TRANSFERRING "TRANSLOCATION SCIENCE" TO WILDLIFE CONSERVATION ON DOD INSTALLATIONS

DEMONSTRATION OF ENVIRONMENTAL ENRICHMENT AND SOFT RELEASE TECHNOLOGY

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Brett DeGregorio, Jinelle Sperry, and Bruce Kingsbury

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14. ABSTRACT

Translocation (the intentional release of captive-propagated or wild-caught animals into the wild) to establish a new population or augment a critically small population often fails to meet its goals. Two promising tools to improve the success of translocation are soft release and environmental enrichment. This work evaluated the augmentation of current translocation efforts to improve its success through (1) the use of soft release to improve the survival and reduce the post-release movement of translocated Eastern Massasaugas (*Sistrurus catenatus*) and Texas Horned Lizards (*Phrynosoma cornutum*), and (2) environmental enrichment to improve the post-release behavior and survival of captive-reared Eastern Box Turtles (*Terrapene carolina*). We found little evidence that soft release improved the survival or reduced the movement of Eastern Massasaugas or adult Texas Horned Lizards. Annual survival rates of soft- and hard-released snakes and lizards were nearly identical and significantly lower than those of residents. Juvenile soft-released horned lizards had high survival relative to hard-released lizards, comparable to resident juvenile lizards; this age class may be the best candidate for soft release. Environmentally enriched turtles grew faster post-release than unenriched turtles. However, overall survival rates were similar; both treatments had higher than expected survival in the wild.

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TABLE OF CONTENTS

Report Documentation Page

LIST OF FIGURES

LIST OF TABLES

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LIST OF ACRONYMS

Term	**Definition**
AFB	Air Force Base
AG	Army Garrison
AIC	Akaike’s Information Criterion
ANSI	American National Standards Institute
BCI	Body Condition Index
CERL	Construction Engineering Research Laboratory
CI	Confidence Interval
DoD	U.S. Department of Defense
DT	See P 41
ERDC	U.S. Army Engineer Research and Development Center
ESA	U.S. Endangered Species Act
ESTCP	Environmental Security Technology Certification Program
GIS	Geographic Information System
GPS	Global Positioning System
GSA	General Services Administration
HR	Hard Release
HSD	Honestly Significant Difference
IUCN	International Union for Conservation of Nature and Natural Resources
MCP	Minimum Convex Polygon
NSN	National Supply Number
OMB	Office of Management and Budget
PCR	Quantitative Polymerase Chain Reaction
PO	Performance Objective
SAR	Same As Report
SAS	Statistical Analysis System
SD	Standard Deviation
SE	Standard Error
SERDP	Strategic Environmental Research and Development Program
SFD	Snake Fungal Disease
SR	Soft Release
SSC	Species Survival Commission
SVL	Snout-to-Vent Length
TAFB	Tinker Air Force Base
USA	United States of America
UTM	Universal Transverse Mercator

EXECUTIVE SUMMARY

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 DoD installations.

The idea that enriched experiences may be necessary for the development of beneficial species-specific brain characteristics is not a new concept although it has received renewed interest recently in relation to translocation science (Rosenzweig and Bennett 1996, 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, 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.

OBJECTIVES OF THE DEMONSTRATION

Our objectives were to augment existing reptile translocation projects occurring on DoD installations using traditional translocation techniques with either soft release or environmental enrichment and to demonstrate the value of these technologies relative to traditional translocation techniques. For example, 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, we can implement environmental enrichment technologies. Our goals are 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.

Our 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 are captured and moved from active training ranges or construction areas and hard released (i.e., direct, unrestrained release without spending time in an acclimation pen) into suitable habitat. Here, we augmented these efforts by adding a soft release component, which allowed us to compare the survival of soft- and hard-released individuals. Similarly, we 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. We 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.

We 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 us to assess how different rearing conditions affected the survival and behavior of translocated animals post-release. We compared the post-release survival of turtles using known-fates modeling. Additionally, we 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, respectively.

TECHNOLOGY DESCRIPTION

Holding the animals in outdoor, naturalistic enclosures at the release site for some period of time before release allowed the individuals to become acclimated to the area and may have encouraged them to develop site fidelity to the area, which may have reduced the tendency to try to disperse and find their way back to their capture locations. Our 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 ES-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, we constructed open-topped soft relase pens. The walls were constructed of 50.8cm tall aluminum flashing trenched approximately 8-10cm 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, we kept the doors 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 2-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.

We constructed a similar enclosure for Texas Horned Lizards, which are also not climbers. In the enclosure, we provided drinking water and created a large sand mound for thermoregulation, burrowing, and oviposition by gravid individuals. Additionally, we 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, we built this pen to provide some protection from avian predators as enclosed lizards could be vulnerable to predation by crows or hawks. Thus, we used fine-mesh wildlife netting to create a ceiling and prevent predators from accessing the pen.

Figure ES-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 2 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 132cm long x 79cm wide x 30cm 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 ES-2). Unenriched turtles were housed individually in comparably simplistic enclosures consisting of a 60cm long x 42cm wide x 28cm tall transparent plastic tub with reptile cage carpet (Zoo Med Eco Carpet; Zoo Med Laboratories, Inc., San Luis Obispo, California) and a 42cm x 42cm 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. 4cm deep) in the lower end for drinking and soaking.

Figure ES-2. Comparison of complex enriched captivity vs. standard unenriched captivity for captive-reared Eastern Box Turtles (*Terrapene carolina*). We 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, we 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. 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 5 days per week, and we dusted food with calcium powder 3 days per week. We also provided enriched turtles with cuttlebones to chew on. Fresh water was provided ad libitum.

PERFORMANCE ASSESSMENT

Soft Release of Massasaugas: Over the course of our demonstration, we captured 55 Eastern Massasauga Rattlesnakes. We implanted transmitters into each snake using a modification of the methods used by Reinert and Cundall (1982), and snakes were surgically implanted with either a 5g or 9g 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 2 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.

We tracked each snake three times per week between May-August and once every 3 weeks between September-November. To assess differences in survival rates between the three treatments, we 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 ES-3). The model-averaged annual survival estimates for resident, soft-, and hard-released Eastern Massasaugas were 0.72 (SE[*] ± 0.21, lower CI[†] = 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).

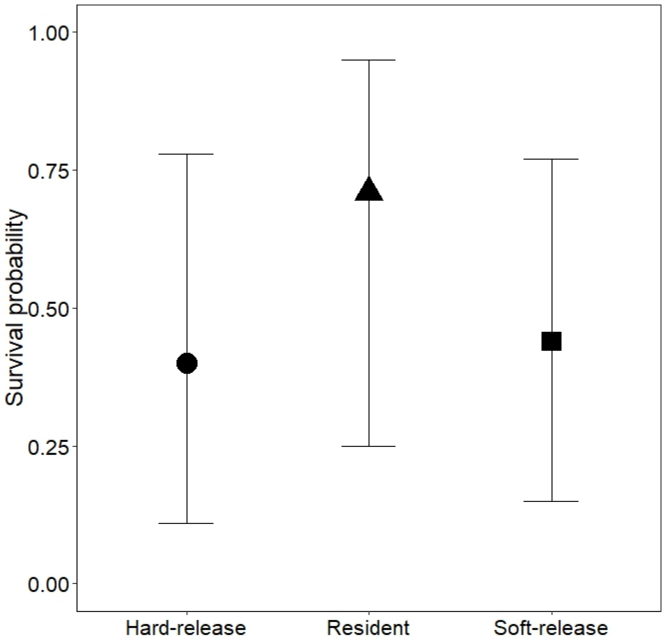

Figure ES-3. Resident Eastern Massasaugas (*Sistrurus catenatus*) had higher estimated annual survival rates (72%, n = 22) than either soft- (40%, n = 16) or hard-released (44%, n = 17) translocated snakes. Error bars represent 95% confidence intervals.

We predicted that translocated snakes would move more frequently and make longer distance movements than resident snakes. However, we 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, we compared four different movement metrics: (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

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[*] Standard Error (SE)

[†] Confidence Interval (CI)

snakes, 333 for soft-released snakes, and 430 for hard-released snakes, we found no evidence that soft releasing Eastern Massasaugas reduced their post-release movements. Using Mood's median and Kruskal-Wallis tests, we found no differences 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 ($\chi2(2) = 0.44$, $P = 0.80$).

We also predicted that soft releasing snakes would reduce their homing behavior. We observed four translocated snakes 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: We tracked 84 Texas Horned Lizards from 2016 – 2018. We dorsally attached radio transmitters (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 we glued harmonic radar diodes (low-barrier-height Schottkey barrier diodes that weighed only 1 mg to 12 mg) to their backs and relocated them using handheld RECCO transmitter/receiver (RECCO Rescue Systems, Lidingo, Sweden). We tracked lizards between 3-5 times per week during the active season (April – November). We soft released 23 Texas Horned Lizards and tracked 61 residents in the same area. We constructed two different soft release pens. Animals were held within pens for approximately 2 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 areas 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: We successfully hatched and reared 32 Eastern Box Turtles, half in enriched captivity and half in unenriched captivity. All turtles that hatched survived in captivity until release. We released two cohorts of captive-reared turtles 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 individual 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. We radio-tracked turtles 5 days per week from May to August and bi-weekly from September to November in each year. We monitored all released turtles 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 ES-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.

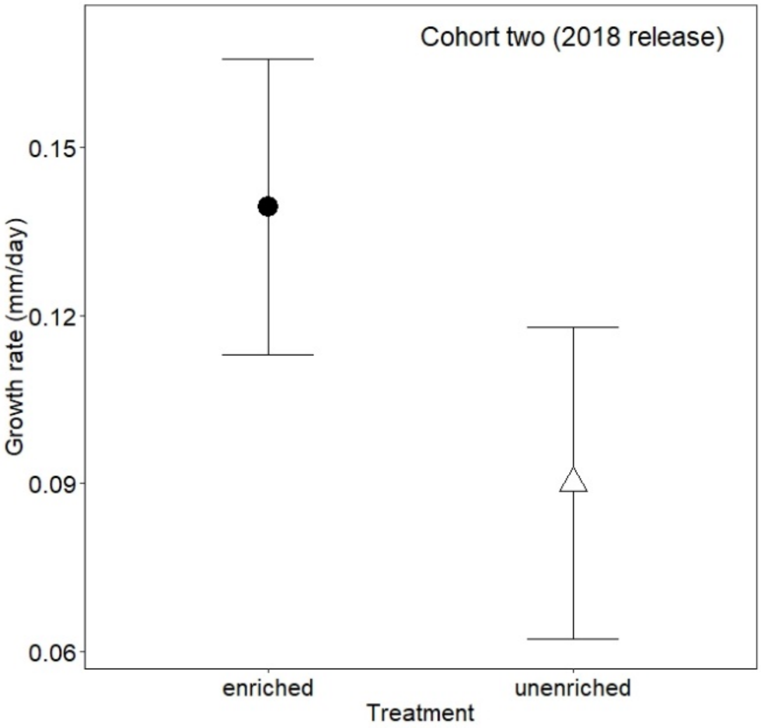

Figure ES-4. Growth rates of enriched (n = 10) and unenriched (n = 10) Eastern Box Turtles (*Terrapene carolina*) reared in captivity for 22 months before being released into the wild on Fort Custer, MI.

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, our 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 10 enriched and six of 10 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.

COST ASSESSMENT

Our demonstration revealed that soft release pens could be built, monitored, and maintained well below our goal of $1000 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 our 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. We calculated that the cost to raise a single enriched box turtle for 1 year was approximately $293 while the cost to raise a single unenriched turtle for a year was $644. We conclude that both soft release and environmental enrichment can be implemented at either a modest increase or even a cost savings over traditional translocation techniques.

IMPLEMENTATION ISSUES

Our 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.

Our demonstration relied on a 2-week soft release time period (i.e., individuals were kept in soft release enclosures for 2 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 we used in our demonstration. Holding animals in pens for longer duration may entail other challenges such as the needs to feed or provide other resources to enclosed animals, more intensive husbandry, or additional permits from regulatory agencies.

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

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 and both technologies have applicability for existing and future wildlife translocation projects on DoD installations.

1.1 BACKGROUND

The idea that enriched experiences may be necessary for the growth of species-specific brain characteristics is not a new concept although it has received renewed interest recently in relation to translocation science (Rosenzweig and Bennett 1996). 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, which shed attention on the deleterious effects of captivity, has caused renewed interest in environmental enrichment. Enrichment entails the practice of 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, companionship, 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 include communal housing, thermal gradients, live prey items, structurally complex enclosures with retreat sites, and temperature manipulation to stimulate hibernation (Roe et al. 2010, 2015; Burghardt 2013; Sacerdote-Velat et al. 2014).

Soft release entails placing individuals in outdoor enclosures at the release site before being released. 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 get acclimated 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.

The DoD is currently engaged in numerous translocation programs to manage threatened and endangered species and to move individuals out of harm's way of training and construction activities. The effectiveness of these programs vary from installation to installation and between species. The adoption of environmental enrichment and soft release technologies is broadly applicable to many of the DoD's translocation projects and may net an overall increase in their effectiveness.

1.2 OBJECTIVE OF THE DEMONSTRATION

Our specific objectives were to augment existing reptile translocation projects occurring on DoD installations using traditional translocation techniques with either soft release or environmental enrichment and to demonstrate the value of these technologies relative to traditional translocation techniques. The decision to incorporate soft release or environmental enrichment was a direct reflection of how the existing translocation programs operated and what their objectives were. For example, translocation programs that captured, moved, and released animals from areas of high-risk to low-risk, provided an opportunity to incorporate soft release technologies. For translocation programs that maintained animals in captivity for prolonged periods, we could implement environmental enrichment. Our 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.

We proposed 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 had been captured and moved from active training ranges or construction areas and hard released into suitable habitat. We augmented these efforts by adding a soft release component that allowed us to directly compare between the survival of soft- and hard-released individuals using known-fates survival analyses with the prediction that soft-released animals will have survival closer to that of resident animals. Similarly, we 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. We predicted that soft-released animals would move less frequently and occupy smaller home ranges than hard-released animals and that these movement parameters and space uses would be similar to those of resident animals.

We also proposed to demonstrate how environmental enrichment could enhance 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 standard, simplistic captive conditions we could assess how different rearing conditions affected the survival and behavior of translocated animals post-release. We compared the post-release survival of turtles using known-fates survival analyses. Additionally, we used general linear models to compare growth rates and dispersal of enriched vs. unenriched turtles post-release to assess the predictions that enriched captivity would better prepare captive individuals to naturally forage and that it would also reduce the propensity for those individuals to leave the release area, respectively.

1.3 REGULATORY DRIVERS

All federal land management agencies are required to comply with federal environmental laws and regulations. This demonstration specifically addresses the compliance challenges posed by the U.S. Endangered Species Act (ESA) of 1973. Protection of threatened or endangered reptiles under the ESA, varies depending on whether the species is located on federal or private property. For example, the ESA no-take provisions prohibit landowners from causing harm to listed species.

