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Preface

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A B S T R A C T

Raising captive animals past critical mortality stages for eventual release (head-starting) is a common conservation tactic. Counterintuitively, post-release survival can be low. Post-release behavior affecting survival could be influenced by captive-rearing duration and housing conditions. Practitioners have adopted environmental enrichment to promote natural behaviors during head-starting such as raising animals in naturalistic enclosures. Enrichment might be especially beneficial for animals held in captivity long-term to prevent degradation of adaptive behaviors. Using 32 captive-born turtles (*Terrapene carolina*), half of which were raised in enriched enclosures, we employed a factorial design to explore how enrichment and rearing duration affected post-release growth, behavior, and survival. Six turtles in each treatment (enriched or unenriched) were head-started for nine months (cohort one). Ten turtles in each treatment were head-started for 21 months (cohort two). At the conclusion of captive-rearing, turtles in cohort two were overall larger than cohort one, but unenriched turtles were generally larger than enriched turtles within each cohort. Once released, enriched turtles grew faster than unenriched turtles in cohort two, but we otherwise found minimal evidence suggesting enrichment affected post-release survival or behavior. Cohort two dispersed farther and had generally higher active season survival than cohort one (0.50 vs. 0.33). Body mass was positively associated with daily survival probability. Our findings suggest attaining larger body sizes from longer captive-rearing periods to enable greater movement and alleviate susceptibility to predation (the primary cause of death) could be more effective than environmental enrichment alone in chelonian head-starting programs where substantial predation could hinder success.

1. Introduction

Wildlife translocation, the deliberate human-facilitated movement and release of animals, is a common management technique aimed at augmenting imperiled populations or reintroducing species to areas where they have been extirpated (Seddon et al., 2007). Despite the potential conservation value of translocations, many fail because animals have low survival when released in novel environments, precluding successful establishment of released populations (Fischer and Lindenmayer, 2000; Germano and Bishop, 2009). In particular, releasing animals from captivity is typically less successful than translocating wild animals directly between natural sites (Griffith et al., 1989; Fischer and Lindenmayer, 2000). This is in part thought to be a function of captive animals lacking the necessary experience to successfully transition to post-release environments (Stamps and Swaisgood, 2007). Head-starting entails rearing captive animals for an extended period of time to allow them to grow until they reach a size threshold in which predation vulnerability is greatly reduced. However, these efforts often fail because individuals disperse from release sites and struggle with avoiding predators, acquiring food, or selecting suitable habitats (Einum and Fleming, 2001; Jule et al., 2008; Le Gouar et al., 2012). Two fundamental factors that might impact head-starting success are how long juveniles are raised before release and the captive-rearing conditions.

The behavior of captive animals often differs from wild-bred conspecifics because captivity can have detrimental effects on development (Mathews et al., 2005; Swaisgood et al., 2018). Potential conflicts could thus exist when deciding how long animals should be held prior to translocation. Longer head-starting periods generally result in larger or more mature animals being released, which could provide a survival advantage if such individuals are less susceptible to predation (Nagy et al., 2015; Daly et al., 2018). However, behaviors critical to survival such as foraging and predator avoidance might degrade with longer captivity duration (DeGregorio et al., 2013, 2017; Swaisgood et al., 2018).

Attempts to promote ecologically relevant behaviors and improve welfare for head-started animals have been accomplished by incorporating environmental enrichment into rearing protocols (Swaisgood, 2010). This could include raising animals in enclosures with naturalistic features simulating release sites, providing foraging opportunities like those experienced in nature, and communally housing conspecifics to promote social skills (Reading et al., 2013). Indeed, enrichment appears to broadly have positive effects on translocations, as post-release survival is generally higher for animals that are enriched compared to unenriched conspecifics (Tetzlaff et al., 2019a).

The effects of environmental enrichment and captivity duration on reptile head-starting success has received minimal attention. Roe et al. (2015) found no differences in post-release growth, behavior, or survival between captive-born common watersnakes (*Nerodia sipedon*), which were provided with enrichment for several months prior to release relative to unenriched conspecifics. However, the same study reported that larger and older snakes had higher survival compared to younger and smaller individuals (Roe et al., 2010), indicating extended head-starting duration to facilitate growth and maturity could be beneficial. Enriching captive-born reptiles from birth might show more pronounced differences. A factorial experiment investigating the impacts of time in captivity and environmental enrichment would better enable mechanistic understanding of post-release behavior (e.g., foraging, movement, exposure) and survival for head-started animals.

Head-starting is an intuitive and attractive option for conserving chelonians (turtles, tortoises, and terrapins; order Testudines) given this is one of the most threatened vertebrate groups globally, with approximately 60% of species threatened with extinction or having gone extinct in recent times (Lovich et al., 2018). However, head-starting efforts have met with mixed success and considerable debate exists regarding the efficacy of this practice (Burke, 2015). Before adopting head-starting practices, rearing facilities should evaluate both the positive and negative effects of various rearing durations and the use of environmental enrichment for promoting behaviors that lead to improved survival once released.

We experimentally tested the effects of time in captivity and enrichment on short-term head-starting success for eastern box turtles (*Terrapene carolina*). We raised one cohort of turtles, half in enriched conditions and half in unenriched conditions, for nine months before release. We chose this duration because it is representative of several published turtle head-starting efforts (e.g., Buhlmann et al., 2015; Daly et al., 2018; Quinn et al., 2018). We raised another cohort of turtles, half in enriched conditions and half in unenriched conditions, for an additional year before release (21 months total). We hypothesized enrichment provides opportunities for head-started turtles to develop behaviors that minimize predation risk, as predation is likely the primary cause of mortality for juvenile turtles due to their small body sizes and incomplete hardening of the shell (Dodd, 2001). Once released we expected enriched turtles would move less, remain less visible, and grow faster due to enhanced foraging skills, leading to higher survival than unenriched turtles. If time in captivity has negative effects on behavior, we expected turtles reared for longer would remain exposed and move more than turtles kept in captivity for a shorter duration, leading to lower survival. Alternatively, if larger body size reduces post-release predation (sensu the “bigger is better” hypothesis; Packard and Packard, 1988; Janzen, 1993), we expected turtles reared for a longer period would have higher survival.

2. Methods

2.1. Study species and site

Eastern box turtles inhabit temperate and subtropical regions over much of the eastern United States (Dodd, 2001). This species is typically associated with forested habitats but also occupies forest edges, shallow wetlands, and grasslands such as

old field and prairie (Dodd, 2001; Gibson, 2009). The species is of conservation concern because populations have declined from habitat loss, road mortality, intense predation (particularly of nests and juveniles), and collection for the pet trade (Kiestler and Willey, 2015). As such, the eastern box turtle is listed as Vulnerable by the IUCN and is included in CITES Appendix II (van Dijk, 2011).

