COMPARING GROWTH AND BODY CONDITION OF INDOOR-REAURED, OUTDOOR-REAURED, AND DIRECT-RELEASED JUVENILE MOJAVE DESERT TORTOISES

JACOB A. DALY¹, KURT A. BUHLMANN¹, BRIAN D. TÓDDO³, CLINTON T. MOORE³, J. MARK PEADE¹, AND TRACEY D. TUBERVILLE¹

¹University of Georgia’s Savannah River Ecology Lab, Post Office Drawer E, Aiken, South Carolina 29802, USA
²Department of Wildlife, Fish and Conservation Biology, University of California, Davis, California 95616, USA
³U.S. Geological Survey, Georgia Cooperative Fish and Wildlife Research Unit, University of Georgia, Athens, Georgia 30602, USA

Abstract.—Desert Tortoise (Gopherus agassizii) populations have declined, and head-starting hatchlings in captivity until they are larger and older, and presumably more likely to survive, is one strategy being evaluated for species recovery. Previous studies have reared hatchlings in outdoor, predator-proof pens for 5–9 y before release, in efforts to produce hatchlings in excess of 100–110 mm midline carapace length that are believed to be predation-resistant. We began a comparative study to evaluate indoor-rearing to shorten this rearing period by facilitating faster initial growth. We assigned 70 neonates from the 2015 hatching season to three treatment groups: (1) indoor-reared (n = 30), (2) outdoor-reared (n = 20), and (3) direct-release (n = 20). We released direct-release hatchlings shortly after hatching in September 2015 and monitored them 1–2 times per week with radio telemetry. We head-started the indoor- and outdoor-reared treatment groups for 7 mo before releasing them in April 2016. Indoor-reared tortoises were fed five times per week (September to March). Outdoor-reared tortoises had access to native forage and we gave them supplemental water and food once per week while active before winter dormancy. Indoor-reared tortoises grew > 16 times faster than direct-release tortoises and > 8 times faster than outdoor-reared tortoises; however, indoor-reared tortoises weighed less and had softer shells than comparatively sized older (3–4 y-old) tortoises raised outdoors. Increasing the duration of the indoor-rearing period or incorporating a combination of both indoor and outdoor husbandry may increase shell hardness among head-starts, while retaining the growth-promoting effect of indoor rearing and shortening overall captivity duration.

Key Words.—body condition; Chelonian; conservation; husbandry; morphology; reptile; threatened; wildlife management

INTRODUCTION

Head-starting seeks to increase the number of animals eventually recruited into a breeding population by raising juvenile animals in protected conditions early in life 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 projects have been initiated with varying success for mammals (Sinn et al. 2014), birds (Cohn 1999), amphibians (Lannoo 2005), and reptiles (Jarvie et al. 2015; Tuberville et al. 2015). Turtles may be particularly suited to head-starting as a recovery tool (Burke 2015) because they have low survival in the wild during their early life stages and high survival as adults under most natural conditions (Gibbons 1987). Turtle head-starting studies have increased recently (see Herpetological Conservation and Biology, Volume 10), and include Blanding’s Turtles (Emydoidea blandingii; Green 2015; Buhlmann et al. 2015), Gopher Tortoises (Gopherus polyphemus; Tuberville et al. 2015; Quinn et al. 2018), Western Pond Turtles (Actinemys marmorata; Vander Haegen et al. 2009), and Kemp’s Ridley Sea Turtles (Lepidochelys kempii; Caillouet et al. 2015), among others.

Head-starting can be a useful tool in turtle conservation. For example, head-starting has been used to reestablish wild populations of Blanding’s Turtles (Buhlmann et al. 2015) and Galapagos Tortoises (Chelonoidis hoodensis; Gibbs et al. 2014) in areas where they had previously been extirpated. Head-starting has also been useful in restoring ecosystem services. By establishing populations of the non-native Aldabra Giant Tortoise (Aldabrachelys gigantea) using head-starting to replace extinct Cylindraspis, conservationists have begun to control the spread of invasive alien species, as the Aldabra tortoises restored grazing and seed dispersal to the ecosystem (Griffiths et
Gopherus agassizii), and Mycoplasma spp., Common Ravens, Corvus corax, and Coyotes, Canis latrans), upper-respiratory tract disease (Mycoplasma spp.), and habitat degradation from disturbance and invasive plants have all been identified as contributing causes (Berry 1986; Esque et al. 2010; Nafus et al. 2013; Peaden et al. 2015). Head-starting has been identified as a possible management action to reinforce diminished populations of Mojave Desert Tortoises (USFWS 2008, 2011; hereafter desert tortoises), provided that the original causes of population decline have been mitigated or are addressed concurrently. 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 et al. 2015; Nagy et al. 2015b). Estimates of size at which juvenile post-release survival substantially increases range from 84 mm (Hazard et al. 2015) to 100 mm mid-line carapace length (MCL; Nagy et al. 2015b). Although supplemental food and water can increase growth and survival of desert tortoises raised outdoors (Nafus et al. 2017, Nagy et al. 2015a), outdoor rearing, as in the wild, may take 5–9 y to produce a juvenile tortoise of 100 mm MCL (Nagy et al. 2015a) because tortoises maintain natural behaviors and are inactive during both the hottest and coldest seasons in the desert. Rearing tortoises indoors may decrease the time needed to raise tortoises to larger size by keeping juveniles active and growing during the winter months, when growth otherwise ceases in the wild. No study has yet evaluated indoor head-starting in desert tortoises.