2.0 TECHNOLOGY/METHODOLOGY DESCRIPTION

2.1 TECHNOLOGY/METHODOLOGY OVERVIEW

Holding animals in outdoor, naturalistic enclosures at the release site for some period of time before release allows the individuals to become acclimated to an area and may help them to develop site fidelity to the area so that they do not later try to disperse and find their way back to their capture locations. Our goal in constructing soft release enclosures was to provide animals with complex, safe enclosures situated within suitable habitat that are escape proof. Soft release enclosures were constructed at the release sites in suitable habitat known to support resident animals. 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, we constructed open-topped soft release pens (Figure 1). The walls were constructed of 50.8cm tall aluminum flashing trenched approximately 8-10cm 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 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 within the enclosure, we kept the doors 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 2-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.

Figure 1. Soft release pen built in 2016 at Camp Grayling, Michigan to improve the translocation success of Eastern Massasaugas (*Sistrurus catenatus*). On the right, an individual Massasauga can be seen exiting the pen through one of the removable doors after having spent 2 weeks acclimatizing inside the soft release pen.

We constructed a similar enclosure for Texas Horned Lizards as they are not climbers. In the enclosure we provided drinking water and created a large sand mound for thermoregulation, burrowing, and oviposition by gravid individuals. Additionally, we used sugar bait to lay trails to attract ants (prey for lizards) to the inside of the enclosure to ensure that enclosed animals had sufficient access to food. However, we built this pen with some protection from avian predators as enclosed lizards could be vulnerable to predation by crows or hawks. Thus, we used fine-mesh wildlife netting to create a ceiling and to prevent predators from obtaining access to the pen (Figure 2).

Figure 2. Soft release pen built in 2017 at Tinker Air Force Base, Oklahoma to improve the translocation success of Texas Horned Lizards (*Phyrynosoma cornutum*). On the right, an individual Texas Horned Lizard can be seen inside the pen. Individuals had access to standing water (plastic dish seen to the right of the lizard), sand and vegetation for cover, and access to food resources while within the pen. Additionally, the pen was equipped with a mesh netting over it to prevent predatory birds from accessing lizards while within the pen. Lizards were released after spending 2 weeks acclimitizing inside of the pen.

Environmental enrichment can be designed to target the growth of many types of 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 (Figure 3).

Enriched box turtles were communally housed in 132cm long x 79cm wide x 30cm 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 4). Unenriched turtles were housed individually in comparably simplistic enclosures consisting of a 60cm long x 42cm wide x 28cm tall transparent plastic tub with reptile cage carpet (Zoo Med Eco Carpet; Zoo Med Laboratories, Inc., San Luis Obispo, California) and a 42cm x 42cm 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. 4cm deep) in the lower end for drinking and soaking.

The type and amount of food provided to individuals at each feeding was similar between rearing treatments. However, we 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. 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 5 days per week, and we dusted food with calcium powder 3 days per week. We also provided enriched turtles with cuttlebones to chew on. Fresh water was provided ad libitum.

Figure 3. Captive-reared Northern Water Snakes (*Nerodia sidpedon*) raised in standard and enriched conditions at Purdue University Fort Wayne. All snakes were released at restored wetland habitats in the Midwest. Enriched snakes were exposed to a thermal gradient, social interaction with other snakes, hunted their prey (fish) and had a complex environment. For details see Roe et al. 2015.

Figure 4. Rearing conditions for enriched and unenriched Eastern Box Turtles (*Terrapene carolina*). The panels to the left show environmental enrichment tubs. Turtles were communally housed in groups of 4-5 individuals, had substrate to burrow in, complex plant structure to hide in and foraged for spatially variable food. In contrast, unenriched turtles were housed individually in plastic tubs that comparatively lacked complexity, ate out of dishes, and had only one retreat site.

2.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY/ METHODOLOGY

Wildlife translocation is a commonly used practice, although the success of translocation varies widely (Seddon et al. 2007). Often, it is used for direct conservation applications to establish or augment an existing population by releasing additional individuals into it (IUCN 2012). Other times, it can be used to simply move animals out of the way of training or construction activities. Here, we demonstrated improvements to translocation by the addition of either soft release or environmental enrichment technology. The advantages of these additions are that these technologies are inexpensive and can be used to augment existing translocation programs and to allow for direct comparison between current and augmented success rates.

Environmental enrichment is a more labor intensive technological addition to wildlife translocation and is only an option for individual animals that spend some period of time in captivity before being released. This is most applicable to conservation projects in which animals are head-started, or born and raised in captivity to a size where they are either sexually mature or large enough to resist predation, and then released into the wild. In these situations, environmental enrichment is likely to be a critical component to ensure that captive-reared animals are able to maintain or learn naturalistic behaviors that will lead to enhanced survival on release (Shepherdson 1994, Roe et al. 2010). Environmental enrichment may also be a valuable addition to rehabilitation programs where animals are kept in captivity for prolonged periods of time as they recover from illness or injury. In these cases, environmental enrichment can be used to ensure that these individuals do not lose their natural behaviors when in captivity (Aaltonen et al. 2009). In some translocation situations, animals are removed from the wild, held in captivity for prolonged periods, and then released. Often these situations arise when animals must be moved from harm's way, but the release site must be restored or managed in some way before the animals can be re-released. Alternatively, animals may be held for long periods when managers are waiting for permits to be acquired, or for animals to be quarantined and physically examined before release. All of these situations make ideal candidates for environmental enrichment. However, environmental enrichment is not a viable option when animals have either never been in captivity, or when they have only been in captivity for very short periods of time. Additionally, environmental enrichment may require expert husbandry skills depending on the needs of the focal animal. Here, we chose the Eastern Box Turtle because it is a generally easy and robust animal to rear in captivity (Dodd 2001). In comparison, environmental enrichment may not be feasible for Texas Horned Lizards because of the difficulty in keeping them alive in captivity and their specialized diet on difficult to obtain ants and termites.

To our knowledge, environmental enrichment had not been applied to juvenile box turtles, but the species has certain behaviors that make it especially suitable for this approach. As juveniles, Eastern Box Turtles are small (2 – 10g in mass) and can be communally housed with limited spatial requirements. In addition, they display highly stereotyped behaviors such as burrowing, and feeding on live prey that can be denied or provided to them easily in captivity. The ability to practice and refine these behaviors in captivity should translate to enhanced survival in the wild for the species.

Soft release has many advantages over traditional hard release translocations. By holding animals in outdoor, naturalistic enclosures at the release site for some period of time before release, the individuals become acclimated to an area. They may develop site fidelity to the area so that they do not try to disperse and find their way back to their capture locations (Kingsbury and Attum 2009). Often, soft release enclosures can be quickly and cost-effectively constructed at release sites and require little to no daily maintenance. For most translocation efforts involving tortoises and other animals that cannot climb, soft release enclosures entail a ring of aluminum flashing constructed around a plot of land (Tuberville et al. 2005). Animals are then allowed to free-roam within the enclosure for some period of time (ranging from weeks to years) until the walls are opened up and animals are allowed to disperse. This method has been shown to work effectively for many species of turtles and tortoises (Kingsbury and Attum 2009). The limitations of this technique are that it can be challenging or costly to construct naturalistic enclosures for species that need large areas or that are adept at escaping. In such cases, soft release enclosures may be expensive and require daily maintenance. However, there are very few translocation situations in which soft release technology cannot be adopted, and the effects of this technique have been largely positive or neutral.

Soft release should be applicable to both of our demonstration sites because existing translocation programs are currently operating, have had low survival of translocated individuals, and have primarily relied on hard release methods. Snakes have been well-studied as translocation targets, and increased movement rates after translocation have been directly identified as the mechanism by which survival decreases for translocated individuals (Nowak 1998, Plummer and Mills 2000, Butler et al. 2005). Thus, Eastern Massasaugas should make excellent candidates for soft release technology aimed at reducing their post-release movements. The Texas Horned Lizard on Tinker Air Force Base also makes an ideal candidate as they are currently hard releasing lizards and seeing low survival. Furthermore, patches of suitable habitat on Tinker are small and isolated, thus individuals exhibiting larger than normal movement patterns often enter into construction or urbanized areas of the installation where survival should be very low. If soft release can reduce post-release movement for lizards on this installation, it will provide land managers with a significant benefit.

3.0 PERFORMANCE OBJECTIVES

The objective of the Eastern Massasauga translocation program on Camp Grayling was to move snakes from areas of human conflict on active training ranges and to release them at a managed natural area of the installation where they could be integrated into the resident population. This relies on the assumption that snakes left in place are at great risk of mortality due to human activity. The current translocation strategy has been to hard-release snakes at the release site, a technique that has been shown to result in large movements of translocated animals and high mortality rates in this species of snake (B.A. Kingsbury, pers. comm.) and others (Nowak 1998, Plummer and Mills 2000, Butler et al. 2005). The goal of soft release was to reduce the post-release movement of individuals in the hopes of increasing their survival and improving their probability of being integrated into the resident population.

Performance Objective 1 (PO1) (see Table 1) addresses the annual survival rates of soft-released, hard-released, and resident snakes. Annual adult survival rates for resident snakes at this site varies from 0.68 (females) to 0.90 (males) and averages 0.67 across the range of the species (Jones et al. 2012). To assess if PO1 was met we compared annual adult survival rates of hard- and soft-released snakes to one another and resident snakes using known-fate models (Program MARK). This approach allowed us to compare a number of competing models, weighted for different amounts of time individuals were tracked, and allowed us to estimate daily, monthly, or annual survival based on radio telemetry data. Survival rates of soft-released snakes should be significantly higher ($\alpha < 0.05$) than those of hard-released snakes. If the annual survival rate of soft-released snakes exceeds or was equal to the overall mean for the species (0.67) and approaches sex-specific survival rates for this site, we accepted that PO1 was met.

PO2 and PO3 are quantitative performance objectives focused on characterizing and comparing the behavior of soft- and hard-released snakes relative to each other and to residents. Translocated snakes often make large dispersal movements after release, attributed to disorientation or homing behavior (Nowak 1998). Soft release is directly aimed at reducing these dispersal movements by constraining their ability to disperse when first translocated and most at-risk. The mean home range size of Eastern Massausaga Rattlesnakes at Grayling is 16.7 ± 8 ha (males = 29.8 ± 10, females = 14.4 ± 6: DeGregorio et al. 2011). To assess if PO2 was met, we compared the space use of soft- and hard-released snakes with the expectation that soft-released snakes occupy smaller home ranges than hard-released snakes and that home ranges were within the range reported (less than 1 Significant Deviation [SD] from mean value) for resident snakes.

PO3 is intended to be an assessment as to whether translocated snakes integrated into the local population. We considered this PO successful if we observed males courting or mating with resident females within 1 year of release or if translocated females were courted or mated with by resident males within 1 year. Due to the relatively high density of snakes at this site and the prolonged mate-search polygyny mating system of the Eastern Massasauga, it was assumed that all released snakes will having mating opportunities post-release.

Table 1. Performance objectives for soft release of Eastern Massasauga Rattlesnakes at Camp Grayling, MI.

Performance Objective	Metric	Data Requirements	Success Criteria	Results
Quantitative performance objectives				
1. Increase survival of soft-released snakes.	Annual survival rates.	a. Survival rates of hard- and soft-released snakes post-release. b. Survival rates of resident snakes.	a. ~68–90% (female vs. male) annual survival of soft-released snakes. b. Soft-released snakes must have ≥ annual survival rates than hard-released snakes ($\alpha \leq 0.05$).	Statistically similar survival rate of soft- and hard-released snakes. Both lower than resident snakes.
2. Decrease dispersal behavior of soft-released snakes.	MCP home range size.	Home range size of soft- and hard-released snakes and resident snakes.	Soft-released snakes occupy home ranges similar to resident animals (less than 1 SD difference: Mean = 16.7 ± 8 ha) and smaller than hard-released snakes ($\alpha \leq 0.05$).	Soft-released snakes do not have smaller home ranges than hard-released snakes.
3. Integration of translocated individuals into resident population.	Social interaction behavior.	Behavioral observations of social interactions.	At least one social interaction (courting or mating) with resident snake in first year after release.	Six soft-released snakes observed mating with residents vs. three hard-released snakes observed mating with residents.
Qualitative performance objectives				
4. Ease and cost-efficiency of soft release implementation.	a. Construction cost. b. Cost per individual. c. Ability of technician to construct and maintain animals in pens.	a. Cost of materials. b. Labor costs of construction. c. Cost per individual snake. d. Feedback from maintenance personnel.	a. ≤ $1000 construction cost per pen. b. ≤ $3000 per individual. c. ≥ 3 years of use from the pen. d. Escape-proof pen. e. 100% survival of animals within pen. f. Ability for one personnel to adequately monitor and maintain pens.	a. Construction and 1 year of maintenance of pen costs less than $741.38. b. We released 16 snakes for an average cost of $72.23 per snake. c. The pen was escape proof. d. All animals in pen survived. e. All animals were easily monitored in pens by one person.

Finally, PO4 is a qualitative metric to assess the ease of adoption and cost of soft release vs. hard release. Although soft release is more expensive than hard release (which requires no prep of animals by simply releasing them unrestrained into a new area), we aimed to assess how difficult and expensive it would be for an installation to adopt the technology. We considered PO4 met if total construction costs of the soft release pen was less than $1000 and the pen lasts in the environment for 3 years, if a single technician could maintain the pen and observe animals within it, that the pen was escape proof, and if the total cost associated with monitoring and maintaining the animal in the pen was less than $3000 per snake (within the range of median costs estimated for per capita wildlife translocations: Finseth and Conrad 2014).

The objective of Texas Horned Lizard translocation on Tinker Air Force Base (AFB) was to move lizards from areas undergoing construction to safe, restored, and managed grasslands on the installation so they can be integrated into the resident population. The current translocation strategy is to hard-released lizards at the release site, although survival rate of hard-released lizards is substantially lower than resident lizards (0.16 vs. 0.57: Hellgren and Bogosian 2009). The POs for soft release of Texas Horned Lizards are redundant with those of Eastern Massasauga Rattlesnakes described above, although the thresholds and movement parameters were different and modeled after baseline estimates of survival and movement patterns of adult lizards on the installation.

PO1 addressed the annual survival rates of soft-released and resident lizards (see Table 2). Mean annual adult survival rates for lizards at this site is 0.57 (Hellgren and Bogosian 2009) and survival of hard released lizards was 0.16 (Bogosian 2010). To assess if PO1 had been met, we compared annual adult survival rates of soft-released lizards to resident lizards using known-fates binomial logistic regression modeling (PROC GENMOD in SAS). This approach allowed us to compare a number of competing models weighted for different amounts of time that different lizards were tracked, and allowed us to estimate daily, monthly, or annual survival based on radio telemetry data. Survival rates of soft-released lizards should be similar to those of resident lizards. If the annual survival rate of soft-released lizards approached mean annual survival rate of resident lizards (0.47-0.57), we accepted that PO1 had been met.