We conducted fieldwork at Fort Custer Training Center, an Army National Guard training facility located in southwest Michigan, USA, near the northern range limit for eastern box turtles. The approximately 3,000 ha installation is comprised primarily of woodlands (2,023 ha), wetlands (485 ha), and old field/prairie (485 ha). Fort Custer is enclosed by chain-link fence, and unpaved dirt roads intersect the site at approximately 1 km intervals. Most of the site has minimal human disturbance and vehicle traffic is limited.

2.2. Husbandry practices

Subjects for this study were acquired as eggs from nests laid by free-ranging females at Fort Custer. We artificially incubated eggs indoors and raised hatchlings ($n = 32$) in a greenhouse on the campus of University of Illinois at Urbana-Champaign. The transparent greenhouse ceiling allowed exposure to natural photoperiods. Similarly, temperature inevitably fluctuated on a daily and seasonal basis, but we attempted to regulate ambient temperature in the greenhouse between 21 and 29 °C. We raised hatchlings in either an enriched or unenriched environment beginning in mid-August 2016 (within two weeks of hatching). Enriched turtles ($n = 16$) were communally housed in 132 cm long x 79 cm wide x 30 cm deep Rubbermaid® stock tanks (n = 4–5 individuals per replicate) with naturalistic features designed to mimic vegetation and substrate commonly utilized by wild eastern box turtles (Dodd, 2001, Fig. 1). Unenriched turtles ($n = 16$) were housed individually in comparably simplistic enclosures consisting of a 60 cm long x 42 cm wide x 28 cm tall transparent plastic tub with reptile cage carpet (Zoo Med Eco Carpet; Zoo Med Laboratories, Inc., San Luis Obispo, CA, USA) and a 42 × 42 cm piece of plastic shelf liner resting on the carpet. We provided these turtles a small plastic hide box and kept tubs on a slight angle to hold fresh standing water (ca. 4 cm deep) in the lower end for drinking and soaking (Fig. 1). We note that although enriched turtles had opportunities for social interactions that unenriched turtles did not, we considered this as a general component of environmental enrichment. We observed no agonistic interactions between enriched turtles, and regardless of treatment, all turtles survived during captive-rearing.

The type and amount of food provided to individuals at each feeding was similar between rearing treatments, but we predominantly fed enriched turtles by scattering food throughout their enclosures to promote active foraging. Unenriched turtles were provided food on 10 cm diameter petri dishes placed in the same spot in enclosures at each feeding. We fed turtles live invertebrate prey such as blackworms (*Lumbriculus variegatus*), mealworms (*Tenebrio molitor*), superworms (*Zophobas morio*), and redworms (*Eisenia foetida*). We also offered thawed frozen berries, fresh mixed greens (excluding spinach), and Zoo Med Gourmet Box Turtle Food—a commercial diet consisting of pellets and dehydrated mealworms, strawberries, and mushrooms. Turtles were offered fresh food daily, five days per week. Fresh water was provided ad libitum. We generally weighed (g) and measured carapace length (mm) of each turtle weekly and conducted brief behavioral trials prior to releasing turtles (Tetzlaff et al., 2019b), but we otherwise limited handling. Additional details of study animal acquisition and husbandry methods have been previously described (Tetzlaff et al., 2018, 2019b).



Fig. 1. Rearing conditions for enriched (left) and unenriched (right) head-started eastern box turtles (*Terrapene carolina*). Enriched turtles were housed in groups of 4–5, provided with coconut fiber substrate to bury in, artificial plants and half logs for additional hiding areas, and naturalistic water dishes. Unenriched turtles were housed individually and provided with carpet for substrate, a small plastic hiding structure, and standing water to soak in.

2.3. Release procedures

We released 12 turtles (six enriched and six unenriched) at Fort Custer in May 2017, after approximately nine months of head-starting. We hereafter refer to this release group as cohort one. We retained the remaining 20 turtles (cohort two: 10 enriched and 10 unenriched) in captivity for an additional year and released these individuals in the same area as cohort one in May of 2018 after approximately 21 months of head-starting. We released turtles into a roughly 450 m² area in hardwood forest dominated by an overstory of maples (*Acer* spp.) and oaks (*Quercus* spp.) and an understory dominated by a diverse community of herbs and shrubs. Several wetlands were adjacent to the forest patch. We chose the release site based on previous work suggesting resident box turtles at Fort Custer prefer these habitat types (Gibson, 2009). It is also adjacent to a heavily-used box turtle nesting site, so the general area is likely occupied by resident juveniles (Laarman et al., 2018).

We anticipated initial post-release mortality might be high, so we placed all turtles in acclimation pens in the forest patch to ease their transition to the wild (Tuberville et al., 2005). This also provided us daily opportunity to observe well-being, behavior, and growth of turtles without the risk of rapid mortality. Pens were 1.8 m long x 1 m tall x 1 m wide and constructed using approximately 4 cm diameter PVC pipe. We enclosed the top and sides of each pen with plastic poultry netting. We buried the legs and netting of each pen approximately 10 cm into the ground to keep predators from entering and prevent turtles escaping. We installed three pens approximately 45 m apart in the release area in April 2017 and placed four turtles in each pen on 19 May. We repeated a similar release procedure for cohort two in 2018 and we built two additional pens for release of this cohort to accommodate the larger number of individuals being released. To track growth rates during the acclimation period, we measured straight carapace length to the nearest 0.01 mm of each turtle once per week using digital calipers (Fisherbrand™ Traceable™ Digital Calipers; Fisher Scientific, Hampton, New Hampshire, USA).

We opened pens at the conclusion of the acclimation period by cutting away an approximately 10 cm tall section of the netting from ground level on each of the pens, which allowed turtles to exit pens at-will. In 2017, we opened the pens after 38 days on 26 June. All turtles in cohort one aside from one individual exited pens within three days after pens were opened; the last turtle left its pen on 5 July. In 2018, we opened the pens after 34 days on 8 June. All, except one turtle, in cohort two left their release pens within one day; the last turtle left its pen on 14 June. Although we released each cohort in different years and at slightly different times within each year, the general climatic conditions were similar between release years. For example, we used loggers (Thermochron iButton model DS1921G, Fondriest Environmental, Inc., Fairborn, OH, USA; ± 0.5 °C error) to monitor hourly temperature in the forest patch where turtles were released, and the mean temperature difference between years when turtles were undergoing acclimation was 1.22 °C. We thus do not expect minimal climatic differences influenced behavior or survival of turtles in pens or once released.

