EXECUTIVE SUMMARY

Transferring Translocation Science to Wildlife Conservation on DoD Installations: Demonstration of Environmental Enrichment and Soft Release Technology

ESTCP Project RC-201616

APRIL 2019

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1.0 INTRODUCTION

Translocation is the intentional release of captive-propagated or wild-caught animals into the wild for the purpose of establishing a new population, augmenting a critically small population, or managing animals that are in harm's way. Despite substantial investments of time, energy, and resources, these endeavors often fail to establish wild populations (Seddon 1999), yet the practice is becoming more common on private, state, and federal lands. The emerging discipline of "translocation science" has grown to address the shortcomings of this popular but troubled practice. Research has focused on mechanisms that may limit the success of translocation associated with the care of animals in captivity pre-release and the manner by which they are released. Both "environmental enrichment" and "soft-release" have been identified as useful techniques to enhance the survival of translocated animals. Both techniques have applicability for existing and future wildlife translocation projects on Department of Defense (DoD) installations.

The idea that enriched experiences may be necessary for the development of beneficial species-specific brain characteristics is not a new concept (Rosenzweig and Bennett 1996) although it has received renewed interest recently in relation to translocation science (Swaisgood 2010). Projects involving wild-to-wild translocations compared to the release of captive animals have generally been more successful (Griffith et al. 1989, Wolf et al. 1996). This disparity has shed attention on the deleterious effects of captivity and has led to enhanced interest in environmental enrichment. Enrichment entails providing captive animals with complex enclosures that stimulate particular brain functions and behaviors. This may be as simple as providing animals with natural substrates, climbing structures, social interaction, realistic retreat sites, or prey that they would naturally encounter in the wild. The behavioral benefits of environmental enrichment have been demonstrated for all vertebrate taxa (Poole 1992; Vargus and Anderson 1999; Dinse 2004; Almli and Burghardt 2006; Kenison and Williams 2018). Maintaining animals pre-release in enriched environments has also been shown to increase natural behaviors and survival of wildlife post-release in translocation projects (Biggins et al. 1999; Nicholson et al. 2007). Several promising avenues of environmental enrichment for reptiles have been identified including communal housing, thermal gradients, live prey items, structurally complex enclosures with retreat sites, and temperature manipulation to stimulate hibernation (Roe et al. 2010; Roe et al. 2015, Burghardt 2013; Sacerdote-Velat et al. 2014).

Soft-release entails placing individuals in outdoor enclosures at the release site before full release. This allows animals to experience local environmental conditions and develop fidelity to a site (Kingsbury and Attum 2009). Soft-release often allows animals to develop, practice, and display natural behaviors such as foraging, mating, thermoregulating, and burrowing and has proven effective for a number of successful translocation projects (Tuberville et al. 2005; Mitchell et al. 2011; Knox and Monks 2014). Soft-release enclosures may be as simple as outdoor pens or fenced in sections of the release site. Limited time in these pens (2-6 weeks) allows animals to acclimate to the local environment and to form an affinity with the area to prevent immediate dispersal into potentially unsuitable surrounding habitats. Release pens can be designed to exclude predators to ensure survival of individuals within the enclosures as they acclimate to the local environment.

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2.0 OBJECTIVES

The objectives of this project were to augment existing reptile translocation projects occurring on DoD installations using traditional translocation techniques with either soft-release or environmental enrichment and demonstrate the value of these technologies relative to traditional translocation techniques. Translocation programs that currently capture, move, and release animals from areas of high-risk to low-risk, provide an opportunity to incorporate soft-release technologies. For translocation programs that maintain animals in captivity for prolonged periods, environmental enrichment technologies can be implemented. The goals were to clearly define success criteria following the suggestions of Hall and Fleischman (2010) and to compare the success of soft-release and environmental enrichment approaches with standard protocols.