PO2 and PO3 are quantitative performance objectives focused on characterizing and comparing the behavior of soft released lizards relative to residents and are mostly redundant with those of the Eastern Massasauga (above) but with different values. To assess if PO2 was met, we compared the space use of soft-released lizards to resident lizards with the expectation that soft-released lizards should have similar homes ranges to resident lizards (0.87: Range 0.3 – 1.35 ha). Similarly, we expected the movement rates (distance moved per day) of soft-released horned lizards to be similar to those of residents (mean = 24.6 m/day). Home range sizes and movement rates of hard released lizards have previously been studied at this site (reported in Hellgren and Bogosian 2009; Bogosian 2010) and we compare soft- to hard-released lizards qualitatively.

Table 2. Performance objectives for soft release of Texas Horned Lizards at Tinker AFB, OK.

Performance Objective	Metric	Data Requirements	Success Criteria	Results
Quantitative performance objectives				
1. Reduce dispersal behavior of soft-released lizards.	Movement patterns, MCP home range size.	Distance moved per day and home range size of soft-released and resident lizards.	Soft-released lizards occupy home ranges similar to resident animals (less than 1 SD difference: Mean = 0.87 ha: Range 0.3–1.35). Soft-released lizards move similar to residents (less than 1 SD difference: Mean = 0.24 m/day) ($\alpha \leq 0.05$).	a. No difference in home range size between soft-released and resident adults (t = -0.84, P = 0.24) but there was for juveniles (t = -2.24, P = 0.02). b. Soft-released adults moved similarly to residents (t = 1.33, P = 0.098) but juveniles moved more per day than residents (t = -1.97, P = 0.03).
2. Increase survival of soft-released lizards.	Annual survival rates.	a. Annual survival rates of hard- and soft-released lizards post-release. b. Annual survival rates of resident lizards.	a. Survival of soft-released lizards > hard-released lizards. b. ≥ 16% annual survival of soft-released lizards.	a. Soft-released adult lizards had poor survival compared to residents (4% vs. 57%). However, soft-released juveniles had higher survival than residents (55% vs. 39%). b. Soft release survival rate was higher than reported for hard release (31% vs. 16%).
3. Integration of translocated individuals into resident population.	a. Breeding behavior. b. Reproductive status of females.	a. Observed mating behavior, b. Presence/absence of eggs in females.	a. At least one mating interaction with resident lizard in first year after release. b. Presence of eggs in females within 16 months after release.	a. Translocated females laid eggs that successfully hatched in release pen. b. No mating observed.
Qualitative performance objectives				
4. Ease and cost-efficiency of implementation.	a. Construction cost. b. Cost per individual. c. Ability of technician to construct and maintain animals in pens.	a. Construction cost. b. Cost per individual translocated. c. Feedback from maintenance personnel.	a. ≤ $1000 construction cost per pen. b. ≤ $3000 per individual. c. ≥ 3 years of use from the pen. d. Escape-proof pen with 100% survival of animals within pen. e. Ability for one personnel to adequately monitor and maintain pens.	a. Pen cost approximately $414.32 to construct. b. Cost per individual was $44.93. c. Pen was escape proof. d. Adequately monitored and maintained by one person.

PO3 was again intended to be an assessment as to whether translocated lizards integrated into the local population. We considered PO3 accomplished if we observed males courting or mating with resident females within 1 year of release or if translocated females were confirmed to be gravid within 1 year of release.

Finally, PO4 is a qualitative metric to assess the ease of adoption and cost of soft release vs. hard release and is identical to PO4 for Eastern Massasauga.

PO1 is a quantitative assessment of the effects of environmental enrichment on post-release growth of Eastern Box Turtles (see Table 3). Often, standard reared animals are inefficient foragers on release because the transition from eating out of a bowl or a similar unnatural situation to actively foraging can be challenging, at least initially. If environmentally enriched turtles are better prepared to forage naturally on release, we expected them to grow more and maintain a higher body condition index than standard reared turtles. PO2 was similar and is a quantitative comparison of the thermoregulatory efficiency of each treatment. Temperature influences all aspects of turtle behavior and physiology. Thus, we monitored the thermoregulatory efficiency (how closely turtles maintain their body temperature to the published preferred temperature range, approximately 25 °C; do Amaral et al. 2002) of treatment and resident turtles. To confirm PO2, we expected that environmentally enriched turtles should more frequently maintain preferred body temperatures compared to standard reared turtles ($\alpha \leq 0.05$) and that this metric should be similar between enriched and resident turtles.

PO3 is a quantitative assessment of annual survival of enriched vs. standard reared turtles. We expected that enriched turtles should have higher annual survival than standard reared turtles ($\alpha \leq 0.05$). Because we had very limited no baseline data for resident juvenile turtles at this site, we could not compare survival of translocated turtles with that of residents.

PO4 and PO5 are qualitative assessments of the ease of adoption and cost of enriched captivity vs. standard captivity. To achieve PO4, we expected the maintenance time between treatments to be similar and survival of turtles in each treatment to be greater than 90%. To achieve PO5 we expected the cost to establish enriched enclosures and maintain enriched animals to be no more than double the cost of standard reared individuals.

Table 3. Performance objectives for environmental enrichment of Eastern Box Turtles on Fort Custer, MI.

Performance Objective	Metric	Data Requirements	Success Criteria	Results
Quantitative performance objectives				
1. Use environmental enrichment to improve growth rates and body condition.	a. Growth rate data. b. Body condition index (BCI).	Repeated body mass and length measures of enriched and standard reared turtles.	a. Statistically higher growth rates of enriched vs. standard reared animals in first year post-release (α ≤ 0.05). b. Higher BCI of enriched compared to standard turtles 1 mo. and 3 mo. post-release (α ≤ 0.05).	a. In cohort 1 (turtles released when 9 months old), growth rates (mm per day) did not differ between enriched turtles (n = 6) and standard turtles (n = 6) (P = 0.73). In cohort 2 (turtles released when 21 months old), enriched turtles (n = 10) grew faster than standard turtles (n = 10) (P = 0.01). b. In cohort 1, BCI did not differ between enriched and standard turtles 1-month post-release (P = 0.26). Because few individuals survived 3 months post-release in cohort 1, we lacked statistical power to compare BCI between treatments. c. In cohort 2, BCI did not differ between enriched and standard turtles 1 month (P = 0.19) or 3 months post-release (P = 0.24).
2. Use environmental enrichment to increase thermoregulatory efficiency of released turtles.	Thermoregulatory efficiency.	a. Continuous body temperature measurements. b. Environmental temperatures.	Enriched turtles should maintain body temperatures within preferred range more often in captivity and wild than standard turtles (α ≤ 0.05).	In cohort 1, average body temperatures of enriched and standard 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 those of resident juveniles (P = 0.71).
3. Use environmental enrichment to improve annual survival.	Annual survival.	Annual survival of enriched and standard turtles.	Higher annual survival of enriched vs. standard reared turtles (α ≤ 0.05).	In cohort 1, three of six turtles in each treatment survived. Annual survival rates of enriched and standard turtles in cohort 1 were thus the same (0.33, 95% CI: 0.08–0.73). In cohort 2, four of 10 enriched and six of 10 standard turtles survived. The survival rate of enriched turtles (0.40, 95% CI: 0.16–0.70) was statistically indistinguishable from standard turtles (0.60, 95% CI: 0.30–0.84).

Performance Objective	Metric	Data Requirements	Success Criteria	Results
4. Use environmental enrichment to improve overwinter survival	Overwinter survival data	Overwinter survival of enriched and standard turtles	Higher overwinter survival of enriched vs. standard reared turtles	Overwinter survival was 100% for all turtles surviving the active season
Qualitative performance objectives				
5. Ease of implementation of enrichment	a. Daily maintenance time, b. Health of captive animals	a. Maintenance time for enriched and standard husbandry, b. Health and survival of captive individuals	a. Similar maintenance time between enriched and standard husbandry. b. > 90% survival of individuals in each group	a. Total maintenance time (feeding and cleaning enclosures) for enriched turtles was 30 min per day and 90 min per day for unenriched turtles. b. 100% survival in captivity and excellent health of all individuals
6. Cost of enriched enclosure	Setup cost	Setup costs	Cost for enrichment no more than double cost for unenriched husbandry	One year of husbandry for each enriched enclosure cost approximately $293 compared to $644 for each unenriched enclosure

4.0 SITE DESCRIPTION

Tinker AFB (TAFB), Oklahoma: TAFB is located in Oklahoma County, Oklahoma and is an approximately 2,000 ha military installation. TAFB is a major maintenance and supply depot for the U.S. Air Force. Since its creation in 1941, urbanized habitat has expanded to cover roughly 66% of the base, leaving 500 ha of native habitat in a highly fragmented matrix. Dominant vegetation types are mixed oak-hardwood forests (*Quercus* spp.) and a mixture of native (*Adropogon gerardii, Schizachyrium scoparium, Bothriochloa ischaemum, Sorghastrum nutans)* and non-native (*Festuca arundinacea, Lolium perenne*) grasslands interspersed with eastern redcedar (*Juniperus virginiana*).

The Texas Horned Lizard, an Oklahoma Species of Special Concern, is found in the grassland habitats of TAFB, primarily in Wildlife Reserve 3 (Figure 4). The behavior, population demography, and habitat requirements of the Texas Horned Lizard have been studied on TAFB since 2003 by installation natural resource managers and biologists from Southern Illinois University.

The construction of new storage hangars and expansion of housing areas has created the need to translocate Texas Horned Lizards since 2008. Researchers have conducted installation-wide habitat modeling to identify areas of suitable Texas Horned Lizard habitat in areas of the installation that will not be developed or trained on. It is to these areas that translocated Texas Horned Lizards are moved. The habitat requirements of the Texas Horned Lizard consist of a matrix of structural complexity (for thermoregulation), but suitable habitat must contain bare ground, shrubs, and be primarily grass dominated.

Camp Grayling, Michigan: Founded in 1913, Camp Grayling is located in the north-central portion of the lower peninsula of Michigan. This installation encompasses over 59,000 ha of land, and over 10,000 troops are scheduled to train at Camp Grayling annually. It is primarily covered in northern hardwood forests, and major tree species include Speckled Alder (*Alnus incana*), Maples (*Acer* spp.), Oaks (*Quercus* spp.), and Quaking Aspen (*Populus tremuloides*). The site also contains conifers such as Cedars (*Thuja* spp.), Pines (*Pinus* spp.), and Spruce (*Picea* spp.), as well as barrens dominated by lichen and blueberry (*Vaccinium* spp.).

Eastern Massasauga Rattlesnakes (*Sistrurus catenatus*), a federally threatened species, occur in high densities in areas of Camp Grayling. Several radio telemetry studies of the Eastern Massasauga have been conducted in the ca. 800 ha area heavily used by the snakes since 2002 and have helped to identify patterns of habitat use, movements, and overwintering site selection. Six large clear-cuts (ca. 6.5 ha acres each) were created in winter 2006 with the aim of creating basking habitat for the snakes, and a large-scale fire passed through the southern portion of the study area in May 2010 in habitat traditionally used for overwintering. The snakes use a mosaic of open, wetland, and upland habitats while active, but they primarily overwinter within root systems and small mammal burrows within the burned area and adjacent forest edges in the southern portion of the site.

Eastern Massasauga Rattlesnakes are frequently encountered by soldiers, maintenance workers and other military personnel at this site. Though these snakes are venomous (and thus pose a potential threat), they are seldom persecuted. They are, however, frequently moved indiscriminately by untrained personnel to areas several kilometers away from their capture locations, leaving the fate of the snakes unknown.

Fort Custer, Michigan: Fort Custer Training Center is an Army National Guard facility located in southwestern Michigan, near Augusta. The installation is approximately 3,035 ha and is primarily woodlands (2,023 ha), wetlands (485 ha), and old field/prairie (485 ha). Fort Custer is unique in that it comprises a large area of continuous, relatively unfragmented habitat. Unpaved dirt roads intersect the installation at approximate 1-mile intervals. The majority of the study site receives minimal human disturbance and vehicular traffic is limited. The boundaries of Fort Custer are enclosed by a 7-ft chain-link fence and is closed to public access.

The ecology and behavior of Eastern Box Turtles has been studied on Fort Custer since 2006. Research indicates that turtles at this site extensively use open herbaceous habitats and forest edges. Juveniles in particular are reliant on open herbaceous areas and forest edges (Gibson 2009). Additionally, turtles at this site select locations with extensive leaf litter and structural cover (Gibson 2009). Choice of a soft release site at Fort Custer was based on finding a location that met all of these specific habitat requirements and that was located away from active prescribed fire management and active training. The soft release sites were chosen based on site selection criteria (see, for example, Figure 5, p 24).

5.0 TEST DESIGN

This chapter provides an outline of the overall test design and results from our two demonstrations of soft release and one demonstration of environmental enrichment.

5.1 CONCEPTUAL TEST DESIGN

Soft Release: To evaluate soft release technology, we will use radio telemetry to track the movements, survival, and social interactions of Eastern Massasaugas and Texas Horned Lizards. We will also use radio telemetry to track the same metrics of hard-released individuals and resident individuals that will serve as controls. We predict that the survival and behavior of soft-released individuals will be more similar to that of resident controls than to that of hard-released animals. Hard-released animals are predicted to move more, interact with residents less, and ultimately have lower survival.

Environmental Enrichment: To evaluate environmental enrichment technology, we will rear Eastern Box Turtles in captivity in either enriched conditions (treatment group) or standard conditions (control). All individuals will be soft released at the selected release sites and tracked with radio telemetry. We will compare the movement, growth, and survival between enriched and standard reared individuals.

5.2 BASELINE CHARACTERIZATION AND PREPARATION

The most important aspect of our demonstrations was selection of suitable sites. However, because we were augmenting existing translocation projects we were limited in our ability to change or alter our sites. As such, we used expert opinion from installation biologists and existing, delineated field sites to choose our release sites for demonstrations. For Eastern Massasaugas, we chose to place our soft release enclosure at the site of a known, high quality hibernacula. Previous studies have shown that the translocation efforts have failed due to complete overwinter mortality of snakes that were unable to find suitable hibernacula. We choose a hibernacula that was adjacent to open areas frequently used by resident Eastern Massasauga and that had a high density of resident snakes. For Texas Horned Lizards, we chose an area far from construction activities in prairie habitat that contained resident lizards. For Eastern Box Turtles, we chose a release site in forest adjacent to the best known nesting site for turtles on the installation. This habitat is an area to which resident juvenile turtles would likely disperse immediately following hatching; it provides a realistic demonstration of the area and characteristics that juvenile turtles would naturally encounter.