2.4. Post-release monitoring

We used radio-telemetry to monitor turtles once they exited pens until they either died during the active season (generally April–October at Fort Custer; Gibson, 2009) or initiated overwintering. Depending on a turtle's size, we affixed a 0.9 or 1.2 g transmitter (Advanced Telemetry Systems, Inc., Isanti, MN, USA) to the carapace of each turtle using epoxy. Transmitters were no more than 7% of a turtle's mass. We generally located individuals five days per week during daylight hours (0700–1800) from June to August and once every 1–2 weeks from September to November each year using a handheld receiver (R-1000, Communications Specialists, Inc., Orange, CA, USA) and 3-element mini Yagi antenna. Each time we located a turtle, we recorded its position in Universal Transverse Mercator units (UTM, North American Datum of 1983) with a handheld GPS (Garmin eTrex 30; 3 m accuracy). We visually confirmed if turtles were alive at least two times per week on non-successive tracking days. During these occasions, we estimated the proportion of a turtle's body that was exposed to the nearest 25% (Harvey and Weatherhead, 2010). To minimize disturbing turtles on days we did not estimate exposure, we radio-tracked individuals to within approximately 1 m of their location but did not visually confirm survival status. We measured each turtle's straight carapace length to the nearest 0.01 mm using digital calipers and mass to the nearest 0.01 g using a digital scale (Sartorius M-PROVE Portable Scale; Sartorius AG, Göttingen, Germany) every two weeks.

We inferred turtles had initiated overwintering by their consistent lack of aboveground activity for more than two weeks each fall. Transmitter batteries would not last through the winter, so we placed approximately 15 cm tall x 100 cm long x 50 cm wide wire cages over each turtle once we were confident they were overwintering, which aided in determining overwinter survival the following spring. We placed cages on turtles in cohort one on 10 November 2017 and turtles in cohort two on 2 November 2018. We replaced transmitters on any turtles from cohort one that survived into the second monitoring season in 2018 and radio-tracked these turtles along with cohort two that year. We ceased monitoring in April 2019 after we confirmed which turtles survived the 2018–19 winter.

2.5. Data analyses

We conducted all statistical analyses in R version 3.5.1 (R Core Team, 2018). Where appropriate, we confirmed data residuals approximated a Gaussian distribution by inspecting quantile-quantile plots and ensured variances were homogeneous using Brown-Forsythe tests.

2.5.1. Growth

We calculated daily growth rate for each turtle while in acclimation pens using the difference of the first and last carapace measurement divided by the number of days between measurements. Using similar methods, we calculated daily growth rate for each turtle post-release—from when pens were opened until death or overwintering initiated. We used linear models to analyze the effects of treatment and cohort on individual daily growth rates while in pens and post-release.

2.5.2. Movement

We calculated daily movement rate for each turtle, defined as the summed distance between subsequent tracking events divided by the number of days monitored. We also calculated how far each turtle dispersed during radio-tracking by measuring the straight-line distance between a turtle's release pen and its furthest location from the pen. We used linear models to analyze the effects of treatment and cohort on these movement metrics. To control for the varying survival rates between turtles, we included individual probability of surviving the active season monitoring period as a covariate in our dispersal model. We also used a linear model to determine if dispersal distance was associated with individual survival probability within each cohort.

2.5.3. Exposure

To analyze exposure of turtles, we used a linear mixed model with the package *nlme* (Pinheiro et al., 2018). We included treatment and cohort as fixed effects and turtle identity as a random effect to account for the repeated measurements per individual. For this and the abovementioned analyses, we initially modeled treatment and cohort as an interactive effect. However, we found no significant interactions (see Results) and thus tested treatment and cohort as main effects for each response (growth, movement rate, dispersal, and exposure).

2.5.4. Survival

To assess the influence of turtle- and study-specific effects on daily survival probability during the active season, we used generalized linear mixed models implemented in the package *lme4* (Bates et al., 2015). Our response variable was the binary response of “survived” or “died” for each individual radio-telemetry location. We implemented a modified version of the traditional logit link function used in binary logistic regression, where models were weighted for the time interval between radio-telemetry locations (Shaffer, 2004). This approach also allowed us to utilize a turtle's most recent mass measurement for a given telemetry location, an important consideration because turtles were presumably experiencing weight changes once released that could be related to survival. We evaluated a candidate model set for the fixed effects of treatment, cohort, time (days since release), and body mass—both independently and as varying additive combinations. Mass and time since release were not highly correlated ($|r| \leq 0.70$). We also included an intercept-only model (i.e., a null model with no predictors) in our candidate set, for a total of 11 models. To control for the non-independent multiple observations per turtle, we used turtle identity as a random effect in all survival models. We used Akaike's Information Criterion (Akaike, 1973) corrected for small sample sizes (AICc) to rank candidate models and evaluated their support based on model weight (i.e., the relative probability a given model is the “best”; Burnham and Anderson, 2002). If a single model was not highly ranked (e.g., received ~90% of the AICc weight), we used multimodel inference and generated model-averaged estimates for parameters of interest. We did not evaluate models for overwinter survival probability because all but one turtle survived winter (see Results).

3. Results

3.1. Growth rates

All turtles survived the on-site acclimation period. Average daily growth rate in pens was modest (0.04 mm/day \pm 0.02 SD). Growth did not differ between treatments while in acclimation pens but differed between cohorts (Table 1). Turtles in cohort two grew faster while in acclimation pens than those in cohort one (Fig. 2).

We radio-tracked 12 turtles, 276 times in 2017 and 24 turtles, 1,459 times in 2018 (including three from the previous year). Post-release growth was on average 0.10 mm/day \pm 0.05 SD. We found evidence that daily growth was influenced by the additive effects of treatment and cohort (Table 1). This relationship appeared to be driven by enriched turtles in cohort two, which grew faster than unenriched turtles in the same cohort (Fig. 3). Growth rates of unenriched turtles in both cohorts and enriched turtles in cohort one were similar (Fig. 3). We were unable to calculate post-release growth rate for one unenriched turtle in cohort two because this individual died shortly after release.

3.2. Movement

Turtles moved 9.78 m/day \pm 6.19 SD on average, but movement rates were quite variable between individuals (range: 4.25–27.28 m). Movement rates did not differ between treatments or cohorts (Table 1). Turtles dispersed an average of 205.98 m \pm 191.79 SD from release pens, but dispersal distances were also highly variable between individuals (range: 31.78–828.90 m). We found no differences in dispersal distance between treatments, but dispersal differed between cohorts (Table 1). Turtles in cohort two dispersed more than twice the distance as those in cohort one on average (Fig. 4). Dispersal distance was not associated with survival probability in cohort one ($R^2 = -0.001$, $P = 0.36$) or cohort two ($R^2 = 0.02$, $P = 0.27$).