The specific objectives were to demonstrate how soft-release could improve the survival and decrease the post-release movements of Eastern Massasauga Rattlesnakes (*Sistrurus catenatus*) on Camp Grayling and Texas Horned Lizards (*Phrynosoma cornutum*) on Tinker Air Force Base. At each of these installations, animals were captured and moved from active training ranges or construction areas and hardreleased (i.e., direct, unrestrained release without spending time in an acclimation pen) into a suitable habitat. Here, the research team augmented these efforts by adding a soft-release component, which allowed them to compare the survival of soft- and hard-released individuals. Similarly, the research team calculated movement indices (home range size and daily movement rate) of soft- and hard-released individuals and compared them with the movements of control resident individuals using generalized linear models or Kruskal-Wallis tests. The research team predicted that soft-released animals will move less frequently and occupy smaller home ranges than hard-released animals and that these space use and movement parameters will be similar to those of resident animals.

The research team also demonstrated how environmental enrichment influenced the survival and growth of Eastern Box Turtles (*Terrapene carolina*) on Fort Custer. By rearing box turtles in complex and challenging enriched captive conditions compared to unenriched, simplistic captive conditions allowed them to assess how different rearing conditions affected the survival and behavior of translocated animals post-release. The research team compared the post-release survival of turtles using known-fates modeling. Additionally, they used general linear models to compare growth rates, temperatures, and dispersal of enriched and unenriched turtles post-release to assess the predictions that enriched captivity better prepares captive individuals to naturally forage and reduced the propensity to leave the release area.

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3.0 TECHNOLOGY DESCRIPTION

Holding the animals in outdoor, naturalistic enclosures at the release site for some period before release allowed the individuals to become acclimated to the area and may have encouraged them to develop site fidelity to the area. This may have reduced the tendency for the animals to try to disperse and find their way back to their capture locations. The goal in constructing soft-release enclosures was to provide animals with complex, safe enclosures situated within suitable habitat that were also escape proof. Soft-release enclosures were constructed at the release sites in suitable habitat known to support resident animals (Figure 1). Because of the different behaviors of the target species, the size and construction of the soft-release pens varied between demonstrations. For instance, because Eastern Massasauga Rattlesnakes are poor climbers, the research team constructed open-topped soft-relase pens. The walls were constructed of 50.8 cm tall aluminum flashing trenched approximately 8-10 cm into the ground and held upright by wooden stakes. The pen was approximately 0.1 ha in size and was built to contain numerous retreat sites (logs, stumps, rodent burrows). Additionally, the pen site contained several active hibernacula used by resident animals. The topography inside the pen allowed for animals to seek higher ground on soil and vegetation hummocks or to go into shallow depressions that often held water. The pen had two removable doors that could be opened and closed as needed. When no animals were in the enclosure, the doors were kept open to allow small mammals and lizards (prey) to move in and out of the enclosure. When animals were contained within, the doors were closed. After the two-week retention period ended, the doors were opened and the animals were allowed to disperse at will, as opposed to forcefully removing them from the enclosure.

The research team constructed a similar enclosure for Texas Horned Lizards, which are also not climbers. In the enclosure, the research team provided drinking water and created a large sand mound for thermoregulation, burrowing, and oviposition by gravid individuals. Additionally, the research team used sugar water to lay trails to attract ants (prey for lizards) to the inside of the enclosure to ensure that soft-released lizards had sufficient access to food. However, this pen was built to provide some protection from avian predators as enclosed lizards could be vulnerable to predation by crows or hawks. Thus, the research team used fine-mesh wildlife netting to create a ceiling and prevent predators from accessing the pen.

Figure 1. Soft-release pens constructed for Eastern Massasaugas (*Sistrurus catenatus*: left) and Texas Horned Lizards (*Phyrnosoma cornutum*: right). Animals captured on active training ranges or in construction sites were removed from harm's way and placed into these pens for approximately two weeks before being released back into the wild. Soft-release is thought to increase the survival and reduce the movement and homing behavior of translocated wildlife.