5.3 DESIGN AND LAYOUT OF TECHNOLOGY AND METHODOLOGY COMPONENTS

Soft release is a simplistic undertaking that relies on two stages: in-situ enclosures and post-release monitoring. Section 2.1 describes the design and construction of soft release enclosures in detail (also see Figures 1 and 2). Post-release monitoring consists of radio telemetry, a standard wildlife research technique that uses methods and equipment tailored to the size, shape, and ecology of the focal species. Section 5.5 describes the equipment and techniques for each focal species in this demonstration in detail. Sections 2.1 and 5.5 describe in detail environmental enrichment entailed rearing turtles in complex tubs (see Figure 3).

5.4 FIELD TESTING

Table 4. Timeline for demonstration of soft release for Eastern Massasauga and Texas Horned Lizard Translocation and environmental enrichment for Eastern Box Turtles (continued onto next page).

Activity	Date Range	Description
Demonstration of Soft Release for Eastern Massasauga and Texas Horned Lizard Translocation		
Site selection	April – May 2016	Study sites chosen and resident individuals confirmed at location.
Construction of soft release pens	June 2016	Each pen was constructed in 1 day of work. One constructed at Camp Grayling and two constructed at TAFB.
Translocation and radio telemetry of study animals	June 2016 – August 2018	Study animals tracked continuously at these sites.
Demonstration of Environmental Enrichment for Eastern Box Turtles		
Site Selection	May 2016	Location for release determined, nesting areas identified for egg collection.
Eggs collected	May – June 2016	Eggs collected from field and brought to incubators.
Eggs hatched	August – Sept 2016	Eggs hatched in incubators and hatchlings assigned to treatments.
Environment enrichment initiated	August – Sept 2016	Half of hatchling assigned to enrichment group, half to unenriched.
First cohort released to wild and tracked with radio telemetry	May 2017	All individuals equipped with transmitters and released in same area.
First cohort monitored in field	May – Oct 2017	Monitored until hibernation.
Second cohort released to wild	May 2018	All individuals equipped with transmitters and released in same area.
Second cohort monitored in field	May – Nov 2018	Monitored until hibernation.

5.5 SAMPLING PROTOCOL

Soft release: Camp Grayling and Eastern Massasaugas. Adult Eastern Massasaugas of all sex classes (males, females, and gravid females) were captured yearly during visual encounter surveys of suitable habitat on or nearby military training areas from May-July 2016-2018. Eastern Massasaugas were also obtained opportunistically during this time if they were located during routine radio-tracking activities or if military or Michigan Department of Natural Resources personnel contacted us to come retrieve snakes that they had discovered at firing ranges, industrial areas, or residences.

When suitable study animals were captured, we surgically implanted radio transmitters using a modification of the methods used by Reinert and Cundall (1982). We implanted each snake with either a 5g or 9g Holohil Systems Ltd. SI-2T temperature-sensitive transmitter that was ≤ 6% of the snake's body mass. During transmitter surgery, we weighed (g), measured (snout-to-vent length, SVL; cm), and sexed via cloacal probing each snake. We also swabbed each animal with sterile, flocked, cotton-tipped applicators to test for the presence of *Ophidiomyces ophiodiicola*, which is believed to be the causative agent of snake fungal disease (SFD; discovered in this population in 2013, Tetzlaff et al. 2015). Snakes exhibiting obvious clinical signs of SFD were not translocated, and any asymptomatic snakes that later developed clinical signs and/or tested positive for *O. ophiodiicola* using qPCR under the methods developed by Allender et al. (2015) were excluded from analysis. To maintain sterile conditions and reduce the risk of transmitting fungal spores, all capture, housing, and surgical equipment that came into contact with the snakes was sterilized in an autoclave, with bleach solution, or with other household cleaners that have proven effective against *O. ophiodiicola* (Rzadkowska et al. 2016). No snakes died during transmitter surgery.

After surgery, Eastern Massasaugas were held for a minimum of 2 days to monitor recovery, after which they were randomly assigned to soft- or hard-released treatments. We also monitored a sample of resident snakes captured at the release site that were simply released back at their capture location after recovery from surgery. Soft-released snakes were placed in outdoor enclosures at the study site before release, hard-released snakes were released within 5m of the peripheries of the enclosures, and resident snakes were released at their exact site of capture. Up to three individuals were placed in enclosures or hard-released together at any given time. We opened the soft release pen after 14 days to allow the animals to exit on their own to further minimize stress that might be induced by handling. A 10-14 day holding period was chosen because soft-released, captive-raised Eastern Massasaugas radio-tracked for another study at this site found that the snakes stopped patrolling the perimeter of the enclosure after approximately 1 week, which suggests that even short periods of time may be enough to curtail dispersal behavior (Kingsbury and Attum 2009).

In addition to the snakes that were monitored during this demonstration we also included a small number of snakes that were soft- (n =2) or hardreleased (n = 3) in 2014 for a pilot study of the technique conducted by co-pi B.A. Kingsbury. The enclosure used was located at the same study site, although in a different location, approximately 500m from the pen used during this demonstration. The pen was smaller and constructed of sediment fencing as opposed to aluminum flashing. The aluminum pen we used during the current demonstration was escape proof, however the sediment fencing pen had two snakes escape. The first escaped after 2 days of confinement and the other within 24 hours of the date that it was supposed to be taken out of the enclosure. The former was returned to the enclosure and made to finish the remainder of its term, while the latter was not returned to the enclosure given the proximity of the escape to the planned release date. Three resident snakes included in analyses were also tracked in 2014.

We tracked Eastern Massasaugas approximately three times per week during daylight hours between May-August and once every 3 weeks between September-November using a handheld receiver (R-1000, Communications Specialists, Inc., Orange, CA) and three-element folding Yagi antenna (Advanced Telemetry Systems, Isanti, Minnesota). Overwintering locations were determined during the winter months, and survival was assessed the following spring after snakes did or did not emerge. Attempts were made to locate snakes in a different order each day to avoid temporal bias. The locations of the snakes were recorded at each sighting with a handheld global positioning system (GPS) unit (Garmin eTrex 20; approx. 3m accuracy) in Universal Transverse Mercator (UTM) coordinates. The causes of any mortalities were determined by visually inspecting the transmitters and carcasses, and it was assumed that a snake had died during the winter if it did not emerge in the spring.

Eastern Massasaugas have been radio-tracked at this study site since 2013 (i.e., Ravesi 2016, Tetzlaff et al. 2017a,b), and these snakes were included in the resident control treatment of the analyses herein to boost sample sizes. All capture, surgery, and radio-tracking methodologies were the same for these snakes.

The fates of the Eastern Massasaugas included in this analysis (i.e., lived, died, censored) were tallied for soft-released, hard-released, and resident controls.

We used Program MARK known-fate models (Version 8.2; White and Burnham 1999) to estimate the annual survival of soft-released, hard-released, and resident control snakes. We also followed methodologies similar to those employed in a study (Jones et al. 2012) that also used known-fate models to analyze Eastern Massasauga survival and that used older data from this site. Specifically, to be consistent over years, relocation intervals were set to 1 week for all analyses so that multiple relocations of an individual were compiled into one relocation for each week. Some Eastern Massasauga mortalities could not be determined until the spring when snakes did not emerge from overwintering underground. Therefore, the fall and winter period when radio-tracking was not conducted on a weekly basis was accounted for in a single interval. Eastern Massasaugas at this site typically begin to emerge in April and return to their hibernacula in September (DeGregorio et al. 2011), so the snakes were active during part of this single quasi-winter interval. In total, each individual encounter history had 16 intervals, with the first 15 representing the 19th-33rd weeks of each year when radio-tracking was regular (i.e., approx. May 8th-August 19th) and the 16th interval representing the portion of the year when radio-tracking was irregular (i.e., approx. August 20th-May 7th). If a snake was not located in an interval, then it was censored for that interval. Soft-released snakes were coded as alive during weeks that they were contained in enclosures, as it was possible that they could have still experienced mortality during their captivity (e.g., from predators).

In addition to evaluating the effect of treatment (soft release, hard release, or resident) on annual survival rates, we also assessed other factors likely to affect survival of Eastern Massasauga. We evaluated models for body condition index (BCI; calculated using the ratio index of initial mass/initial SVL; Stevenson and Woods Jr 2006) and distance translocated (i.e., the Euclidean distance from the capture site to the release site). We also attempted to evaluate models for sex class, year, and season. We used Akaike's Information Criterion corrected for small sample sizes (AICc; Akaike 1998, Burnham and Anderson 2002) to rank candidate models within Program MARK to determine whether survival differed between the treatments, sexes, time intervals, seasons, BCI, and distance translocated. We conducted analyses using all snakes tracked and again only for male snakes (because we had the most robust sample size for this group). We also did a direct comparison between hard- and soft-released snakes by excluding all resident snakes. Models that had a ΔAICc > 8 were considered to have negligible support and were eliminated before model averaging, as were any models that had "masquerading" variables (e.g., models with one more parameter than the highest ranked model that had a ΔAICc < 2 and a reduction in deviance of < 0.5, models with two more parameters that had a ΔAICc < 4 and a reduction in deviance of < 1, and so on; Burnham and Anderson 2002, Arnold 2010). Any parameters that remained in the models had enough support to potentially be important to the survival of the snakes, and parameters within models that had a ΔAICc ≤ 2 were considered the most competitive.

After removing inadequate models, model-averaged estimates of annual survival, SEs, and 95% confidence intervals (CIs) were calculated for each of the sex and treatment groups directly by Program MARK. If parameters in top models (i.e., models with ΔAICc ≤ 2) were categorical covariates (i.e., time, season), then effect sizes between the categories were generated by rerunning the models containing those covariates using the identity link function and examining the beta parameter estimates output by Program MARK. Again, there was no significant effect if the CIs overlapped zero. If parameters in top models were continuous covariates (i.e., BCI, DT), then individual covariate plots were made in RStudio (Version 3.4.3; RStudio Team 2016) using data generated by Program MARK to assess their potential relationships with survival.

We calculated maximum dispersal distances from the release site after 1, 2, 4, and 8 weeks. We calculated dispersal distance by determining the furthest Euclidean distance between snake release and tracking UTM locations during each time interval. Mean distance moved per day, activity range size, and activity range length during the first spring and summer post-release (i.e., approx. May 8th-August 19th when radio-tracking was regular) were calculated for all Eastern Massasaugas that were radio-tracked for a minimum of 8 weeks and 24 tracking events. Tracking events and movements for soft-released snakes that took place while they were inside enclosures were not counted towards the minimum threshold for inclusion and were not incorporated in analyses. Activity range sizes and lengths for each snake were computed in Quantum GIS (Version 2.18.13; Quantum GIS Development Team 2017) using the 100% MCP method (Jennrich and Turner 1969) and by measuring the Euclidean distance between the two tracking locations that were the furthest apart from one another, respectively. Mean distance moved per day for each snake was calculated by dividing the total distance moved between each tracking occasion by the number of days between each location and then averaging those distances across the entire season. We tested all assumptions of normality and homogeneity of variances, and, if they were not met, data were transformed to approximate normal distributions or homogeneity of variances. If assumptions were met, averages were compared using one-way analysis of variance and Tukey's honestly significant difference (HSD) post-hoc tests. Kruskal-Wallis or Mood's median tests were used with appropriate post-hoc tests to compare means if these assumptions were not met after transformation. For all tests, $\alpha = 0.05$ unless stated otherwise.

Homing and release site fidelity were compared qualitatively among translocated Eastern Massasaugas. The numbers of soft- and hard-released Eastern Massasaugas that returned to within 300m of their capture locations at any time before hibernation were tallied to see if the treatments exhibited different homing propensities. Hibernation locations were examined for snakes released at the second enclosure in 2016-17, and the numbers of those that returned to within a 300m radius of it within each translocation treatment were tallied to see if they exhibited different release site fidelities. This distance encompasses the estimated size of the hibernaculum at this site and therefore was used as an approximation of how close a homing snake would have to be to its capture location to definitively have homed. Fidelity to release site could not be assessed for snakes released at the first enclosure as it was not at a hibernaculum, and there was not sufficient second-year post-release data to assess if translocated snakes tended to return to that area during a second active season.

Soft release of Texas Horned Lizards on Tinker AFB. We used radio telemetry to monitor the survival and movement of soft-released and resident Texas Horned Lizards on Tinker AFB. We captured lizards by hand during visual encounter surveys of residential, construction, and industrial locations. To ensure that we had monitored resident lizards at the release site, we conducted regular visual encounter surveys in the prairie surrounding the soft release pen. We also relied on fortuitous encounters by our field crew or maintenance personal on the installation. We conducted surveys from March–September 2016-2018. We permanently marked all lizards ≥ 10 g by inserting an AVID PIT tag (Biomark Inc., Boise, ID) into the ventral side of the animal. To conduct radio telemetry, we dorsally attached radio transmitters (model BD-2, 0.95-1.95 g, Holohil Systems Ltd., Ontario, Canada) to individuals using silicone epoxy and small elastic collars placed around individuals' necks (total encumbrance was ≤ 10% of an individual's mass). We used R-1000 receiver (Communication Specialists, Orange, CA) and Yagi 3-element antennae (Wildlife Materials Inc., Murphysboro, IL) to track these lizards between 3-7 times per week. To track juveniles we glued harmonic radar diodes (low-barrier-height Schottkey barrier diodes that weighed only 1 mg to 12 mg) to their backs and relocated them using handheld RECCO transmitter/receiver (RECCO Rescue Systems, Lidingo, Sweden). We tracked juveniles as frequently as possible, although relocations for juveniles were more unreliable for adults and they were typically relocated between 3-5 times per week during the active season (April – November). All lizard relocations were stored in a Geographic Information System (GIS) database using handheld GPS units (Trimble GeoXT, Terrasync 2.3, Strategic Consulting International, Oklahoma City, OK).

To calculate survival differences between soft-released and resident lizards, we used known-fate models within a binomial logistic regression framework. Each time we tracked a lizard, we determined whether it was alive or not (1 or 0). We used these data to calculate weekly probability of survival and used these weekly encounter histories to calculate annual survival rates. We compared annual survival rates of soft-released and resident lizards. During this demonstration, we did not hard release lizards, however a previous study on Tinker Air Force had (Bogosian et al. 2010) so we compared our results to these reported survival estimates for hard-released lizards. We calculated and did separate comparisons for adults and juveniles as adults are expected to have significantly higher annual survival than juveniles.

To compare movement metrics between soft-released and resident lizards, we calculated and compared two movement metrics. We calculated mean distance moved per day and home range size for each lizard tracked during each year. We only calculated home range size for lizards with greater than 15 relocations. Tracking events and movements for soft-released lizards that took place while they were inside enclosures were not counted towards the minimum threshold for inclusion and were not incorporated in analyses. Home range sizes and movement distances for each lizard were computed in Quantum GIS (Version 2.18.13; Quantum GIS Development Team 2017) using the 95% minimum convex polygon (MCP) method (Jennrich and Turner 1969) and by measuring the Euclidean distance between consecutive tracking locations. Mean distance moved per day for each snake was calculated by dividing the total distance moved between each tracking occasion by the number of days between each location and then averaging those distances across the entire season. We compared mean home range size and mean distance moved per day for soft-released and resident lizards using non-parmetric Kruskal-Wallis tests. We did two comparisons for each metric, the first using only adults and the second using only juveniles.