Table 1

Parameter estimates with 95% lower (LCL) and upper (UCL) confidence limits and associated *P*-values for growth and behavior metrics for head-started eastern box turtles (*Terrapene carolina*). Estimates are mean differences between rearing treatments (enriched or unenriched) or cohorts (released after nine or 21 months of head-starting). Unenriched turtles are the reference group for treatment, and turtles released when 21 months old (cohort two) are the reference group for cohort. Turtles were held in acclimation pens for approximately one month before being fully released and radio-tracked. An asterisk indicates an interactive effect.

Metric	Predictor	Estimate	LCL	UCL	<i>P</i>
Growth rate (mm/day) in acclimation pens	Treatment	-0.01	-0.02	0.01	0.41
	Cohort	0.02	0.01	0.04	0.01
	Treatment*Cohort	0.01	-0.03	0.04	0.71
Post-release growth rate (mm/day)	Treatment	-0.03	-0.07	0.00	0.04
	Cohort	0.04	0.00	0.07	0.03
	Treatment*Cohort	-0.04	-0.10	0.02	0.21
Movement rate (m/day)	Treatment	-0.47	-5.04	4.10	0.84
	Cohort	3.03	-1.66	7.72	0.20
	Treatment*Cohort	-3.82	-13.27	5.63	0.41
Dispersal distance (m)	Treatment	-30.03	-157.53	97.47	0.63
	Cohort	132.91	-8.41	274.23	0.06
	Treatment*Cohort	-167.42	-432.57	97.72	0.21
Exposure	Treatment	0.82	-5.93	7.56	0.81
	Cohort	-6.17	-13.74	1.39	0.11
	Treatment*Cohort	7.22	-7.70	22.14	0.33

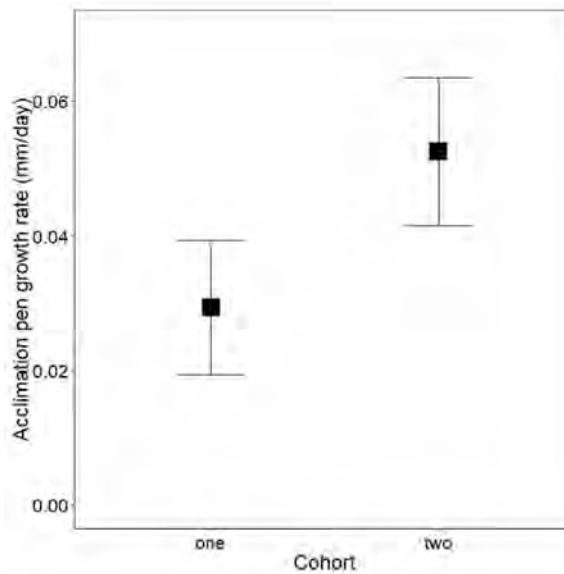


Fig. 2. Average daily growth rates of head-started eastern box turtles (*Terrapene carolina*) while being held in acclimation pens for approximately one month before release. Turtles in cohort one and two had been head-started for nine and 21 months, respectively, prior to being placed in pens. Points are means and bars are 95% confidence intervals.

3.3. Exposure

We recorded exposure 146 times for turtles in cohort one and 420 times for turtles in cohort two. Exposure levels did not differ between treatments or cohorts (Table 1). In cohort one, enriched turtles were fully exposed on 45 of 84 (54%) locations, and unenriched turtles were fully exposed on 31 of 62 (50%) locations. In cohort two, enriched turtles were fully exposed on 73 of 185 (39%) locations, and unenriched turtles were fully exposed on 93 of 235 (40%) of locations.

3.4. Survival

In total, 14 of the 32 (0.44, 95% confidence interval [CI]: 0.26–0.62) released turtles survived their first active season post-release. Two of six turtles in each treatment (0.33, 95% CI: 0.04–0.78) of cohort one survived the 2017 active season. These four turtles all survived winter and emerged in spring 2018. Three of these individuals (one enriched and two unenriched) also survived the 2018 active season and were alive at the end of this study in April 2019. The proportion of surviving enriched turtles was higher than unenriched turtles for several weeks post-release in 2017, but their survival eventually fell to levels

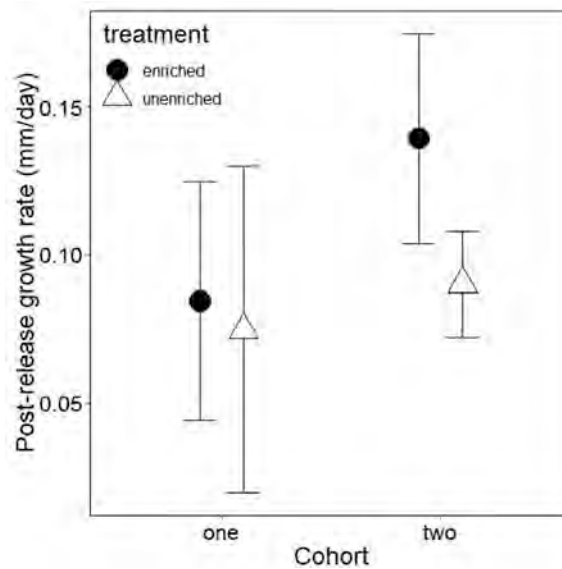


Fig. 3. Daily post-release growth rates of head-started eastern box turtles (*Terrapene carolina*). Turtles were captive-reared in either an enriched or unenriched condition for nine months (cohort one) or 21 months (cohort two). Points are means and bars are 95% confidence intervals.

similar to unenriched towards the end of the active season (Fig. 5). In cohort two, four of 10 (0.40, 95% CI: 0.12–0.74) enriched turtles and six of 10 (0.60, 95% CI: 0.26–0.88) unenriched turtles survived the 2018 active season. The proportion of enriched turtles surviving post-release was always lower than unenriched turtles in this cohort as the season progressed (Fig. 5). All turtles in cohort two that survived the 2018 active season were alive in April 2019 except for one enriched individual; only its intact shell with the transmitter attached was found in its post-overwintering cage on the surface after the 2018–19 winter, so we could not determine when it died.

The main cause of active season mortality in both cohorts was presumed to be predation, accounting for all mortality in cohort one and most in cohort two. Two individuals (one from each treatment) in cohort two were run over by vehicles on dirt road edges. One enriched turtle in this cohort died of unknown causes but was not depredated as its intact, undamaged carcass was recovered. We rarely recovered intact carcasses of depredated turtles but frequently found either shell fragments or only a carapace as well as teeth impressions in epoxy coating transmitters suggestive of mesopredators and rodents as likely dominant predators.

