Tradeoffs with Growth and Behavior for Captive Box Turtles Head-Started with Environmental Enrichment

Sasha J. Tetzlaff [1,*] **, Jinelle H. Sperry** [1,2] **and Brett A. DeGregorio** [1,2]

[1] Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, 1102 South Goodwin Avenue, Urbana, IL 61801, USA; Jinelle.Sperry@usace.army.mil (J.H.S.); badegregorio@gmail.com (B.A.D.)

[2] Engineer Research and Development Center, 2902 Newmark Drive, Champaign, IL 61822, USA

[*] Correspondence: sashatetzlaff@gmail.com; Tel.: +1-217-333-2770

Abstract: Head-starting is a conservation strategy that entails releasing captive-reared animals into nature at sizes large enough to better resist post-release predation. However, efforts to maximize growth in captivity may jeopardize development of beneficial behaviors. Environmental enrichment can encourage natural behaviors before release but potentially comes with a tradeoff of reduced growth in complex enclosures. We compared growth and behavior of enriched and unenriched captive-born juvenile box turtles (*Terrapene carolina*). Enriched turtles grew slower than unenriched turtles during the first eight months in captivity, although growth rates did not differ between treatments from 9–20 months old. After five months post-hatching, unenriched turtles became and remained larger overall than enriched turtles. During two foraging tasks, unenriched turtles consumed more novel prey than enriched turtles. In a predator recognition test, eight-month-old enriched turtles avoided raccoon (*Procyon lotor*) urine more than unenriched turtles of the same age, but this difference was not apparent one year later. The odds of turtles emerging from a shelter did not differ between treatments regardless of age. Although our results suggest turtles raised in unenriched environments initially grew faster and obtained larger overall sizes than those in enriched conditions, tradeoffs with ecologically-relevant behaviors were either absent or conditional.

Keywords: antipredator behavior; behavioral assay; growth rate; captive-rearing; foraging behavior; *Terrapene carolina*

1. Introduction

Head-starting, a practice that involves captive-rearing animals for release into nature, is a popular approach for augmenting or reintroducing imperiled wildlife populations. A common focus in these programs involves rapidly growing individuals to reduce susceptibility to predation or be closer to reproductive age or size upon release [1–3]. However, emphasis on rapid growth may have undesirable consequences, such as the degradation or loss of normal behaviors. Behavior may be especially compromised the longer individuals are kept in captivity [4,5]. Empirical evidence is needed to explore if maximizing growth does indeed come at the cost of beneficial behaviors, which could reduce head-starting success. We investigated this potential tradeoff by comparing growth and behavior of environmentally enriched and unenriched captive-reared juvenile eastern box turtles (*Terrapene carolina*).

Head-started animals are often raised in simplistic enclosures which can be cost-efficient, are relatively easy to maintain, and allow caretakers to closely monitor food intake. With easy access to food, individuals housed in such conditions often experience rapid growth [6]. However, because captivity can have detrimental effects on development, behavior of captive animals often

differs from wild-bred conspecifics [5]. Such findings have implications for outcomes of wildlife translocations using captive animals. For instance, carnivores translocated from captivity may have reduced ability to capture prey compared to those directly translocated from the wild [7], suggesting captive animals are often unprepared for life in nature.

Practitioners attempting to promote ecologically relevant behaviors and improve welfare for head-started animals have incorporated environmental enrichment into rearing protocols, such as 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 [8–11]. Assessing behavior of head-started animals prior to release often suggests enrichment can prepare animals to succeed in post-release environments [12,13].

A potential conflict with using environmental enrichment to condition adaptive behavior is that it may come at the cost of accelerated growth rates in captivity. If the enrichment regime requires animals to search for spatially variable food in structurally complex enclosures and/or compete for food with conspecifics, growth may be reduced compared to unenriched individuals [14]. Animals in complex enclosures may simply also choose to spend more time hidden or exploring their surroundings and less time eating than conspecifics raised in simplistic enclosures. Thus, maximizing growth and promoting ecologically relevant behaviors could be mutually exclusive goals, presenting potentially conflicting rationale for deciding how individuals should be reared.

Tradeoffs between growth and behavior may be particularly relevant to turtle head-starting programs due to the life-history attributes of most Chelonians [15]. Juvenile turtles (order Testudines) often have lower estimated survival rates than adults, with predation assumed to be the primary cause of mortality [16,17]. Older, larger animals are less vulnerable to predation, in part due to a larger and thicker shell that can be a successful deterrent for many predators [3,18]. Additionally, wild turtles often do not reach reproductive sizes for upwards of 1–2 decades, so increasing juvenile growth rates should allow individuals to be closer to reproductive size upon release [19]. Success of turtle head-starting efforts has been mixed. Although this approach involves considerable time and expense and survival of released juveniles can be low [20], in some cases it may be one of the most effective methods for re-establishing viable populations [21]. Studies explicitly evaluating a tradeoff between a naturalistic rearing environment and one that maximizes growth rate on behaviors that are important for survival in the wild are lacking.