Environmental enrichment can be designed to target development of many types of beneficial species-specific brain characteristics (Rosenzweig and Bennett 1996). Here, we demonstrated how environmental enrichment enclosures can be simply and easily designed to target ecologically-relevant, species-specific behaviors to improve individual survival post-release. Previous efforts have shown that environmental enrichment can provide reptiles with social interaction, structural complexity, thermal heterogeneity, and spatially dispersed, live prey items.

Enriched box turtles 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 used by wild box turtles (Dodd 2001, Figure 2). Unenriched turtles 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, California) and a 42 cm x 42 cm piece of plastic shelf liner resting on the carpet. The research team 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.

Figure 2. Comparison of complex enriched captivity vs. standard unenriched captivity for captive-reared Eastern Box Turtles (*Terrapene carolina*). The research team explored whether being raised in enriched conditions would improve the survival, growth, and thermoregulation of turtles after being released into the wild.

The type and amount of food provided to individuals at each feeding was similar between rearing treatments. However, the research team predominantly fed enriched turtles by scattering food throughout their enclosures to promote active foraging, whereas unenriched turtles were provided food on 10cm diameter petri dishes, placed in the same spot in enclosures at each feeding. The research team initially fed live blackworms (*Lumbriculus variegatus*) and mealworms (*Tenebrio molitor*). The turtles were then transitioned to live superworms (*Zophobas morio*) and then solely to live redworms (*Eisenia foetida*) after several months. Fresh mixed greens (excluding spinach) were also offered 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 food was dusted with calcium powder three days per week. Enriched turtles were also offered cuttlebones to chew on. Fresh water was provided ad libitum.

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4.0 PERFORMANCE ASSESSMENT

Soft-Release of Massasaugas: Over the course of the demonstration, the research team captured 55 Eastern Massasauga Rattlesnakes. Transmitters were implanted into each snake using a modification of the methods used by Reinert and Cundall (1982). The snakes were surgically implanted with either a 5 g or 9 g Holohil Systems Ltd. SI-2T temperature-sensitive transmitter that was ≤ 6% body mass. Snakes were randomly assigned to either a hard-release (n = 17) or soft-release translocation treatment (n = 16). Twenty-two of the snakes were residents and were never moved but instead tracked as a baseline comparison. Snakes in the soft-release treatment were placed in the holding pen for approximately two weeks before being allowed to leave the pen. Hard-released snakes were released just outside the pen but were never confined. Resident snakes were released at their point of capture 2 - 3 days after surgery to ensure they had recovered.

Each snake was tracked three times per week between May-August and once every three weeks between September-November. To assess differences in survival rates between the three treatments, the research team used Program MARK known-fate models (Version 8.2; White and Burnham 1999). Translocated snakes were at a survival disadvantage relative to resident control snakes (Figure 3). The model-averaged annual survival estimates for resident, soft-, and hard-released Eastern Massasaugas were 0.72 (SE[1] ± 0.21, lower CI[2] = 0.25, upper CI = 0.95), 0.44 (SE ± 0.18, lower CI = 0.15, upper CI = 0.77), and 0.40 (SE ± 0.20, lower CI = 0.11, upper CI = 0.78).