Accelerating growth of captive tortoises by raising them indoors may have unknown consequences for the overall health and condition of the animals. Thus, the monitoring of metrics like body condition and shell hardness within head-start studies would provide a comprehensive assessment of robustness of captive-reared tortoises. Body condition (BC) is expressed as the mass of an animal relative to an appropriate size metric (approximated volume in our case) and can reflect nutritional condition, stored fat, and water balance (Shine et al. 2001; Nagy et al. 2002; Loehr et al. 2007; Nagy et al. 2015a). Shell hardness increases with body size and age in juvenile desert tortoises (Nagy et al. 2011), and the hardness of the shell of a turtle likely plays a major role in its protection against predators.

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. The research presented here is part of a larger effort to evaluate indoor head-starting through long-term, post-release monitoring. We established three treatment groups: indoor-reared head-started tortoises, outdoor-reared head-started tortoises, and direct-release hatchlings (all 2015 cohort). We reared indoor and outdoor head-start animals for seven months (September to April) prior to release, and direct-release animals were released in the natural environment days after hatching (September) to serve as a control. We compared growth, body condition, and survival among the three treatment groups at the end of the 7-mo period (a short time relative to the potential lifespan of > 50 y of desert tortoises). We also evaluated the indoor-reared tortoise group for shell hardness at the end of the rearing period relative to similarly sized, but older (3–4 y-old of 2011 and 2012 cohorts) outdoor-reared captive tortoises from an earlier study.

**Materials and Methods**

**Study site.**—The Mojave National Preserve (MNP) is a 650,000 ha preserve in San Bernardino County, California, USA, in the eastern Mojave Desert managed by the U.S. National Park Service (NPS). We conducted all experiments and observations 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 (3.8 tortoises per km$^2$ in 2008, Allison 2012; 77–85 tortoises per km$^2$ in 1977–1980, Turner et al. 1984). The area into which we released juvenile tortoises was a 0.7-km$^2$ unfenced plot centered 850 m from a powerline service road in the Ivanpah Valley of the MNP, the exact location not disclosed for security (Lindemayer and Scheele 2017; Litgusz, J. 2017. The illegal turtle trade: why scientists keep secrets. Available from http://theconversation.com/the-illegal-turtle-trade-why-i-keep-secrets-85805. [Accessed 29 November 2017].

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Other human disturbances in our study area include abandoned cattle grazing infrastructure (fencing and corrals), and a railroad track 4.5 km away.

Obtaining hatchlings.—In May 2015 we captured our previously radio-tagged female desert tortoises (Nafus et al. 2015), brought them to the Ivanpah Desert Tortoise Research Facility (hereafter, facility), and radiographed them to determine gravidity. We placed females with at least three calcified eggs in predator-proof nesting pens at the facility; we returned all others to their capture sites. The nesting enclosure measured 30 × 30 m and was permanently subdivided with metal siding into 18 smaller pens (5 × 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 310-mm (12-in) diameter cardboard Quik-Tube building forms® (Quikrete International, Atlanta, Georgia, USA) that were cut in half longitudinally and buried at a 20° angle underground. We kept gravid females until they laid their eggs (within 30 d in most cases) and then returned them to their last burrow location. As hatching approached (70 d into the estimated 90-d incubation period), we began monitoring pens for hatchlings several times daily.