We hypothesized a tradeoff exists between growth and behavior for turtles raised with and without environmental enrichment. We predicted enriched turtles reared in structurally complex enclosures, which entailed foraging for spatially variable food in the presence of conspecifics, would grow slower and attain overall smaller body sizes than unenriched turtles. However, we anticipated this form of enrichment would allow turtles to be more proficient at foraging for novel prey than unenriched turtles, which were housed individually and fed out of simple dishes. We predicted enriched turtles would also show more risk-aversion than unenriched turtles by avoiding a predator cue more as well as being less likely to emerge from a shelter into a novel environment.

2. Materials and Methods

2.1. Study Species and Animal Husbandry

Eastern box turtles inhabit temperate and subtropical regions over much of the eastern United States [16]. 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 [22]. As such, the species is listed as Vulnerable by the IUCN and is included in CITES Appendix II [23].

This research was conducted under an approved protocol (#16017) by the University of Illinois Institutional Animal Care and Use Committee and Scientific Collector's Permits granted by the States of Michigan and Illinois (#NH17.5980). Subjects for this study were acquired as eggs from nests

laid by free-ranging female eastern box turtles at Fort Custer Training Center, an Army National Guard training facility located near Battle Creek, Michigan, USA. 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 21–29 °C. We raised neonates 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 × 79 cm wide × 30 cm deep Rubbermaid® stock tanks (Rubbermaid Commercial Products, Huntersville, NC, USA) (n = 4–5 individuals per replicate) with naturalistic features designed to mimic vegetation and substrate commonly utilized by wild eastern box turtles [16] (Figure 1). Unenriched turtles (n = 16) were housed individually in comparably simplistic enclosures consisting of a 60 cm long × 42 cm wide × 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 gave 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 (Figure 1). We provided all turtles with UVB light daily from 0630–1630 h using Zoo Med ReptiSun® 5.0 bulbs (Zoo Med Laboratories, Inc.). We affixed a 25 cm diameter light hood with a 13 W compact fluorescent bulb to each enriched tank. We hung a continuous row of 117 cm fluorescent linear tubes housed in fixtures (Zoo Med T5 High Output Terrarium Hood) spanning the lower (wet) ends of the rows of unenriched tubs. We used Zoo Med Reptitherm® (Zoo Med Laboratories, Inc.) under tank heat pads from October–March to maintain temperatures and facilitate activity and foraging throughout the winter months.

Figure 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 and a small plastic hiding structure.

The type and amount of food provided to individuals at each feeding was similar between rearing treatments. However, we fed enriched turtles in a manner that we anticipated would entail challenges leading to improved foraging, as this is a skill frequently cited as being deficient for translocated animals released from captivity [11]. Because wild box turtles actively forage for items such as invertebrates, vegetation, and fungi in complex terrestrial microhabitats [16], we predominantly fed

enriched turtles by scattering food throughout their enclosures. Unenriched turtles were provided food on 10 cm diameter petri dishes, placed in the same spot in enclosures at each feeding. We initially fed live blackworms (*Lumbriculus variegatus*) and mealworms (*Tenebrio molitor*). We then transitioned turtles to live superworms (*Zophobas morio*) and then solely to live redworms (*Eisenia foetida*) after several months. We also offered 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 five days per week, and we dusted food with calcium powder three days per week. Because wild box turtles often scavenge animal carcasses and consume bones to obtain minerals [24,25], we provided enriched turtles with commercially available cuttlebones to chew on as an additional component to their enrichment. Fresh water was provided ad libitum. Further details of study animal acquisition and husbandry methods are described elsewhere [26].

2.2. Growth

We recorded individuals' mass (g) using a digital scale (Sartorius M-PROVE Portable Scale; Sartorius AG, Göttingen, Germany) approximately once per week. To compare growth between rearing treatments at the time behavior was assessed in captivity (see below), we calculated daily growth rate for all 32 turtles when eight months old using each turtles' mass after hatching in August 2016 and mass in April 2017, divided by the number of days between measurements. Twelve turtles (six enriched and six unenriched) were randomly selected for release into the wild in May 2017. We recalculated daily growth rates for the 20 turtles (10 in each treatment) retained in captivity for an additional year, when 20 months old. We compared growth rates between treatments for each age range (0–8 and 9–20 months) with linear models in R version 3.4 [27], which we used for all analyses. We analyzed the interactive effect of treatment and time (number of months post-hatching) on mass using a linear model.

2.3. Behavioral Assays

2.3.1. General Information

We conducted several behavioral assays in the greenhouse where turtles were reared during late March–early April of 2017 and 2018 when turtles were eight and 20 months old, respectively. All 32 turtles (16 in each treatment) were tested in 2017. The twenty remaining individuals (10 in each treatment) were re-tested in the same assays one year later. We randomly selected the testing order of individuals. Turtles were tested individually, placed back in primary enclosures once a trial was completed, and were tested in only one assay per day. We affixed covers around the sides and bottom of testing arenas to reduce the influence of external stimuli. Between trials, we cleaned arenas with ethanol and rinsed them with hot water. We conducted assays during daylight hours (0800–1600) because this species is predominantly diurnal [16]. Relevant substrates and food items were novel to all turtles at the first time of testing. For trials involving food, each turtle was fasted for 48 h prior to testing to encourage an appetitive response, but all individuals were offered their normal diet in primary enclosures for five consecutive days before the fasting period began.