Environmental enrichment of Eastern Box Turtles: 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. We artificially incubated eggs indoors and raised hatchlings (n = 32) in a greenhouse on the campus of University of Illinois at Urbana-Champaign. We raised neonates in either an enriched or unenriched environment beginning in mid-August 2016 (within 2 weeks of hatching). Enriched turtles (n = 16) were communally housed in 132cm long x 79cm wide x 30cm deep Rubbermaid® stock tanks (n = 4–5 individuals per replicate) with naturalistic features designed to mimic vegetation and substrate commonly used by wild Eastern Box Turtles (Dodd 2001, Figure 4). Unenriched turtles (n = 16) were housed individually in comparably simplistic enclosures consisting of a 60cm long x 42cm wide x 28cm tall transparent plastic tub with reptile cage carpet (Zoo Med Eco Carpet; Zoo Med Laboratories, Inc., San Luis Obispo, California) and a 42cm x 42cm piece of plastic shelf liner resting on the carpet. We provided these turtles with a small plastic hide box and kept tubs on a slight angle to hold fresh-standing water (ca. 4cm deep) in the lower end for drinking and soaking (Figure 4).

The type and amount of food provided to individuals at each feeding was similar between rearing treatments. However, we 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. 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 5 days per week, and we dusted food with calcium powder 3 days per week. We also provided enriched turtles with cuttlebones to chew on. Fresh water was provided ad libitum.

We recorded individuals' mass (g) using a digital scale (Sartorius M-PROVE Portable Scale; Sartorius Army Garrison [AG], Göttingen, Germany) generally once per week during rearing. Being raised in a greenhouse, all turtles were exposed 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 °C – 29 °C. Further details of study animal acquisition and husbandry methods are described elsewhere (Tetzlaff et al. 2018).

We released two cohorts of captive-reared turtles to their capture sites on Fort Custer Training Center, Michigan. Twelve turtles (six in each treatment) were released at Fort Custer in May 2017 after 9 months in captivity. The remaining individuals (10 in each treatment) 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 (Figure 5) for approximately 30 days. Three release pens were used in 2017 and five were used in 2018.All turtles had a 0.9 or 1.2 g radio-transmitter (Advanced Telemetry Systems, Inc., Isanti, Minnesota) affixed to their carapace using epoxy. We radio-tracked turtles 5 days per week from May to August and bi-weekly from September to November in each year.

Figure 5. Acclimation pen used to soft release head-started Eastern Box Turtles at Fort Custer Training Center.

Using a handheld GPS, we recorded UTM locations for each turtle when they were radio-tracked. These data were used to calculate dispersal as the maximum distance (m) moved from release pen between treatments (interactive effect of rearing condition and time in captivity) using general linear models. We also recorded various microhabitat variables during the second and fourth tracking events each week for each turtle, such as substrate type selected, distance to and heights (cm) of vegetation near turtles, cover type (e.g., woody debris, leaf litter, live vegetation), and litter depth (cm). Additionally, we recorded variables related to turtle behavior when microhabitat data are recorded, such as whether a turtle is buried or on the surface and the estimated percentage of the turtle that is visible.

To calculate survival differences between the groups, we used known-fate models in Program MARK. Each time we tracked a turtle, we determined whether it was alive or not (1 or 0). We used these data to calculate weekly probability of survival and compared these values between enriched and unenriched turtles in each cohort. We also evaluated the effects of other models on the probability of survival to assess whether rearing treatment, week since release, their interaction, or a constant (i.e., null) model had the largest effect on survival.

We compared dispersal distance between enriched and unenriched turtles using a general linear model.

5.6 SAMPLING RESULTS

5.6.1 Soft release of Eastern Massasauga

5.6.1.1 Survival
We tracked and included 55 Eastern Massasaugas in survival analyses: 22 resident controls, 16 soft-released, and 17 hard-released snakes. Of the 55 snakes, 21 died during the demonstration. Snake mortalities were attributable to vehicles, predators, overwintering, and unknown causes.

To determine whether soft-released, hard-released, and resident male, female, and gravid female Eastern Massasaugas had different survival during their first year post-release and to evaluate what factors might influence survival, we compared competing models using known-fates survival analyses in Program MARK. Because the movement ecology and survival of Massasaugas varies by sex and reproductive condition (Jones et al. 2012), we repeated our analyses several times, first by including all of the snakes that we tracked, then by using only males (our most robust sample size for a sex class), and the finally by only directly comparing the soft- and hard-released males.

When we evaluated the effects of all of the factors on the survival of all 55 snakes tracked, we did not find compelling evidence that any of the models adequately explained patterns in survival at this site. We found that, where survival was constant, the null model had the strongest support. Effect sizes calculated between groups from the model-averaged annual survival estimates indicated that there were no significant differences between the treatments or sexes. Likewise, BCI and season did not have much support, and time had none whatsoever. Given that the constant model was the only model that had a ΔAICc < 2 and that Eastern Massasaugas of different sexes at this site have been found to have different survival in the past (Jones et al. 2012), the estimates from this dataset are not likely very accurate or informative.

When we confined our analyses to only male snakes, the top models included the treatment only model, the null model, and the treatment and BCI interactive model (Table 5). The model-averaged survival estimates for resident, soft-, and hard-released Eastern Massasaugas were 0.72 (SE ± 0.21, lower CI = 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). Effect sizes calculated between treatments from the model-averaged annual survival estimates indicated that the resident treatment had a significant, positive effect on survival relative to the soft-released treatment (effect size = 0.29, SE ± 0.09, lower CI = 0.11, upper CI = 0.47), but there was no significant effect of the resident treatment relative to the hard-released treatment (effect size = 0.33, SE ± 0.33, lower CI = -0.33, upper CI = 0.99) or of the soft-released treatment relative to the hard-released treatment (effect size = 0.04, SE ± 0.05, lower CI = -0.05, upper CI = 0.13). The treatment and BCI interactive model suggested that snakes in all treatments had lower annual survival with higher BCI, with translocated snakes experiencing lower survival at higher BCI than residents (Figure 6). This could suggest that snakes in good condition might be more active while engaging in activities such as searching for females to mate with, thus making them more visible to predators than snakes in poorer condition (Jellen et al. 2007). However, this finding should be interpreted with caution given that the confidence intervals were especially large for the smallest and largest body conditions, that the seasonal estimates were not significantly different, and that the quasi-winter season examined here occurs over a period twice as long (approximately 37 weeks) as the spring/summer active season (15 weeks). Regardless, this result may help inspire future efforts that are specifically designed to more rigorously test the influence of such covariates on survival in general or in combination with other translocation efforts. Neither season nor time had much support (Table 5).

Table 5. Model selection results for predicting survival of translocated Eastern Massasaugas at Camp Grayling, MI based on translocation treatment (soft released, hard released, or resident), BCI, or season.

Model	ΔAICc	AICc w_i	Deviance
S(Treatment)	0.00	0.29	56.06
S(.)	0.84	0.19	60.97
S(Treatment + BCI)	1.47	0.14	55.49
S(BCI)	2.20	0.10	60.30
S(Treatment x Season)	2.54	0.08	52.41
S(Season)	2.85	0.07	60.95
S(Treatment x BCI)	2.95	0.07	52.82
S(BCI + Season)	4.18	0.04	60.24
S(BCI x Season)	5.27	0.02	59.28
S(Treatment x BCI x Season)	7.34	0.01	44.46

ΔAICc is the difference in AICc values from a given model to the top model.
AICc weight shows the relative likelihood a given model is the most supported.
Deviance provides a relative measure of goodness of fit.
The notation (.) refers to the constant (i.e., null) model, (+) an additive effect, and (x) signifies an interaction.

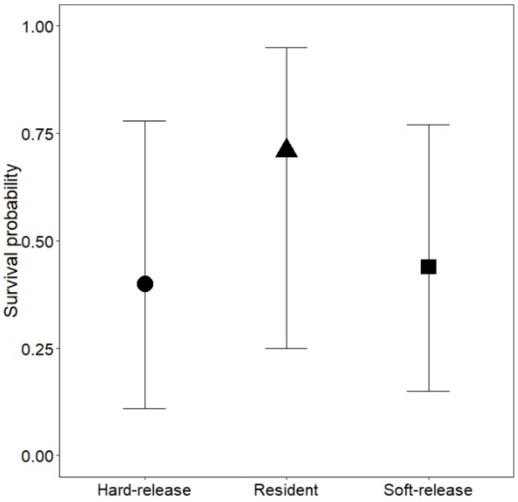

Figure 6. Resident Eastern Massasaugas (*Sistrurus catenatus*) had higher estimated annual survival rates (72%, n = 22) than either soft- (40%, n = 16) or hard-released (44%, n = 17) translocated snakes.

When we only compared survival of translocated snakes (soft vs. hard release), the model-averaged survival estimates for soft- and hard-released males were 0.33 (SE ± 0.17, lower CI = 0.10, upper CI = 0.69) and 0.32 (SE ± 0.17, lower CI = 0.09, upper CI = 0.69), respectively, and the effect size for soft relative to hard release showed that the survival probabilities were not significantly different (effect size = 0.01, SE ± 0.13, lower CI = -0.24, upper CI = 0.27). The top models affecting survival of translocated snakes included the BCI only model, the null model, the season-only model, the BCI and season additive model, and the treatment only model. As with the results from the second dataset, the BCI only model and the BCI and season additive model suggested that translocated snakes with higher BCI had lower survival than those with lower BCI. The season-only model indicated that translocated snake survival during the spring and summer active season might have been higher than during the quasi-winter season (spring/summer survival estimate = 0.47, SE ± 0.21, lower CI =0.15, upper CI = 0.81; quasi-winter survival estimate = 0.25, SE ± 0.16, lower CI = 0.06 , upper CI = 0.64), but the effect size showed that the survival estimates were not significantly different (effect size = 0.21, SE ± 0.26, lower CI = -0.30, upper CI = 0.73).

5.6.1.2 Movement Behavior

To determine whether soft-released, hard-released, and resident Eastern Massasaugas had different movement behaviors during most of the spring and summer active season post-release (i.e., from date of first release to August 19th when radio-tracking was regular), we compared four different movement metrics: (1) maximum dispersal distance (m) from release site after 1, 2, 4, and 8 weeks, (2) mean distance moved per day (m), (3) 100% MCP activity range size (ha), and (4) activity range length (m). There were totals of 616 spring/summer active season tracking events for resident snakes, 333 for soft-released snakes, and 430 for hard-released snakes. There were insufficient data to statistically compare dispersal distances for females among treatments for all but the 2-week time frame. There were no significant differences in maximum dispersal distances from release sites after any number of weeks between any treatments within a sex class (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: $\chi2(2) = 0.43$, $P = 0.81$), although the means and medians for soft-released groups were higher than those of hard-released and resident snakes in almost all instances (Figure 7). Sample sizes were also too small to evaluate any of the remaining movement behavior metrics for females or gravid females (i.e., $n < 2$ for all but hard-released gravid females). There were 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 ($\chi2(2) = 0.44$, $P = 0.80$), although soft-released male means and medians were higher than hard-released or resident snakes for all metrics except mean distance moved per day (Figure 7).

Four translocated snakes homed to within 300m of their capture locations, and all of them were released at the second soft release enclosure. One was a soft-released gravid female who homed in the fall approximately 134 days post-enclosure release after giving birth within 300m of the enclosure during late summer. The other three snakes that homed were hard-released males, and they did so after 20, 52, and 163 days post-release, although they may have been within their established summer activity ranges before returning to within 300m of their capture locations, which are assumed to have been relatively close to their hibernacula. The hard-released male that returned within 52 days post-release was within 300m of his capture location for approximately 3 days, although he may not have realized it because he backtracked his movements and ultimately overwintered less than 100m from the second soft release enclosure. Four of six soft-released snakes (66.7%) and four of seven hard-released snakes (57.1%) that were released at the second enclosure in 2016-17 and survived to overwintering used hibernacula within 300m of the enclosure. Of the soft-released snakes that returned, two were males, one was female, and one was a gravid female; of those that did not return, one was a male and one was a gravid female. The male that did not return did not home, and the gravid female that did not return was the one that did home. Of the hard-released snakes that returned, one was male (the aforementioned snake that homed), one was female, and two were gravid females; all three of those that did not return were males. Of these three males that did not return, one still overwintered on the core study area 464m from the enclosure, and neither of the other two homed.

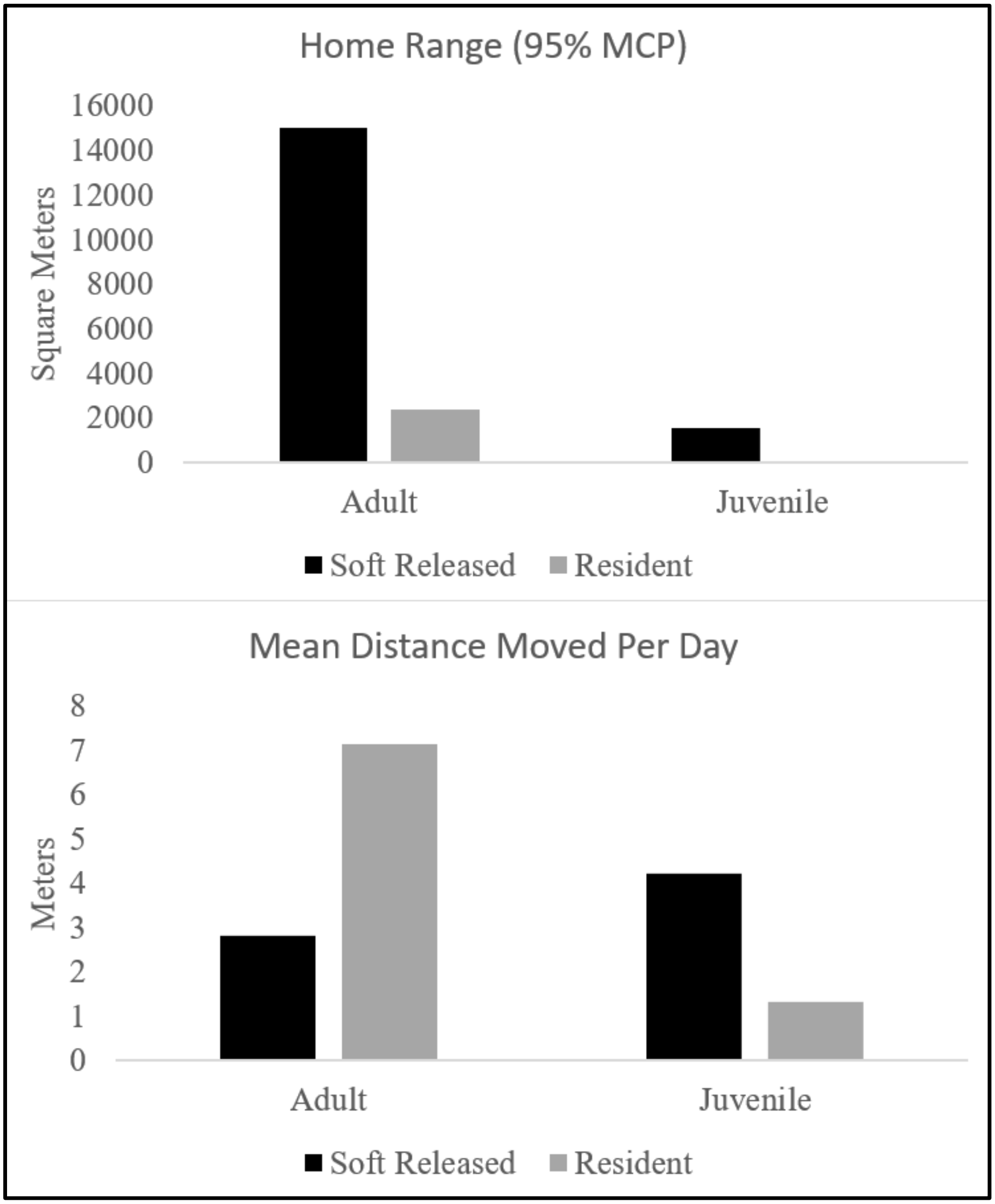

Figure 7. Home range size (A) and average distance moved per day (B) of adult and juvenile Texas Horned Lizards (*Phrynosoma cornutum*) that have either been soft released or are resident at the site. We tracked 17 resident adults and 6 soft-released adults and 47 resident juveniles and 17 soft-released juveniles.

