INVESTIGATING THE SUITABILITY OF RELEASING CONFISCATED EASTERN BOX TURTLES (*TERRAPENE CAROLINA CAROLINA*) BACK INTO THE WILD

by

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(Under the Direction of Tracey D. Tuberville and Olin E. Rhodes)

ABSTRACT

Reptile and amphibian populations are drastically declining on a global scale. Many factors contribute to their risk of extinction, including unsustainable use due to the illegal wildlife trade. Confiscated turtles are often not returned to the wild due to concerns over disease or because their collection location is unknown. As more confiscations take place, there is urgency to evaluate whether seized turtles can contribute to conservation of wild populations, but post-release monitoring data are lacking. I radio-tracked 39 released confiscated eastern box turtles and 10 sympatric resident turtles for two years on the Savannah River Site (SRS), South Carolina. Confiscated turtles established home ranges, which were larger in Year 1 but comparable to residents in Year 2. Confiscated turtles had a 60.0% - 65.0% survival rate in Year 1 but their survival increased to 95.8% - 100% in Year 2. They also had high pathogen prevalence and complex coinfections.

INDEX WORDS: Translocation; Reintroduction; Reptile Conservation; Illegal Wildlife Trade, Eastern Box Turtle; Pathogens

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Reptiles and amphibians are declining globally due to habitat loss, invasive species, pollution, disease and parasitism, climate change, and unsustainable use (Gibbons et al 2000). Turtles are especially sensitive to these factors because of their delayed reproductive maturity, low juvenile survival, and reliance on high adult survival for population persistence (Congdon et al. 1993; Rhodin et al. 2018). This ancient taxon is currently made up of 360 species found around the world, 187 species (51.9%) are considered threatened, 127 species (35.3%) are endangered, and 7 species and 10 taxa of turtles and tortoises have gone extinct (Stanford et al. 2020).

Turtles are the most vulnerable major groups of vertebrates, even more so than birds, mammals, and fishes (Lovich et al. 2018), and wild individuals are often targeted and collected to serve as food or pets in the illegal wildlife trade (Turtle Conservation Coalition, 2011). Poaching has had a negative impact on the native populations of both aquatic and terrestrial turtles worldwide (Stanford et al. 2020). Although confiscations by law enforcement do occur, it is hard to determine what portion of the illegal trade these confiscations represent, as smugglers find inventive ways to hide exports, use false Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permits, and falsely claim wild-caught animals to be captive-bred ones (Rosen and Smith 2010). Thus, only a fraction of illegal shipments are likely detected. Even when turtles are seized, however, often they are not returned to the wild because their locality of origin is unknown, or due to the potential risk of introducing pathogens to

established wild populations (D'Cruze and Macdonald 2016). Confiscated turtles are instead placed in facilities or, when no holding facilities are available, euthanized. However, as illegal collection increases in frequency and more illegal exports are detected, there is a growing need to find a possible solution whereby confiscated turtles can be returned to the wild and contribute to the persistence of wild populations.

Translocation, the human-mediated movement of living organisms from one area for release in another (IUCN/SSC 2002), is one potential outcome for turtles confiscated from the illegal wildlife trade. Many considerations need to be addressed prior to actual translocations. These include knowledge of the original geographic locations of the confiscated turtles, availability of release sites, genetic knowledge, and concern for release of novel pathogens. As mentioned above, introduction of novel pathogens into wild populations is one major risk associated with translocation (Cunningham 1996; Kock et al. 2010). Both released animals and resident wild animals can be at risk of pathogen transfer associated with translocation (Cunningham 1996). Translocated animals may be immunologically naïve to pathogens that are endemic to the resident population. Released animals also may harbor novel pathogens, particularly confiscated animals – which are often held in captivity with other species and under poor conditions (IUCN, SSC 2002). Translocation can also cause stress, which can have negative effects on immunity, making stressed animals more vulnerable to disease, thus affecting their survival (Dickens et al. 2010). Pathogens or clinical disease can also affect movement patterns. For example, gopher tortoises (*Gopherus polyphemus*) with severe clinical symptoms associated with *Mycoplasma* infection had significantly larger home ranges and were more likely to travel large distances than uninfected tortoises or those with only mild clinical symptoms (McGuire et al. 2014). Dispersal from the release site is also the most common reason for failure of

amphibian and reptile translocations (Germano and Bishop 2009). Thus, post-release movement is an important criterion for evaluating translocation outcome. There have been only a few releases of confiscated turtles back to the wild and there is little to no post-release monitoring data to evaluate the outcome of those releases.

In August 2019, South Carolina Department of Natural Resources (SCDNR) seized 208 eastern box turtles (*Terrapene carolina carolina*) from the illegal wildlife trade. The University of Georgia's Savannah River Ecology Laboratory (SREL), SCDNR, and United States Forest Service-Savannah River (USFS) forged a partnership to release and monitor the seized animals on a protected area within the Savannah River Site (SRS). We affixed a subset of 40 adult turtles with radio transmitters to monitor their movement and survival for two years following release. We also radio-tracked 10 adult resident box turtles as a control. Our objectives for this research project are to evaluate the suitability of confiscated eastern box turtles for release back into the wild by comparing a suite of health and movement metrics between confiscated and resident eastern box turtles. Results from this study will help guide decisions regarding future confiscations of illegally collected turtles and how they can contribute to species conservation efforts.

Background and Literature Review

Vulnerability of Chelonians

Most turtles possess a suite of life history traits that make persistence of their populations heavily dependent on high adult survival. These traits typically include low annual reproductive rates, low juvenile survival rates, and delayed reproductive maturity (Iverson 1991, Congdon et al. 1993, Howell et al. 2019, Tetzlaff et al. 2020). Turtle populations persistence generally

depend on high adult survivorship—over 80% in some species (Iverson 1991). Both chronic increases in adult mortality and acute short-term mortality events can cause severe population declines. Mass mortality events can eliminate large portions of the population (Gasbarrini et al. 2021) and cause long-term population declines. For example, a disease outbreak reduced a flattened musk turtle (*Sternotherus depressus*) population by 50% within a year (Dodd 1988), and after a decade, the population still had not recovered to its original abundance (Bailey and Guyer 1998). Similarly, a population of common snapping turtles (*Chelydra serpentina*) experienced a three year period of unusually high predation by river otters during which survival decreased from 94% to 76% and 50% of the adult population died. Although annual survivorship over the next 23 years subsequently averaged 94% to 97%, the population continued to decline (Keevil et al. 2018). Thus, even naturally occurring mortality events can put a strain on turtle populations, which are unlikely to be able to sustain increased loss of adults resulting from harvest for the wildlife trade (CEC 2019).

Impacts of the Illegal Wildlife Trade

The illegal wildlife trade can be defined as all illicit activities accompanying the commercial exploitation and trade of wildlife (Sas-Rolfes et al., 2019). It is one of the largest forms of illegal trade and is a multi-billion-dollar industry that is steadily expanding globally (Sollund 2013), thereby threatening the world's biodiversity. Alongside habitat loss, overexploitation of wildlife is driving the global biodiversity crisis as it promotes the collection of wild animals and provides potential for disease spread and negative impacts from invasive species (Rosen and Smith 2010, Romero-Munoz et al. 2021). Reptiles are the second-most species rich taxa to be sold and exported in the international trade (legal and illegal trade; Bush

et al. 2014). Even the legal trade is poorly regulated and wild herpetofauna are heavily targeted. During 2000 – 2019, 59.2% of live reptiles from legal exports were wild-sourced, resulting in almost 12 million individuals removed from their native habitat. These numbers do not include illegal exports that were confiscated during the same period (Marshall et al., 2020). Reptiles represented over 20% of animals seized during 1999 – 2018 (UNODC/WWCR 2020). Chelonians are especially vulnerable and are becoming among the most trafficked species (Rhodin et al. 2018, UNODC/WWCR 2020).

When seizures do occur, there are often few options available for confiscated animals: 1) keep individuals in permanent captivity for the rest of their lives; 2) euthanize individuals; or 3) return individuals to the wild (IUCN, SSC 2002). Euthanasia and placing animals in captivity are identified as the most preferable options by the IUCN because returning individuals to the wild can come with complications such as high mortality rates, introduction of novel pathogens to native populations, and unknown genetic origin (IUCN, SSC 2002). While confiscated animals placed in captivity could potentially serve as breeding stock for future reintroduction efforts, few such captive facilities are available, and the need for captive facilities greatly exceeds current capacity. In earlier documents produced by IUCN (IUCN, 2002), euthanasia is recommended, but that is clearly not a satisfactory outcome given the greatly expanding problem of illegally poached animals from the wild. It also prevents confiscated animals from contributing to conservation of wild populations in any meaningful way as only 6% of confiscated animals are returned to the wild (Rivera et al. 2021). There is growing interest, however, in determining whether confiscated turtles can be safely released back into the wild and, if so, building the capacity and expertise necessary to do so (Collins 2021).

Confiscated turtles are only rarely reintroduced to the wild and few post-release monitoring results have been published in the literature. Wimberger et al (2009) released rehabilitated leopard tortoises (*Stigmochelys pardalis*) back into the wild. These individuals included escaped, unwanted pets or individuals from confiscations. This repatriation was considered to be unsuccessful as only three of the 25 turtles released survived after 25 months. Attum et al (2007) released two Egyptian tortoises (*Testudo kleinmanni*) seized from the illegal wildlife trade into their native habitat. Both individuals showed large dispersal rates and abnormal movements (Attum et al 2007). Attum et al. (2010) reintroduced over 100 confiscated Egyptian tortoises into their native habitat and monitored them for two years after their release. Recapture rates in translocated tortoises were low, thus making it hard to determine success or failure of the project. Moore et al. (2013) assessed the suitability of repatriating 249 adult alligator snapping turtles (*Macrochelys temminckii*) back to the wild that were seized from the illegal wildlife trade. After two years of mark and recapture efforts, released individuals presented high survival in the first year and successfully reproduced. Even though several years of post-release monitoring are needed for long-lived species to determine the success of a translocation, this particular study is encouraging. More data is needed to assess if released confiscated turtles can help the recovery of wild turtle populations.

Translocation as a Conservation Tool

Translocation is relocation of an animal or group of animals from one area to another outside of their original established territory (IUCN, SSC 2013). The most common types of translocation are reintroductions when an animal is released within its native range from where it has disappeared, and reinforcements (sometimes termed “augmentation” or “supplementation”)

where animals are translocated to an existing population of conspecifics (Berger-tal et al. 2019). Other types of translocation include assisted colonization where animals are intentionally released outside of their native range to avoid complete extinction, non – lethal nuisance animal removal, and ecological replacement whereby animals are released outside of their range to fill a specific ecological niche (IUCN/SSC 2013).

Translocations were first introduced as a conservation tool in 1895 (Morris et al. 2021). It has been a widely recognized practice for managing game species but is gradually being implemented in non-game species. Although, over the last 40 years the success rate of translocations has increased from 50% to 75% (Morris et al. 2021), there are pros and cons to this approach in management. A recent review of translocations reported that the primary challenges encountered across all taxa related to behavior of released animals, including abnormal dispersal and movement (Berger-tal et al. 2019). Similarly, one of the primary factors attributed to failure of herpetofaunal translocations is dispersal from the release site (Germano and Bishop 2009), making post-release movement one of the important metrics of translocation success.

Historically, the translocation of reptiles and amphibians have not been common as other groups like birds and mammals (Seddon et al, 2005) and success rates in herpetofauna were historically lower than other taxonomic groups (Dodd and Seigel 1991). Herpetofauna, especially reptiles, are generally long-lived species and long-term monitoring after release is important in determining if a translocation was successful or not (Germano and Bishop 2009). Thus, the lower reported success rate may in part be due to the length of time required to document release outcome, as suggested by a review by Germano and Bishop (2009), that showed success rates had doubled over the course of 15 years. The reported success rates for

herpetofauna translocations may continue to rise as more post-release monitoring data become available.

Risk of Spreading Pathogens and Parasites Via Translocation

Any translocation that includes individuals of unknown origin can increase the risk of spreading or introducing pathogens to wild populations (Woodford 1993). There is potential that pathogens can be introduced to native populations or to immunologically naïve individuals released in an area where pathogens are endemic (Cunningham 1996). For example, mass mortality events occurred in a wild population of red-listed Mallorcan midwife toads (*Alytes muletensis*) in central Spain following their exposure to *Batrachochytrium dendrobatidis* due to the release of animals from a captive breeding facility (Walker et al. 2008). Contrastingly, several viral and parasitic infections were acquired by reintroduced grey wolves (*Canis lupus*) and their offspring caused by spillovers from resident canid populations within Yellowstone National Park (Almberg et al. 2012). Translocation can also cause stress, which can compromise otherwise healthy immune systems, increasing translocated individual's vulnerability to disease and mortalities (Dickens et al. 2010, Tarszisz et al. 2014). The risk of pathogen transfer may be heightened in confiscated animals, which are of unknown origin and commonly kept in crowded conditions with poor husbandry, with no clarity of what other species they may have been exposed to (IUCN, SSC 2002). Confiscations have generally high pathogen and mortality rates. Ashley et al. (2014) evaluated a confiscation from a Texas animal wholesale dealership that consisted of over 26,400 animals. Due to the poor conditions found upon their discovery, 872 animals were lost per day after their seizure with reptiles making up 41.6% of deaths. Infection, stress, and overcrowding contributed to their mortalities (Ashley et al. 2014).

