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## Predators May Lose Interest in Turtle Acclimation Pens: Implications for Translocations Using Soft Release

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**ABSTRACT.** – We opportunistically investigated predator visits to enclosed acclimation pens containing juvenile eastern box turtles (*Terrapene carolina*) over a 34-d acclimation period using motion-triggered cameras. Daily visitation probability by raccoons (*Procyon lotor*) and fox squirrels (*Sciurus niger*; the only predator species observed visiting) decreased from a peak of 36% probability of a visit on the first day turtles were penned to nearly zero probability by day 34 (the release day). Our findings indicate predators lose interest in pens over time, and we suggest animals should be released from acclimation pens after predators have lost interest in pens to avoid heightened predation risk of translocated animals.

Translocation is a common management strategy in chelonian conservation programs, whether moving wild turtles directly between natural sites (Attum et al. 2013) or releasing captive-reared (head-started) juveniles into nature (Burke 2015). However, such efforts are often unsuccessful because released animals have low survival, and postrelease predation is a common cause of mortality (Fischer and Lindenmayer 2000). As an alternative to simply being relocated and released unrestrained at the recipient site (“hard” release), practitioners have implemented “soft” release, which entails temporarily confining animals in acclimation pens at release areas. Soft release can reduce immediate dispersal propensity from the release area, decrease activity range size, increase site fidelity, and enhance survival (Tuberville et al. 2005; Frederick 2006; Tetzlaff et al. 2019a). Despite the postrelease benefits of soft release, predators often interact

with acclimation pens (Keiter and Ruzicka 2017), which can increase predation risk for animals during acclimation or after they are released from pens. Further research is therefore needed to better understand predator–prey interactions during acclimation penning of turtles.

Like many chelonians, eastern box turtles (*Terrapene carolina*) are generally in decline as a result of habitat loss, road mortality, collection for the pet trade, and intense nest predation (van Dijk 2011). As such, the species is listed as Vulnerable by the International Union for Conservation of Nature and included in Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendix II (van Dijk 2011). We were provided an opportunity to monitor a cohort of captive-reared, juvenile eastern box turtles in acclimation pens as part of a larger research project evaluating the effectiveness of experimental head-starting techniques (Tetzlaff et al. 2019b). Our objectives were to identify potential predators at the release site, quantify frequency of predator visitation with pens while turtles were enclosed, and explore whether predator visitations changed over time or differed among pens. Our ultimate goal was to determine if visits to pens by predators decreased as predators became accustomed to the pens and thus determine an optimal time to release animals from pens when predation risk would be reduced.

**Methods.** — We conducted this study at Fort Custer Training Center in Michigan, USA. Natural habitats at this approximately 3,000-ha Army National Guard training facility consist primarily of deciduous woodlands, wetlands, and grasslands. Predator species observed at Fort Custer include those frequently implicated as predators of North American juvenile turtles, such as raccoon (*Procyon lotor*), Virginia opossum (*Didelphis virginiana*), coyote (*Canis latrans*), red fox (*Vulpes vulpes fulva*), American crow (*Corvus brachyrhynchos*), sandhill crane (*Antigone canadensis*), and Sciurid rodents such as eastern chipmunk (*Tamias striatus*) and squirrels (*Sciurus* spp.; Belzer et al. 2000; Dodd 2001; Jones and Sievert 2012; Tetzlaff et al. 2018). Animal subjects for this study were acquired as eggs from nests laid by free-ranging females at Fort Custer. We incubated eggs and raised 20 hatchlings for 21 mo in a greenhouse on the University of Illinois at the Urbana–Champaign campus beginning in August 2016.

We placed the 20 captive-reared turtles into 5 acclimation pens at Fort Custer on 5 May 2018, where they were held for 34 d before being released. We installed the pens roughly 50 m apart encompassing an approximately 450-m<sup>2</sup> area within a hardwood forest patch dominated by an overstory of oak (*Quercus* spp.) and maple (*Acer* spp.). The pens were an average of 116 ± 11 m standard deviation (SD; range, 103–130 m) from the nearest dirt road and located 4.5 km from substantial human activity. Each pen was 1.8 m long × 1 m tall × 1 m wide and constructed using 4-cm-diameter polyvinyl chloride. The top and sides of each pen were enclosed with 2 × 2-cm plastic poultry netting, and the legs and netting



**Figure 1.** Visits by predators (circled) to acclimation pens containing juvenile eastern box turtles (*Terrapene carolina*) at Fort Custer Training Center, Michigan, USA, documented with motion-triggered cameras. Counter-clockwise from top right: raccoons (*Procyon lotor*) in the first three images and fox squirrel (*Sciurus niger*) in the fourth image.

were buried approximately 10 cm into the ground to keep predators from entering and prevent turtles escaping. Four turtles were placed in each pen. We provided fresh water daily in a shallow ceramic dish within each pen. Further details of study animal acquisition, husbandry methods, and the acclimation penning procedure are described elsewhere (Tetzlaff et al. 2019b).