We obtained 74 hatchlings 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) with codes assigned to us by USFWS. We excluded four hatchlings from the study due to especially low body mass at hatching and/or developmental defects. We assigned healthy hatchlings to one of three treatment groups (described further below): (1) direct-release (control group, n = 20); (2) outdoor-reared (n = 20), and (3) indoor-reared (n = 30; Fig. 1). 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. When more than one hatching from a clutch was assigned to a treatment, we distributed clutch-mates among replicate enclosures receiving the same treatment.

Experimental treatment groups.—On 24 September 2015 (21–46 d after hatching), we moved the indoor-reared head-start (HS) treatment group to mesocosms inside the climate-controlled facility. We set ambient temperature inside the facility to a constant 24.4° C. We used 189-L (50-gallon) Rubbermaid (Atlanta, Georgia, USA) stock tanks (132 × 79 × 30.5 cm) filled with a 5-cm layer of natural desert sand as substrate. 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, USA) 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 nighttime heat. Lights were connected to automatic timers. We timed basking lights to operate 0600–1830, and the infrared lights were timed to operate 1900–0530 (an interval set to approximate the natural photoperiod at the beginning of the study, but not adjusted seasonally thereafter). The lights created basking spots of 37° C during the day and 32° C at night. The daytime basking bulbs provided ultraviolet long-wave light (UVA) but not ultraviolet short-wave (UVB) light. Windows on two sides of the room also provided some natural light during the day.

We outfitted each tank with three cover items constructed from halved plastic pipe (11.5 cm in diameter and cut into 12-cm linear segments) and a paper feeding plate. Because inadequate humidity has been linked to unnatural shell growth 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 hide boxes by cutting burrow-shaped entrance holes (one hole per hide box) into the sides of lidded plastic tote boxes (Rubbermaid Roughnecks; Rubbermaid, Atlanta, Georgia, USA; 40 × 26 × 18 cm) and we lined each tote box with 7 cm of moist peat moss that was re-moistened every 3–4 d.

We fed indoor-reared hatchlings ad libitum five times per week and soaked them weekly for 15 min in 1–2 cm
of water to allow them to drink. Diet was a mixture, by mass, of leafy greens (50%) supplemented with commercially available food pellets (25%, ZooMed® Grassland Tortoise Diet, ZooMed® Laboratories Inc., San Luis Obispo, California, USA), 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 natural forage of desert tortoises. Collectively, they provide the ratio of phosphorus to potassium that facilitates assimilation of calcium (Jarchow et al. 2002). The 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 locally, we used Kale (a cultivar of Brassica oleracea) or Swiss Chard (Beta vulgaris cicla) as a substitute. On 11 December 2015, we also began adding Rep-Cal® Calcium with Vitamin D3 (Rep-CaL Research Labs, Los Gatos, California, USA) to the food mixture twice per week.

On 23 September 2015, we placed outdoor-reared hatchlings into predator-proof, semi-natural pens at the facility. The 30 × 30-m enclosure was constructed of chain-link fence (buried to exclude digging mammals) and was covered with netting to exclude avian predators. Within the larger enclosure, we placed hatchlings into two 10 × 10-m pens at a density of 10 hatchlings per pen. The pens mimicked the local natural environment and contained native vegetation, sand substrate, rocks, dead woody structure, and starter burrows. The starter burrows were constructed from halved PVC pipe (13-cm diameter) buried in a 0.5–1.0-m trench at a 20° angle from the surface.

We provided artificial rain weekly during the active season (until late October) for 30 min with rotating sprinklers to allow hatchlings to drink and to stimulate growth of native vegetation (Beatley 1974). Within the outdoor rearing pens, we inventoried 11 annual species on which tortoises are known to forage (Jennings and Berry, 2015; Abella and Berry, 2016) including, Wingnut Cryptantha (Cryptantha pterocarya), Common Stork’s-bill (Erodium cicutarium), Common Mediterranean Grass (Schismus barbatus), Desert Indianwheat (Plantago ovata), Booth’s Evening Primrose (Camissonia boothii), Brittle Spineflower (Chorizanthe brevicornu), Devil’s Spineflower (C. rigida), Pepperweed (Lapidium spp.), Desertsnow (Linanthus demissus), Desert DanDELion (Malacothrix glabrata), and Whitestem Blazingstar (Mentzelia albicaulis). Although natural vegetation was readily available for outdoor-reared tortoises, we also provided supplemental food on watering days because watering stimulated hatchling exploration and feeding (Nafus et al. 2017). The food mixture was the same as described above for the indoor-reared hatchlings. While tortoises were housed within pens, we frequently observed foraging behavior on both natural vegetation and supplemental food; however, we collected no data on their foraging preferences. The amount of supplemental food fed was 5% of the total tortoise biomass in each pen, which was functionally ad libitum, but which minimized waste to avoid attracting ants (Formicidae). We ceased supplemental watering and feeding during the fall and winter when hatchlings are normally inactive outdoors.