There are several pathogens that are cause for concern in captive collections and wild populations of chelonians. Ranaviruses cause severe systemic lesions and multi-organ failure followed quickly by death (Adamovicz et al. 2020). Ranaviruses are the suspected cause of mass mortality events in some turtle populations and can be found in 21 species of turtles and tortoises, including the eastern box turtle (Adamovicz et al. 2018, 2020). This pathogen has a high mortality rate (71% - 100%) and short time interval between infection and death (30 days) making it difficult to develop successful treatments (Adamovicz et al. 2020). Treatment and release are not often considered in asymptomatic diseased individuals or those who have recovered because they serve as carriers and may risk infecting other individuals, potentially causing a damaging mass mortality event (Adamovicz et al. 2020).

Mycoplasma sp. and herpesviruses are pathogens that are also cause for concern. Upper Respiratory Tract Disease in turtles is caused by *Mycoplasma spp*. and first discovered in connection with mortality events in gopher tortoises and desert tortoises (*Gopherus agassizii*; Brown et al. 1994, Brown et al. 1999). While the respiratory disease has never been associated with high mortality rates, it has been documented in captive collections and wild box turtles where outbreaks seem to occur in combination with other stressors (Sim et al. 2016, Adamovicz et al. 2018). Captive eastern box turtles have successfully recovered from *Mycoplasma sp*. with long-term treatment (34 days), however even when clinical signs were not present, individuals still shed the virus. (Sim et al. 2016). Herpesvirus infections trigger an assortment of clinical symptoms, including stomatitis, encephalitis, conjunctivitis, hepatitis, and lesions (Okoh et al. 2021). Herpesvirus has been found in wild and captive eastern box turtles and in wild bog turtles (*Glyptemys muhlenbergii*), spotted turtles (*Clemmys guttata*), and wood turtles (*Glyptemys insculpta*; Ossiboff et al. 2015, Sim et al. 2016, Adamovicz et al. 2018). However, herpesvirus

infections seem to be mild and mortality rates are generally low in turtles (Ossiboff et al. 2015). Although, *Mycoplasma* spp., ranavirus, and herpesvirus can present similar clinical symptoms, they can be distinguished on a molecular scale by screening oral and/or cloacal swabs via polymerase chain reaction using pathogen-specific primers (Adamovicz et al. 2020). Both captive collections and wild populations of multiples species of chelonians can have a co-infection by more than one pathogen (Sim et al. 2016, Archer et al. 2017, Cozad et al. 2020).

The Eastern Box Turtle (Terrapene carolina carolina)

The eastern box turtle can be found in mesic woodlands consisting of hardwood forests, mixed hardwood-pine forests, and hardwood swamps. They range from southern Maine along the east coast to the Piedmont and Coastal Plain of southern Georgia, and west to the Mississippi River (Dodd 2002). It is a long-lived chelonian, often living 50 - 70 years (Kiester and Willey 2015), but individuals have been recorded to live to 138 years in the wild (Nigrelli 1954). They exhibit delayed reproduction, taking at least 10 years to reach sexual maturity (Altobelli et al. 2021). Several populations of box turtles across the eastern United States have been the focus on long-term monitoring projects that indicate the species has been declining rapidly for decades. For example, a population in Maryland declined by 75% over a 30 year period (Stickel 1978).

Box turtles are increasingly sought by poachers as they are popular as pets (Dodd 2002), contributing to the species' decline. For example, between the years of 1990 and 1993, an estimated 81,000 box turtles, including eastern box turtles, were exported from the U.S. (Farrell et al. 2006). In response, CITES listed all North American box turtles under Appendix II in 1995. Appendix II requires the exporting country to set an annual quota and then enforce it. Unfortunately, quota limits are often exceeded by illegal exports which mostly go undetected,

making it hard to regulate. This allows the continuing collection of adults, contributing to the decline of their populations.

Box turtles are a good candidate species for reintroductions and have been the focus of numerous translocation efforts, involving wild-to-wild translocations (Hester et al. 2008, Rittenhouse et al. 2008, Farnsworth and Seigel 2013) as well as the release of rehabilitated animals (Cook 2004, Henriquez et al. 2017). However, success of such efforts has been limited. Cook (2004) released 335 eastern box turtles onto a protected area in New York and monitored 53 individuals for up to seven years. Individuals involved in the study were mostly wild turtles displaced by development, but some had been kept previously in captivity as pets prior to their release. Only 47.2% established home ranges, while 24.6% dispersed from the release area, and 28.3% died without establishing home ranges (Cook 2004). Two studies compared movements and home range sizes of hard-released translocated wild eastern box turtles to that of resident turtles already found at the study site. Translocated individuals had significantly larger home range sizes and their rates of survival were lower than that of residents (Hester et al. 2008, Farnsworth and Seigel 2013). In the closely related three-toed box turtles (*Terrapene carolina triunguis*), distance moved, and home range size of translocated turtles was not significantly different from those of wild resident box turtles, suggesting that the translocation was successful (Samuelson 2012). Still, no studies have evaluated movement patterns or fate in eastern box turtles seized from the illegal wildlife trade and repatriated back to the wild.

Objectives and Outline of Thesis Research

The objective of my thesis is to evaluate the suitability of releasing confiscated eastern box turtles back into the wild by monitoring movement, survivorship, and health in both resident and

released confiscated eastern box turtles. My thesis focuses on two main subjects: post-release movement and survival of confiscated and sympatric resident eastern box turtles. Chapter 2 evaluates settling behavior, movement patterns, and site fidelity of confiscated and resident eastern box turtles. Chapter 3 assesses body condition, pathogen prevalence, and hematology and their effects on survival of confiscated and eastern box turtles. Chapter 4 presents a summary of results and gives recommendations and potential guidelines regarding future confiscations

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CHAPTER 2

HOME RANGE AND SITE FIDELITY IN RELEASED CONFISCATED AND SYMPATRIC RESIDENT EASTERN BOX TURTLES ON THE SAVANNAH RIVER SITE, SOUTH CAROLINA

Introduction

Translocation – the movement by humans of animals from one location to another (IUCN, SSC 2013) – is a widely accepted practice for managing game species and is increasingly being implemented for non-game species (Griffith et al. 1989, Fischer and Lindenmayer 2000, Morris et al. 2021). Historically, reintroductions of herpetofauna were not as common as those conducted for other taxa such as mammals and birds (Seddon et al. 2005). A review by Dodd and Seigel (1991) suggested that translocation success rates were lower in herpetofauna than for other taxonomic groups, with only 19% of herpetofauna translocations deemed successful compared to 44% reported for mammals and birds (Griffith et al. 1989). Dodd and Seigel (2000) even recommended that reintroductions be considered as a last resort conservation strategy in herpetofauna.

However, as reptiles and amphibian species continue to decline (Gibbons et al. 2000), many are becoming increasingly reliant on population manipulations such as translocation to ensure their continued persistence. A successful translocation results in a viable and self-sustaining population in the wild but requires monitoring for an adequate period of time to verify that it is self-sustaining (Griffith et al. 1989, Dodd and Seigel 1991). A recent review of translocation studies published from 1991 to 2006 by Germano and Bishop (2009) found that

success rates for reptiles and amphibians had doubled since the review by Dodd and Seigel (1991) and were on par with success rates for other taxa (Griffith et al. 1989, Fischer and Lindenmayer 2000). In part, the higher success rate was attributed to having sufficient duration of post-release monitoring available to evaluate the outcome of individual projects. In addition, implementation of techniques such as soft-release has been effective in increasing site fidelity, further contributing to success rates (Germano et al. 2014, Tetzlaff et al. 2019). Nonetheless, one of the primary factors attributed to failure of herpetofaunal translocations is dispersal from the release site (Germano and Bishop 2009, Berger-tal et al. 2020), making post-release movement an important early metric of translocation success.

Turtles are the most vulnerable major class of vertebrates, with >60% of the approximately 360 species worldwide being endangered or already extinct (Lovich et al. 2018). Major threats to chelonians include habitat loss, climate change, invasive species, disease, and pollution (Gibbons et al. 2000). In addition, wild individuals are often targeted and collected as food or pets (Turtle Conservation Coalition, 2011). The illegal wildlife trade, with an estimated 138,000 tortoises and freshwater turtles taken from the wild annually in the U.S., is a growing threat to chelonian populations and their conservation efforts, and it is thought that much of the trade likely goes undetected (CITES 2016). Wild-to-wild translocations and head-starting are becoming increasingly common and recommended recovery tools for turtle populations (Turtle Conservation Fund 2002, Burke 2015), but confiscated turtles are generally excluded from conservation translocations due to concerns about their unknown origin and disease status. Consequently, most confiscated turtles are placed into permanent captivity or euthanized when no holding facilities are available (IUCN SSC 2002, D'Cruze et al. 2016, IUCN SSC 2019). Due to both declining populations in the wild and the increasing illegal wildlife trade, there is

heightened need to find a potential solution whereby confiscated turtles can be released and contribute to the persistence of wild populations.

To date, there have been only a few releases of confiscated turtles back to the wild and there are little to no post-release monitoring data available to evaluate the outcome of those releases. Wimberger et al (2009) investigated the suitability of repatriating rehabilitated leopard tortoises (*Stigmochelys pardalis*) that included unwanted pets or confiscated turtles. Only three of the 25 turtles released survived the 25-mo. monitoring period, suggesting that this specific release was unsuccessful. Attum et al. (2007) released two Egyptian tortoises (*Testudo kleinmanni*) that were confiscated from the illegal wildlife trade into their native habitat; both individuals exhibited large and irregular movements. Attum et al. (2010) subsequently released >100 confiscated Egyptian tortoises into their native habitat and monitored them through a survey conducted two years after their release. Recapture rates of translocated tortoises were low, and the success of the project is questionable. Moore et al. (2013) released 249 adult alligator snapping turtles (*Macrochelys temminckii*) that were confiscated from the illegal wildlife trade and monitored them for two years. Mark and recapture revealed that released individuals exhibited high survival in the first year and successfully reproduced. Although several years of post-release monitoring are needed to deem a translocation successful, this particular case is promising. More data, however, are clearly needed to evaluate whether released confiscated turtles can contribute to the recovery of wild turtle populations and specifically what strategies for repatriation to the wild would be most successful.

North American box turtles (*Terrapen*e spp.) are popular as pets and are increasingly sought after by poachers (Dodd 2002, Kiester and Olson 2011), contributing to their decline. Several populations of box turtles across the eastern United States have been the focus of long-

term monitoring projects that indicate the species has experienced chronic declines. For example, Kemp et al (2022) reported that a population of eastern box turtles (*T. carolina carolina*) in Pennsylvania declined by ≥70% over a 40-year period, and one of the factors hypothesized as contributing to the population's deterioration was collection for the pet trade. Box turtles are among the most highly trafficked chelonian species. For example, it is estimated that 81,000 box turtles, including eastern box turtles and ornate box turtles (*T. carolina ornata*), were exported from the U.S. between the years of 1990 and 1993 alone (Farrell et al. 2006). In response, CITES listed all North American box turtles under Appendix II in 1995. Appendix II requires the exporting country to set an annual quota and then enforce it. Unfortunately, quota limits are often exceeded by illegal exports which mostly go undetected, making it hard to enforce quota limits. This allows the continuing collection of adults, contributing to the decline of their populations.

Box turtles have been the focus of several translocation efforts, including wild-to-wild translocations (Hester et al. 2008, Rittenhouse et al. 2008, Farnsworth and Seigel 2013) and the release of rehabilitated animals (Cook 2004, Henriquez et al. 2017). To date, such efforts have had limited success in terms of demonstrating that released turtles survive, become established at release area, and functionally contribute to sustaining wild populations through reproduction. Cook (2004) radio-tracked 53 of 335 eastern box turtles released onto a protected area in New York for up to seven years. Most were wild turtles displaced by development, but some had been kept as pets in captivity prior to release. Only 47.2% established home ranges, 24.6% dispersed from the study site, and 28.3% died before establishing a home range. Two studies have compared movements and home ranges of hard-released translocated wild eastern box turtles to that of resident turtles. Although translocated individuals did not disperse off the study sites, their home ranges were still significantly larger and their survival rates lower than that of

resident individuals (Hester et al. 2008, Farnsworth and Seigel 2013). Samuelson (2012) found that in the closely related three-toed box turtles (*T. carolina triunguis*), distance moved, and home range size of translocated turtles did not significantly differ from wild resident box turtles, giving no early indication that the translocation was unsuccessful. To date, however, no studies have followed the fate of eastern box turtles confiscated from the illegal wildlife trade and subsequently released back into the wild.

In August 2019, South Carolina Department of Natural Resources (SCDNR) seized 208 eastern box turtles from the illegal wildlife trade. The University of Georgia's Savannah River Ecology Laboratory (SREL), SCDNR, and United States Forest Service-Savannah River (SRSFS) subsequently forged a partnership to release and monitor the seized animals on the Savannah River Site (SRS) in west-central South Carolina. My objectives were to evaluate the suitability of confiscated eastern box turtles for release back into the wild by comparing a suite of movement and site fidelity metrics between confiscated and resident eastern box turtles. I predicted that 1) confiscated turtles would exhibit greater movement and have larger home range sizes compared to resident turtles during Year 1 post-release but exhibit similar movements as residents in Year 2; 2) adult male confiscated box turtles would take longer to settle than adult female confiscated box turtles as male box turtles typically exhibit greater movement than females; 3) following settlement, males would exhibit greater movement and have larger home range sizes than females in both resident and confiscated turtles; and 4) residents would exhibit greater between-year site fidelity compared to released confiscated turtles. Results from this study will help guide decisions regarding future confiscations of illegally collected turtles and how they can contribute to species conservation efforts.