For trials requiring direct observation to record response variables, we placed video cameras (Lorex LNB3163B bullet surveillance camera; Lorex Technology, Inc., Markham, ON, Canada) above testing arenas and filmed trials using a digital video recorder (Yoko Technology RYK-9122 DVR; Yoko Technology Corp., New Taipei City, Taiwan). Observers recorded data while situated out of view from turtles by watching trials on a television monitor (Eyoyo 20.32 cm portable HDMI LCD monitor; Shenzhenshi cai hui Technology Co., Ltd, Shenzhen, China). All timed response variables were recorded in seconds using stopwatches. Observers were at times aware of a turtle's identity. To ensure this did not bias our results, an observer unaware of turtles' identities scored a randomly selected sample of the same response variables from recorded trials. We found strong agreement between

response variables recorded at the time of testing and those scored from video footage (Pearson's r > 0.9 in all cases).

2.3.2. Foraging Behavior

We designed two assays to test turtles' foraging efficiency in contrasting environments so that each generally differed from a respective treatment's rearing conditions. The first tested their ability to detect and capture relatively immobile prey but in a more challenging environment (hereafter "simple prey, complex environment"). We placed turtles in a 35 cm long × 21 cm wide × 12 cm tall, open-topped plastic arena with 2 cm of cypress mulch uniformly spread over the floor. We put a turtle in the center of the arena under a dark 10 cm^3 cup, where it acclimated for 5 min. We then placed one commercially-raised live black soldier fly (*Hermetia illucens*) larva (0.5–2 cm in length) slightly hidden in each of three corners of the arena just before removing the cup covering the turtle. We recorded the number of larvae consumed by each turtle within 4 h once unconstrained to move about the arena. Because our only response variable for this assay were count data, we did not collect video footage for this trial. For each age group (eight and 20 months), we used generalized linear models assuming a Poisson distribution to examine if the number of larvae consumed differed between treatments.

To complement the simple prey, complex environment test, we conducted a second assay to test turtles' ability to procure more mobile prey but in a relatively simplistic environment (hereafter "complex prey, simple environment"). We used a slightly larger plastic arena (60 cm long × 42 cm wide × 17 cm) than that used in the other foraging task because we used more agile prey for this assay. We placed turtles in the center of the arena and allowed them to explore their surroundings for 5 min. We then placed an individual under a dark 10 cm^3 cup at one end of the arena, where it was held for an additional 5 min. We placed two commercially-raised live dubia cockroaches (*Blaptica dubia*; ca. 0.5 cm in length) at the opposite end of the arena (one in each corner) after the acclimation period and immediately removed the cup covering the turtle. We chose dubia cockroaches because they were fast, active prey that would represent a challenge for turtles to capture and were morphologically and behaviorally different from any live prey they had previously encountered. For this test, we provided no substrate in the arena for prey to hide in, but we roughed the floor with sandpaper to better enable turtles and prey to locomote. Within a 30 min time-limit, we recorded latency to strike at a cockroach as well as the number consumed. Any individual that did not strike at prey within the time-limit during this assay was given the full trial length as its strike latency. We used generalized linear models assuming a Poisson distribution to test if the number of cockroaches consumed differed between treatments in each age group. We used linear models to examine if strike latency differed between treatments at each age.

2.3.3. Antipredator Behavior

We designed this assay to test turtles' recognition and avoidance of an olfactory predator cue. Although neither group of turtles had ever been exposed to predators, we predicted enriched turtles may be more likely to recognize and respond to this cue than unenriched turtles because they were continually stimulated by their surroundings. We placed two evenly-sized paper towels covering all but approximately 4 cm of the center of the floor in a 35 cm long × 21 cm wide × 12 cm tall, plastic arena. One paper towel was sprayed with diluted raccoon (*Procyon lotor*) urine (hereafter "scented"). Raccoons are a well-known predator of eastern box turtles [16] and are abundant at the site where subjects in this study were collected (unpublished data). The other paper towel was sprayed with distilled water as an unscented liquid control (hereafter "unscented"). Similar approaches have been used to test antipredator behavior in other taxa [28,29]. This 20 min trial began immediately after placing a turtle in the center of the arena, where their plastron was not in contact with either paper towel. Turtles thus had opportunity to inspect either side before making a choice but had to fully move onto one of the paper towels to have been considered choosing. Any individual that did not respond within the time-limit for this assay (i.e., remained motionless) was excluded from analysis. We used

generalized linear models assuming a binomial distribution to examine if the odds of choosing the unscented side of the arena differed between treatments at each age. We also recorded how much time individuals spent away from the scented side of the arena as a measure of avoidance of the cue. We tested if this response differed between treatments at each age using linear models.