On 28 September 2015, we released hatchlings assigned to the direct-release treatment group (hereafter DR) into the natural environment in Ivanpah Valley. We released the hatchlings within a 0.7-km² rectangular study area of unmanipulated and unfenced natural desert that was imbedded in the general area where their mothers had been captured. We used radio-telemetry to monitor these free-ranging hatchlings for post-release growth and survivorship. We attached 2.1-g radio transmitters (BD-2, Holohil Systems Ltd., Ontario, Canada) with 7-mo batteries to each tortoise on the fourth vertebral scute with 5-min epoxy. The mass of radio transmitters was generally <10% of the mean body mass for released hatchlings (range = 7.7–11.9%, with only 3/20, or 15%, of individuals with transmitters > 10% of their body mass). Prior to release, we monitored animals for any signs of stress or abnormal behavior. Radio transmitter placement and type were approved prior to release by USFWS and California Department of Fish and Wildlife. We used a 3-element Yagi antenna (AF Antronics, Inc., Urbana, Illinois, USA) and a R1000 receiver (Communications Specialists, Inc., Orange, California, USA) 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. We resumed twice per week tracking in March 2016.

Morphometrics.—We measured and weighed (hereafter measured) hatchlings immediately after hatching (hatching size) and then again prior to treatment group assignment on 22 September 2015 (initial size). We measured indoor-reared tortoises approximately every 30 d until their release in late April 2016. We measured 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. We measured the indoor and outdoor HS animals again prior to their release in late April 2016. We recorded mass with a digital 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.

**Body condition.**—We calculated body condition (BC) for all surviving animals from all treatment groups based on measurements taken 5.8 mo into the rearing period (11–16 March 2016) using the formula described by Loehr et al. (2004), where \( BC = \text{body mass (g)} / \text{shell volume (cm}^3 \)\). We computed shell volume (for the BC calculations and for analysis of volume-corrected mass) using the standard formula for a half-ellipsoid (Loehr et al. 2004), where all input sizes are in mm and the product is in cm\(^3\):

\[
\text{Shell volume (cm}^3\) = \left(\pi \times \text{MCL} \times \text{width} \times \text{height} \right) / 6000
\]

To make our data easily comparable with metrics most often reported in the literature, we also present body condition in the formula described by Nagy et al. (2002), where shell volume, approximated as a box, is calculated as \( \text{MCL} \times \text{width} \times \text{height} \) (Tables 1–2).

**Shell hardness.**—To measure shell hardness, we used a 10.2-cm (4-in) tension-calibrated micrometer (model 3732XFL-4; L.S. Starrett Company, Athol, Massachusetts, USA) to measure normal, uncompressed shell height (UCSH) at the center of the third vertebral scute (Nagy et al. 2011). We then turned the micrometer spindle, compressing the shell of each tortoise between the two measuring faces until a point where the spindle ratchet began to slip continually for approximately 240° of further turning. 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:

\[
\text{Shell Hardness Index (SHI)} = \left(\frac{\text{CSH}}{\text{UCSH}}\right) \times 100
\]

We measured shell hardness of surviving indoor HS animals (\( n = 29 \)) just prior to release in April 2016. Prior to analysis, we discarded an unrealistically low measurement attributed to one indoor HS individual, which we suspect was due to misreading the instrument. Of the 2015 cohort juveniles, we were only able to measure the indoor HS group for shell hardness. The 2015 cohort outdoor HS and DR juveniles were too soft to safely measure, raising concern that compressing these smaller tortoises would cause injury. Therefore, we compared the shell hardness of the 2015 cohort indoor HS juveniles with shell hardness data taken in September 2015 from similar-sized, but older (3–4 y-old), outdoor-reared animals (2011–2012 cohorts) from other enclosures at the facility (Nafus et al. 2017). Similarly, we also compared body condition among indoor HS 2015 cohort juveniles and similar-sized but older 2011 and 2012 cohort juveniles in case any differences observed among the 2015 treatment groups (indoor HS, outdoor HS, and DR) were a result of allometric effects. These 2011 and 2012 cohort juveniles were reared in similar conditions to the 2015 cohort, outdoor HS animals in our current study, except approximately half of them (19/38: 2012 cohort; 14/26: 2011 cohort) were rain supplemented half as often (one time per two weeks vs. one time per one week), as part of a previous investigation on the effects of differing artificial rainfall regimens on head-starting (Nafus et al. 2017).