Materials and Methods

Study Site

The Savannah River Site (SRS) is an 802 km^2 National Environmental Research Park owned and operated by the Department of Energy (DOE), located near Aiken, South Carolina. The SRS was established in 1951 by the Atomic Energy Commission, which constructed nuclear production facilities and promoted reforestation of abandoned agricultural fields (White et al 2000). The SRS lies in the Upper Coastal Plain physiographic province of west-central South Carolina bordering the Savannah River and supports a variety of terrestrial and aquatic habitats, including bottomland swamp forests, upland hardwood forests, pine plantations, upland sandhills, abandoned farm ponds, Carolina Bay wetlands, and several stream systems (White et al. 2000).

I conducted my study in a Research Set Aside on the SRS in a mature hardwood forest dominated by oaks (*Quercus* spp.) and hickories (*Carya* spp.) that transitions to bottomland floodplain habitat as it nears Upper Three Runs Creek. The surrounding habitat consists of mature slash pine (*Pinus elliottii*) stands with pockets of hardwood forests, floodplains, and small streams. Winter temperatures for this area are generally mild with the average low temperatures being slightly above freezing (DeGregorio et al. 2016). Summer temperatures average ~26ºC and annual rainfall on the SRS is approximately 120 cm (White et al. 2000).

Acquisition and Handling of Confiscated Turtles

On 16 August 2019, SCDNR confiscated 208 eastern box turtles from the illegal wildlife trade and transported them to the Savannah River Ecology Laboratory (SREL), where SREL marked individuals with a unique ID by notching their marginal scutes (Cagle, 1939), and

weighed them to the nearest 2 g. SREL then held the confiscated turtles in a predator-proof outdoor aviary (10 m x 15 m), where they had access to shallow water and vegetative cover and were provided with supplemental food. This temporary holding period provided turtles the opportunity to recover from the poor conditions in which they had been held prior to confiscation and allowed time for the partners to identify and prepare a suitable release site. In late October 2019, in preparation for placing turtles in a soft release pen at the selected release site, SREL painted ID numbers onto the carapace with a non-toxic paint pen and epoxied PIT tags (AVID, Norco, CA, USA) to the anterior carapace to facilitate monitoring by allowing for identification of individuals while minimizing handling. SREL measured each turtle (midline carapace length [MCL], width [CW], and shell height to the nearest 1 mm), and weighed them to the nearest 2 g. I calculated an initial body condition index (BCI) for individuals using the following formula developed by dePersio et al (2019) specifically for eastern box turtles to estimate fat reserves:

$$\textit{Fat Volume (BCI)} = \text{Exp}(\text{Ln}(\text{Weight})* 0.400336 + \text{Ln}(\text{CW}/10)*0.985831 - 2.26447)$$

Soft Release of Confiscated Turtles

On 15 October 2019, SREL and SRSFS installed a 1-ha soft-release pen constructed of 66 cm tall silt fencing at the release site and reinforced the fencing with wood stakes. A total of 156 confiscated turtles were placed in the soft-release pen during 2-3 November 2019 and were held there until they emerged from winter dormancy in the spring of 2020. In April 2020, I attached 13-mo radio transmitters (model # R1860; transmitter 3.5g; Advanced Telemetry Systems, MN, USA) to the left or right rear carapace of 40 adult confiscated turtles with epoxy putty (Waterweld ; J-B Weld Company, LLC; Sulphur Springs, TX, USA) and placed turtles

back into the pen. I reweighed and remeasured each transmittered turtle and used the morphometric data to calculate their pre-release BCI.

On 15 May 2020, I opened the pen to allow confiscated turtles to move freely. If any transmittered confiscated turtles died before the pen was opened, I substituted other confiscated turtles into the study. At the time the pen was opened, a total of 39 (19 female, 20 male) confiscated box turtles were included in the telemetry study.

Capture and Handling of Resident Turtles

When I opportunistically encountered resident eastern box turtles at the release site, I added them to the radio-telemetry study as the field season progressed. I handled residents similarly to confiscated turtles as each individual was measured, marked, and affixed with a VHF radio transmitter. I used the morphometrics data to calculate a baseline BCI for residents. I added a total of 10 resident turtles (5 females, 5 males) to the telemetry study between 24 May and 29 June 2020.

Post-Release Monitoring

During the first year following release, I tracked both 39 confiscated and 10 resident turtles twice per week from 15 May – 25 Aug 2020 via radiotelemetry and visually confirmed their locations. I then tracked them weekly until turtles initiated winter dormancy in December 2020 (end of Year 1). During winter dormancy, I tracked turtles every two weeks. Once turtles emerged from winter dormancy in Spring 2021, I replaced radio-transmitters to allow radio-tracking to continue for another year (until winter dormancy 2021), and I repeated the collection of body measurements to compute BCI at the start of Year 2. During Year 2, I tracked turtles

once weekly from transmitter replacements until December 2021. At each tracking event, I recorded the following data: date, time of day, location to nearest ± 5m with a Garmin GPS (GPSMAP 64s; Garmin, KS, USA), distance (m) from previous location, health status (bright/alert/responsive, lethargic, or dead), activity (basking, buried, digging, foraging, interacting, resting, walking, or inactive/not seen), habitat type (hardwood forest, floodplain, pine forest, ephemeral wetland, upland sandhill, or stream), and microhabitat type (leaf litter, herbaceous cover, woody cover, exposed, grasses, pine straw, or other).

Statistical Analysis

Settling Metrics in Confiscated Turtles

Translocated chelonians often make large movements from their release site in the first weeks to months following release (Germano and Bishop 2009, Berger-tal et al. 2020). I wanted to evaluate settling behavior in translocated confiscated turtles to establish a timeline of settling dates, evaluate what factors influenced their settling behavior, and determine whether after excluding the settling period home range sizes of confiscated turtles differed from home ranges of resident turtles. To understand the settling period in confiscated turtles, I divided telemetry locations into Year 1 (May 2020 – Dec 2020) and Year 2 (January 2021 – December 2021). For each confiscated turtle, I used Year 1 telemetry data to estimate settling date based on two different movement metrics: daily step length (settling $date_{DSL}$; meters travelled between tracking locations divided by the number of days between tracking events) and daily net displacement (settling $date_{DND}$; the linear distance of each tracking location from the release pen, standardized by the number of days since release). I was interested in both metrics as they describe different biologically relevant movement behaviors. Settling $date_{DSL}$ served as an index of overall

movement of individual turtles, which I would expect to decrease over time as they cease their exploratory behavior and establish home ranges. Settling $date_{DND}$ captured the extent to which each individual turtle continued to move away from the release pen. I used linear piecewise splines to identify an inflection point representing the point in time (days since release) when movement behavior changed and began to decrease for each individual turtle. I created separate candidate model sets for each response variable (settling $date_{DSL}$, settling $date_{DND}$) in R (R Core Team 2021). Within each model set, I included a linear model, a one-knot model, and a two-knot model, with a knot representing an inflection point of time in movement behavior (Wold 1974, Kohl et al. 2019). I compared different candidate models using Akaike's Information Criterion, corrected for small sample sizes (△AICc, Burnham and Anderson 2004). If one-knot models fell within the △AICc criterion of <2, it suggested two distinct trends in the movement metric separated by one time point. Similarly, if two-knot models fell within the same criterion, it indicated three distinct trends in the movement metric separated by two time points. For every individual, the △AICc for between the one-knot and two-knot models was <2, suggesting either of the two models were suitable for describing the data pattern. I dropped the linear model from consideration as it fell outside of the difference threshold for every individual. Because AICc was smallest for the two knot model for both settling metrics in most confiscated turtles, I chose the two-knot model to estimate values of settling $date_{DSL}$ and settling $date_{DND}$ for each turtle, with the settling date corresponding to the second knot. Although I report settling dates based on each movement metric, I only used settling $date_{DSL}$ for subsequent analyses (described below) because the estimated settling date based on daily net distance resulted in extreme values for two individuals and did not align with field observations.

To understand the factors influencing individual settling times in translocated turtles, I then constructed linear models with settling $date_{DSL}$ as the response variable and Sex (male, female) and pre-release body condition (BCI) as predictor variables. My candidate model set consisted of five models which included one null model, two single predictor models, one model additive model with both predictors, and one model interactive between the predictors. I evaluated candidate models using Akaike's Information Criterion corrected for small sample sizes (△AICc, Burnham and Anderson 2004) in R, applying the "AICcmodavg" package (Mazerolle et al. 2020). I used the lowest AIC to identify the top model and considered any models with △AIC criterion <2 from the top model as having greatest support.

As additional metrics of settling behavior, for each individual confiscated turtle I also calculated the distance between the release pen and its winter dormancy location in Year 1 and the distance between the release pen and Year 2 dormancy location. Wild eastern box turtles spend an average of 115 days in winter dormancy (range 45-168 days; DeGregorio et al. 2016) and their survival is dependent on finding a suitable dormancy location. Thus, we considered the winter dormancy site selection in released confiscated turtles to be an important early metric of their settling behavior in the release site. To account for having two years of data for individual turtles, I analyzed data using linear mixed models for repeated measures using the "lme4" package (Bates et al. 2015) in R. I used distance from pen to dormancy location as the response variable, turtle ID as a random effect, and Sex (male, female), pre-release BCI, and Year (Year 1, Year 2) as fixed predictor variables. The candidate set of eight models included one global model, a null model, three models with individual fixed effects, two additive models with two fixed effects (Sex + BCI; Sex + Year), and one interactive model (Sex*Year). All models

contained the random effect, and response variables were transformed on a log 10 scale to improve normality prior to analysis.

Movement Metrics

To evaluate potential differences in movement patterns between confiscated and resident turtles, I quantified several movement metrics for each turtle for both Year 1 and Year 2. I converted all coordinates (decimal degrees) to the Universal Transverse Mercator (UTMs) grid system, and I only included data collected from the active season (roughly May-Nov), with the dormancy location represented only once during a given tracking year. From these data, I calculated a suite of linear and area movement metrics. Linear metrics included cumulative distance, mean daily movement, and maximum displacement. Cumulative distance was the distance between consecutive tracking locations, summed over the active season. I estimated mean daily movement for each turtle in each year by dividing the cumulative distance by the tracking duration (number of days between the turtle's first and last tracking event in a given year; Otten et al. 2021). I defined maximum displacement for confiscated turtles as the linear distance between the release pen and their furthest tracking location in each tracking year. I defined maximum displacement for residents as the linear distance between the two most distant telemetry locations for each individual within a tracking year. As a measure of home range size, I calculated 95% minimum convex polygons for each turtle in each year (MCPs; Silverman 1986), applying the "adehabitatHR" package in R. For characterizing Year 1 home ranges for confiscated turtles, I calculated one estimate of 95% MCP based on all active season locations of the turtle (i.e., including the settling period) and another estimate considering only the subset of locations following its settling $date_{DSL}$.

Prior to analysis, data were transformed on a log 10 scale to improve normality. For each movement metric response variable, I constructed a set of 14 separate candidate models using linear mixed models for repeated measures in the "lme4" package (Bates et al. 2015) in R. I chose linear mixed models for repeated measures because there were two years of data, and I wanted to account for potential changes in response variables from Year 1 to Year 2 in individual turtles and I wanted to account for continuous predictor variables that changed between years, such as BCI. All models included turtle ID as a random effect and Turtle Origin (confiscated, resident), Sex (male, female), BCI (Year 1 BCI, Year 2 BCI) and Year (Year 1, Year 2) as fixed predictor variables. I was careful in the selection of models to avoid overfitting and also constructed models that made the most biological sense when looking at my predictor variables. The candidate set of models included a global model, a null model, four models with individual fixed effects, seven additive models with two or more fixed effects, and one interactive model (Turtle origin*Sex).

Between-Year Site Fidelity Metrics

I evaluated between-year site fidelity in both confiscated and resident turtles using two different metrics: home range overlap and distance between winter dormancy locations. I calculated home range overlap as the proportion of Year 1 home range that was reused in Year 2. I first calculated the area of overlap using the intersect tool provided by the "raster" package in R. I then divided the area of overlap by the area contained in Year 1 home range to obtain a proportion, with 0 corresponding to no overlap and 1 indicating complete home range overlap between years. For confiscated turtles, I used only post-settling locations to assess home range

overlap. I also characterized site fidelity by calculating the linear distance (m) between Year 1 and Year 2 winter dormancy locations for each turtle.

To investigate site fidelity, I built 14 separate fixed effects linear models that included categorical predictor variables of Turtle origin and Sex and continuous variables of pre-release BCI and the change in BCI between Year 1 and Year 2. I considered both pre-release body condition and change in body condition because body condition is important for fueling winter dormancy and other activities such as movement (Price 2016). Body condition might also be expected to change between years, particularly in confiscated turtles. I was careful in the selection of models to avoid overfitting and also built models that made the most biological sense when looking at my predictor variables. Candidate models included one null model, four single predictor models, eight additive models, and one interactive model.

For all settling, movement, and site fidelity analyses, I compared different candidate models using Akaike's Information Criterion corrected for small sample sizes (△AICc, Burnham and Anderson 2004) in R using the "AICcmodavg" package (Mazerolle et al. 2020) and report the results for all candidate models with a △AIC < 2. Raw data are presented as means ± 1 standard error in Table 2.1.

Results

I was able to calculate movement metrics for a total of 41 eastern box turtles (31 confiscated, 10 residents). Of the 39 confiscated turtles included in the study at the time of pen removal, eight had <10 tracking locations due to turtle death (n=7) or radio transmitter failure (n=1) prior to 11 June 2020 and were excluded from analysis. An additional six confiscated turtles died from 15 Jun – 21 Sept 2020 but they had sufficient locations to calculate movement

metrics for Year 1. One confiscated turtle was lost from the study in September 2020 but had sufficient locations to calculate Year 1 movement metrics. No turtles died during Year 2. One confiscated turtle was lost from the study due to radio transmitter failure in August 2021 but had sufficient numbers of telemetry locations to calculate its Year 2 movement metrics.