2.3.4. Shelter Emergence

This assay was designed to evaluate propensity to emerge from shelter into a novel environment. We placed turtles in a clear 60 cm long × 42 cm wide × 17 cm plastic arena with a 10 cm^3 PVC shelter placed at one end. There was no substrate in the arenas. We put turtles inside shelters facing the entrance and allowed them to acclimate for 5 min by covering the entrance with cardboard. We then removed the cover, allowing the turtles the option of exiting the shelter. Our response variable was a binary effect of "emerged" or "did not emerge". We used generalized linear models assuming a binomial distribution to examine if the odds of emerging differed between treatments at each age.

For general linear models, we ensured data residuals approximated a Gaussian distribution by inspecting quantile-quantile plots, and we assessed homogeneity of variances using Brown-Forsyth tests. We assessed overdispersion for generalized linear models by dividing the residual deviance by the degrees of freedom. We considered effects to be statistically significant when $P \leq 0.05$.

3. Results

3.1. Growth

We recorded mass an average of 28.72 ± 1.37 standard deviation (SD) times for 32 turtles from August 2016 to April 2017. We recorded mass an average of 44.15 ± 0.99 SD times for 20 individuals kept in captivity for an additional year. Enriched turtles grew significantly slower than unenriched turtles during the first eight months in captivity ($P < 0.001$), but we found no difference in growth rates between treatments for turtles kept in captivity an additional year ($P = 0.27$; Figure 2a). We found a significant interaction between treatment and time for predicting body mass ($P < 0.001$). Unenriched turtles became larger than enriched turtles after the first five months in captivity and remained overall larger for the duration of rearing (Figure 2b).
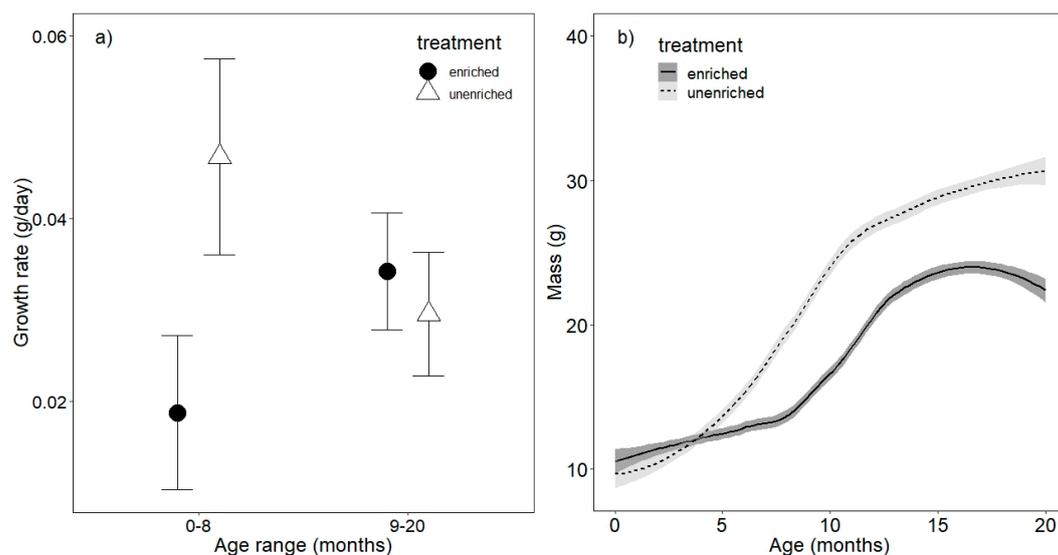

Figure 2. (**a**) Growth rates (g/day) of enriched and unenriched eastern box turtles (*Terrapene carolina*) during captive-rearing. Thirty-two turtles (16 in each treatment) were raised for eight months. Twenty turtles (10 in each treatment) were kept in captivity for an additional year. Symbols represent means and bars are 95% confidence intervals. (**b**) Mass (g) of enriched and unenriched turtles during captive-rearing. Lines are means fit by loess smoothing, and ribbons represent 95% confidence intervals.