**Statistical methods.**—We performed all statistical tests in Program R (R Core Team 2014), using \( \alpha = 0.05 \) as the acceptable threshold of Type I error. We present data as means ± 1 SE. We used the Kaplan-Meier estimator to estimate the survival of each treatment group (Pollock et al. 1989). To test for MCL differences among treatment groups (2015 cohort), we used linear mixed effects models with MCL as the response variable and the unique ID code of the mother as a random effect. Visual assessment of histograms of model residuals showed that model residuals generally approximated the normal distribution, with the exception of one outlier (the smallest individual in the indoor-reared treatment group). We ran our analyses with and without this outlier, and outcomes were unchanged; thus, we felt justified modeling our full dataset (including the outlier).

To analyze volume-corrected mass (a way of analyzing body condition) by treatment group (2015 cohort), we used analysis of covariance (ANCOVA) with log-transformed mass as the response variable and log-transformed shell volume as the covariate (García-Berthou, 2001), and we included maternal identity as a random effect. We also used ANCOVA to test for treatment group (indoor HS 2015 cohort, outdoor HS 2011 cohort, and outdoor HS 2012 cohort) effects on shell hardness and volume-corrected mass. When analyzing shell hardness, we included MCL as a covariate, and when analyzing volume-corrected mass we used log-transformed mass as the response variable and log-transformed shell volume as the covariate (García-Berthou, 2001). For each ANCOVA, we tested for interactions between treatment variables and covariates and retained interactive terms when significant. When treatment effects were detected, we performed Tukey’s post-hoc multiple comparisons using the glht function in the multcomp package in R to further investigate treatment group differences.
Twenty-five of 31 captured females were gravid with at least three eggs and were placed in predator-proof nesting pens at the facility. They collectively laid 123 eggs, from which 74 hatchlings successfully emerged (60.2% emergence success). 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. On 8 April 2016, however, we found one indoor HS dead of unknown causes in its mesocosm; thus, survival for the indoor HS group was 96.7% (29/30) through the head-start period. Fifteen of the 20 (75%) DR juveniles survived from their release on 28 September 2015 until spring measurements in mid-March 2016; we found four dead and we never found one (unknown fate) before mid-March. Throughout the rearing period, survival estimates among the treatment groups did not differ significantly \((P > 0.05\) based on overlapping confidence intervals; Daly 2017).

Mean initial MCL for the 2015 cohort was 46.9 ± 0.2 mm \((n = 70);\) range, 40.5–50.2) and MCL did not differ significantly \((F_{2,55} = 2.67, P = 0.078)\) among the three treatment groups \((DR: 46.5 ± 0.4 \text{ mm}; \text{ indoor HS: } 45.8 ± 0.3 \text{ mm}; \text{ outdoor HS: } 46.8 ± 0.4 \text{ mm})\). After 5.8 mo (mid-March 2016), mean MCL differed significantly \((F_{2,47} = 249.3, P < 0.001; \text{ Fig. 2; Table 1})\) among treatment groups \((DR: 48.8 ± 1.4 \text{ mm}; \text{ indoor HS: } 78.2 ± 1.0 \text{ mm}; \text{ outdoor HS: } 50.6 ± 1.2 \text{ mm})\). Indoor HS tortoises were larger \((\text{ MCL})\) than either outdoor HS \((z = 18.67, P < 0.001)\) or DR juveniles \((z = 18.16, P < 0.001)\), whereas outdoor HS and DR juveniles did not differ significantly in MCL \((z = 1.05, P = 0.543)\). Among the 2015 cohort, indoor HS tortoises grew over 16 times faster in length than DR tortoises \((70.7 ± 2.0\% \text{ vs. } 4.3 ± 2.7\%)\) and over eight times faster during the first 5.8 mo than outdoor HS tortoises \((70.7 ± 2.0\% \text{ vs. } 8.3 ± 2.4\%)\).