To monitor pens during acclimation, we placed either a Bushnell Trophy Cam or Reconyx Hyperfire high-definition trail camera affixed to a 65-cm-tall tripod placed 3 m from each pen. Depending on the camera model, each camera was programmed to take either an image or a 10-sec video clip when its motion sensor was triggered. When reviewing camera media to document predator identities and visitation frequencies, we considered a predator as “visiting” when interacting with the pen (e.g., standing on or grasping the pen) or showing obvious interest in a pen at close ( $< 0.5$  m) proximity (e.g., peering in or patrolling the perimeter of pens). We considered visits to be unique if separated by  $> 30$  min for a given species (Keiter and Ruzicka 2017).

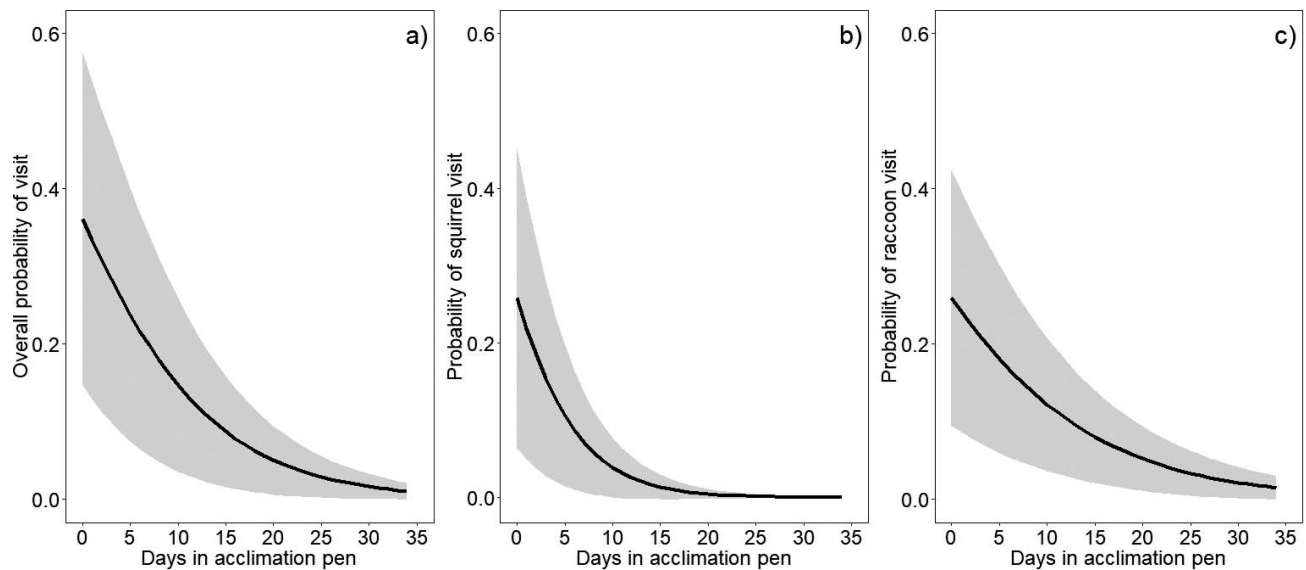
We conducted analyses using Program R version 3.4 (R Core Team 2017). We used a generalized linear mixed model assuming a binomially distributed error to analyze the daily probability of predators visiting pens predicted by the number of days turtles had been penned. To control for the repeated sampling of each pen, we treated pen identity as a random intercept. To determine whether daily visitation probability differed among pens, we used a generalized linear model with pen identity as a predictor. One pen (Pen 5) was not visited, so we eliminated it from this analysis

because its inclusion in the model caused convergence issues. We made post hoc comparisons of visitation probability among Pens 1 to 4 using Tukey contrasts.