Survival analyses were based on 2,740 turtle “exposure” or “tracking” days. Model selection using AICc suggested body mass was more important for predicting daily survival than treatment, cohort, or time since release (Table 2). There was a modest positive association between survival probability and body mass (model-averaged regression coefficient and 95% unconditional CI: 0.05, 0.01–0.1; Fig. 6). The model with mass as a sole predictor received 33% of the weight of evidence and was 3.5 delta AIC units above the first model that did not include body mass (intercept-only model). All models that included the effects of mass and other additive variables cumulatively received 71% of the weight of evidence and were all ranked above the null model. Models for the independent or additive effects of treatment, cohort, or time since release received little support ($\leq 6\%$) and were all ranked below the null model.

4. Discussion

We explored if head-starting juvenile eastern box turtles with environmental enrichment could lead to behaviors that would enhance survival post-release compared to turtles raised in more traditional, simplistic (unenriched) conditions. We expected the potential deleterious effects of captivity would be offset with enrichment the longer turtles were held in captivity. However, overall we found limited evidence of enrichment improving post-release behavior and survival. Instead, turtles head-started for 21 months grew faster in acclimation pens on the release site, dispersed farther, and had generally higher active season survival probability (0.50, 95% CI: 0.28–0.72) than turtles head-started for nine months (0.33, 95% CI: 0.07–0.60), which we attribute to body size.

Our findings are largely in line with the “bigger is better” hypothesis, which suggests larger juvenile body size increases survival and performance (Packard and Packard, 1988; Janzen, 1993; Kissner and Weatherhead, 2005). Given nest predation can be a serious threat to chelonian population viability (Dodd, 2001; Spencer et al., 2017), head-starting might be an effective strategy for enhancing recruitment (Carstairs et al., 2019). Estimated annual adult survival for *T. carolina* can be very high (e.g., >0.95 , Currylow et al., 2011), whereas at another study site in Michigan, Altobelli (2017) found estimated survival probability of radio-tracked hatchling *T. carolina* was zero at upwards of one-year in age. Similar to our results, larger hatchlings were more

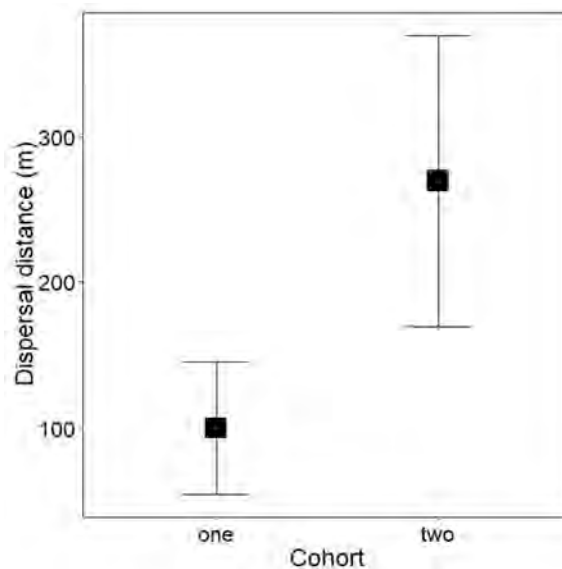


Fig. 4. Mean dispersal distances of head-started eastern box turtles (*Terrapene carolina*). Turtles in cohort one and two had been head-started for nine and 21 months, respectively, prior to release. Points are means and bars are 95% confidence intervals.

likely to survive over a longer time period (Altobelli, 2017). At the time of each cohort's release, turtles in cohort two were generally larger than cohort one, but unenriched turtles were overall larger than enriched turtles at each release point (Tetzlaff et al., 2019b). However, there was still variation in body sizes among turtles in each treatment within cohorts. This variation could partially explain why the effects of treatment and cohort were not ranked as highly in model selection predicting daily survival probability as models containing the effect of body mass. Although confidence intervals for survival estimates overlapped considerably, we suggest there were likely still biologically relevant effects of treatment and cohort on survival; in cohort two, the proportion of surviving enriched turtles was nearly ten percent higher than cohort one (0.40, 95% CI: 0.12–0.74 vs. 0.33, 95% CI: 0.04–0.78), and the proportion of surviving unenriched turtles (0.60, 95% CI: 0.26–0.88) was nearly twice that of cohort one. Additionally, turtles with the highest daily survival probability were the heaviest (approximately 70 g) and therefore were from cohort two because no turtle in cohort one exceeded 50 g in the first season post-release.

Accumulating evidence from this and other studies suggests attaining larger body sizes over a longer rearing period could be an effective method for increasing survival of head-started reptiles. For instance, head-started common watersnakes had greater survival if reared for a longer period (Roe et al., 2010, 2015). Size at release might be especially important for turtles because juveniles are vulnerable to numerous predator species (Dodd, 2001; Nagy et al., 2015). We randomly selected turtles for release in each cohort to reduce potential biases related to behavior and survival. However, our results collectively suggest intentionally selecting the largest individuals for release at a given point may be most beneficial for increasing survival, and this may require rearing smaller individuals for longer periods before release. Additionally, juvenile turtles have softer shells than adults and full hardening of the carapace does not naturally occur until several years after birth (Arsovski et al., 2018). By growing turtles at an advanced rate in captivity, their carapace hardens earlier and might provide resistance to predation leading directly to higher survival (Daly et al., 2018).

We predicted enriched turtles would be more efficient foragers and thus grow faster than unenriched turtles once released because of their experience searching for spatially variable prey in their more complex rearing environments (Reading et al., 2013). Although growth rates did not differ between treatments in cohort one, enriched turtles in cohort two grew faster than unenriched turtles despite being overall smaller at release (Tetzlaff et al., 2019b). In addition to the potential behavioral benefits conferred by enrichment, being able to eat more diverse prey from having a larger gape could also explain why turtles in cohort two generally grew faster in acclimation pens than those in cohort one (Tucker et al., 1995).

Although unenriched turtles were able to seek cover in an artificial shelter, we expected more naturalistic hiding opportunities during captive-rearing would better condition enriched turtles to remain hidden more often once released. In turn, we predicted this could be a mechanism for enhancing survival because juvenile terrestrial turtles have limited defenses from predators aside from exhibiting cryptic behavior (Dodd, 2001). However, exposure levels did not differ between treatments or cohorts, and turtles were frequently observed fully exposed. Wild-caught adult captive ratsnakes provided enrichment prior to translocation were also no less visible overall than unenriched conspecifics (DeGregorio et al., 2017). Snakes released from captivity were found exposed more often than wild conspecifics, which seemingly increased vulnerability to predators (DeGregorio et al., 2017). If an extended rearing duration to maximize body size is not logistically or financially feasible when head-starting reptiles, practitioners might consider implementing training programs such as those that have been successful for conditioning antipredator behavior in other taxa (Reading et al., 2013; Tetzlaff et al., 2019a).