A body condition index was calculated for each confiscated and resident turtle and used as a predictor variable throughout the analysis. Pre-release Year 1 body condition was calculated from morphometric measurements collected in April of 2020 during the initial transmitter attachment at the beginning of Year 1 active season in confiscated turtles and at initial capture (24 May-29 June 2020) for resident turtles. Year 2 BCI for both confiscated and resident turtles was calculated from metrics collected during transmitter replacements in April 2021 at the beginning of Year 2 active season (Figure 2.1).

Settling Date

Both movement metrics used to estimate settling date revealed high variability among individual confiscated turtles, with settling date derived from daily distance moved ranging from 20 May 2020 (5 days since release) to 10 Aug 2020 (97 days) and settling date derived from daily net displacement ranging from 20 May 2020 (5 days since release) to 30 Oct 2020 (155 days; Table 2.1). One confiscated female's settling date could not be determined as she had inconsistent tracking sessions due to radio-transmitter issues directly after her release from the pen, thus settling date could only be calculated for 30 confiscated turtles. In contrast to my prediction, males and females took similar times to settle (average of 35-36 days based on DSL, average of 43-44 days based on DND). Based on settling $date_{DSL}$, the top model included only pre-release BCI, which had a weak but positive coefficient, suggesting that turtles with a lower

body condition settled earlier than turtles with higher body condition. However, pre-release BCI was not a significant predictor and the null model garnered almost equal support (Table 2.2).

Confiscated females tended to select winter dormancy locations further from the pen (Year 1: 1035.8 ± 196.5 m; Year 2: 1057.1 ± 189.0 m) than did confiscated males (Year 1: 687.4 ± 168.5 m; Year 2: 600.6 ± 171.4 m), but in both sexes distance from pen to dormancy location was similar between years (Table 2.1). However, the top model was the null model, indicating that none of the predictor variables (Year, Sex, pre- release BCI) we investigated exerted a strong influence on log-transformed distance to dormancy locations. (Table 2.3).

Home Range

As predicted, when including all telemetry locations (including the settling period), confiscated turtles had much larger home ranges in Year 1 (11.8 ± 2.9 ha; n=31) than in Year 2 (4.6 ± 1.2 ha; n=24) and this pattern was evident in both sexes (Table 2.1). Interestingly, resident turtles also had larger home ranges in Year 1 (6.9 ± 1.5 ha; n=10) than in Year 2 (4.5 ±1.4 ha; n=10). Confiscated turtles had similar home ranges as resident turtles in Year 2 (4.6 ± 1.2 ha and 4.5 ± 1.4 ha, respectively: Figure 2.2). When including all active season locations, Year had a significant negative effect on log-transformed home range size (95% MCPs) and the single predictor model that only included Year was the highest ranking model and garnered the most AICc weight (Table 2.4). Turtle origin was not a significant predictor in our models, but the high degree of variability among confiscated turtles make have masked differences in movement behavior between confiscated and resident turtles.

Year 1 home ranges of confiscated turtles were much smaller when the settling period was excluded (4.4 ± 1.3 ha; n=31) than when all locations were included (11.8 ± 2.9 ha) and

actually tended to be smaller than Year 1 home range sizes for residents (6.9 ± 1.5 ha; n=10; Figure 2.3). When including only post-settling locations, the top model included only Turtle origin (confiscated or resident) but it was not a significant predictor. However, the null model ranked was within △AIC criterion of the top model and no model garnered > 0.2 model weight (Table 2.5), indicating high model uncertainty. Thus, none of the factors we investigated, including Turtle Origin, exerted strong influence on log-transformed home range size when the settling period was excluded for confiscated turtles.

Linear Movement Metrics

In Year 1, mean daily movement (based on all locations) differed between confiscated males (66.32 ± 8.8 m) and resident males (88.6 ± 9.3 m) but mean daily movement in females was similar between the two origins (confiscated: 74.2 ± 8.2 m; residents: 72.2 ± 5.0 m; Table 2.1; Figure 2.4). In Year 2, mean daily distance moved changed by sex in both turtle origins. Confiscated females (63.9 ± 5.5 m), and resident males (70.4 ± 9.5 m) movement decreased, and confiscated males (93.2 ± 16.6 m) and resident females (82.8 ± 12.6 m) mean daily movement increased from Year 1. However, the null model ranked was within △AIC criterion of the top model and no model garnered > 0.22 model weight (Table 2.6), indicating high model uncertainty. This suggests that there is uncertainty between models and none of the predictor variables have any significant influence on mean daily movement.

In evaluating cumulative distance, however, I found that the top model was the model that included only Year, which was a significant predictor (p = .0002), suggesting that Year influenced log-transformed cumulative distance moved regardless of turtle origin (Table 2.7; Figure 2.5). Two bivariate models fell within the △ AICc criterion, with one being the additive

model including Turtle origin and Year and the other being the additive model that included Sex and Year as predictors (Table 2.7). Across all three of the top candidate models, Year was the only significant predictor, further indicating that of the predictors we evaluated, Year exerted the strongest influence on cumulative distance moved.

Maximum displacement tended to be greater in Year 2 than in Year 1 (Figure 2.6), except resident males, which exhibited the opposite trend, having greater maximum displacement in Year 1 than Year 2 (Table 2.1). The top model explaining log-transformed maximum displacement was the additive model that included both Sex and Turtle origin, with Sex being the only significant predictor. Seven models fell within the < 2 $\triangle$AICc criterion including the null model, and none garnered >0.16 of model weight, indicating high model uncertainty (Table 2.8).

Site Fidelity

I hypothesized that residents would exhibit greater between-year site fidelity than confiscated turtles. However, both mean home range overlap (0.50 in confiscated turtles, 0.48 in residents; Table 2.1; Figure 2.7) and mean distance between Year 1 and Year 2 winter dormancy locations (100.3 m, 79.6 m, respectively; Table 2.1; Figure 2.8) were similar between confiscated and resident turtles. Accordingly, the top model for both site fidelity metrics was the null model (Tables 2.9–2.10).

Discussion

My study is one of the first to assess post soft-release movement in eastern box turtles confiscated from the illegal pet trade and repatriated back into the wild. I monitored released confiscated turtles for two years, calculated a suite of movement metrics, and compared them to

resident box turtles that occurred sympatrically at the release site. I found that: 1) confiscated turtles on averaged settled within the first six weeks following release, with turtles with lower body condition at release settling sooner that turtles having higher body condition at release; 2) during the first year following release, confiscated turtles had larger home ranges than resident turtles, particularly when including the settling period, but in the subsequent year confiscated turtles had similar home ranges as resident turtles; and 3) between-year site fidelity was similarly high between confiscated and resident turtles, with both groups reusing about 50% of their home range and selecting winter dormancy sites with <100 m of the previous year.

Unlike my prediction, sex did not play a role in settling behavior of confiscated turtles. Of the factors we examined, only body condition influenced settling behavior, with turtles with a higher body condition taking longer period of time to settle than those with a lower body condition. The body condition index I used had been developed specifically for estimating fat reserves in eastern box turtles and was validated using computed tomography – a medical imaging technique (dePersio et al. 2019). Body condition can have important consequences for turtles, with animals having higher fat stores exhibiting greater probability of reproduction (Congdon and Tinkle 1982), having higher reproductive output (Litzgus et al. 2008), and being better able to survive periods of dormancy (Price 2016). High body mass has also been linked to larger home range sizes in turtles (Slavenko et al. 2015), possibly explaining why animals with lower body condition settling sooner than turtles with higher body condition. Even though some individuals took upwards of three months to settle, all individuals who survived established home range within the first year of release.

As predicted, confiscated turtles had larger home range sizes and greater cumulative distances moved in the first year of release. Unexpectedly though, resident turtles also exhibited

larger home ranges and greater cumulative distances in Year 1. Movement in both confiscated and resident turtles decreased in the second year of data collection and became comparable, making Year a significant predictor in home range size and cumulative distance in both turtle origins in the analysis. This suggests that the primary differences in movement behavior between confiscated and resident turtles was due to the exploratory movements made by confiscated turtles during the settling phase. Translocated turtles, including previously captive and wild individuals, often exhibit larger movements after their reintroduction compared to residents found at the release site (Hester et al. 2008, Rittenhouse et al. 2008, Farnsworth and Seigel 2013), resulting in inflated home range estimates. Although larger movement patterns in translocated individuals are common, studies have reported an establishment and some reduction in home range size in individuals comparable to sympatric residents. In fact, this trend was observed in two eastern box turtle relocation projects, in which 33.3% and 72.7% of translocated turtles established home ranges within 2 years of release (Hester 2008, Farnsworth and Seigel 2013, respectively). My study observed 95.8% of released confiscated turtles establishing home ranges within the first year. One explanation for more rapid and higher settling rates exhibited in my study compared to the previous relocation projects may be due to the soft-release method I used for the reintroduction of confiscated turtles. Soft-release translocation in chelonians has proven to reduce large movements and increase site fidelity (Germano et al. 2014, Tetzlaff et al. 2019). Tuberville et al (2005) found that repatriated gopher tortoises (*Gopherus polyphemus*) penned for 12 months exhibited higher likelihood of site fidelity in comparison to those only penned for 9 months and for individuals who did not receive any penning treatment. The confiscated turtles in my study were held in the 1 hectare soft-release pen for seven months prior to their release. The soft-release pen was also placed in suitable habitat on private, contiguous

land which is highly recommended for successful translocations (Germano and Bishop 2009). Using the penning technique as well as placing confiscated turtles in good quality habitat potentially played a role in the promising outcome of this study.

Home range overlap and distance between dormancy locations of confiscated turtles between Year 1 and Year 2 were fairly similar to those of resident turtles indicating that the confiscated turtles exhibited similar between-year site fidelity as resident turtles. Site fidelity to overwintering locations has been documented in box turtle species including resident ornate and eastern box turtles (Refsnider et al. 2012, DeGregorio et al. 2016), thus the results indicate potential benchmarks for success within my research project.

Natural variability of movement in individual confiscated turtles was notable compared to the SRS resident box turtles as home range sizes varied from 0.01 ha – 32.6 ha within the first year of release even after the settling period was excluded. SRS residents home ranges varied from 1.6 ha – 17.7 ha, showing less variability. Home range sizes in box turtles vary depending on habitat and geographical location across wild population within the eastern box turtle's range (Habeck et al. 2019) The origin of the confiscated turtles was unknown, but individuals may have been collected from different wild populations in the southeast, perhaps contributing to the observed variability. More likely, though, is that individual turtles respond differently to the translocation, resulting in greater variability in confiscated turtles than exhibited by resident turtles. Despite this variability, however, the main behavioral differences exhibited by confiscated turtles compared to resident turtles was during their exploratory phase prior to establishing a home range.

Overall, the results from this study strongly suggest that confiscated and resident turtles behaved similarly in the way they moved on the landscape. Making the decision to add residents

as a control to the study was essential in order to have a baseline to compare movement data from the confiscated turtles. Without this control group, it would have been difficult to understand if the confiscated turtles moved naturally throughout the study site. Evaluating movement patterns in repatriated confiscated turtles is essential in determining whether individuals can contribute to the conservation of vanishing populations. Soft release can positively influence the outcome of translocation and increase site fidelity, and it allows a period of recovery from captivity and confinement for individuals. Selection of good quality and contiguous habitat is also essential in successful reintroductions. Long-term monitoring is highly recommended for long-lived species and will ultimately be required to evaluate whether a translocation was truly successful. While my sample size of residents was small and the research only evaluated one case study, the findings suggest that this study is at least promising. I found that translocated confiscated box turtles behaved similarly to resident turtles found on site when assessing their movement patterns and site fidelity. This study is the first to evaluate movement patterns and site fidelity of soft-released confiscated eastern box turtles potentially providing critical information and can be used as guidance in future confiscations.

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Table 2.1. Summary table of linear distances in meters and area metrics in hectares of confiscated and resident eastern box turtles on Savannah River Site, Aiken, SC during 2020-2021. Raw data represented by the means with ± 1 standard error (n = # of individuals) and minimum and maximum values are listed below.