3.2. Foraging Behavior

In the simple prey, complex environment task, where turtles were placed in enclosures with mulch substrate and given 4 h to find and consume black soldier fly larvae, they consumed an average of 1.13 ± 1.33 SD larvae (range 0–3). Eight-month-old enriched turtles consumed significantly fewer larvae on average compared to unenriched turtles of the same age ($P < 0.001$; Figure 3). We found a similar pattern between treatments when turtles were 20 months old ($P < 0.01$; Figure 3). The average number of larvae eaten during a trial by enriched turtles was low due to only three enriched turtles consuming any prey during trials, whereas only five unenriched turtles did not consume larvae.
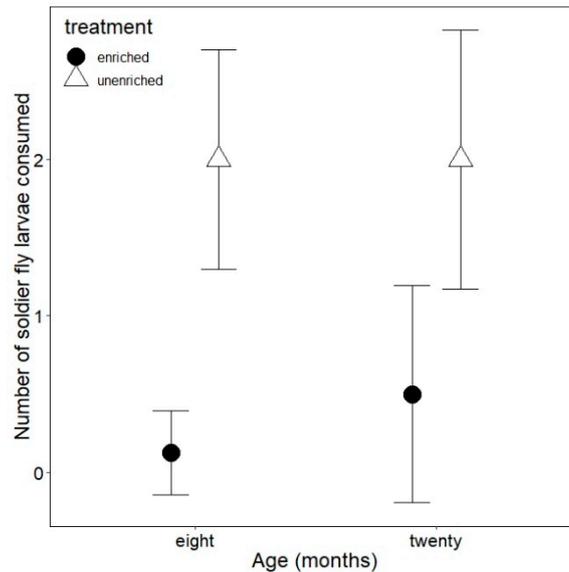
Figure 3. Number of black soldier fly larvae (*Hermetia illucens*) consumed by enriched and unenriched captive-reared eastern box turtles (*Terrapene carolina*) when eight and 20 months old. Symbols represent means and bars are 95% confidence intervals.

In the complex prey, simple environment test, where turtles were placed in simplistic enclosures and given 30 min to strike at and consume dubia cockroaches, they consumed an average of 1.06 ± 0.96 SD larvae (range 0–2). Like the previous foraging assay, many enriched turtles (n = 12) did not respond to the prey and remained motionless during trials, whereas only three unenriched turtles did not consume a cockroach. Eight-month-old enriched turtles consumed significantly fewer cockroaches on average compared to unenriched turtles of the same age ($P < 0.001$; Figure 4a). Again, we found a similar pattern between treatments when turtles were 20 months old, where enriched turtles consumed few cockroaches and all ten unenriched turtles consumed both cockroaches ($P < 0.01$; Figure 4a). Unenriched turtles were significantly quicker to strike at prey than enriched turtles when eight ($P < 0.001$) and 20 months old ($P < 0.001$; Figure 4b).
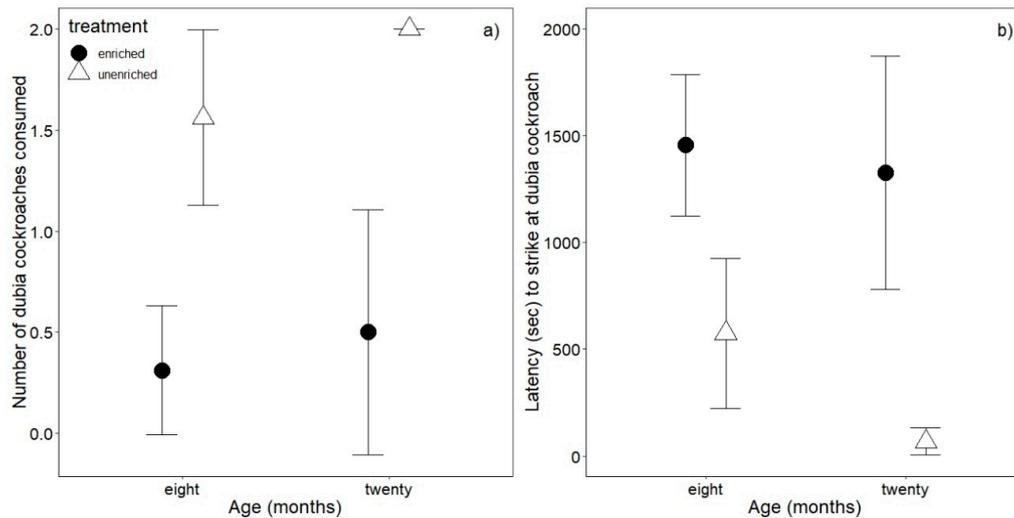
Figure 4. Number of dubia cockroaches (*Blaptica dubia*) consumed (**a**) and latency to strike at a cockroach (**b**) by enriched and unenriched eastern box turtles (*Terrapene carolina*) when eight or 20 months old. Symbols represent means and bars are 95% confidence intervals. There is no confidence interval for twenty-month-old unenriched turtles in panel (**a**) because all these turtles consumed both cockroaches.

