing (as raven nestlings fledge by mid summer). Furthermore, fall release would also allow managers to identify areas of raven activity and nesting (to avoid) in the spring and summer, prior to head-start release in the fall. Therefore, fall may be a better time to release head-started desert tortoises than spring.

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