Movement metrics	Confiscated females	Confiscated males	Resident females	Resident males
Settling date				
Settling Date$_{DSL}$ (days)	36 days ± 7.5 (5 – 97; n=13)	35 days ± 7.2 (5 – 87; n =17)	N/A	N/A
Settling Date$_{DND}$ (days)	44 days ± 11.9 (10– 155; n = 13)	43 days ± 10.6 (5 – 168; n = 17)	N/A	N/A
Distance to dormancy location – Year 1 (m)	1035.8 m ± 196.5 (102.3 – 2886.6; n= 13)	687.4 m± 168.5 (229.1 - 1842.1; n = 11)	N/A	N/A
Distance to dormancy location – Year 2 (m)	1057.1 m ± 189.0 (173.5 – 2809.8; n = 13)	600.6 m ± 171.4 (250.6 - 2084.1; n = 10)	N/A	N/A
Home range size				
95% MCP all locations - Year 1 (ha)	15.5 ± 5.3 (0.1 – 60.5; n = 14)	8.7 ± 3.0 (0.03 - 43.7; n = 17)	8.5 ± 2.7 (1.6 - 17.7; n = 5)	5.3 ± 1.1 (1.8 - 7.9; n = 5)
95% MCP after settling date – Year 1 (ha)	5.3 ± 2.3 (n=14) (0.03 – 32.6; n = 13)	3.6 ± 1.4 (0.01 - 22.9; n = 17)	N/A	N/A
95% MCP - Year 2 (ha)	3.09 ± 0.6 (0.5 – 7.0; n = 13)	6.5 ± 2.4 (0.7 - 27.9; n = 11)	6.2 ± 2.6 (1.02-15.69; n = 5)	2.8 ± 0.6 (1.10 - 4.95; n = 5)
Linear movement metrics				
Mean Daily Movement – Year 1 (m)	74.2 ± 8.2 (17.6 – 128.4; n = 14)	66.3 ± 8.8 (16.5 - 131.1; n = 17)	72.2 ± 5.0 (61.0 - 85.7; n = 5)	88.6 ± 9.3 (62.8 - 119.0; n = 5)
Mean Daily Movement – Year 2 (m)	63.9 ± 5.5 (35.3 – 92.4; n = 13)	93.2 ± 16.6 (39.9 - 216.7; n = 11)	82.8 ± 12.6 (61.8 - 32.0; n = 5)	70.4 ± 9.5 (46.6 - 97.1; n = 5)
Cumulative distance – Year 1 (m)	3204.3 ± 365.0 (352.1 – 5262.6; n = 14)	2643.0 ± 433.7 (149.2 - 6032.8; n = 17)	2517.2 ± 208.8 (2046.1 – 3238.7; n = 5)	3217.3 ± 536.4 (2072.7 - 5238.9; n = 5)
Cumulative distance -Year 2 (m)	2126.4 ± 188.6 (1092.6 – 3050.2; n = 13)	2803.5 ± 439.8 (1318.6 - 6069.4; n = 11)	2853.4 ± 473.5 (1855.9 - 4620.0; n = 5)	2062.5 ± 319.2 (1339.1 - 2915.0; n = 5)
Maximum displacement – Year 1 (m)	1097.4 ± 187.8 (81.5 – 2903.1; n = 14)	690.7 ± 131.0 (106.9 - 2098.3, n = 17)	509.8 ± 56.1 (411.8 - 679.4, n = 5)	420.1 ± 57.4 (202.2 - 536.1, n = 5)
Maximum displacement – Year 2 (m)	1200.0 ± 173.9 (447.5 – 2890.5, n = 13)	928.5 ± 250.6 (332.9 - 2960.4; n = 11)	589.6 ± 75.0 (408.3-857.5; n = 5)	352.1 ± 53.6 (220.0 - 524.9; n = 5)
Site fidelity				
Distance between. Yr 1 – Yr 2 dormancy (m)	87.7 ± 23.7 (6.0 – 302.5; n = 13)	116.7 ± 49.9 (8.3 - 527.1; n = 10)	103.5 ± 55.70 (16.8 - 316.2; n = 5)	49.9 ± 24.4 (16.4 - 121.9; n = 4)
Proportion of home range overlap	0.41 ± 0.09 (.04 – 1.0; n = 12)	0.63 ± 0.10 (0.21 - 0.95; n = 9)	0.49 ± 0.11 (0.17-0.81; n = 5)	0.48 ± 0.14 (0.16 - 0.91; n = 5)

Table 2.2. Candidate models used to detect predictors that had an influence on days to settling since release in confiscated eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. The days to settling response variable was determined through analysis of daily step length. There is one categorical variable, Sex (male, female) and one continuous variable, pre-release BCI (Body condition index). The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Sex	BCI	BCI*Sex
BCI	0.50	3	35.71	0	0.35		0.08	
Null	1.39	2	35.81	0.1	0.69			
BCI+Sex	0.43	4	37.27	1.55	0.85	-0.16	0.09	
SEX	1.43	3	37.99	2.28	0.96	-0.08		
BCI*Sex	0.49	5	40.16	4.45	1	-0.26	0.09	0.009

Table 2.3. Candidate models used to detect predictors that had an influence on log-transformed distance of over wintering location from pen including pre-settling data in confiscated eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Year (Yr) and Sex (male, female). Pre-release BCI serves as a continuous variable. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Yr	Sex	BCI	Sex* Year
Null	2.83	3	-31.16	0	0.25				
Sex	2.92	4	-30.71	0.45	0.44		-0.18		
Year	2.80	4	-30.46	0.7	0.62	0.02			
Sex+Year	2.88	5	-29.87	1.29	0.75	0.02	-0.18		
BCI	2.71	4	-28.92	2.24	0.83			0.01	
Sex*Year	2.86	6	-28.78	2.38	0.9	0.04	-0.12		-0.04
Sex+BCI	2.76	5	-28.47	2.69	0.97		-0.19	0.01	
ALL	2.87	6	-27.23	3.93	1	0.02	-0.18	0.001	

Table 2.4. Candidate models used to detect predictors that had an influence on log-transformed home range size (95% MCPs – all active season locations) in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Year (Yr), Turtle origin (Origin), and Sex (male, female). BCI (Year 1, Year 2)serves as a continuous variable. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Yr	Sex	Origin	BCI	Origin* Sex
Yr	0.92	4	137.04	0	0.34	**-0.28**				
Sex+Yr	1.02	5	138.15	1.11	0.54	**-0.29**	-0.2			
Origin+Yr	0.88	5	138.65	1.61	0.69	**-0.29**		0.17		
Origin+Sex	0.99	6	139.86	2.82	0.78	**-0.29**	-0.2	0.17		
All	0.29	7	141.37	4.33	0.83	**-0.29**	-0.21	0.11	0.0	
Null	0.53	3	141.38	4.34	0.86					
BCI	-0.18	4	142.47	5.43	0.9				0.0	
Sex	0.61	4	142.63	5.58	0.93		-0.16			
Origin	0.5	4	143.15	6.11	0.95			0.12		
Sex+BCI	-0.16	5	143.52	6.47	0.96		-0.18		0.0	
Origin+Sex	0.58	5	144.49	7.45	0.98		-0.16	0.12		
Origin+BCI	-0.13	5	144.6	7.56	0.99			0.08	0.0	
Origin+Sex	-0.11	6	145.76	8.72	0.99		-0.18	0.07	0.0	
Origin*Sex	0.58	6	146.85	9.81	1		-0.15	0.14		- 0.03

Table 2.5. Candidate models used to detect predictors that had an influence on log-transformed home range size (95% MCPs – locations only after settling date) in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Year (Yr), Turtle origin (Origin), and Sex (male, female). BCI (Year 1, Year 2) serves as a continuous variable. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Yr	Sex	Origin	BCI	Origin* Sex
Origin	0.12	4	139.37	0	0.2			0.50		
Origin+Sex	0.29	5	139.89	0.53	0.36		-0.30	0.49		
Null	0.25	3	140.64	1.28	0.46					
Sex	0.42	4	141.05	1.69	0.55		-0.32			
Origin+Yr	0.19	5	141.36	1.99	0.63	-0.04		0.51		
Origin+BCI	0.22	5	141.66	2.29	0.69			0.51	-	
Origin+Sex+Yr	0.36	6	141.91	2.54	0.75	-0.05	-0.31	0.49		
Origin*Sex	0.31	6	142.17	2.81	0.8		-0.34	0.41		0.16
Origin+Sex+BCI	0.25	6	142.26	2.9	0.84		-0.30	0.48	0.003	
Yr	0.31	4	142.56	3.2	0.88	-0.04				
BCI	-0.008	4	142.8	3.43	0.92				0.02	
Sex+Yr	0.49	5	143	3.63	0.95	-0.05	-0.32			
Sex+BCI	0.03	5	143.17	3.8	0.98		-0.32		0.03	
ALL	0.25	7	144.34	4.97	1	-0.05	-0.31	0.48	0.01	

Table 2.6. Candidate models used to detect predictors that had an influence on log-transformed mean daily movement including pre-settling data in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Year (Yr), Turtle origin (Origin), and Sex (male, female). BCI (Year 1, Year 2) serves as a continuous variable. The coefficient estimate for each predictor in a model are given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Yr	Sex	Origin	BCI	Origin* Sex
Null	1.81	3	-28.52	0	0.22					
Origin	1.78	4	-28.16	0.36	0.4			0.10		
BCI	1.55	4	-27.26	1.26	0.52				0.02	
Yr	1.82	4	-26.44	2.08	0.59	-0.01				
Sex	1.81	4	-26.35	2.16	0.67		-0.02			
Origin+BCI	1.61	5	-26.33	2.18	0.74			0.09	0.02	
Origin+Yr	1.80	5	-26.04	2.47	0.81	-0.01		0.10		
Origin+Sex	1.80	5	-25.91	2.6	0.87		-0.01	0.10		
Sex+BCI	1.56	5	-25.09	3.43	0.9		-0.02		0.02	
Sex+Yr	1.83	5	-24.23	4.29	0.93	-0.01	-0.02			
Origin+Sex+BCI	1.61	6	-24.05	4.46	0.95		-0.02	0.09	0.02	
Origin+Sex+Yr	1.81	6	-23.74	4.78	0.97	-0.01	-0.02	0.10		
Origin*Sex	1.80	6	-23.58	4.93	0.99		-0.02	0.09		0.03
ALL	1.62	7	-21.89	6.63	1	-0.02	-0.02	0.09	0.02	

Table 2.7. Candidate models used to detect predictors that had an influence on log-transformed cumulative distance moved including pre-settling data in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Year (Yr), Turtle origin (Origin), and Sex (male, female). BCI (Year 1, Year 2) serves as a continuous variable. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Yr	Sex	Origin	BCI	Origin* Sex
Yr	3.50	4	9.68	0	0.4	**-0.12**				
Origin +Yr	3.50	5	10.83	1.15	0.62	**-0.12**		0.13		
Sex+Yr	3.55	5	10.86	1.18	0.84	**-0.12**	-0.11			
Origin+Sex+Yr	3.52	6	12.13	2.45	0.96	**-0.12**	-0.10	0.12		
ALL	3.47	7	14.55	4.87	0.99	**-0.12**	-0.11	0.12	0.004	
Null	3.32	3	19.63	9.95	0.99					
Origin	3.30	4	20.89	11.21	1			0.11		
Sex	3.37	4	20.94	11.26	1		-0.09			
BCI	3.29	4	21.85	12.18	1				0.003	
Origin+Sex	3.34	5	22.31	12.63	1		-0.09	0.10		
Origin+BCI	3.36	5	23.16	13.49	1			0.11	-0.005	
Sex+BCI	3.30	5	23.2	13.52	1		-0.09		0.006	
Origin*Sex	3.35	6	24.53	14.85	1		-0.11	0.06		0.08
Origin+Sex+BCI	3.36	6	24.67	15	1		-0.09	0.10	-0.002	

Table 2.8. Candidate models used to detect predictors that had an influence on log-transformed maximum displacement including pre-settling data in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Year (Yr), Turtle origin (Origin), and Sex (male, female). BCI (Year 1, Year 2) serves as a continuous variable. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Yr	Sex	Origin	BCI	Origin* Sex
Origin+Sex	2.94	5	-23.17	0	0.16		**-0.19**	-0.19		
Origin+Sex+Yr	2.90	6	-22.86	0.31	0.3	0.03	-0.19			
Sex	2.88	4	-22.47	0.7	0.41		-0.19			
Sex+Yr	2.85	5	-22.11	1.06	0.5	0.03	-0.18			
Origin	2.83	4	-21.56	1.61	0.57			-0.19		
Origin+Yr	2.79	5	-21.38	1.79	0.64	0.03		-0.19		
Origin+Sex+BCI	2.71	6	-21.37	1.8	0.7		**-0.20**	-0.21	0.02	
Null	2.79	3	-21.31	1.86	0.77					
Yr	2.75	4	-21.08	2.1	0.82	0.03				
Origin*Sex	2.94	6	-20.84	2.34	0.87		-0.20	-0.21		0.04
ALL	2.79	7	-20.55	2.62	0.91	0.03	**-0.19**	-0.21	0.01	
Sex+BCI	2.77	5	-20.31	2.87	0.95		-0.19		0.01	
Origin+BCI	2.66	5	-19.56	3.61	0.98			-0.20	0.02	
BCI	2.72	4	-19.12	4.05	1				0.00	

Table 2.9. Candidate models used to detect predictors that had an influence on log-transformed distance between overwintering locations in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Turtle origin (Origin), and Sex (male, female). Pre-release BCI (Year 1) and BCI△ serves as a continuous variable. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Sex	Origin	BCI △	BCI (Yr1)	Origin* Sex
Null	1.70	2	51.54	0	0.36					
BCI△	1.68	3	53.68	2.14	0.48			0.01		
Sex	1.73	3	53.86	2.32	0.6	-0.06				
Origin	1.72	3	53.95	2.41	0.71		-0.04			
BCI(Yr1)	1.69	3	53.98	2.44	0.81				0.001	
Sex+BCI△	1.70	4	56.22	4.68	0.85	-0.05		0.01		
Origin+BCI△	1.69	4	56.3	4.76	0.88		-0.02	0.01		
Origin+Sex	1.74	4	56.46	4.91	0.91	-0.06	-0.04			
Sex+BCI(Yr1)	1.71	4	56.49	4.95	0.94	-0.06			0.002	
Origin+BCI(Yr1)	1.66	4	56.57	5.03	0.97		-0.04		0.005	
Sex+BCI(Yr1)+BCI△	1.44	5	58.94	7.4	0.98	-0.05		0.02	0.02	
Origin+BCI(Yr1)+BCI△	1.39	5	58.99	7.45	0.99		-0.04	0.02	0.03	
Origin*Sex	1.72	5	59.01	7.47	1	-0.003	0.05			-0.21
All	1.42	6	61.96	10.41	1	-0.05	-0.04	0.02	0.03	

Table 2.10. Candidate models used to detect predictors that had an influence on home range overlap in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include Turtle origin (Origin), and Sex (male, female). Pre-release BCI (Yr1) and BCI△ serves as a continuous variable. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Sex	Origin	BCI△	BCI(Yr1)	Origin* Sex
NULL	0.06	2	71.93	0	0.28					
Sex	-0.10	3	72.58	0.65	0.48	0.37				
BCI△	0.09	3	74.09	2.17	0.58			-0.02		
BCI(Yr1)	-0.30	3	74.3	2.37	0.67				0.03	
Origin	0.05	3	74.4	2.48	0.75		0.006			
Sex+BCI△	-0.06	4	75.03	3.1	0.81	0.36		-0.02		
Sex+BCI(Yr1)	-0.32	4	75.21	3.28	0.86	0.36			0.02	
Origin+Sex	-0.10	4	75.25	3.33	0.92	0.37	0.0008			
Origin+BCI△	0.11	4	76.77	4.84	0.94		-0.02	-0.02		
Origin+BCI(Yr1)	-0.31	4	76.97	5.04	0.96		-0.02		0.03	
Origin*Sex	-0.14	5	77.92	5.99	0.98	0.45	0.12			-0.28
Sex+BCI(Yr1)+BCI△	0.02	5	77.93	6	0.99	0.36		-0.02	-0.007	
Origin+BCI(Yr1)+BCI△	0.06	5	79.66	7.74	1		-0.02	-0.02	0.004	
ALL	0.01	6	81.07	9.15	1	0.36	-0.02	-0.02	-0.007	

Figure 2.1. A comparison in pre-release body condition in Year 1 and Year 2 body condition between soft-released confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA.