5.6.2 Soft release of Texas Horned Lizards

We tracked 87 Texas Horned Lizards from 2016 – 2018. We soft released 23 Texas Horned Lizards (6 adults and 17 juveniles) and tracked 64 residents (17 adults and 47 juveniles) in the same area accumulating 1,041 tracking events for the 87 individuals. We constructed two different soft release pens. Animals were held within pens for approximately 2 weeks before being released at the study site.

Survival analyses indicated that resident adult lizards had far higher survival than soft-released adults. The estimated annual survival rate of soft-released lizards was only 5% compared to estimated survival of 57% for residents at this site. The estimate survival rate of 5% was statistically similar to that reported for hard-released lizards from a previous study at this site (16% survival). Our results suggest that adult lizards fare poorly when translocated at this site.

However, soft-released juveniles had remarkably high annual survival (55%) statistically similar to residents (29%). The statistically similar estimate survival rates for soft-released and resident juveniles indicates that soft release may be a viable strategy for this age class. 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.

Soft released juveniles move 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 Figure 7).

5.6.3 Environmental enrichment of Eastern Box Turtles

We successfully hatched and reared 32 Eastern Box Turtles. Half were raised in enriched captivity and half in unenriched captivity. All turtles that hatched survived in captivity until release. Half of the turtles were released after approximately 10 months in captivity and the other half of the turtles were released after an additional year of captivity. We successfully attached radio transmitters to each of the turtles and tracked them throughout at least one active season until hibernation. Four turtles (two in each treatment) released in 2017 that survived into 2018 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 8).

In cohort 1, BCI (body condition index) did not differ between enriched and unenriched turtles 1-month post-release (*P* = 0.26). Because few individuals survived 3 months post-release in cohort 1, we lacked statistical power to compare BCI between treatments. In cohort 2, BCI did not differ between enriched and unenriched turtles 1 month (*P* = 0.19) or 3 months post-release (*P* = 0.24).

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 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; Figure 9).

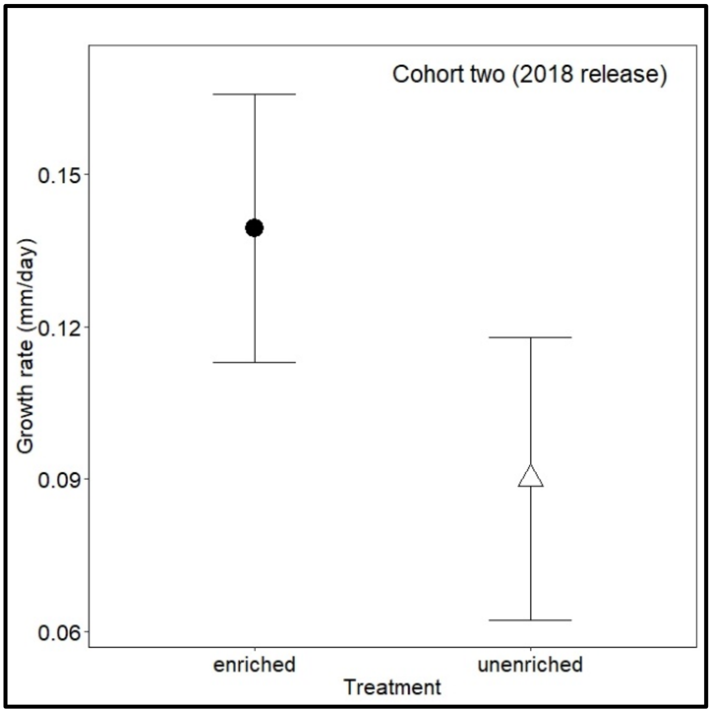

Figure 8. Post-release growth rates for enriched (n = 10) and unenriched (n = 10) Eastern Box Turtles released at Fort Custer Training Center, MI when 21 months old. Enriched turtles from cohort 2 (21 months in captivity before release) grew faster in the wild than unenriched turtles from the same cohort suggesting that enrichment better equipped turtles to forage in the wild relative to unenriched conditions.

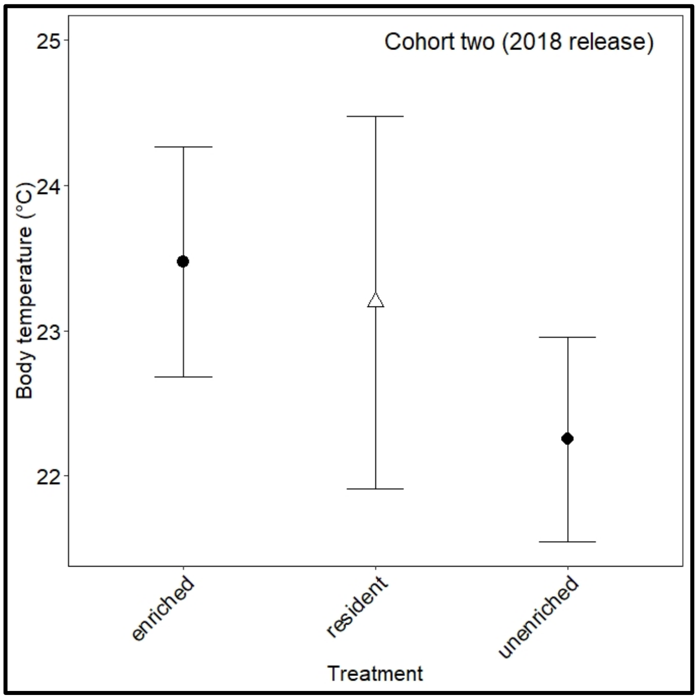

Figure 9. Mean carapace temperatures of enriched (n = 10) and unenriched (n = 10) juvenile Eastern Box Turtles released at Fort Custer Training Center, MI when 21 months old compared to resident juvenile turtles (n = 4). Enriched turtles maintained temperatures closer to that of resident turtles suggesting that enrichment better prepared turtles for thermoregulatory challenges in wild conditions.

In general, our observed survival rates were higher than anticipated and higher than has been reported for similarly aged box turtles (Altobelli 2017). Furthermore, survival for cohort 2 was considerably higher than in cohort 1. The constant survival (i.e., null) model was the most supported for survival of cohort 1, the predictor "treatment" received some support, but "time" and the interaction of "time and treatment" received none (Table 6). 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 unapparent later in the season when turtle activity declined and both groups experienced similar survival (Figure 10). All four turtles that hibernated at the site survived until the following spring emergence.

In cohort 2, four of 10 enriched and six of 10 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 indistinguishable (Figure 10). The constant survival (i.e., null) model was the most supported for survival of cohort 2, the predictor "treatment" received some support, but "time" and the interaction of "time and treatment" received none (Table 7).

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 (Figure 11).

Table 6. Model selection results for predicting survival of head-started Eastern Box Turtles (*Terrapene carolina*) released on Fort Custer Training Center, MI when 9 months old (cohort 1).

Model	ΔAICc	AICc weight	Deviance
S(.)	0.00	0.72	57.34
S(g)	1.93	0.28	57.20
S(t)	16.64	0.00	35.36
S(g*t)	68.45	0.00	28.09

ΔAICc is the difference in AICc values from a given model to the top model.
AICc weight shows the relative likelihood a given model is the most supported.
Deviance provides a relative measure of goodness of fit.
The notation (g) refers to treatment group (enriched or unenriched), (t) refers to time, the asterisk signifies an interaction, and (.) refers to the constant (i.e., null) model.

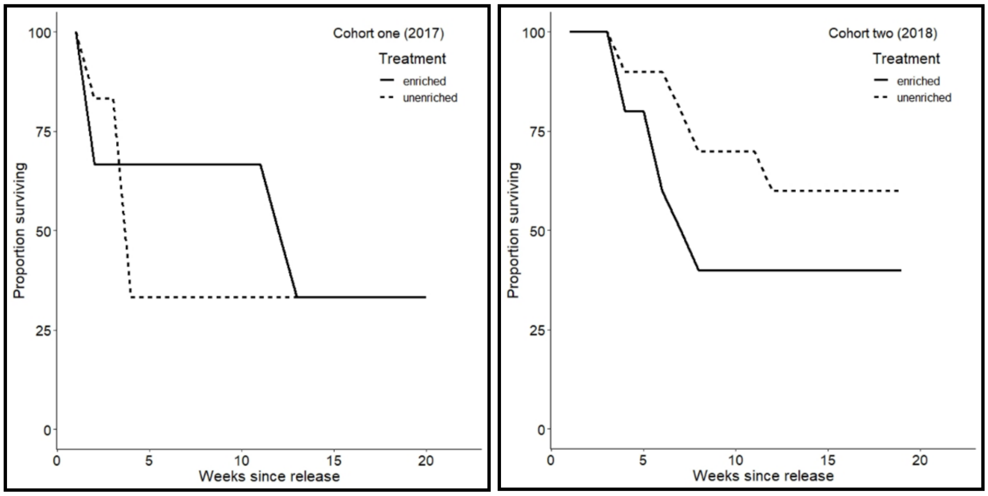

Figure 10. Proportion of juvenile Eastern Box Turtles surviving over time post-release based on being raised in enriched or unenriched conditions for either nine months (left panel, n = 12) or 21 months (right panel, n = 20) before release at Fort Custer Training Center, MI.

Table 7. Model selection results for predicting survival of head-started Eastern Box Turtles (*Terrapene carolina*) released on Fort Custer Training Center, MI when 21 months old (cohort 2).

Model	ΔAICc	AICc weight	Deviance
S(.)	0.00	0.61	29.76
S(g)	0.93	0.39	28.67
S(t)	22.49	0.00	8.72
S(g*t)	67.28	0.00	0.00

ΔAICc is the difference in AICc values from a given model to the top model.
AICc weight shows the relative likelihood a given model is the most supported.
Deviance provides a relative measure of goodness of fit.
The notation (g) refers to treatment group (enriched or unenriched), (t) refers to time, the asterisk signifies an interaction, and (.) refers to the constant (i.e., null) model.

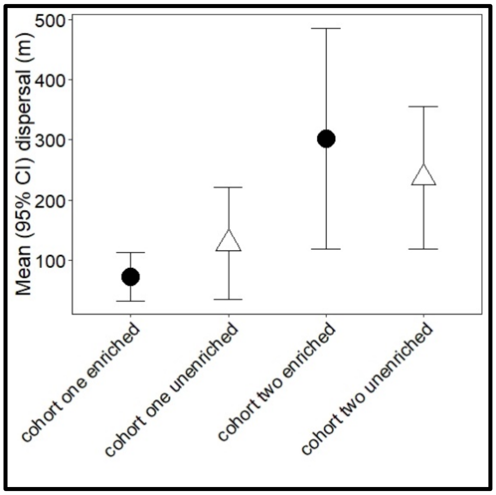

Figure 11. Average dispersal distance of enriched and unenriched juvenile Eastern Box Turtles released when 9 months (cohort 1) or 21 months old (cohort 2). Six turtles in each treatment were released in cohort one and 20 individuals in each treatment were released in cohort two.

6.0 PERFORMANCE ASSESSMENT

6.1 SOFT RELEASE OF EASTERN MASSASAUGA

PO1: Increase survival of soft-released snakes: To test this performance objective we used Program MARK known-fate models (Version 8.2; White and Burnham 1999) to estimate the annual survival of soft-released, hard-released, and resident control Eastern Massasaugas. We used Akaike's Information Criterion corrected for small sample sizes (AICc; Akaike 1998, Burnham and Anderson 2002) to rank candidate models within Program MARK to determine whether survival differed between the treatments. We calculated weekly survival intervals for each snake tracked and averaged across the treatments. We found that resident snakes had higher survival rates than either hard- or soft-released translocated snakes and that there was no measurable difference in survival between soft- and hard-released snakes. The model-averaged survival estimates for resident, soft-, and hard-released Eastern Massasaugas were 0.72 (SE ± 0.21, lower CI = 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).

PO2: Decrease dispersal behavior of soft-released snakes: To test the post-release movement behavior of soft- and hard-released snakes in relation to each other and in relation to resident snakes we calculated 100% MCPs (Jennrich and Turner 1969) for each snake using Quantum GIS (Version 2.18.13; Quantum GIS Development Team 2017). We averaged MCPs for each snake within each treatment (resident, soft release, and hard release). Using RStudio (RStudio Team 2016), we tested the assumptions of normality and homogeneity of variances, and, if they were not met, data were transformed to approximate normal distributions or homogeneity of variances. We then compared average MCP between the three treatments using Kruskal-Wallis median tests with appropriate post-hoc tests to compare means if these assumptions were not met after transformation. For all tests, $\alpha = 0.05$ unless stated otherwise. We found no statistical differences in the home range size of any of the three treatments ($\chi2(2) = 1.10$, $P = 0.58$).

PO3: Integration of translocated individuals into resident population. For this performance object we relied on behavioral observations conducted at each radio telemetry location. We categorized the behavior of each snake tracked at the time of the tracking event. When we observed snakes interacting with other snakes, we categorized these interactions as either antagonistic male-male combat interactions or courtship and mating. Our goal with this performance objective was simply to confirm that translocated snakes engaged in courtship and mating with resident snakes at the release site and to qualitatively compare the frequency with which hard-released and soft-released snakes interacted with residents. We observed nine instances in which translocated snakes were mating or engaged in courtship with resident snakes, which indicated that we had met the goals of the performance objective. Furthermore, in six of the nine observed instances, soft-released snakes were mating with residents and hard-released snakes were observed in only three of the nine instances.