Mean initial mass was 22.6 ± 0.3 g \((n = 70)\) and mass did not differ significantly \((F_{2,51} = 1.95, P = 0.153)\) among treatment groups \((DR: 22.6 ± 0.6 \text{ g}; \text{ indoor HS: } 22.3 ± 0.5 \text{ g}; \text{ outdoor HS: } 23.1 ± 0.8 \text{ g})\). After 5.8 mo (mid-March 2016) the indoor HS were heavier than the outdoor HS or DR tortoises \((DR: 24.5 ± 3.7 \text{ g}; \text{ indoor HS: } 94.7 ± 2.7 \text{ g}; \text{ outdoor HS: } 30.2 ± 3.3 \text{ g})\); however, after taking into account shell volume, relative mass did not differ significantly among the treatment groups \((P > 0.05)\) (indoor HS vs. DR: \(t = -0.387, df = 46, P = 0.913\); indoor HS vs. outdoor HS: \(t = -1.198, df = 46, P = 0.432\); outdoor HS vs. DR: \(t = 1.676, df = 46, P = 0.202\)). Mean ratio values for body condition \((\text{ box and half-ellipsoid type})\) from mid-March 2016 were similar among treatment groups \((\text{ Table 1})\). When comparing volume-corrected mass of 2015 indoor HS tortoises with older, but similar-sized \((2011 \text{ and } 2012 \text{ cohort})\) outdoor-reared tortoises from a previous study, we found a significant difference among treatment groups \((F_{2,87} = 660.83, P < 0.001)\) and a significant interaction between treatment group and log-transformed shell volume \((F_{2,87} = 3.66, P = 0.030; \text{ Fig. 3})\). Among all smaller-size tortoises, volume-adjusted mass of indoor HS tortoises was less than that of comparatively sized but older outdoor HS tortoises \((\text{ Fig. 3})\); however, this difference diminished as tortoises approached 95 mm in MCL \((\text{ Fig. 3})\).

Midline carapace length was a significant predictor of shell hardness \((\beta = 0.208, P < 0.001)\). For every 1.0-mm increase in MCL, shell hardness increased by 0.21% \((\text{ Fig. 4})\), and there was no evidence \((F_{2,58} = 1.66, P = 0.196)\) that this relationship differed by cohort. Indoor HS tortoises \((2015 \text{ cohort})\) had a 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 the 2012 cohort (n = 38; Fig. 4; Table 2). At a fixed size of 80 mm in MCL, outdoor-reared 2012 and 2011 cohort tortoises both had predicted shell hardness index of 88%, whereas the 2015 cohort indoor-reared tortoises had a predicted shell hardness index of 82% (Fig. 4). There was a significant difference in shell hardness among the groups ($F_{2,88} = 38.32, P < 0.001$). With MCL included as a covariate, indoor HS tortoises had significantly softer shells (lower shell hardness index) than did either 2011- ($t = -8.93, df = 88, P < 0.001$) or 2012-cohort ($t = -7.32, df = 88, P < 0.001$) outdoor HS animals. In other words, a 2015 indoor HS tortoise (at 7.5 mo age) 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 ($t = 0.57, df = 88, P = 0.837; Fig. 4; Table 2).

**Discussion**

Over their first 7 mo of life, tortoises reared indoors grew much faster than siblings from the same cohort either released immediately into the wild after hatching (DR) or reared outdoors in protected enclosures. Survival during indoor and outdoor head-starting was high, 97% and 100%, respectively, compared to 75% survival of direct-release hatchlings over the same period. After 7 mo, indoor-reared tortoises reached the same mean size (87 mm in MCL) as 5–6-y-old wild desert tortoises (Turner et al. 1987; Curtin et al. 2009). Additionally, indoor-reared tortoises reached and exceeded, in some cases, MCL of both 2011 and 2012 outdoor-reared...
tortoises, which had ranges of 75–99 mm (2011) and 63–88 mm (2012). At this rapid rate of growth (4.3 mm/mo), indoor-reared tortoises would have reached the 100 mm MCL release-size threshold recommended by Nagy et al. (2015b) in just three more months (by July 2016; a hot month, and not ideal for release). However, by September 2016, when temperatures would again be favorable for release, they would have been 109 mm in average MCL.

Of the 2015 cohort animals, only the indoor head-starts had shells hard enough to perform shell hardness measurements at 7.5 mo of age. Thus, we compared shell hardness of 2015 indoor head-starts to that of older 2011 and 2012 outdoor-reared tortoises. The younger indoor-reared tortoises had significantly lower shell hardness values than the older cohorts, but this finding is not entirely unexpected. Younger juveniles have less ossified shells than adults and desert tortoise shells become harder with age (Boarman 2003; Nagy et al. 2011). Our indoor-reared tortoises had shell hardness values (SHI = 83%) similar to 1-y-old 40-mm MCL outdoor-reared tortoises reported by Nagy et al. (2011), but not surprisingly were softer than similar-sized, but older, outdoor reared tortoises (87.1%, 3 y-old; 89.9%, 4 y-old). Collectively, our findings and those of Nagy et al. (2011) suggest that age, not just size, plays an important role in juvenile shell hardness.