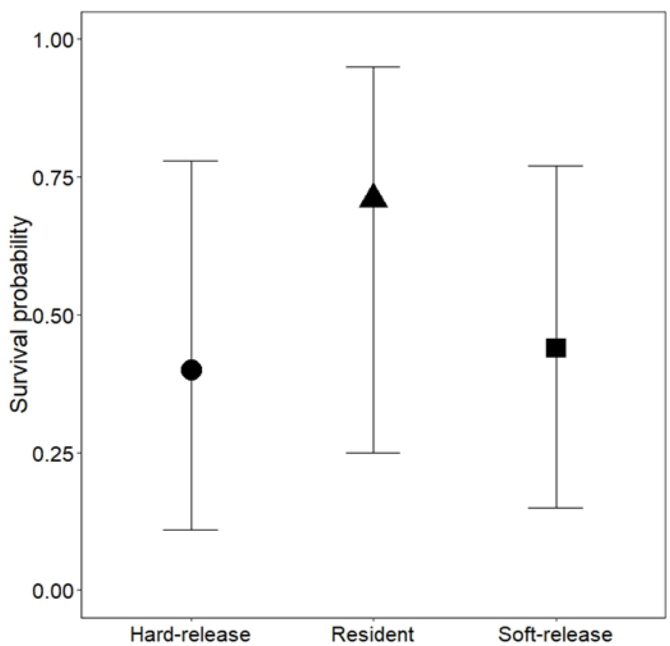

Figure 3. Resident Eastern Massasaugas (*Sistrurus catenatus*) had higher estimated annual survival rates (72%, n = 22) than either soft- (40%, n = 16) or hard-released (44%, n = 17) translocated snakes. Error bars represent 95% confidence intervals.

[1] Standard Error (SE)
[2] Confidence Interval (CI)

The research team predicted that translocated snakes would move more frequently and make longer distance movements than resident snakes. However, it was predicted that soft released snakes would move more similarly to resident snakes than hard released snakes. To determine whether soft-released, hard-released, and resident Eastern Massasaugas had different movement behavior, four different movement metrics were compared: (1) maximum dispersal distance (m) from release site after 1, 2, 4, and 8 weeks, (2) mean distance moved per day (m), (3) 100% minimum convex polygon (MCP) activity range size (ha), and (4) activity range length (m). Based on 616 tracking events for resident snakes, 333 for soft-released snakes, and 430 for hard-released snakes, no evidence was found that soft-releasing Eastern Massasaugas reduced their post-release movements. Using Mood's median and Kruskal-Wallis tests, no differences were found in maximum dispersal distances from release sites after any number of weeks between any treatments (males after 1wk: $p = 0.39$; 2wks: $\chi2(2) = 1.34$, $P = 0.51$; 4wks: $\chi2(2) = 0.13$, $P = 0.94$; 8wks: $\chi2(2) = 1.02$, $p = 0.60$; females after 2wks: $\chi2(2) = 0.56$, $p = 0.76$; gravid females after 1wk: $P = 0.22$; 2wks: $\chi2(2) = 2.35$, $P = 0.31$; 4wks: $P = 0.61$; 8wks: $\chi2(2) = 0.43$, $P = 0.81$). Using Kruskal-Wallis tests, we found no significant differences between resident (n = 8), soft-released (n = 6), or hard-released (n = 6) males in mean stepwise distance moved per day ($\chi2(2) = 0.10$, $P = 0.95$), activity range size ($\chi2(2) = 1.10$, $P = 0.58$), or activity range length ($\chi2(2) = 0.44$, $P = 0.80$).

It was also predicted that soft-releasing snakes would reduce their homing behavior. Four translocated snakes were observed returning to within 300m of their capture locations and one was a soft-released individual and the other three were hard-released.

Soft-Release of Texas Horned Lizards: The research team tracked 84 Texas Horned Lizards from 2016 – 2018. Radio transmitters were dorsally attached (model BD-2, 0.95 - 1.95 g, Holohil Systems Ltd., Ontario, Canada) to adult lizards using silicone epoxy and small elastic collars placed around the neck (total encumbrance was ≤ 10% of an individual's mass). To track juveniles, harmonic radar diodes (low-barrier-height Schottkey barrier diodes that weighed only 1 mg to 12 mg) were glued to their backs and relocated them using handheld RECCO transmitter/receiver (RECCO Rescue Systems, Lidingo, Sweden). The lizards were tracked between 3-5 times per week during the active season (April – November). Tenty-three Texas Horned Lizards were soft-released and 61 residents were tracked in the same area. Two different soft-release pens were constructed. Animals were held within pens for approximately two weeks before being released at the study site.

Survival analyses indicated that soft-release was a viable technique for juveniles but was ineffective for adults. Soft-released juveniles had remarkably high annual survival (55%) compared to residents (29%). However, only 5% of soft-released adult lizards survived the year compared to an estimated annual survival rate of 57% for resident adults. These results suggest that juveniles may be a better age class to target for soft-release because they have yet to develop an affinity to an area whereas adults may display homing behavior after being translocated.