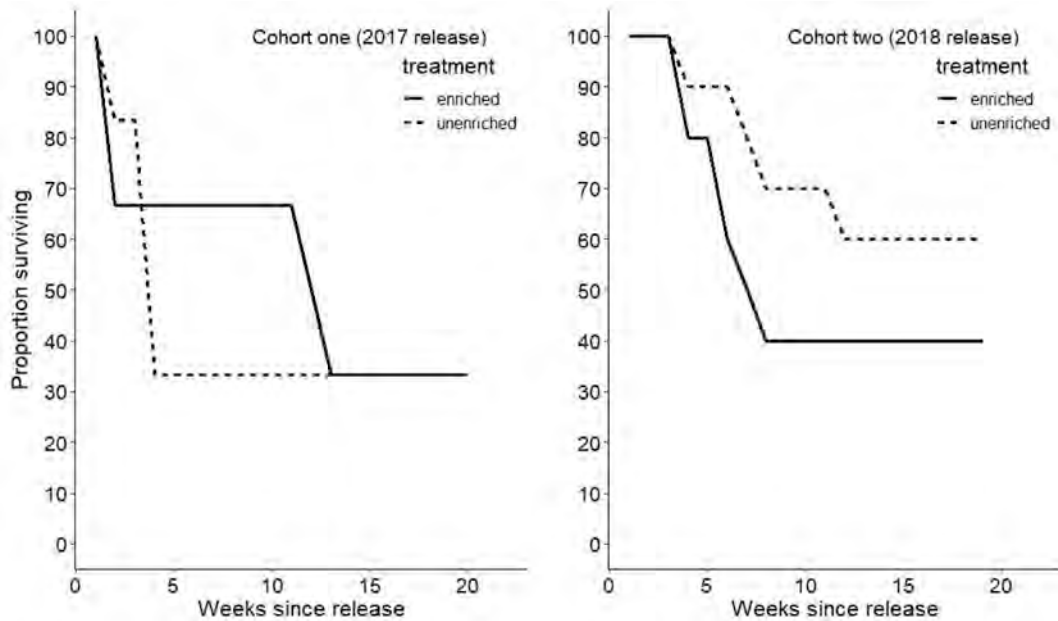


Fig. 5. Proportion of head-started eastern box turtles (*Terrapene carolina*) surviving over time during the active season based on being raised in enriched or unenriched conditions for either nine (left panel) or 21 months (right panel) before release.

Table 2

Model selection results for predicting daily survival of head-started eastern box turtles (*Terrapene carolina*) based on body mass, time (days since release), rearing treatment (enriched or unenriched), cohort (released in 2017 or 2018), and an intercept-only (i.e., null) model. K is the number of parameters in each model. Δ AICc is the difference in AICc values from a given model to the top-ranked model. AICc weight shows the relative likelihood a given model is the most supported. + indicates an additive effect.

Model	K	Δ AICc	AICc weight	Log likelihood
mass	3	0.00	0.33	-85.66
mass + time	4	1.72	0.14	-85.51
mass + cohort	4	1.99	0.12	-85.65
mass + treatment	4	2.01	0.12	-85.66
intercept-only	2	3.49	0.06	-88.41
cohort	3	3.64	0.05	-87.48
time	3	3.77	0.05	-87.55
cohort + time	4	4.09	0.04	-86.70
treatment	3	5.19	0.02	-88.26
treatment + cohort	4	5.39	0.02	-87.35
treatment + time	4	5.41	0.02	-87.36

Daily movement rates did not differ between rearing treatments or release cohorts, but turtles in cohort two dispersed farther on average than those in cohort one, regardless of rearing treatment. This suggests all turtles had similar activity patterns, but those in cohort two moved farther across the landscape. Larger overall body sizes from a longer rearing period likely facilitated greater movement (Janzen et al., 2000), as we found no evidence suggesting captivity duration affected boldness or exploratory behavior of turtles prior to release (Tetzlaff et al., 2019b).

Dispersal-related mortality is commonly linked with translocation failure because animals could be more susceptible to predators or vehicle mortality, expend energy reserves while forgoing feeding, or leave high-quality release sites and move to lower quality areas (Le Gouar et al., 2012; Attum and Cushall, 2015). Although two (10%) turtles in cohort two were killed by vehicles when dispersing, an unexpected finding in our study was that dispersal largely did not come at a short-term cost to survival. Similar results were also found for translocated voles (*Microtus rossiaemeridionalis*), which were thought to benefit from dispersal by reducing odor concentrations near the release site that attracted predators hunting via olfaction (Banks et al., 2002). Olfactory-hunting mammals are also major predators of turtles (Dodd, 2001). Dispersal of translocated juvenile turtles could thus not only confer survival benefits but also enhance gene flow in populations with low genetic diversity (Kimble et al., 2014). Further, terrestrial chelonians have important movement-dependent ecological functions such as seed dispersal (Lovich et al., 2018). For example, eastern box turtles are the only known effective dispersal agent of mayapple (*Podophyllum peltatum*) (Braun and Brooks, 1987).

Head-starting is a common management practice in turtle conservation programs, but traditional captive-rearing practices thought to increase success are often based on intuition rather than quantitative evidence (Seddon et al., 2007). Our

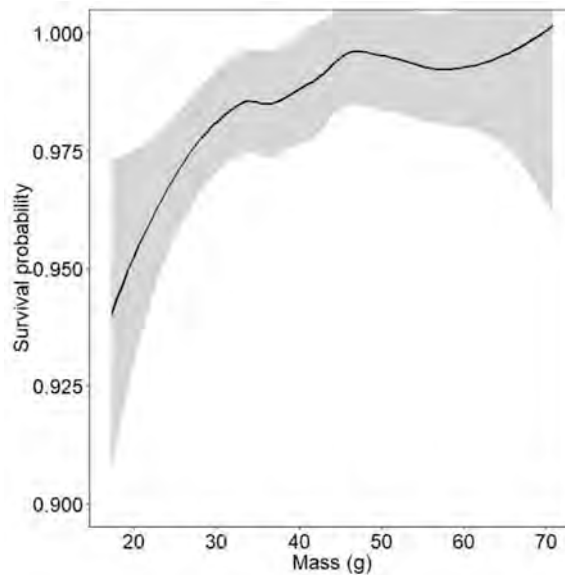


Fig. 6. Estimated daily survival probability (solid line) fit by loess smoothing with 95% confidence interval (ribbon) of head-started eastern box turtles (*Terrapene carolina*) as a function of body mass.