6.2 SOFT RELEASE OF TEXAS HORNED LIZARDS

PO1: Increase survival of soft-released lizards: To test this performance objective we used Program MARK known-fate models (Version 8.2; White and Burnham 1999) to estimate the annual survival of soft-released and resident control Texas Horned Lizards. We used radio telemetry data recorded between 3 and 5 times per week to calculate annual survival rates for each tracked lizard.

We followed similar procedures from other studies done on Tinker AFB to compare our results to previous tracking and translocation efforts. We qualitatively compared survival rates of soft-released and resident animals. Because annual survival rates are often very different for adults and juveniles of this species, we calculate annual survival rates for juveniles (hatchlings and 1-year olds) and adults. We found that resident adult lizards had higher annual survival rates (57%) than soft-released adults (4%). Previous studies at this location used hard release translocation and documented a 16% annual survival rate for these lizards. This rate was qualitatively similar to what we recorded for soft-released lizards, and both measures are below those of resident lizards. However, annual survival rates of juvenile lizards that were soft released were higher than resident juveniles (55% vs. 35%) suggesting that soft release confers a survival advantage to juvenile lizards but not adults.

PO2: Decrease dispersal behavior of soft-released lizards. To compare the post-release movement behavior of soft-released and resident lizards at this site we calculated home range estimates for each individual (95% MCP) and movement rates (mean distance moved per day). We calculated averages for adults and juveniles separately and compared the mean differences between resident and translocated lizards using t-tests. We found no difference in home range size between soft-released and resident adults (Chi Square = 0.17, df = 1, $P = 0.68$), but there was a difference for juveniles (Chi Square = 9.17, df = 1, $P = 0.003$), with translocated juveniles occupying more space than resident lizards. Soft-released adults moved similarly to residents (Chi Square = 1.75, df = 1, $P = 0.19$) but juveniles moved more per day than resident juveniles (Chi Square = 10.21, df = 1, $P = 0.001$).

PO3: Integration of translocated lizards into population: For this performance objective we relied on behavioral observations conducted at each radio telemetry location. We categorized the behavior of each lizard tracked at the time of the tracking event. When we observed lizards interacting with other lizards, we categorized these interactions as courtship and mating or egg laying. We rarely observe lizards mating at this study site, although we frequently observe females nesting and by obtaining daily weights on the female lizards as the laying season approaches, we can determine if they are gravid or not. Our goal with this performance objective was simply to confirm that translocated lizards engaged in courtship and mating with resident lizards at the release site, or that they nested at the site, thereby potentially contributing to future generations and to the establishment of a population at the new site. While we never observed mating of our soft-released lizards, at least one translocated adult lizard nested at the new location. Unfortunately, survival of translocated lizards was very low so our opportunity to document gravidity was limited.

6.2.1 Environmental enrichment of Eastern Box Turtles

PO1: Use environmental enrichment to improve growth rates and body condition. To calculate post-release growth rate (mm/day) for each turtle, we used the first and last straight carapace length measurements for each individual divided by the number of days between measurements. We compared growth rate between enriched and unenriched turtles in each cohort using a general linear model with $\alpha = 0.05$. In cohort 1 (turtles released when 10 months old), growth rates 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 ($n = 10$) ($P = 0.01$).

To calculate a BCI for each turtle, we used the residuals of log body mass and log length for each turtle 1 and 3 months post-release (if an individual survived that long). We then compared BCI between enriched and unenriched turtles in each cohort using a t-test. In cohort 1, BCI (body condition index) did not differ between enriched and unenriched turtles 1-month post-release ($P = 0.26$). Because few individuals survived 3 months post-release in cohort 1, we lacked statistical power to compare BCI between treatments. In cohort 2, BCI did not differ between enriched and unenriched turtles 1 month ($P = 0.19$) or 3 months post-release ($P = 0.24$).

PO2: Use environmental enrichment to increase thermoregulatory efficiency of released turtles. Each time we radio-tracked turtles, we used an infrared thermometer to record the carapace temperature of each turtle. We then compared average carapace temperatures between enriched and unenriched turtles in each cohort using a linear mixed model. We used treatment as a fixed effect and turtle ID as a random effect to control for repeated temperature measurements of individuals. Next, we compared each average temperature to the reported preferred temperature range for the species reported in the literature (approximately 25 °C; do Amaral et al. 2002). 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 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 ($P = 0.71$).

PO3: Use environmental enrichment to improve annual survival rates. To compare survival rates between enriched and unenriched box turtles we generated survival estimates for enriched and unenriched turtles in each cohort using known-fate models in Program MARK (Version 8.2; White and Burnham 1999). We used Akaike's Information Criterion corrected for small sample sizes (AICc; Akaike 1998, Burnham and Anderson 2002) to rank candidate models within Program MARK to determine whether survival differed between the treatments. We calculated weekly survival intervals for each turtle tracked and averaged across the treatments. In general, our observed survival rates were higher than anticipated and higher than has been reported for similarly aged box turtles (Altobelli 2017). Furthermore, survival for cohort 2 was considerably higher than for 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 diminished later in the season when turtle activity declined and both groups experienced high survival. In cohort 2, four of 10 enriched and six of 10 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 indistinguishable.

PO4: Use environmental enrichment to improve overwinter survival. For this performance objective, we intended to use known-fate models in Program MARK (Version 8.2; White and Burnham 1999) with our timeframe reduced to only the overwinter period. However, analyses were unnecessary because 100% of the turtles that entered hibernation survived until the spring. We currently are monitoring 10 turtles through this winter and will use the proposed analyses when they emerge from hibernation in the spring.

7.0 COST ASSESSMENT

7.1 COST MODEL

Table 8. Cost model for construction of open-topped 0.1 ha release pen.

Cost Element	Data Tracked	Formula	Soft Release	Total Cost
Material Costs	Aluminum Flashing (115 m)	Quantity X unit cost	8 X $42.68 =	$ 341.44
	Wooden Stakes ~50	Quantity X unit cost	2 X $14.48	$28.96
	Tools (shovel, pickaxe, hammer)	Cost	$45.00	$45.00
	Plastic nail caps (1000)	Quantity X unit cost	$25.98	$25.98
				$441.38
Installation Costs	Labor, time	Labor X Hours	$15.00 hr X 20 hr	$300.00
Maintenance	Repairs per year, labor, time, material	Repairs X Labor rate X Hours + Material	$15.00 hr X 10 hr + $57.16	$207.16
Pen Lifetime	Survival of pen	# years pen was functional X annual repair costs	2 yrs. X 207.16	$414.32
Total Cost to Build				$741.38
Cost for 2 yrs. of use				$948.54
Cost for 3 yrs. of use				$1155.70
Cost Per Snake	Total Cost, # snakes released	Total Cost / # snakes soft released	$1155.70 / 16	$72.23

Materials. The soft release pens used in our demonstrations were simply constructed enclosures of 40cm aluminum flashing encircling an area of approximately 0.1 ha. Construction of these pens requires very little expertise. The most time and labor intensive step in the construction process is digging a 5cm to 10cm deep trench around the entire footprint of the enclosure. Then placing the aluminum flashing in the trench, back filling, and installing wooden stakes every 3m to 5m to hold the flashing upright. Because the species chosen to demonstrate soft release are small-bodied and are not capable climbers, these structures were escape proof and all of the resource needs of the species (water, food, shelter, sun) were provided naturally within each enclosure. All of the equipment needed was easily purchased at commercial hardware stores and the prices we used were through General Services Administration (GSA) suppliers.

Installation: Installation of a 0.1 ha enclosure took approximately 20 hrs of labor. With a crew of five people, we finished installing the pen in half a day. We are far from skilled labor and most of the crew consisted of hourly field technicians hired to track the snakes on release.

Maintenance: We found that the aluminum flashing would come up from the ground creating areas for animals to escape. Far more seriously, each winter that the pen was in the woods, trees would fall and crush sections of the aluminum flashing. Each spring we found that we needed to dedicate approximately 10 hrs to fixing the pens and ensuring that the walls were sunk into the ground. Maintenance typically entailed cutting smashed sections of fencing out and replacing it with fresh sections. Maintenance could be accomplished with one to two people. Often, the pen required no maintenance throughout the active season and maintenance was only required in the spring to repair damage caused by winter storms and weathering.

Pen Lifetime: Our goal was to ensure that the pen we constructed could be used over the 3-year lifespan of the demonstration. We found that with minimal maintenance, this was certainly possible. The pen remains at Camp Grayling and will likely be used in future studies.

Table 9. Cost model for construction of closed-top 0.05 ha release pen.

Cost Element	Data Tracked	Formula	Soft Release	Total Cost
Material Costs	Aluminum Flashing (56 m)	Quantity X unit cost	4 X $42.68 =	$170.72
	Wooden Stakes ~25	Quantity X unit cost	1 X $14.48	$14.48
	Tools (shovel, pickaxe, hammer)	Cost	$45.00	$45.00
	Plastic nail caps (1000)	Quantity X unit cost	$25.98	$25.98
	Wildlife netting	Quantity X unit cost	4 X $15.75	$63.00
				$319.18
Installation Costs	Labor, time	Labor X Hours	$15.00 hr X 20 hr	$300.00
Maintenance	Repairs per year, labor, time, material	Repairs X Labor rate X Hours + Material	$15.00 hr X 10 hr + $57.16	$207.16
Pen Lifetime	Survival of pen	# years pen was functional X annual repair costs	2 yrs. X 207.16	$414.32
Total Cost to Build				$619.18
Cost for 2 yrs. of use				$826.34
Cost for 3 yrs. of use				$1033.50
Cost Per Lizard	Total Cost, # lizards released	Total Cost / # lizards soft-released	$1033.50 / 23	$44.93

Materials. The soft release pens used in our demonstrations were simply constructed enclosures of 40cm aluminum flashing encircling an area of approximately 0.05 ha. Construction of these pens requires very little expertise. The most time and labor intensive step in the construction process is digging a 5 cm to 10 cm deep trench around the entire footprint of the enclosure. Then placing the aluminum flashing in the trench, back filling, and installing wooden stakes every 3m to 5m to hold the flashing upright. Because the species we chose to demonstrate soft release with are small-bodied and are not capable climbers, these structures were escape proof and all of the resource needs of the species (water, food, shelter, sun) were provided naturally within each enclosure. All of the equipment needed was easily purchased at commercial hardware stores and the prices we used were through GSA suppliers.

Installation: Installation of a 0.05 ha enclosure took approximately 20 hrs of labor. With a crew of two people, we finished installing the pen in a full day. The difference in the pens constructed for release of lizards was that we needed to protect the enclosed lizards from avian predators who could pluck them out of the pen. We purchased netting used to prevent deer from browsing garden foliage. This was available in local hardware stores and was easily affixed over the pen creating a ceiling and barrier to avian predators. Additionally, this pen was comparatively more difficult to construct than the release pen for massasaugas since it was placed in clay substrate with deep prairie plant root systems.

Maintenance: We found that the aluminum flashing would come up from the ground creating areas for animals to escape. Far more seriously, each winter that the pen was in the woods, trees would fall and crush sections of the aluminum flashing. Each spring we found that we needed to dedicate approximately 10 hrs to fixing the pens and ensuring that the walls were sunk into the ground. Maintenance typically entailed cutting smashed sections of fencing out and replacing it with fresh sections. Maintenance could be accomplished with one to two people. Often, the pen required no maintenance throughout the active season and maintenance was only required in the spring to repair damage caused by winter storms and weathering. We did lose an entire pen to a tornado between year 1 and year 2 of the demonstration indicating that leaving pens in the field throughout the year can have risks and entailed higher costs if those risks are experienced.

Pen Lifetime: Our goal was to ensure that the pen we constructed could be used over the 3-year study period.

Table 10. Cost model for 1 year of environmental enrichment vs. unenriched captivity and maintenance for 32 Eastern Box Turtles (half in enriched, half in unenriched).lifespan of the demonstration. We found that with minimal maintenance, this was certainly possible (assuming it is not taken by a tornado).

Cost Element	Data Tracked	Formula	Enriched	Unenriched
Material Costs: Initial Setup	Containers / Housing	Quantity X unit cost	$92.29 X 4 = $369.16	$15.00 X 16 = $240.00
	Substrate	Quantity X unit cost	Coconut fiber: $14.99 X 4 = $59.96	Reptile carpet: $7.99 X 16 = $127.84
	Plants, Shelter Caves, and other Decorations	Quantity X unit cost	Plants and shelters: $25.00 X 4 = $100	shelters: $5.00 X 16 = $80
	Food and Water Dishes	Quantity X unit cost	Not Applicable	Dishes: $2.00 X 16 = $32.00
	Total Setup Costs	Sum of Costs / # turtles	$529.12 ($33.07 / turtle)	$479.84 ($29.99 / turtle)
Annual Material Replacement	Containers replaced annually	# replaced annually X cost	Never. $0/yr	$15.00 X 16= $240.00
	Substrate replacement annually	# replaced X cost	4X per year: $179.98	2X per year= $127.84
	Total annual replacement costs		$179.98	$367.84
Maintenance and Daily Husbandry	Hours, labor rate	Hourly rate X # hours per day X 365	$15/hr X 0.5 hrs day X 365 = $2737.50 yr	$15/hr X 1.5 hrs day X 365 = $8212.50
Food and Water	Total cost of all meal worms, worms, produce, and chow	Total amount spent over year	$1250/yr	$1250/yr
Total Annual Cost	All listed costs	Setup + Replacement + Husbandry + Food	$4696.60	$10,310.18
Total Cost per Turtle	Total Cost, # turtles released	Total Cost / # Turtles soft released	$293.53	$644.38

We conducted a direct comparison between raising turtles in enriched and unenriched captivity. We assumed that environmental enrichment would be less cost effective than standard captivity because of the additional accessories needed to set up a challenging environment. We did not expect the large time discrepancy in day-to-day maintenance between environmental enrichment and unenriched captivity. The following paragraphs break down the specific cost elements.

Materials—initial setup: We found that environmental enrichment setups were more costly than standard enclosures. However, this difference was less than expected and was mitigated by the fact that turtles in enriched captivity were communally housed whereas those in unenriched captivity were individually housed and thus this treatment ended up taking up more space and requiring a full complement of enclosures. The initial purchase of 50-gallon stock tanks for the enriched turtles was expensive but they never needed to be replaced and can likely be used for decades. To house 16 enriched turtles, we only had to purchase four of these large stock tanks. Comparatively, unenriched turtles were housed individually so we needed to purchase 16 plastic tubs to house an equal number of unenriched turtles. Furthermore, we found that the plastic tubs used to house unenriched turtles became very brittle from repeated washing and needed to be replaced at least every 6 months, making them more expensive than anticipated. Overall, environmental enrichment cost, on average, only $3.00 per turtle more to set up than standard captivity. If communal housing is a desired component of enrichment, practitioners may realize substantial cost savings in the setup of captive enclosures.