**Results.** — All turtles survived the acclimation period. We observed 38 predator visits to the pens on 20 of the 34 d turtles were penned. Raccoons and fox squirrels (*S. niger*) were the only potential predators detected visiting pens (Fig. 1). Raccoons made 26 visits and squirrels made 12 visits. The overall odds of predator visitation decreased with time (log odds with 95% confidence interval [CI]:  $-0.12$ ,  $-0.18$  to  $-0.07$ ;  $p < 0.001$ ). The probability of visitation on the first day turtles were penned was approximately 0.36 but declined to nearly zero by day 34 (Fig. 2a). When analyzing each predator species separately, daily visit probability declined with time for raccoons (log odds with 95% CI:  $-0.09$ ,  $-0.15$  to  $-0.04$ ;  $p < 0.001$ ) and fox squirrels (log odds with 95% CI:  $-0.22$ ,  $-0.38$  to  $-0.11$ ;  $p = 0.001$ ). Probability of a fox squirrel visit was essentially nonexistent after approximately 15 d into the acclimation period (Fig. 2b), whereas probability of raccoon visitations persisted longer (Fig. 2c). Visitation probability varied among some pens, ranging from one (Pen 2) being visited 22 times on 15 d to another having no detected visits (Pen 5). Visitation probability for Pen 2 (0.52) was higher than Pens 1 (0.22,  $p = 0.04$ ), 3 (0.03,  $p = 0.003$ ), and 4 (0.19,  $p = 0.01$ ), but we found no differences among other pens ( $p \geq 0.15$ ).

**Discussion.** — By monitoring acclimation pens with cameras, we were able to directly identify predators visiting pens. Raccoons accounted for the majority of





**Figure 2.** Daily probability (black line) with standard error (grey shading) of predators visiting acclimation pens containing juvenile eastern box turtles (*Terrapene carolina*) at Fort Custer Training Center, Michigan, USA. The daily probability of both predator species (raccoon [*Procyon lotor*] and fox squirrel [*Sciurus niger*]) observed visiting pens is shown in panel a, of only fox squirrels is shown in panel b, and of only raccoons is shown in panel c.

visits. These widespread habitat generalists are major predators of box turtles and other chelonians (Dodd 2001) and previously have been observed visiting sites with acclimation enclosures. For example, Quinn et al. (2018) reported raccoons searched footprints of removed acclimation pens that had contained head-started gopher tortoises (*Gopherus polyphemus*). Additionally, raccoons were documented as the most frequent predator visiting acclimation pens holding gallinaceous birds (Keiter and Ruzicka 2017). Fox squirrels made fewer visits to, and seemingly lost interests in, pens earlier than raccoons, but Sciurid rodents are also widespread and have been documented consuming chelonians as well as numerous other vertebrates (Callahan 1993; Jones and Sievert 2012). For example, Belzer et al. (2000) reported an outdoor enclosure containing juvenile eastern box turtles was repeatedly breached by eastern chipmunks, which presumably depredated several penned turtles. However, their pen was screened on the top and sides but not underground. We observed no predation while turtles were penned, which we attribute to the predator-proof nature in which enclosures were constructed, such as using small-gauge mesh netting and substantially burying the legs and netting on pens into the ground.

Deciding when to allow enclosed animals to leave acclimation enclosures can be guided by when individuals behaviorally demonstrate acclimation to their surroundings, such as gradual reduction of activity while penned (Kingsbury and Attum 2009). We documented several instances of enclosed turtles patrolling pen perimeters early in the acclimation period, as was noted in a similar study of ornate box turtles (*T. ornata*; Sievers 2015). However, our results also suggest acclimation duration could be guided by when predators drastically reduce

visits to pens. This was after approximately 20 d in our study, but the benefits extended to > 1 mo into the acclimation period. We acknowledge that the proximity of pens to one another could have influenced our results, especially if individual predators were repeatedly visiting pens. However, the causes of variation in visits among some pens, such as Pen 2 being visited relatively frequently and Pen 5 not having any documented visits, is unclear. This was possibly affected by factors such as proximity to game trails, water or other resources, or subtle microhabitat variation within or around pens. Future studies might place replicate pens farther apart at release sites to determine if results similar to ours are found. Additionally, monitoring pens in place well before translocated animals are introduced to them could help determine whether the novelty of pens or cues from enclosed animals elicit stronger predator interactions. We suggest further evaluation of predator–prey interactions during and after acclimation could lead to techniques that improve postrelease survival of soft released turtles.

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