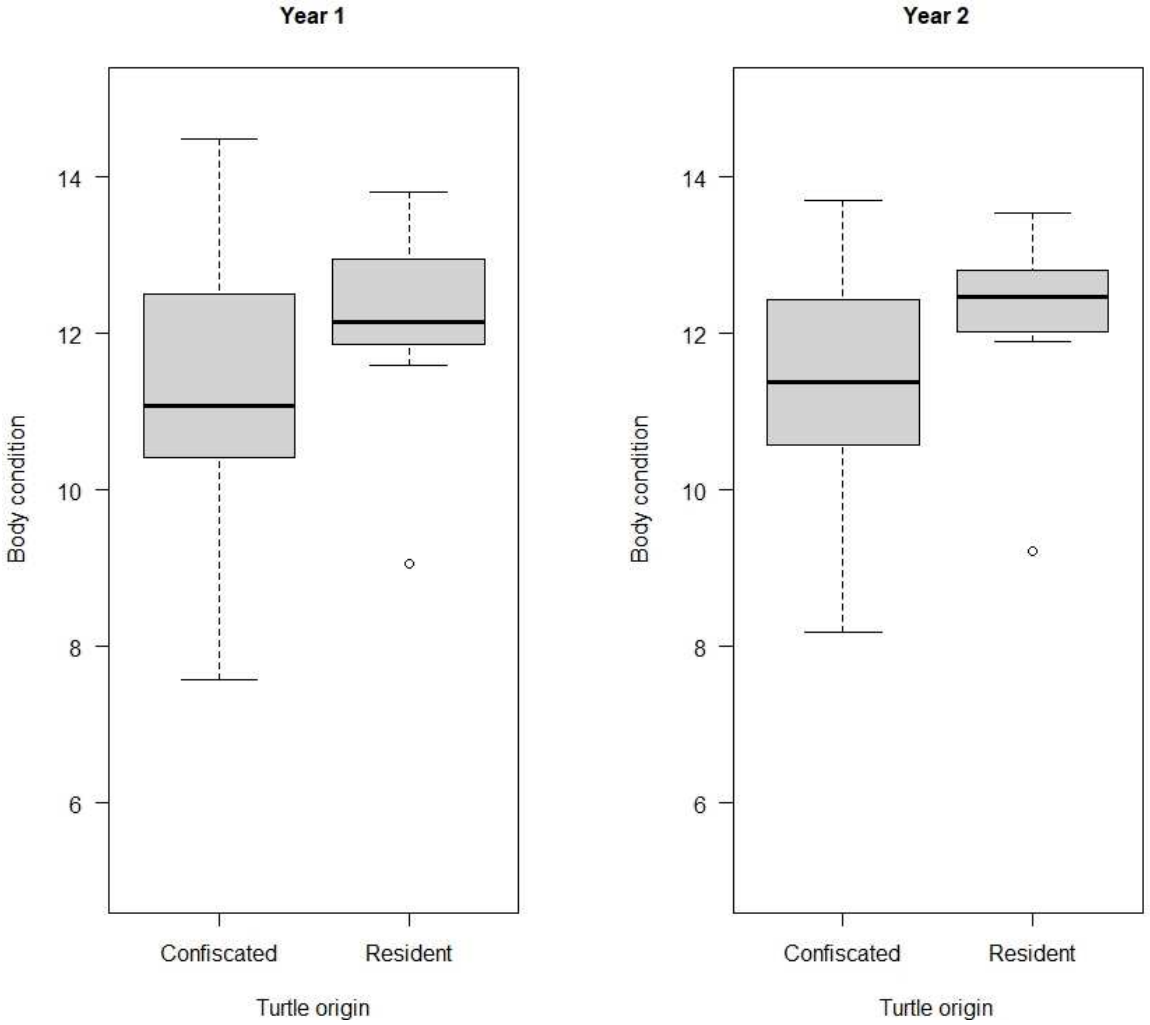


Figure 2.2. A comparison in Year 1 and Year 2 log transformed home range sizes in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA, with pre-settling data included for confiscated turtles in Year 1.

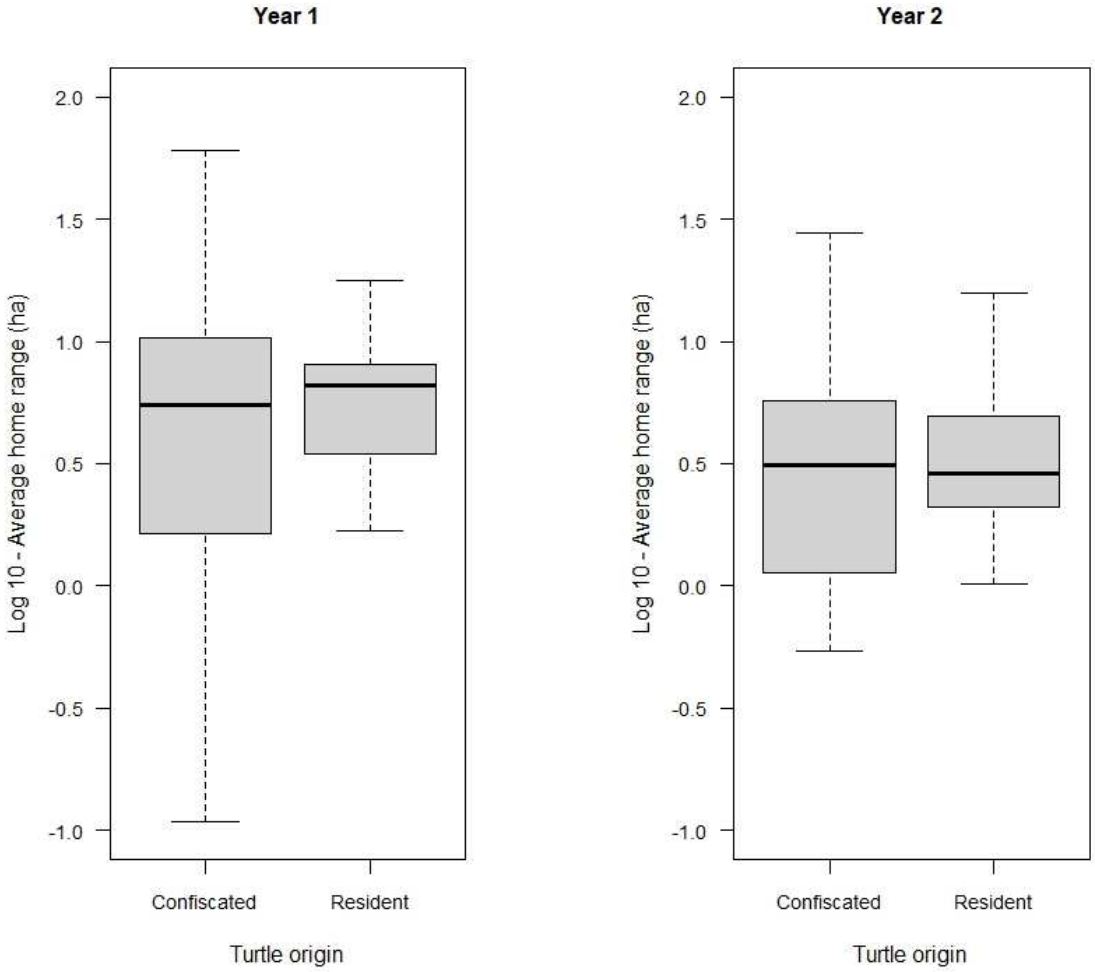


Figure 2.3. A comparison in Year 1 and Year 2 log-transformed home range sizes in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA, with pre-settling data excluded for confiscated turtles in Year 1.

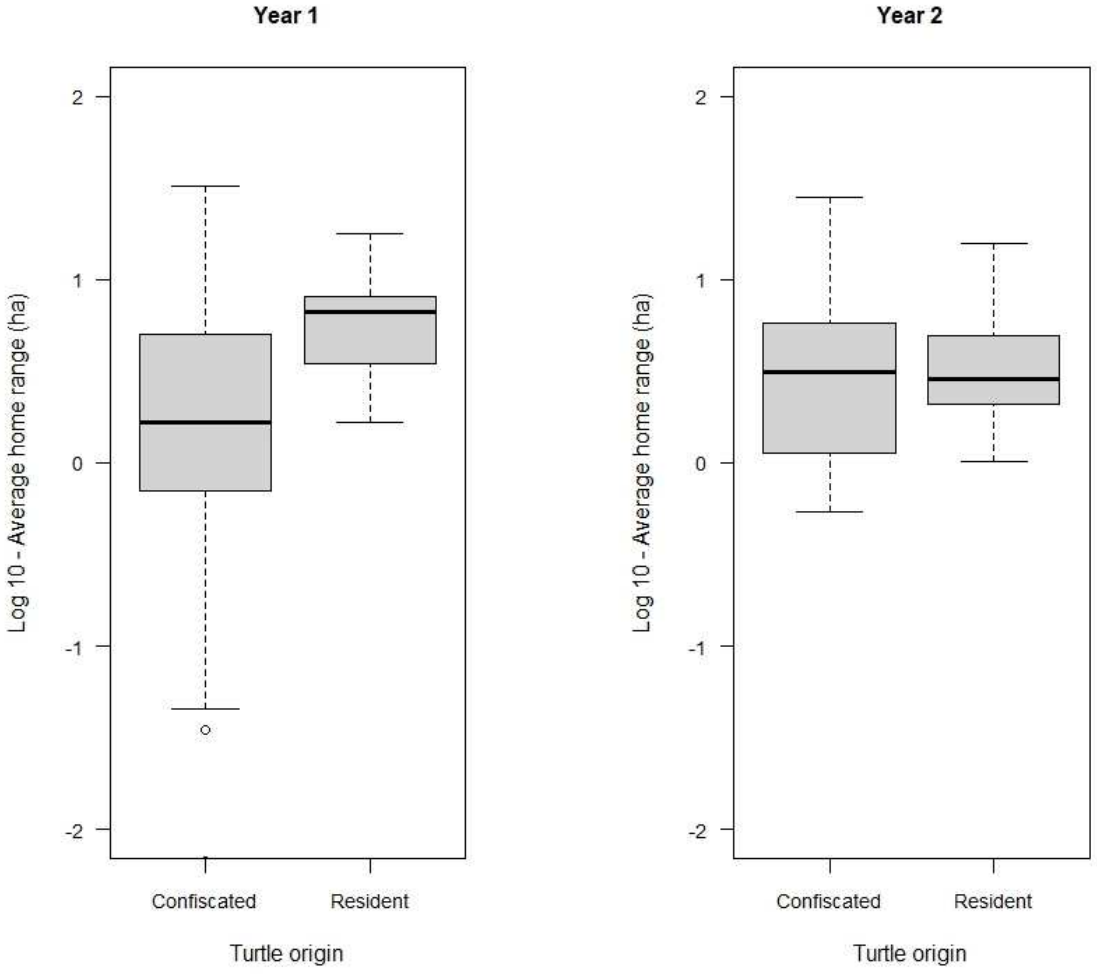


Figure 2.4. A comparison in log-transformed mean daily movement in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA, with pre-settling data included for confiscated turtles in Year 1