Materials—replacement: The most expensive replacement item for enriched containers was the coconut fiber we provided as a substrate. We provided a deep layer of this substance to encourage turtles to burrow and hunt food while submerged in a substrate. We needed to replace this material every 3 months as food items that were scattered and not eaten could begin to rot. In the unenriched enclosures, the plastic tubs themselves were costly to replace approximately every 6 months when they became brittle from cleaning and holding water for extended periods. Combined with the need to replace the reptile carpet substrate at least once every 6 months as it became brittle or rotten, the annual replacement costs for unenriched containers exceeded those for the more elaborate enrichment tubs. We suggest that practitioners that shy away from enrichment due to the initial costs look beyond this period and evaluate the recurring costs that may be higher across multiple enclosures.

Maintenance and Husbandry: Our husbandry routine consisted of daily spot cleanings and weekly full-cleanings. When averaged across the week, it took only 30 min to clean each of the four enrichment tubs and approximately 90 minutes to clean each of the 16 unenriched tubs. The more complex but easier to clean and monitor enrichment tubs saved us a lot of time and money. Over the course of the year, we estimate that the labor required to clean and monitor unenriched tubs was exactly three-fold what was needed to maintain enrichment tubs. We estimated labor at approximately $15/hr, which is the hourly rate of a graduate research assistant and is slightly higher than the hourly student technicians ($12/hr) we hired to assist the graduate student. Federal employees would cost substantially more to maintain. Consequently, the cost difference between enriched and unenriched husbandry would grow even more dramatically in favor of enriched husbandry.

7.2 COST DRIVERS

7.2.1 Soft release

The cost drivers of soft release will almost be entirely driven by the sophistication and size of the pen needed. Our demonstration chose herpetofauna that need relatively small areas of confinement and that are easily confined within simple, aluminum flashing walls. Soft release endeavors focusing on reintroducing large, free-roaming animals such as many mammal species or difficult-to-contain species such as birds will find that their costs and logistics will rapidly expand. Rather than relying on field technicians and graduate, which are inexpensive, practitioners would likely resort to using contractors to build professional quality enclosures. Although enclosures build in such a manner would last for many years, which would reduce their lifetime costs, such enclosures would entail a large upfront cost that many translocation projects would be unable to afford.

7.2.2 Environmental enrichment

The cost drivers of environmental enrichment are likely: (1) the size and complexity of enrichment enclosures, (2) the level of expertise needed for husbandry of the focal species, and (3) the space requirements for rearing the focal species. Our focal species, the Eastern Box Turtle, was easily housed within a single greenhouse bay using only two work benches. We could easily scale this operation up with relatively few additional needs or requirements. However, for larger bodied species that may require large enclosures, husbandry professionals or zoos may be necessary. The contracts associated with bringing in professional husbandry would be substantial. Similarly, our focal species was relatively easy to maintain. Some species may have specialized diets or other needs that may be difficult or impossible to provide in captivity. Other species may be easily stressed in captivity. Most herpetofauna make good candidates for captive rearing due to their small area needs and ease to maintain. Many species of large-bodied mammals or birds will require significantly more resources that may be beyond the ability of installation or university biologists to provide. While the species we chose is an attractive option for environmental enrichment we believe that many threatened and endangered and at-risk species occurring on DoD facilities would be ideal candidates for cost-effective environmental enrichment including most plants, fish, invertebrates, small mammals, amphibians, and reptiles.

7.3 COST ANALYSIS AND COMPARISON

The ultimate goal of wildlife translocation is the establishment of a self-sustaining population at the release site. Our demonstration is not advocating for translocation but rather for replacing traditional translocation methods (hard release and/or traditional captive rearing) with soft release and environmental enrichment. Because we operate under the premise that translocation actions are already occurring at a site we avoid the calculation of costs associated with surveying for animals to be moved as well as the costs associated with post-release monitoring because those costs, equipment, and needs are identical regardless of the translocation technique used (the novel techniques demonstrated here or traditional techniques).

Soft Release of Eastern Massasauga: The primary cost driver of soft release is the labor and raw equipment required to build and maintain a soft release pen. If we assume that the goal is to establish a population of 100 adult Eastern Massasauga at a site over 3 years and that survival of soft-released snakes is approximately 40%, we would need to translocate 250 snakes. The cost to build a single 0.1 ha pen and maintain it for 2 subsequent years is $1156 (Table 8). The number of pens required will depend on how many animals can comfortably be maintained within a single pen and how synchronized their capture and release are. Ideally, an operational scale reintroduction project would rely on multiple pens to spread out snakes on release to reduce density and density dependent effects. If we assume that a single reintroduction site could support the presence of three pens and that these pens could each be maintained for the duration of the project, the total pen cost would be approximately $3468. If each pen contained snakes for a maximum duration of 1 month before release, each pen could release three cohorts of snakes per year (May, June, and July). Density within a single pen would be approximately 9-10 snakes at a given time and this density could be reduced in half if one were to reduce the animals' duration in pens by half (the protocol we adhered to for this demonstration). Practitioners could also provide resources such as mice and water to offset any detrimental competition related effects. Because this species of snake is not territorial, a temporary density this high would be unlikely to be detrimental to the individuals. In this scenario, with approximately 82-83 snakes being soft released per year out of three pens over 3 years, we are looking at a cost of $14 per snake above what it would cost to hard release them. Because hard-released snakes at this site have a statistically equivalent survival rate, the same number would need to be hard released.

Soft Release of Texas Horned Lizards: The primary cost driver of soft release is the labor and raw equipment required to build and maintain a soft release pen. If we assume that the goal is to establish a population of 100 Texas Horned Lizards at a site over 3 years and that survival of soft-released juvenile lizards (which is higher than adults) is approximately 55%, we will need to translocate 181 juvenile lizards. The cost to build a single 0.05 ha pen and maintain it for 2 subsequent years is $1034 (Table 9). The number of pens required will depend on how many animals can comfortably be maintained within a single pen and how synchronized their capture and release are. Ideally, an operational scale reintroduction project would rely on multiple pens to spread out lizards on release to reduce density and density dependent effects. If we assume that a single reintroduction site could support the presence of three pens and these pens could each be maintained for the duration of the project, the total pen cost would be approximately $3102. If each pen contained lizards for a duration of 1 month before release, each pen could release three cohorts of lizards per year (May, June, and July). Density within a single pen would never exceed seven lizards at a given time. Because juvenile Texas Horned Lizards are not territorial and naturally occur in high densities near nesting sites, this level of density would be easy to maintain and would be unlikely to have detrimental effects on enclosed lizards. In this scenario, with approximately 60 lizards being soft released per year out of three pens over 3 years, we are looking at a cost of $17 per lizard above what it would cost to hard release them. Because hard-released lizards at this site experience a much lower survival rate (on average 16%), a concurrent translocation study would need to hard release 625 lizards to establish a population of 100. This would come with increased costs associated with finding the lizards for translocation and any costs associated with post-release monitoring.

Environmental Enrichment of Eastern Box Turtles: If our goal is once again to establish a population of 100 turtles at a reintroduction site, we need to compare the costs associated with housing turtles in enriched vs. unenriched captivity for the duration of the project. We found that the overall survival of enriched and unenriched turtles was statistically similar (approximately 40% annual survival). However, there was a significant increase in survival if turtles were reared in captivity for 22 months as opposed to only 10 months. Thus, a reintroduction program should rely on the longer time in captivity to enhance survival on release and reduce the number of turtles released. A program looking to establish 100 turtles in an area with 40% annual survival would need to release 250 turtles. The average annual cost for an enriched turtle in our demonstration was $294. Therefore, each turtle would be reared for 2 years before release for a per turtle cost of $588. Rearing 250 individuals at $588 would cost $72,000. The total cost for a similar number of turtles raised via a traditional approach in unenriched captivity, in individual housing, with a similar survival estimate of 40% post-release would be $322,000.

8.0 IMPLEMENTATION ISSUES

8.1 SOFT RELEASE

Soft release pens should be situated in high quality habitat, be escape proof, predator-proof, and provide an opportunity to make translocated animals aware of the location of an important resource. The following paragraphs discuss each of these factors individually.

Habitat quality: The concept of soft release is to anchor translocated animals to an area. Obviously, practitioners should attempt to establish translocated animals in areas that lead to high survival rates and establishment of or integration into a population. Thus, soft release pens must be established in patches of high quality habitat. We suggest that before soft release is undertaken, resident animals of the same species are studied to understand the components of habitat that contribute to survival and increased fitness of the target species. Not only must practitioners understand the habitat that featured animals in this region seek out and choose, but they must understand how these factors contribute to improved fitness over standard or low quality habitat. Often, species will rely on different habitats to satisfy different requirements across their lives. For instance some animals switch between habitats throughout the season or their life according to their needs for foraging, predator avoidance, mating, nesting, or hibernating. Practitioners must understand these different needs and choose the location of a soft release pen accordingly. The timing of the release and the confinement should influence the habitat need that is chosen. Similarly, practitioners releasing large numbers of animals should keep in mind that there may be density-dependent costs. If more animals are released than an area's resources can support, both resident and translocated animals are likely to suffer (Osborne and Seddon 2012). Carrying capacity and density of resident animals and release sites should be kept in mind – this will be particularly important for large or territorial animals and is less of a consideration for most reptile species which are not territorial and have low resource needs.

Escape proof: Soft release pens should be escape proof, meaning that animals confined in pens are not able to leave until allowed to do so. This can be easier said than done. When confined, many wildlife species will travel the perimeter of the enclosure constantly looking for escape. Our anecdotal observations indicate that Eastern Massasauga reduced this perimeter travel after approximately 2 weeks of confinement. Presumably, animals constantly trying to escape will not settle in the area of the pen when released. However, if animals have settled down in the pen and have gained familiarity with the habitat features in the pen, they may be more likely to settle in similar habitat near the pen when they are allowed to disperse. Animals leaving the pen and dispersing before desired are less likely to become integrated into the local population.

Predator-free: For wildlife species that are small or particularly vulnerable to predators in the wild, soft release pens should be constructed to be predator-proof. Translocation endeavors are unlikely to be successful if animals are lost before release at the chosen site. Small-bodied animals such as Texas Horned Lizards should be confined in pens that prevent aerial predators or mesopredators from accessing them as they become acclimated to the release area.

Resource awareness: This aspect of soft release is similar to the habitat quality aspect. Practitioners using soft release have the opportunity to situate pens such that enclosed animals become aware of the presence of a particular resource. For many herpetofauna, the location of high quality nesting locations or overwintering locations can be of the utmost importance and determine whether a translocation effort is going to be successful or not. Thus, practitioners have the opportunity to force contact of enclosed animals with a resource. For instance, the use of particular hibernacula are necessary for the overwintering survival of Eastern Massasauga at our study location. We intentionally chose to place the soft release enclosure in an area containing many of the high quality overwintering burrows to ensure that snakes were aware of their presence. Prior research indicates that snakes translocated to sites without knowledge of suitable hibernacula suffered extreme overwinter mortality. We can imagine similar situations in which translocated animals must be made aware of particular food resources, nesting grounds, or other landscape features that can be incorporated into or adjacent to soft release pens.

8.2 ENVIRONMENTAL ENRICHMENT

Environmental enrichment is a fairly vague term that can entail providing captive animals with a wide-range of experiences or stimuli. It can refer to providing relatively modest stimulation in the form of a varied diet or a toy or a complex environment that simulates natural settings. The use of environmental enrichment to enhance wildlife translocation efforts should entail preparing the captive animals for survival on release. Thus, practitioners would benefit most from understanding the composition of the habitat of the release site, the behaviors that animals should display to enhance survival, and the factors most likely to lead to mortality after release.

Most wildlife translocation projects attempt to release the greatest number of individuals they can and are hampered by restricted budgets. Thus, enrichment that can be applied for modest expense are likely to be of the most utility. Thus, environmental enrichment that provides captive animals with a setting most similar to what they are likely to experience in the wild can prepare captive animals to maneuver, hide in, and forage in conditions similar to those in which they will be living on release. Although set-up costs of complex enclosures like this can be initially expensive, they can house groups of animals and can take up less space and require less maintenance than traditional housing (glass aquaria). Practitioners should strive to provide the habitat features that are of the most importance to survival on release. For instance, we ensured that enriched box turtles had a deep, loose substrate to burrow in because, when they are released, they spend most of their time deep in the leaf litter and duff layer where they avoid predators and forage for invertebrates.

Enrichment also provides the opportunity for practitioners to prepare captive animals for particular dangers they may face on release. For instance, in New Zealand, the presence of non-native predators is a critical challenge for many herpetofauna. Thus, practitioners are able to provide exposure to these novel predators in a way that prepares animals to avoid them on release and gives them a higher chance to survive in the wild in the presence of the predators. The application of environmental enrichment must be well-thought out and target the behaviors and stimuli most likely to lead to successful survival in the wild.

We should note our results might be site-specific and thus perhaps not generalizable to all study areas and systems. We were limited by the fact that we used a small number of release sites. This is often a consequence of limited habitat availability for imperiled species. Or at Tinker AFB, this is a consequence of having very little restored prairie available across the installation. Most translocations focus on imperiled species and thus, practitioners will often have very few suitable release sites available to them and the success of released animals will be influenced by site-specific factors.

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10.0 APPENDICES

Appendix A: Points of Contact

POINT OF CONTACT Name	ORGANIZATION Name Address	Phone Fax E-mail	Role in Project
Brian Huggett	Fort Custer Training Center 2501 26th St. Bldg. 2510 Augusta, MI 49012	269-731-6570, 269-731-6572, brian.w.huggett.nfg@mail.mil	Demonstration site liaison, Technology end-user
James Langerveld	Fort Custer Training Center 2501 26th St. Bldg. 2510 Augusta, MI 49012	269-731-6570, 269-731-6572, james.c.langerveld.nfg@mail.mil	Demonstration site liaison, Technology end-user
Jinelle Sperry	U.S. Army Corps Engineers: ERDC-CERL 2902 Newmark Dr. Champaign, IL 61822	217-373-4543 Jinelle.sperry@usace.army.mil	Co-PI
Bruce Kingsbury	Purdue at Fort Wayne Science Building G56 2102 E Coliseum Fort Wayne, IN 46805	260-481-5755 Kingsbur@ipfw.edu	Co-PI
Raymond Moody	U.S. Air Force Tinker AFB Natural Resources 72 ABW/CEIEC 7701 Arnold Street, B-1, RM 109 Tinker AFB, OK 73145	405-739-7065 raymond.moody@us.af.mil	Demonstration site liaison, Technology end-user
Michael Ravesi	Natural Resources Specialist Environmental Office Building 100A Camp Grayling Joint Maneuver Training Center Grayling, MI 49738	989-344-6175 michael.j.ravesi.civ@mail.mil	Demonstration site liaison, Technology end-user