Despite having higher survival, soft-released juveniles moved more per day than resident juveniles (Chi Square = 10.21, df = 1, $P = 0.001$) and had larger overall home ranges (Chi Square = 9.17, df = 1, $P = 0.003$). There was no evidence that there was a difference in home range size between adult soft-released and resident lizards (Chi Square = 0.17, df = 1, P = 0.68) or distance moved per day (Chi Square = 1.75, df = 1, P = 0.19).

Environmental Enrichment of Eastern Box Turtles: Thirty-two Eastern Box Turtles were successfully hatched and reared, half in enriched captivity and half in unenriched captivity. All turtles that hatched survived in captivity until release. Two cohorts of captive-reared turtles were released to their capture sites on Fort Custer, Michigan. Half of the turtles were released at Fort Custer Training Center in May 2017 after 9 - 10 months in captivity. The remaining individuals were released after an additional year and released at the same site in May 2018. Because available evidence suggests acclimation pens increase site fidelity for wild-to-wild translocated turtles (Tuberville et al. 2005), all turtles were soft-released by placing four turtles per pen in 1.8m long x 1m tall x 1m wide pens for approximately 30 days. All turtles had a 0.9 or 1.2 g radio-transmitter (Advanced Telemetry Systems, Inc., Isanti, Minnesota) affixed to their carapace using epoxy. The turtles were radio-tracked five days per week from May - August and bi-weekly from September - November in each year. All released turtles were monitored for at least one active season until hibernation. Some turtles were tracked for two activity seasons.

In cohort 1 (turtles released when ~10 months old), growth rates (mm per day) did not differ between enriched turtles (n = 6) and unenriched turtles (n = 6) ($P = 0.73$). In cohort 2 (turtles released when 22 months old), enriched turtles (n = 10) grew faster than unenriched turtles (n = 10) ($P = 0.01$: Figure 4). Although enriched turtles grew faster post-release than unenriched turtles we found no significant different in body condition index between treatments in either cohort ($P > 0.19$), suggesting that all released turtles were able to successfully forage and maintain healthy body mass.

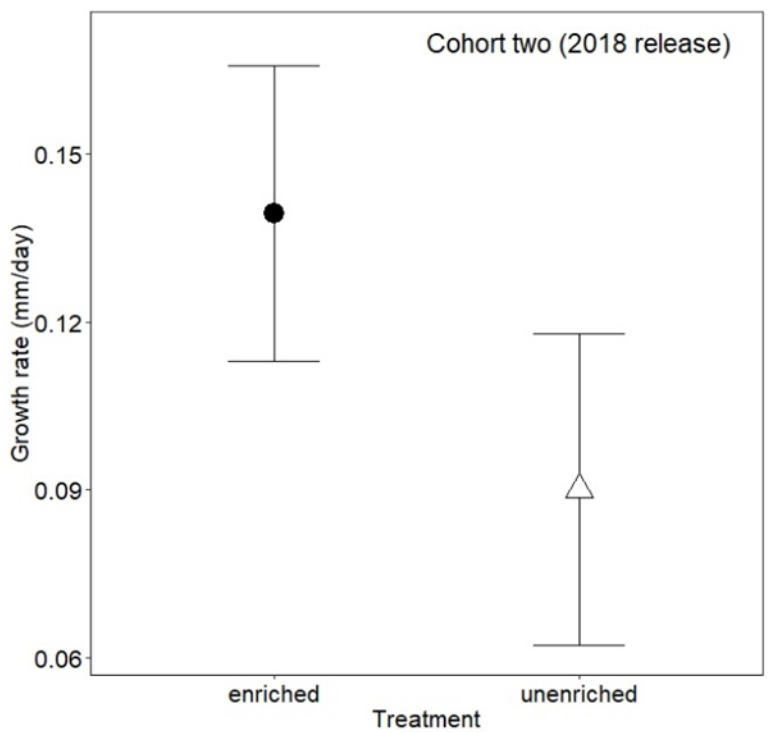

Figure 4. Growth rates of enriched (n = 10) and unenriched (n = 10) Eastern Box Turtles (*Terrapene carolina*) reared in captivity for 22 months before being released into the wild on Fort Custer, MI.