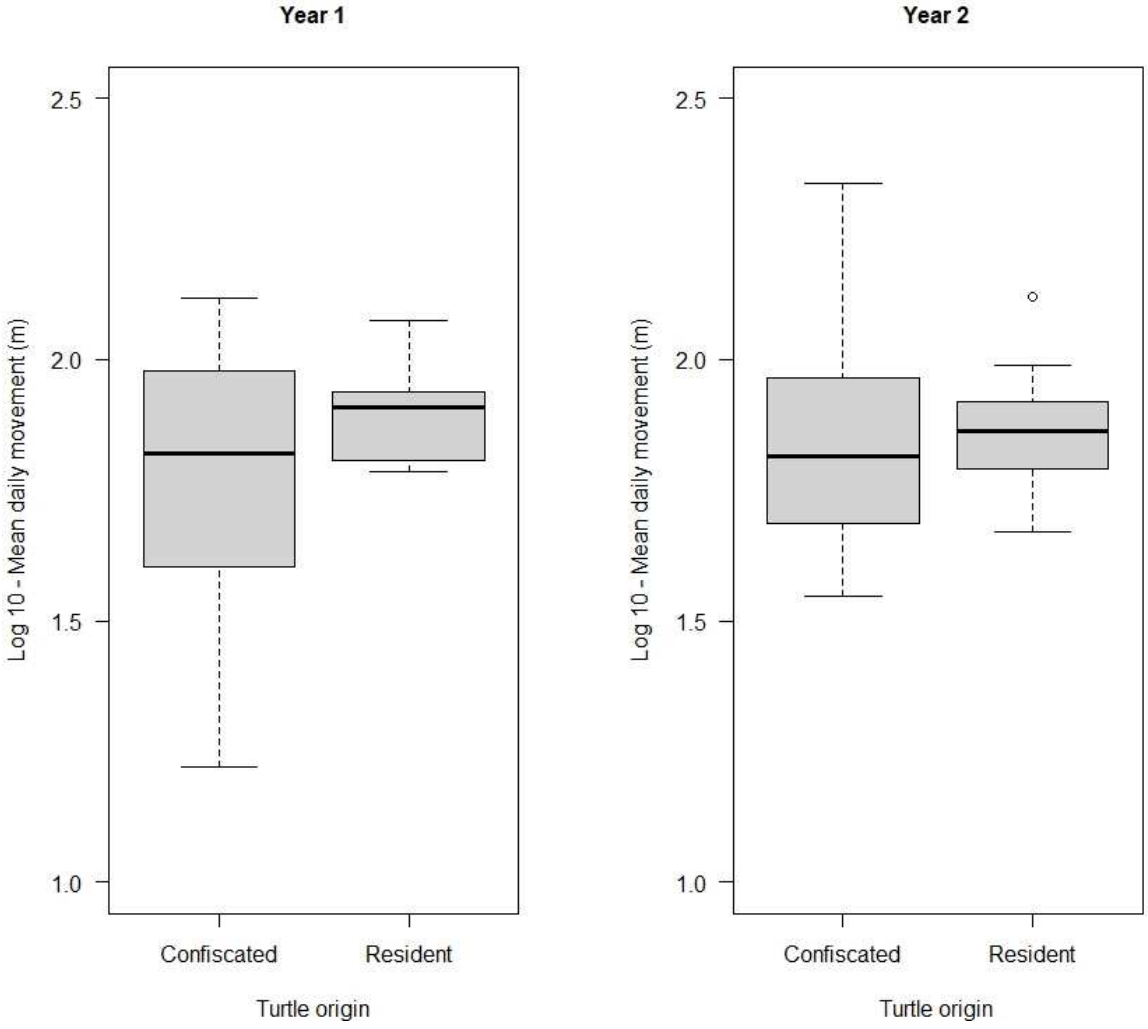


Figure 2.5. A comparison in log-transformed cumulative distance in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA, with pre-settling data included for confiscated turtles in Year 1

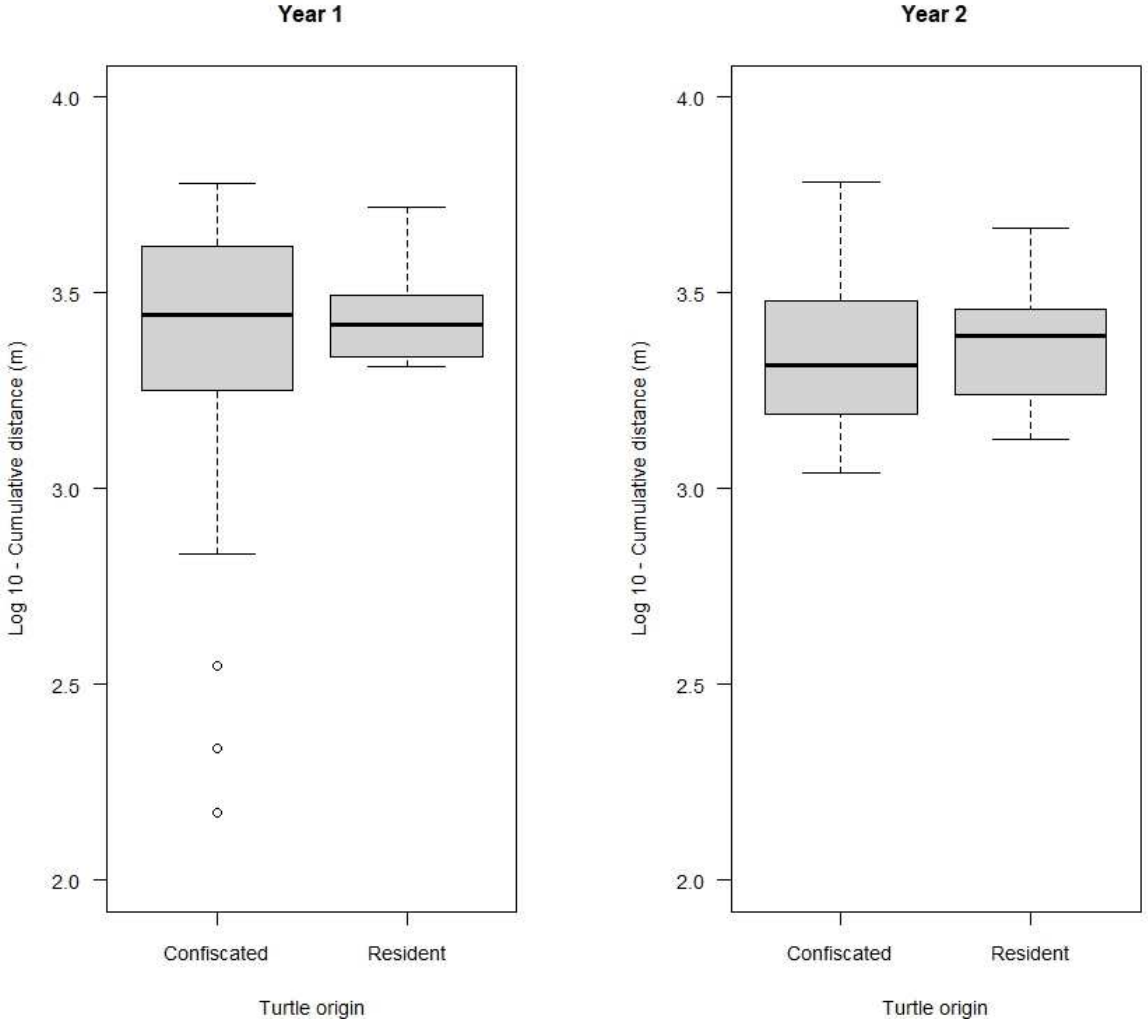


Figure 2.6. A comparison in log-transformed maximum displacement in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA, with pre-settling data included for confiscated turtles in Year 1

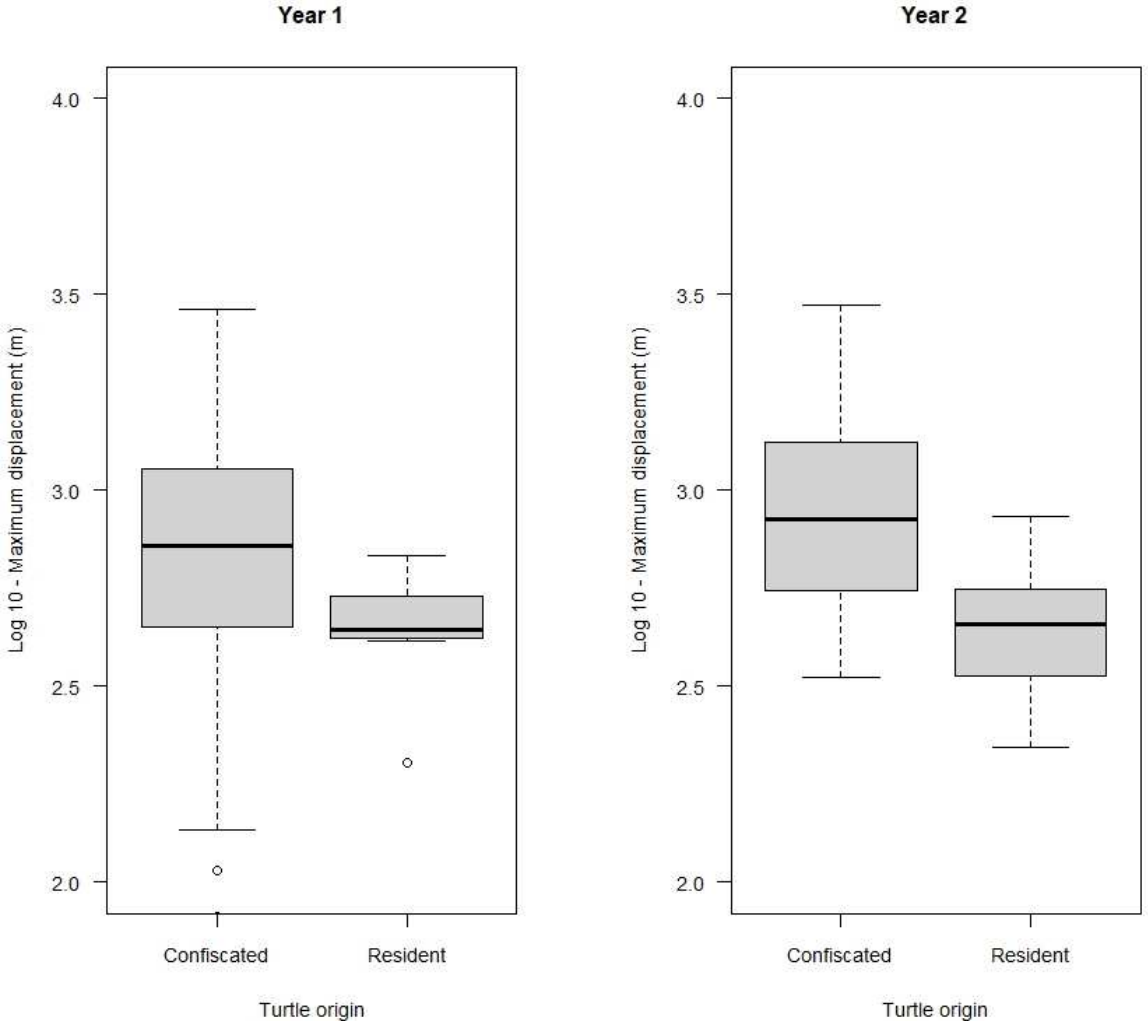


Figure 2.7. A comparison in home range (MCP) overlap between confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA, with pre-settling data excluded for confiscated turtles in Year 1