3.3. Antipredator Behavior

When given 20 min to choose between initially occupying a side of an arena scented with raccoon urine or sprayed with a water control, turtles appeared to choose randomly with no apparent initial distinction between sides. When eight months old, eight of fifteen (53%) enriched turtles (one enriched turtle did not make a choice) chose the unscented side and six of sixteen (37.5%) unenriched turtles chose the unscented side. When tested at 20 months in age, five of 10 (50%) enriched turtles chose the unscented side, and four of 10 (40%) unenriched chose the unscented side. Thus, the odds of selecting the unscented side did not differ significantly between treatments at eight ($P = 0.38$) or 20 months old ($P = 0.65$). Eight-month-old enriched turtles spent significantly more time away from the scented area than unenriched turtles of the same age ($P = 0.05$), but we found no difference in time spent away from the scented area between treatments when turtles were 20 months old ($P = 0.35$; Figure 5).
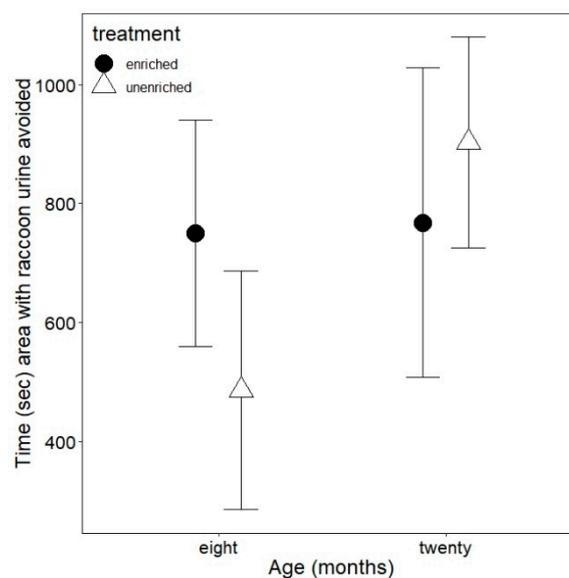

Figure 5. Time (seconds) enriched and unenriched captive-reared eastern box turtles (*Terrapene carolina*) spent away from a side of an experimental arena scented with northern raccoon (*Procyon lotor*) urine when eight and 20 months old. Symbols represent means and bars are 95% confidence intervals.

3.4. Shelter Emergence

When given 20 min to emerge from shelter into a novel environment, two of 16 (12.5%) enriched turtles emerged, and six of sixteen (37.5%) unenriched turtles emerged when eight months old. When 20 months old, five of 10 (50%) enriched and seven of 10 (70%) unenriched emerged. Thus, the odds of emerging did not differ significantly between treatments when eight ($P = 0.12$) or 20 months old ($P = 0.37$).

4. Discussion

Although experiments investigating the effects of environmental enrichment on behavior and morphology for captive reptiles are increasing, this taxon has been historically neglected compared to others such as birds or mammals [10]. Furthermore, studies investigating such effects in the context of informing wildlife reintroductions in general are lacking [11]. We explored a potential tradeoff between growth and ecologically relevant behaviors in captivity for head-started box turtles and found this tradeoff is most apparent for younger turtles. Our prediction that enriched turtles would grow slower than unenriched turtles was supported only during the first eight months of rearing. Growth rates did not differ between rearing treatments from 9–20 months in age, suggesting growth lags from experiencing more natural foraging conditions began to dissipate for enriched turtles towards the end of the study. In a similar study, head-started smooth green snakes (*Opheodrys vernalis*) experiencing restricted foraging during simulated winter in captivity to prepare them for natural conditions exhibited compensatory growth and were of similar size compared to snakes kept active year-round by the time of release [30]. However, it is important to note that our enriched turtles had consistently smaller body sizes than unenriched turtles from a few months after hatching to the end of the study and were ultimately a third smaller, on average. If larger body size allows head-started turtles to be less vulnerable to certain predators once released, as has been suggested, raising them in conditions to facilitate growth may be a preferred rearing method [3].

We predicted enriched turtles would excel over unenriched turtles in foraging assays because they had been raised to find spatially variable food in complex enclosures. However, unenriched turtles consumed more novel prey than enriched turtles in both tests and were quicker to strike at cockroaches in the complex prey, simple environment task. We suggest the novelty of active foraging during assays may have promoted unenriched turtles to have such a strong response. Although subadult enriched ratsnakes (*Elaphe obsoleta*) did not differ from unenriched conspecifics in a laboratory foraging task [31], findings similar to ours indicative of behavioral deprivation were reported for domestic animals raised in unstimulating environments. Rats reared in unenriched conditions pressed a bar to turn on a light in an operant conditioning chamber more often than enriched ones, even if the light did not come on [32]. Pigs (*Sus scrofa domesticcus*) raised in unenriched conditions had abnormally greater dendritic growth in the somatosensory cortex than enriched individuals, which was thought to be a function of environmental deprivation leading pigs to exhibit hyper stimulus-seeking behavior [33]. It is unclear why there was a general lack of response by enriched turtles, but their behavior could indicate they were more willing than unenriched turtles to forgo foraging in unfamiliar environments.

Although all our captive turtles had never been exposed to predators, we presumed enriched turtles would show greater response to a predator cue given the cognitive benefits enrichment can provide [8–11]. Contrary to our prediction, turtles did not show aversion to the area scented with raccoon urine when making a primary choice, which could suggest a lack of ability in naïve individuals to recognize predator urine as a potential risk. We acknowledge this result could be related to limitations of our methodology in that we used small testing apparatuses, so odor from the urine may have been broadly distributed and detectable throughout the arena. Additionally, we did not provide food or refuge in this assay that may motivate turtles to assess tradeoffs between predator cue avoidance and other survival attributes. Enrichment may provide earlier developmental advantages for avoiding predators because eight-month-old enriched turtles avoided the urine more than unenriched turtles of the same age, providing some evidence of aversion to this scent. Treatments did not differ

in how long they avoided raccoon urine when 20 months old; enriched turtles' behavior did not change from the previous year, whereas older unenriched individuals exhibited avoidance behavior like enriched turtles. The mechanisms of predator recognition for captive-born turtles are not well understood, but general stimulation from enrichment may be beneficial in this regard [34], at least in the short-term.