In cohort 1, average body temperatures of enriched and unenriched turtles did not differ ($P = 0.36$). In cohort 2, average body temperatures of enriched turtles were closer to the species' preferred range (25 °C) on average than standard turtles ($P = 0.03$). Body temperatures of enriched turtles did not differ from the small number of resident juvenile turtles (N = 4) that we tracked at this site ($P = 0.71$).

In general, the observed survival rates were higher than anticipated and higher than has been reported for similarly aged box turtles in the literature. Furthermore, survival for cohort 2 was considerably higher than in cohort 1. In cohort 1, two of six turtles in each treatment survived (33% apparent survival). Annual survival rates of enriched and standard turtles in cohort 1 were thus the same (0.33, 95% CI: 0.08 – 0.73). Initially, enriched turtles had a higher survival rate and were more likely to survive past an initial wave of mortality, although this difference was washed out later in the season when turtle activity declined and both groups experienced high survival. Interestingly, all four turtles that hibernated at the site survived until the following spring emergence.

In cohort 2, four of ten enriched and six of ten unenriched turtles survived into hibernation. Although the apparent survival of unenriched turtles was higher than that of enriched turtles, the survival rates of enriched (0.40, 95% CI: 0.16–0.70) and unenriched turtles (0.60, 95% CI: 0.30–0.84) were statistically similar.

All turtles in cohort 2 dispersed farther from release pens on average than enriched turtles in cohort 1, but dispersal otherwise did not differ between turtle groups.

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5.0 COST ASSESSMENT

The demonstration revealed that soft-release pens could be built, monitored, and maintained well below the goal of $1,000 per pen (estimated cost of $620 - $740 depending on size). Because of the durability and longevity of pens, the cost per soft-releasing each animal relative to hard-releasing them was extremely modest (~ $72 per snake and ~ $45 per lizard). Contrary to the research team's prediction, environmental enrichment was a more cost-effective method than raising animals in unenriched enclosures. Although the initial setup of enriched captivity was higher due to the complexity of enclosures, daily husbandry and maintenance costs were actually lower due to the ease with which enriched containers could be cleaned and the ability to house multiple individuals together. The research team calculated that the cost to raise a single enriched box turtle for one year was approximately $293 while the cost to raise a single unenriched turtle for a year was $644. In conclusion, both soft-release and environmental enrichment can be implemented at either a modest increase or even a cost savings over traditional translocation techniques.

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6.0 IMPLEMENTATION ISSUES

The demonstration found relatively modest benefits from soft-release translocation. Both hard-released and soft-released individuals were at a survival disadvantage relative to resident animals. However, there was strong evidence from Texas Horned Lizards that soft-release may be most beneficial to juvenile individuals relative to adults. This may be because juveniles have yet to establish site fidelity and on release at a new site, do not try to home to their capture location. Future endeavors may experience the most effective results focusing on young age classes for translocation studies.

The demonstration relied on a two-week soft-release time period (i.e., individuals were kept in soft-release enclosures for two weeks before being released). This may have been an insufficient amount of time for animals to acclimate to the new study area. Efforts aimed at using soft-release for longer periods of time might benefit from larger pens, more complex pens, and a larger number of pens that can accommodate more individuals than the pens used in the demonstration. Holding animals in pens for longer duration may entail other challenges such as the need to feed or provide other resources to enclosed animals, more intensive husbandry, or additional permits from regulatory agencies.

The research team also found modest benefits to environmental enrichment. While enriched animals were less expensive to care for, they experienced relatively modest increases in growth post-release, and no increase in survival was documented. Because this method was less expensive than traditional methods and there were no costs to the enriched animals, the research team does not see any reason not to adopt this methodology. However, a species that was relatively easy to care for in captivity was specifically chosen. Animals with more complex life histories, larger body sizes, and greater space needs may be far more challenging to enrich in captivity.