Analysis of volume-corrected mass (a way of analyzing body condition) showed that there were no differences among the 2015 cohort, and ratio values of body condition of all treatment groups fell within the range expected for healthy animals. Based on the Loehr et al. (2004) formula, mean body condition of the three treatment groups ranged from 1.06–1.12 g/cm$^3$, with both the lowest (0.90 g/cm$^3$) and highest values (1.22 g/cm$^3$) across all treatments coming from direct-release animals. Using the same formula, body condition of wild, free-ranging juvenile Namaqualand Speckled Tortoises (Homopus signatus signatus) ranged from 0.99–1.11 g/cm$^3$, with the lower values attributed to lower seasonal rainfall, but none considered to be in poor condition (Loehr et al. 2007). Similarly, body condition indices for adult Western Pond Turtles (Actinemys marmorata) ranged from 1.07–1.09 g/cm$^3$ in high quality streams but

<table>
<thead>
<tr>
<th>Metric</th>
<th>Treatment</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
<th>Lower CI</th>
<th>Upper CI</th>
<th>Min</th>
<th>Max</th>
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<tr>
<td>Final MCL (mm)</td>
<td>Indoor</td>
<td>29</td>
<td>87.2</td>
<td>1.0</td>
<td>85.2</td>
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<td>Outdoor</td>
<td>20</td>
<td>51.9</td>
<td>1.6</td>
<td>49.6</td>
<td>54.2</td>
<td>46.7</td>
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<tr>
<td>Final mass (g)</td>
<td>Indoor</td>
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<td>122.6</td>
<td>3.7</td>
<td>115.1</td>
<td>130.0</td>
<td>77.6</td>
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<td></td>
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<td>30.9</td>
<td>4.5</td>
<td>22.3</td>
<td>39.8</td>
<td>22.4</td>
<td>42.5</td>
</tr>
<tr>
<td>Final body cond. (half-ellipsoid)</td>
<td>Indoor</td>
<td>29</td>
<td>1.01</td>
<td>0.01</td>
<td>1.00</td>
<td>1.03</td>
<td>0.94</td>
<td>1.11</td>
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<tr>
<td>(g/cm$^3$) (Loehr et al. 2007)</td>
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<td>1.03</td>
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<tr>
<td>Final body cond. (box)</td>
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<td>0.525</td>
<td>0.539</td>
<td>0.492</td>
<td>0.582</td>
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<tr>
<td>(g/cm$^3$) (Nagy et al. 2011)</td>
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<td>0.005</td>
<td>0.537</td>
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<th>Upper CI</th>
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<td>85.1</td>
<td>95.4</td>
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<td>(g/cm$^3$) (Loehr et al. 2007)</td>
<td>Outdoor 2012</td>
<td>38</td>
<td>1.10</td>
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<td>Outdoor 2011</td>
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<td>1.10</td>
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<td>Body condition (box)</td>
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<td>(g/cm$^3$) (Nagy et al. 2011)</td>
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<td>0.011</td>
<td>0.554</td>
<td>0.596</td>
<td>0.521</td>
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**Table 2.** Summary statistics of growth metrics from measurements of juvenile Mojave Desert Tortoises (*Gopherus agassizii*) taken prior to head-start release in late April 2016. Shell hardness and body condition 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. Abbreviations are n = sample size, SE = standard error, LCI = lower 95% confidence limit, UCI = upper 95% confidence limit, Min = minimum, Max = maximum, and MCL = midline carapace length.
Based on the index developed specifically for desert tortoises (Nagy et al. 2002), mean body condition for our three treatment groups ranged from 0.554–0.586 g/cm³. Although none of our mean body conditions fell within values considered to reflect prime body condition in wild desert tortoises (0.60–0.70 g/cm³), they were well above values for dehydrated wild animals (0.40 g/cm³; Nagy et al. 2002). The direct-release animals in our study exhibited the greatest range of values (0.48–0.64 g/cm³), likely reflecting variation in availability or distribution of resources (shelter, forage, water) in the wild. It is worth noting that we were only able to obtain body condition measurements of DR animals that survived the first 6 months post-release, so we do not know to what extent body condition contributed to mortality of DR animals. In contrast, the indoor and outdoor head-starts exhibited much less variation in body condition (0.51–0.60 g/cm³ and 0.54–0.63 g/cm³, respectively). Both indoor and outdoor-reared tortoises had frequent access to drinking water, which likely contributed to the more consistent body condition of animals from those treatments.