Because enriched turtles had greater hiding opportunities in their enclosures, we expected this group would be less likely to emerge from shelter. However, we did not find any treatment difference in turtles' propensity to emerge, perhaps because there was no potential cost associated with not emerging. Additionally, hiding in cover is likely innate for this species, as this is probably the most efficient antipredator behavior for juveniles which are smaller and have less rigid shells than adults [3,18,26]. As with our predator recognition assay, future experiments might show greater treatment differences if shelter emergence tests incorporate aspects of our other assays, such as determining if turtles will differ in emergence propensity based on whether food or predator cues are present.

Despite mixed success and considerable expense [35], head-starting is likely to remain popular given its intuitive nature and public support [21]. Given the increasing global reduction of turtle populations [36], experimentally evaluating captive-rearing practices as we did here is vital for advancing head-starting efforts [3,9]. Our results suggest environmental enrichment may come at the cost of growth and total size attainment for turtles, and the expected behavioral benefits were modest or absent. However, our investigation looked at only a small number of behaviors in a laboratory setting, and it remains to be seen if enriched and unenriched turtles exhibit differing behaviors or survival post-release.

References

1. Roe, J.H.; Frank, M.R.; Gibson, S.E.; Attum, O.; Kingsbury, B.A. No place like home: An experimental comparison of reintroduction strategies using snakes. *J. Appl. Ecol.* **2010**, *47*, 1253–1261. [CrossRef]

2. Wines, M.P.; Johnson, V.M.; Lock, B.; Antonio, F.; Godwin, J.C.; Rush, E.M.; Guyer, C. Optimal husbandry of hatchling eastern indigo snakes (*Drymarchon couperi*) during a captive head-start program. *Zoo Biol.* **2015**, *34*, 230–238. [CrossRef] [PubMed]

3. Daly, J.A.; Buhlmann, K.A.; Todd, B.D.; Moore, C.T.; Peaden, J.M.; Tuberville, T.D. Comparing growth and body condition of indoor-reared, outdoor-reared, and direct-released juvenile Mojave desert tortoises. *Herpetol. Conserv. Biol.* **2018**, *13*, 622–633.

4. DeGregorio, B.A.; Weatherhead, P.J.; Tuberville, T.D.; Sperry, J.H. Time in captivity affects foraging behavior of ratsnakes: Implications for translocation. *Herpetol. Conserv. Biol.* **2013**, *8*, 581–590.

5. Swaisgood, R.R.; Martin-Wintle, M.S.; Owen, M.A.; Zhou, X.; Zhang, H. Developmental stability of foraging behavior: Evaluating suitability of captive giant pandas for translocation. *Anim. Conserv.* **2018**, *21*, 474–482. [CrossRef]

6. King, R.B.; Stanford, K.M. Headstarting as a management tool: A case study of the plains gartersnake. *Herpetologica* **2006**, *62*, 282–292. [CrossRef]