experimental approach allowed for stronger inference regarding mechanisms influencing success and provides insight for future efforts. Because longer rearing duration came at no apparent cost to adaptive behavior or survival, our results indicate raising turtles for several years in captivity to maximize size at release could be valuable for practitioners attempting to restore imperiled populations. Future studies might investigate if post-release survival rates asymptote or perhaps even decline again after a given length of time of captive-rearing. This could be important for determining if survival rates are not improved after turtles exceed a certain size threshold before release or whether captivity duration eventually has negative effects on behavior and post-release survival (DeGregorio et al., 2013, 2017). Such investigations could aid in striking a balance between maximizing success with cost-effectiveness for head-starting efforts (Fischer and Lindenmayer, 2000; Canessa et al., 2016). Finally, we suggest longer term (i.e., >1 year) post-release monitoring should be conducted when possible. This is necessary for measuring more ultimate outcomes of conservation translocations, particularly for long-lived species such as chelonians and because post-release effects may last for several years (Bertolero et al., 2018).

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References

- Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. Pages 267–281. In: Petrov, B.N., Csaki, F. (Eds.), *Proceeding of the Second International Symposium on Information Theory*. Akademiai, Kiado, Budapest.
- Altobelli, J., 2017. Nest-site Selection and Neonate Survival of Eastern Box Turtles (*Terrapene Carolina Carolina*) in Michigan's Northern Lower Peninsula. Master's Thesis. Grand Valley State University.
- Arsovski, D., Olivier, A., Bonnet, X., Drilhollé, S., Tomović, L., Béchet, A., Golubović, A., Besnard, A., 2018. Covariates streamline age-specific early life survival estimates of two chelonian species. *J. Zool.* 306, 223–234.
- Attum, O., Cutshall, C.D., 2015. Movement of translocated turtles according to translocation method and habitat structure. *Restor. Ecol.* 23, 588–594.
- Banks, P.B., Norrdahl, K., Korpimäki, K., 2002. Mobility decisions and the predation risks of reintroduction. *Biol. Conserv.* 103, 133–138.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.