We found that smaller 2015 indoor-reared tortoises weighed less than outdoor-reared tortoises of similar size from the 2011 and 2012 cohorts; however, this difference diminished as tortoises in all groups approached 95 mm in MCL. The differences between treatments among smaller animals are likely due to differences in bone mass and shell ossification between the age groups, making the younger indoor head-starts lighter with respect to their volume compared to older outdoor head-starts (Arendt and Wilson 2000). Loehr et al. (2007) also noted that juvenile shells of H. signatus are less well-ossified (i.e., softer) than adults, giving juveniles a lower body mass to volume ratio than adults, which are similar in size to juvenile desert tortoises. In contrast, younger indoor head-starts that have attained 95 mm MCL in our study show no evidence of deficiency in volume-corrected mass compared to older outdoor-reared tortoises of the same size.

Management implications.—Indoor head-starting was successful in reducing the time required for desert tortoises to reach sizes approaching published recommendations for release (Nagy et al. 2015b; Hazard et al. 2015). Future studies that evaluate indoor-rearing for longer periods 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) may reveal the conditions needed to increase shell hardness among head-started tortoises while retaining the growth-promoting benefit of indoor-rearing in the first year of life. Furthermore, use of short-wave ultraviolet (UVB) lights coupled with calcium and vitamin D₃ supplementation during indoor head-starting may promote better bone development and shell hardness in future efforts.

Because rapid growth may come with fitness costs (Jackson et al. 1976, Olsson and Shine, 2002), future efforts are likely to be most successful if they consider additional morphological metrics rather than size alone. Body condition and shell hardness indices are valuable metrics for evaluating head-starting efforts. Future head-start studies that monitor body condition can provide information to better evaluate the relationship between body condition and shell hardness, as body condition may increase as shells ossify. Both metrics will be important for helping to assess survival and growth in the wild and in linking release strategies with environmental conditions (i.e., seasonal/annual rainfall) and thus fine-tuning head-starting to benefit species recovery. 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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Herpetological Conservation and Biology


Kurt A. Buhlmann (second from the right) is a Conservation Ecologist whose research interests include life history and evolutionary ecology with application for recovery and management of amphibians and reptiles. He holds a Ph.D. in Ecology from the University of Georgia, Athens, USA, M.S. in Wildlife Sciences from Virginia Tech, Blacksburg, USA and B.S. in Environmental Studies from Stockton State College, Galloway Township, New Jersey, USA. He is a Senior Research Associate at the University of Georgia’s Savannah River Ecology Laboratory, near Aiken, South Carolina, USA, and manages Buhlmann Ecological Research and Consulting, LLC. Brian D. Todd (second from the left) is an Associate Professor in the Department of Wildlife, Fish and Conservation Biology at University of California, Davis, USA. His research interests include understanding the factors that affect the persistence of wildlife species and populations and in understanding how wildlife respond to environmental change. He received his Ph.D. in Ecology (2008), his Master’s in Conservation Ecology and Sustainable Development (2003) and B.S. in Ecology (2000) from the University of Georgia, Athens, USA. J. Mark Peaden (left) is a Postdoctoral Research Associate at the University of California, Davis, USA, where he received his Ph.D. in 2017. His dissertation focused on how roads and road mitigation measures affect habitat use and behavior of Mojave Desert Tortoises. He has a diversity of field and research experiences with a broad range of wildlife, including bats, avifauna, mesocarnivores, reptiles, amphibians, and fish. Tracey D. Tuberville (right) is an Associate Research Scientist at the University of Georgia’s Savannah River Ecology Laboratory, near Aiken, South Carolina, USA. Tracey received her Ph.D. in Ecology (2008) and her M.S. in Conservation Ecology and Sustainable Development (1998) from University of Georgia, Athens, USA, and her B.S. in Biology from Furman University, Greenville, South Carolina, USA (1993). Tracey’s research interests are in applied conservation and management research for reptiles and amphibians. (Photographed by Mark Peaden).

Clinton T. Moore is a Research Wildlife Biologist with the U.S. Geological Survey and serves as Assistant Unit Leader of the Georgia Cooperative Fish and Wildlife Research Unit at the University of Georgia, Athens, USA, where he holds an appointment of Adjunct Associate Professor in the Warnell School of Forestry and Natural Resources. His research focuses on the estimation and modeling of demographic relationships in wildlife populations, with emphasis on their use in formal decision-making applications under uncertainty. (Photographed by Wade Newbury).