7. Jule, K.R.; Leaver, L.A.; Lea, S.E.G. The effects of captive experience on reintroduction survival in carnivores: A review and analysis. *Biol. Conserv.* **2008**, *141*, 355–363. [CrossRef]
8. Swaisgood, R.R. Current status and future directions of applied behavioral research for animal welfare and conservation. *Appl. Anim. Behav. Sci.* **2007**, *102*, 139–162. [CrossRef]
9. Swaisgood, R.R. The conservation-welfare nexus in reintroduction programmes: A role for sensory ecology. *Anim. Welf.* **2010**, *19*, 125–137.
10. Burghardt, G.M. Environmental enrichment and cognitive complexity in reptiles and amphibians: Concepts, review, and implications for captive populations. *Appl. Anim. Behav. Sci.* **2013**, *147*, 286–298. [CrossRef]
11. Reading, R.P.; Miller, B.; Shepherdson, D. The value of enrichment to reintroduction success. *Zoo Biol.* **2013**, *32*, 332–341. [CrossRef] [PubMed]
12. Vargas, A.; Anderson, S.H. Effects of experience and cage enrichment on predatory skills of black-footed ferrets (*Mustela nigripes*). *J. Mammal.* **1999**, *80*, 263–269. [CrossRef]
13. Kenison, E.K.; Williams, R.N. Training for translocation: Predator conditioning induces behavioral plasticity and physiological changes in captive eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) (Cryptobranchidae, Amphibia). *Diversity* **2018**, *10*, 13. [CrossRef]
14. Krech, D.; Rosenzweig, M.R.; Bennett, E.L. Effects of environmental complexity and training on brain chemistry. *J. Comp. Physiol. Psychol.* **1960**, *53*, 509–519. [CrossRef] [PubMed]
15. Heppell, S.S. Application of life-history theory and population model analysis to turtle conservation. *Copeia* **1998**, *1998*, 367–375. [CrossRef]
16. Dodd, C.K., Jr. *North American Box Turtles: A Natural History*; University of Oklahoma Press: Norman, OK, USA, 2002; 256 p.
17. Pike, D.A.; Pizzatto, L.; Pike, B.A.; Shine, R. Estimating survival rates for uncatchable animals: The myth of high juvenile mortality in reptiles. *Ecology* **2008**, *89*, 607–611. [CrossRef] [PubMed]
18. Arsovski, D.; Olivier, A.; Bonnet, X.; Drilholle, S.; Tomović, L.; Béchet, A.; Golubović, A.; Besnard, A. Covariates streamline age-specific early life survival estimates of two chelonian species. *J. Zool.* **2018**, *306*, 223–234. [CrossRef]
19. Congdon, J.D.; Nagle, R.D.; Kinney, O.M. Front-loading life histories: The enduring influence of juvenile growth on age, size, and reproduction of primiparous female freshwater turtles. *Evol. Ecol. Res.* **2018**, *19*, 353–364.
20. Burke, R.L. Head-starting turtles: Learning from experience. *Herpetol. Conserv. Biol.* **2015**, *10*, 299–308.
21. Buhlmann, K.A.; Koch, S.L.; Butler, B.O.; Tuberville, T.D.; Palermo, V.J.; Bastarache, B.A.; Cava, Z.A. Reintroduction and head-starting: Tools for Blanding's turtle (*Emydoidea blandingii*) conservation. *Herpetol. Conserv. Biol.* **2015**, *10*, 436–454.
22. Kiester, A.R.; Willey, L.L. *Terrapene carolina* (Linnaeus 1758)–Eastern box turtle, common box turtle. *Chel. Res. Monogr.* **2015**, *5*. [CrossRef]
23. van Dijk, P.P. *Terrapene carolina* (errata version published in 2016). The IUCN Red List of Threatened Species, 2011. e.T21641A97428179. Available online: http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T21641A9303747.en (accessed on 7 March 2019).
24. Platt, S.G.; Hall, C.; Liu, H.; Borg, C.K. Wet-season food habits and intersexual diet overlap of Florida box turtles (*Terrapene carolina bauri*) on National Key Deer Refuge, Florida. *Southeast. Nat.* **2009**, *8*, 335–346. [CrossRef]
25. Tetzlaff, S.J.; Schiltz, N.G. *Terrapene carolina* (Eastern Box Turtle) Scavenging. *Herpetol. Rev.* **2016**, *47*, 453–454.
26. Tetzlaff, S.J.; Sperry, J.H.; DeGregorio, B.A. Captive-reared juvenile box turtles innately prefer naturalistic habitat: implications for translocation. *Appl. Anim. Behav. Sci.* **2018**, *204*, 128–133. [CrossRef]
27. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2017.
28. Chivers, D.P.; Wildly, E.L.; Kiesecker, J.M.; Blaustein, A.R. Avoidance response of juvenile pacific treefrogs to chemical cues of introduced predatory bullfrogs. *J. Chem. Ecol.* **2001**, *27*, 1667–1676. [CrossRef] [PubMed]
29. Stapley, J. Differential avoidance of snake odours by a lizard: Evidence for prioritized avoidance based on risk. *Ethology* **2003**, *109*, 785–796. [CrossRef]
30. Sacerdote-Velat, A.B.; Earnhardt, J.M.; Mulkerin, D.; Boehm, D.; Glowacki, G. Evaluation of headstarting and release techniques for population augmentation and reintroduction of the smooth green snake. *Anim. Conserv.* **2014**, *17*, 65–73. [CrossRef]

31. Almli, L.M.; Burghardt, G.M. Environmental enrichment alters the behavioral profile of ratsnakes (*Elaphe*). *J. Appl. Anim. Welf. Sci.* **2006**, *9*, 85–109. [CrossRef] [PubMed]

32. Ehrlich, A. The effects of past experience on the rat's response to novelty. *Can. J. Exp. Psychol.* **1961**, *15*, 15–19. [CrossRef]

33. Grandin, T. Effect of Rearing Environment and Environmental Enrichment on Behavior and Neural Development in Young Pigs. Ph.D. dissertation, University of Illinois at Urbana-Champaign, Champaign, IL, USA, 1989.

34. Volkmar, F.R.; Greenough, W.T. Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science* **1972**, *176*, 1445–1447. [CrossRef] [PubMed]

35. Burke, R.L. Relocations, repatriations, and translocations of amphibians and reptiles: Taking a broader view. *Herpetologica* **1991**, *47*, 350–357.

36. Lovich, J.E.; Ennen, J.R.; Agha, M.; Gibbons, J.W. Where have all the turtles gone, and why does it matter? *Bioscience* **2018**, *68*, 771–781. [CrossRef]