- Bertolero, A., Pretus, J.L., Oro, D., 2018. The importance of including survival release costs when assessing viability in reptile translocations. *Biol. Conserv.* 217, 311–320.
- Braun, J., Brooks Jr., G.R., 1987. Box turtles (*Terrapene carolina*) as potential agents for seed dispersal. *Am. Midl. Nat.* 117, 312–318.
- Buhlmann, K.A., Koch, S.L., Butler, B.O., Tuberville, T.D., Palermo, V.J., Bastarache, B.A., Cava, Z.A., 2015. Reintroduction and head-starting: tools for Blanding's turtle (*Emydoidea blandingii*) conservation. *Herpetol. Conserv. Biol.* 10, 436–454.
- Burke, R.L., 2015. Head-starting turtles: learning from experience. *Herpetol. Conserv. Biol.* 10, 299–308.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: a Practical Information-Theoretic Approach*. Springer-Verlag, New York, New York.
- Canessa, S., Genta, P., Jesu, R., Lamagni, L., Oneto, F., Salvadio, S., Ottonello, D., 2016. Challenges of monitoring reintroduction outcomes: insights from the conservation breeding program of an endangered turtle in Italy. *Biol. Conserv.* 204, 128–133.
- Carstairs, S., Paterson, J.E., Jager, K.L., Gasbarrini, D., Mui, A.B., Davy, C.M., 2019. Population reinforcement accelerates subadult recruitment rates in an endangered freshwater turtle. *Anim. Conserv.* <https://doi.org/10.1111/acv.12503>.
- Currylow, A.F., Zollner, P.A., MacGowan, B.J., Williams, R.N., 2011. A survival estimate of Midwestern adult eastern box turtles using radiotelemetry. *Am. Midl. Nat.* 165, 143–149.
- Daly, J.A., Buhlmann, K.A., Todd, B.D., Moore, C.T., Peaden, J.M., Tuberville, T.D., 2018. Comparing growth and body condition of indoor-reared, outdoor-reared, and direct-released juvenile Mojave desert tortoises. *Herpetol. Conserv. Biol.* 13, 622–633.
- DeGregorio, B.A., Sperry, J.H., Tuberville, T.D., Weatherhead, P.J., 2017. Translocating ratsnakes – does enrichment offset negative effects of time in captivity? *Wildl. Res.* 44, 438–448.
- DeGregorio, B.A., Weatherhead, P.J., Tuberville, T.D., Sperry, J.H., 2013. Time in captivity affects foraging behavior of ratsnakes: implications for translocation. *Herpetol. Conserv. Biol.* 8, 581–590.
- Dodd Jr., C.K., 2001. *North American Box Turtles: A Natural History*. University of Oklahoma Press, Norman, Oklahoma.
- Einum, S., Fleming, I.A., 2001. Implications of stocking: ecological interactions between wild and released salmonids. *Nord. J. Freshw. Res.* 75, 65–70.
- Fischer, J., Lindenmayer, D.B., 2000. An assessment of the published results of animal relocations. *Biol. Conserv.* 96, 1–11.
- Germano, J.M., Bishop, P.J., 2009. Suitability of amphibians and reptiles for translocation. *Conserv. Biol.* 23, 7–15.
- Gibson, J., 2009. Influence of Prescribed Fire on a Midwestern Population of the Eastern Box Turtle (*Terrapene C. Carolina*). Master's Thesis. Purdue University, Fort Wayne.
- Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C., 1989. Translocation as a species conservation tool: status and strategy. *Science* 245, 477–480.
- Harvey, D.S., Weatherhead, P.J., 2010. Habitat selection as the mechanism for thermoregulation in a northern population of Massasauga Rattlesnakes (*Sistrurus catenatus*). *Ecoscience* 17, 411–419.
- Janzen, F.J., Tucker, J.K., Pankstis, G.L., 2000. Experimental analysis of an early life stage: selection on size of hatchling turtles. *Ecology* 81, 2290–2304.
- Janzen, F.J., 1993. An experimental analysis of natural selection on body size of hatchling turtles. *Ecology* 74, 332–341.
- Jule, K.R., Leaver, L.A., Lea, S.E.G., 2008. The effects of captive experience on reintroduction survival in carnivores: a review and analysis. *Biol. Conserv.* 141, 355–363.
- Kiester, A.R., Willey, L.L., 2015. *Terrapene carolina* (Linnaeus 1758)—eastern box turtle, common box turtle. *Chelonian Res. Monogr.* 5, 1–25.
- Kimble, S.J.A., Rhodes Jr., O.E., Williams, R.N., 2014. Unexpectedly low rangewide population genetic structure of the imperiled eastern box turtle *Terrapene c. carolina*. *PLoS One* 9, e92274.
- Kissner, K.J., Weatherhead, P.J., 2005. Phenotypic effects on survival of neonatal northern watersnakes *Nerodia sipedon*. *J. Anim. Ecol.* 74, 259–265.
- Laarman, P.B., Keenlance, P.W., Altobelli, J.T., Schumacher, C.M., Huber, P., Jacquot, J.J., Moore, J.A., 2018. Ecology of neonate eastern box turtles with prescribed fire implications. *J. Wildl. Manag.* 82, 1385–1395.
- Le Gouar, P., Mihoub, J., Sarrazin, F., 2012. Dispersal and habitat selection: behavioral and spatial constraints for animal translocations. Pages 138–164. In: Ewen, J., Armstrong, D.P., Parker, K.A., Seddon, P.J. (Eds.), *Reintroduction Biology: Integrating Science and Management*. Wiley-Blackwell Publishing Ltd., Oxford.
- Lovich, J.E., Ennen, J.R., Agha, M., Gibbons, J.W., 2018. Where have all the turtles gone, and why does it matter? *Bioscience* 68, 771–781.
- Mathews, F., Orros, M., McLaren, G., Gelling, M., Foster, R., 2005. Keeping fit on the ark: assessing the suitability of captive-bred animals for release. *Biol. Conserv.* 121, 569–577.
- Nagy, K.A., Hillard, L.S., Tuma, M.W., Morafka, D.J., 2015. Head-started desert tortoises (*Gopherus agassizii*): movements, survivorship, and mortality causes following their release. *Herpetol. Conserv. Biol.* 10, 203–215.
- Packard, G.C., Packard, M.J., 1988. Physiological ecology of reptilian eggs and embryos. In: Gans, C., Huey, R.B. (Eds.), *Biology of the Reptilia*, vol. 16. Liss, New York, pp. 523–605.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2018. *Nlme: Linear and Nonlinear Mixed Effects Models*. R package version, 3, 1–137.
- Quinn, D.P., Buhlmann, K.A., Jensen, J.B., Norton, T.M., Tuberville, T.D., 2018. Post-release movement and survivorship of head-started gopher tortoises. *J. Wildl. Manag.* 82, 1545–1554.
- R Core Team, 2018. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>.
- Reading, R.P., Miller, B., Shepherdson, D., 2013. The value of enrichment to reintroduction success. *Zoo Biol.* 32, 332–341.
- Roe, J.H., Frank, M.R., Gibson, S.E., Attum, O., Kingsbury, B.A., 2010. No place like home: an experimental comparison of reintroduction strategies using snakes. *J. Appl. Ecol.* 47, 1253–1261.
- Roe, J.H., Frank, M.R., Kingsbury, B.A., 2015. Experimental evaluation of captive-rearing practices to improve success of snake reintroductions. *Herpetol. Conserv. Biol.* 10, 711–722.
- Seddon, P.J., Armstrong, D.P., Maloney, R.F., 2007. Developing the science of reintroduction biology. *Conserv. Biol.* 21, 303–312.
- Shaffer, T.L., 2004. A unified approach to analyzing nest success. *Auk* 121, 526–540.
- Spencer, R.J., Van Dyke, J.U., Thompson, M.B., 2017. Critically evaluating best management practices for preventing freshwater turtle extinctions. *Conserv. Biol.* 31, 1340–1349.
- Stamps, J.A., Swaisgood, R.R., 2007. Someplace like home: experience, habitat selection and conservation biology. *Appl. Anim. Behav. Sci.* 102, 392–409.
- Swaisgood, R.R., 2010. The conservation-welfare nexus in reintroduction programmes: a role for sensory ecology. *Anim. Welf.* 19, 125–137.
- Swaisgood, R.R., Martin-Wintle, M.S., Owen, M.A., Zhou, X., Zhang, H., 2018. Developmental stability of foraging behavior: evaluating suitability of captive giant pandas for translocation. *Anim. Conserv.* 21, 474–482.
- Tetzlaff, S.J., Sperry, J.H., DeGregorio, B.A., 2018. Captive-reared juvenile box turtles innately prefer naturalistic habitat: implications for translocation. *Appl. Anim. Behav. Sci.* 204, 128–133.
- Tetzlaff, S.J., Sperry, J.H., DeGregorio, B.A., 2019a. Effects of antipredator training, environmental enrichment, and soft release on wildlife translocations: a review and meta-analysis. *Biol. Conserv.* 236, 342–331.
- Tetzlaff, S.J., Sperry, J.H., DeGregorio, B.A., 2019b. Tradeoffs with growth and behavior for captive box turtles head-started with environmental enrichment. *Diversity* 11, 40.
- Tuberville, T.D., Clark, E.E., Buhlmann, K.A., Gibbons, J.W., 2005. Translocation as a conservation tool: site fidelity and movement of repatriated gopher tortoises (*Gopherus polyphemus*). *Anim. Conserv.* 8, 349–358.
- Tucker, A.D., FitzSimmons, N.N., Gibbons, J.W., 1995. Resource partitioning by the estuarine turtle *Malaclemys terrapin*: trophic, spatial, and temporal foraging constraints. *Herpetologica* 51, 167–181.
- van Dijk, P.P., 2011. *Terrapene Carolina* (errata version published in 2016) The IUCN Red List of Threatened Species 2011: e.T21641A97428179.

REPORT DOCUMENTATION PAGE

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14. ABSTRACT Raising captive animals past critical mortality stages for eventual release (head-starting) is a common conservation tactic. Counterintuitively, post-release survival can be low. Post-release behavior affecting survival could be influenced by captive-rearing duration and housing conditions. Practitioners have adopted environmental enrichment to promote natural behaviors during head-starting such as raising animals in naturalistic enclosures. Using 32 captive-born turtles (<i>Terrapene carolina</i>), half of which were raised in enriched enclosures, we employed a factorial design to explore how enrichment and rearing duration affected post-release growth, behavior, and survival. Six turtles in each treatment (enriched or unenriched) were head-started for nine months (cohort one). Ten turtles in each treatment were head-started for 21 months (cohort two). At the conclusion of captive-rearing, turtles in cohort two were overall larger than cohort one, but unenriched turtles were generally larger than enriched turtles within each cohort. Once released, enriched turtles grew faster than unenriched turtles in cohort two, but we otherwise found minimal evidence suggesting enrichment affected post-release survival or behavior. Our findings suggest attaining larger body sizes from longer captive-rearing periods to enable greater movement and alleviate susceptibility to predation (the primary cause of death) could be more effective than environmental enrichment alone in chelonian head-starting programs where substantial predation could hinder success.					
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