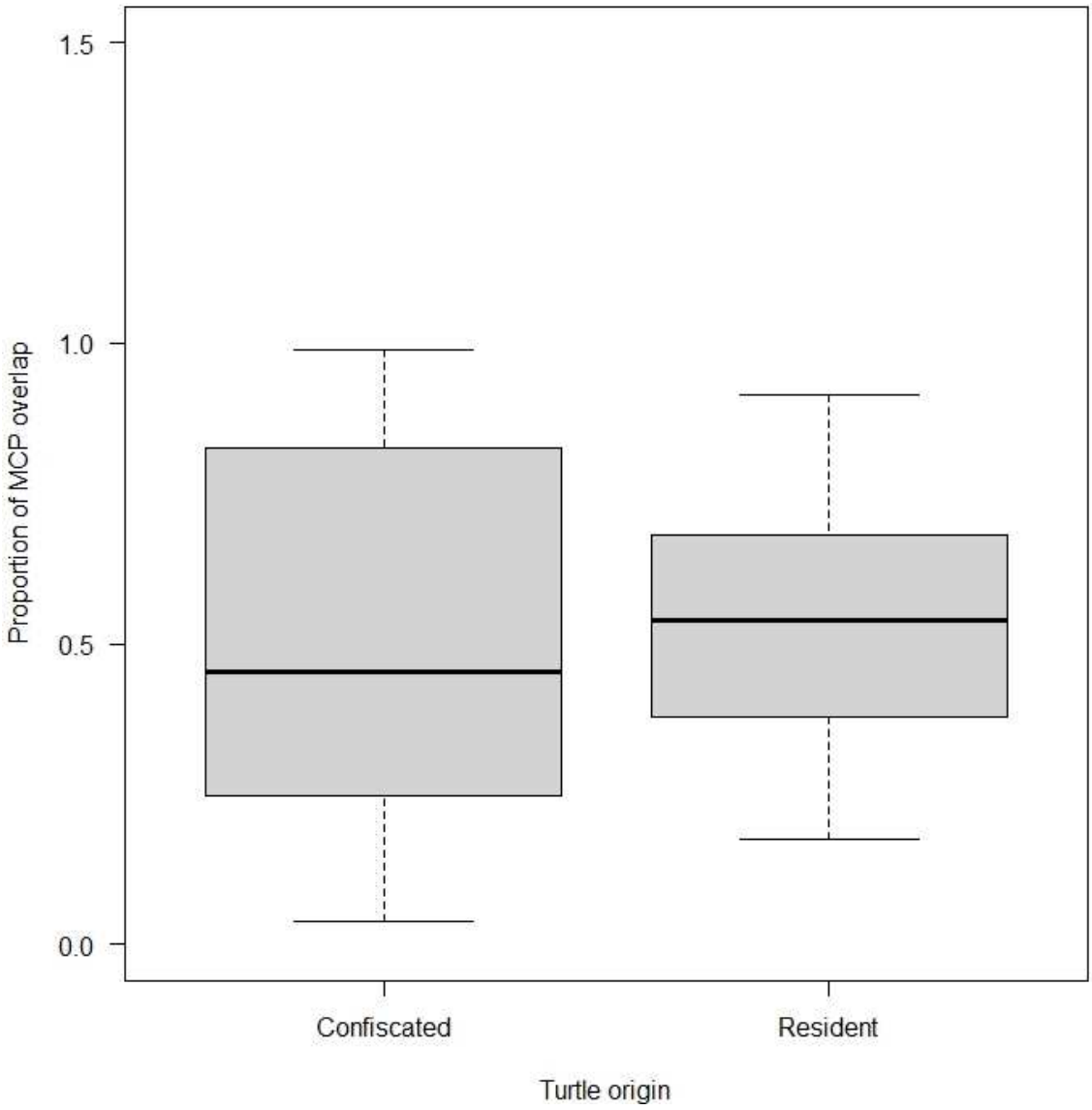
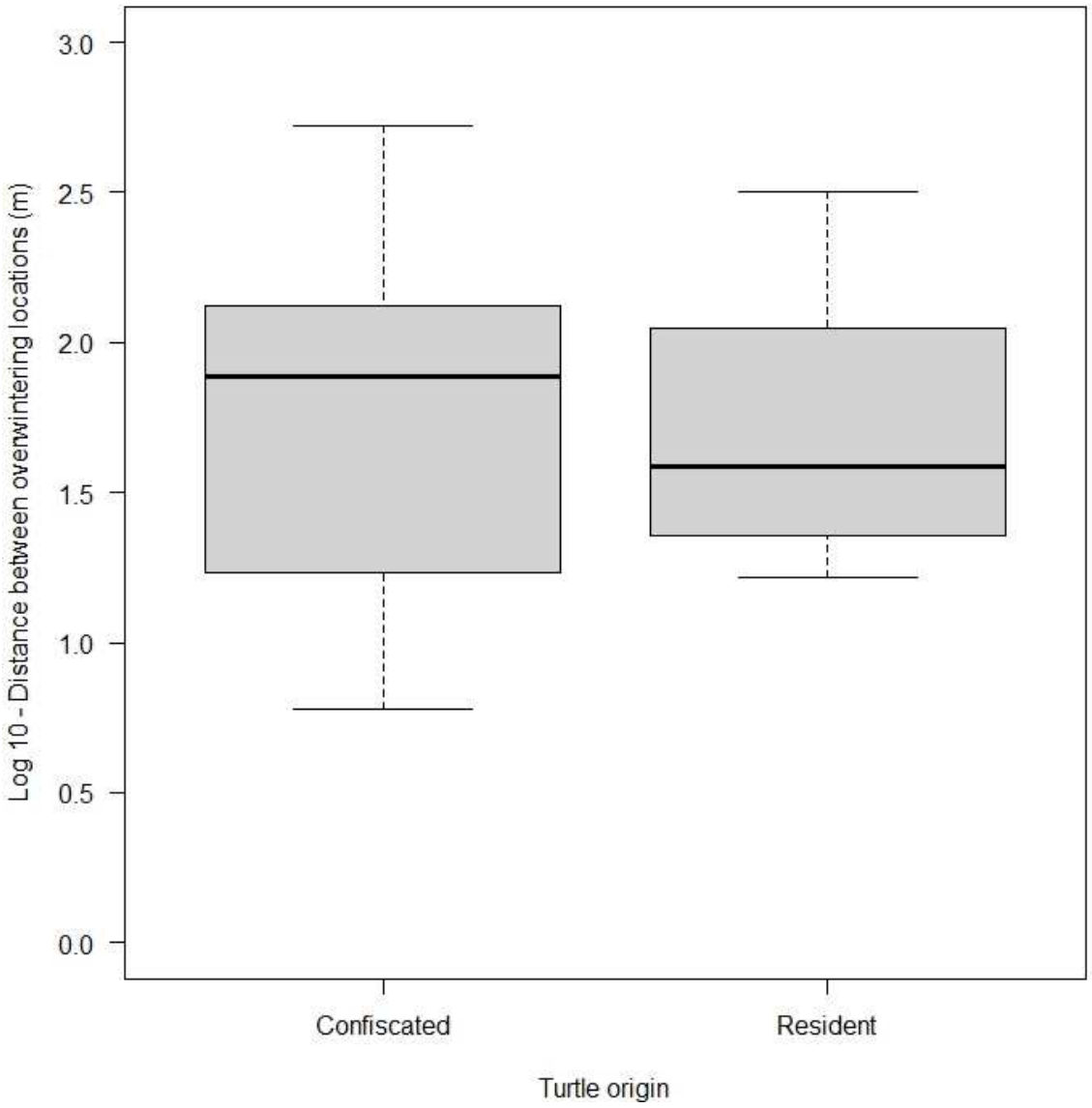


Figure 2.8. A comparison in log-transformed distance between overwintering locations in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA, with pre-settling data included for confiscated turtles in Year 1.



CHAPTER 3

BODY CONDITION, PATHOGEN PREVALENCE, AND HEMATOLOGY AND THEIR EFFECTS ON SURVIVAL OF CONFISCATED AND RESIDENT EASTERN BOX TURTLES

Introduction

Translocation is the purposeful movement of animals by humans from one area to another outside of their original home range or established territory (IUCN, SSC 2013). Translocation is commonly used for managing game species and has been increasingly used in the management of non-game species (Griffith et al. 1989, Fischer and Lindenmayer 2000 Morris et al. 2021). Although the success rate of translocations (translocations potentially qualifying as successful if survival, reproduction, and population growth occur) has increased from 50% to 75% over the last 40 years (Morris et al. 2021), there are concerns associated with this management strategy, including the risk of pathogen spread.

Any translocation carries the risk of pathogen spread or introduction to wild populations (Woodford 1993). Pathogens can be introduced to recipient populations through contact with translocated animals, but immunologically naïve individuals released into an area where pathogens are endemic can also be at risk (Cunningham 1996). For example, *Batrachochytrium dendrobatidis*—the fungal pathogen causing chytridiomycosis—was introduced into a wild population of the red-listed Mallorcan midwife toad (*Alytes muletensis*) in central Spain through release of animals from a captive-breeding facility, causing mass mortality events (Walker et al. 2008). On the other hand, reintroduced grey wolves and their offspring acquired several viral and

parasitic infections due to spillover from native canid populations within Yellowstone National Park (Almberg et al. 2012). In addition to the risk of pathogen transfer, translocation can also cause stress, which can compromise otherwise healthy immune systems and increase vulnerability of translocated animals to disease and mortality (Teixeira et al. 2007, Dickens et al. 2010).

Animals confiscated from the illegal wildlife trade are typically excluded from translocations as their geographic origin is usually not known and it is unclear to what potential pathogens they may have been exposed (IUCN SSC 2002, IUCN SSC 2019). Mortality and pathogen prevalence are generally high in confiscations, with individuals stressed and weak – often becoming clinically ill or even dying. For example, in 2009, over 26,400 animals were confiscated from a Texas animal wholesale dealership and within 10 days after their seizure an average of 872 animals were lost per day, due to the poor conditions they were found in, with reptiles making up 41.6% of those mortalities (Ashley et al. 2014). Contributing factors to the mortalities included infection, stress, and overcrowding (Ashley et al. 2014).

The illegal trade in turtles is a growing threat to persistence of population and species, with an estimated 138,000 tortoises and freshwater turtles taken from the wild annually in the U.S., although much of the trade likely goes undetected (CITES 2016). Although more confiscations are being intercepted, the concern over risk of pathogens places a constraint on potential management options for individuals seized. Several pathogens can be found in captive collections and in wild populations of chelonians that are of special concern in the context of repatriating confiscated turtles. Ranaviruses are particularly problematic as they are highly transmissible between taxa and can cause severe systemic lesions and multi-organ failure followed quickly by death (Adamovicz et al. 2020). Ranaviruses have been found in 21 species

of turtles and tortoises around the world and are the suspected cause of mass mortality events in populations of some turtle species, including the eastern box turtle (*Terrapene carolina carolina*; Adamovicz et al. 2018, 2020). Developing treatments for this pathogen has been challenging because of the high mortality rate (71%-100%) and the short time interval between infection and death (30 days; Adamovicz et al. 2020). Often, when chelonians are infected with ranavirus, treatment and release are not pursued as a mitigation option because asymptomatic individuals and those who have overcome the infection remain carriers that can then spread infection to others in the population, risking a mass mortality event and collapse of the entire population (Adamovicz et al. 2020).

Other pathogens that are cause for concern in chelonian populations are *Mycoplasma* and herpesviruses. *Mycoplasma agassizii* and *M. testudineum* are the causative agents for Upper Respiratory Tract Disease in turtles. *Mycoplasma* was first detected in association with mortality events in gopher tortoises (*Gopherus polyphemus*) and desert tortoises (*G. agassizii*; Brown et al 1994, Brown et al. 1999). It has also been reported in captive and wild box turtles where outbreaks seem to occur in combination with other stressors, although the pathogen is associated with low mortality rates in eastern box turtles (Sim et al. 2016, Adamovicz et al. 2018). Long-term treatment (34 days) has been successful in treating captive eastern box turtles, but treated individuals still shed the virus even when no clinical signs are present (Sim et al. 2016). Herpesvirus infections cause an array of clinical symptoms, including stomatitis, encephalitis, conjunctivitis, hepatitis, and ulcerative lesions of skin and shell (Okoh et al. 2021). Herpesviruses have been detected in wild populations and captive collections of eastern box turtles and in wild bog turtles (*Glyptemys muhlenbergii*), spotted turtles (*Clemmys guttata*), and wood turtles (*Glyptemys insculpta*; Ossiboff et al. 2015a, Sim et al. 2016, Adamovicz et al.

2018). Generally, however, herpesviruses in chelonians are linked to mild infections and mortality rates seem to be low (Ossiboff et al. 2015a).

All three pathogens (*Mycoplasma* spp., ranavirus, herpesvirus) can present with similar clinical symptoms but can be differentiated on a molecular level, such as through screening of oral and/or cloacal swabs via polymerase chain reaction using pathogen-specific primers (Adamovicz et al. 2020). Co-infection by more than one pathogen has been found to occur in both captive collections and wild populations of multiple species of chelonians, including eastern box turtles and gopher tortoises (Sim et al. 2016, Archer et al. 2017, Cozad et al. 2020). Understanding pathogen diversity and prevalence in confiscated turtles is important for informing decisions regarding their suitability for release back into the wild.

Here I present a case study of a large group of eastern box turtles confiscated and subsequently released on the Savannah River Site, near Aiken, South Carolina. I performed comprehensive pre-release health assessments of confiscated turtles, including body condition evaluation, hemogram assessment, and pathogen screening and then monitored their survival for two years following release. My objectives were to characterize the baseline health of confiscated turtles, compare their health to that of wild resident box turtles, and to identify which health metrics best predict post-release survival. I predicted that: 1) confiscated turtles would have lower and more variable body condition than resident turtles; 2) confiscated turtles would harbor a greater diversity of pathogens than residents; 3) for those pathogens detected, prevalence would be higher in confiscated turtles compared to resident turtles; and 4) confiscated turtles would experience lower survival than residents during Year 1, but survival would be comparable between the groups in Year 2. I also predicted that post-release survival of confiscated turtles would be positively associated with pre-release body condition. Finally, I

predicted that pathogen infection status would negatively influence survival such that animals that test positive for one or more pathogens would be less likely to survive the monitoring period. Results from this study will serve to help make decisions involving future confiscations of illegally collected turtles and inform how confiscations can contribute to species conservation efforts.

Materials and Methods

Study Site

The Savannah River Site (SRS) is an 802 km^2 National Environmental Research Park owned and operated by the Department of Energy (DOE) and located near Aiken, South Carolina. The SRS lies in the Upper Coastal Plain physiographic province of west-central South Carolina bordering the Savannah River and supports a variety of terrestrial and aquatic habitats, including bottomland swamp forests, upland hardwood forests, pine (*Pinus* spp.) plantations, upland sandhills, abandoned farm ponds, former nuclear cooling reservoirs, Carolina Bay wetlands, and several stream systems (White et al. 2000).

I conducted my study in mature hardwood forest located between Upper Three Runs Creek and Tinker Creek. The forest is dominated by oaks (*Quercus* spp.) and hickories (*Carya* spp.) and transitions to bottomland floodplain habitat as it nears the creeks on either side. The surrounding habitat consists of mature slash pine (*Pinus elliottii*) stands with pockets of hardwood forests, floodplains, and small streams. Winter month temperatures for this area are generally mild with the average low temperatures being slightly above freezing (DeGregorio et al. 2017). Summer temperatures average ~26ºC and annual rainfall on the SRS is approximately 120 cm (White et al. 2000).

Animal Collection, Handling, and Tracking

Confiscated Turtles

In August 2019, South Carolina Department of Natural Resources (SCDNR) seized 208 illegally collected eastern box turtles from a private citizen and transferred them to the University of Georgia's Savannah River Ecology Laboratory near Aiken, South Carolina for temporary holding and eventual repatriation. SREL permanently marked each turtle with a unique ID by notching their marginal scutes (Cagle, 1939), recorded their sex based on the presence of sexually dimorphic characteristics (enlarged tail and concave plastron in males; Nichols 1939), and measured their midline carapace length (MCL) to the nearest 1 mm. SREL then held them in a predator-proof outdoor aviary (12 m x 15 m), where they had access to shallow water and vegetative cover and were provided with supplemental food. This temporary holding period provided the opportunity for turtles to recover from the poor conditions in which they had been held prior to confiscation and allowed time to identify and prepare a suitable release site. In late October 2019, SREL collected baseline blood samples from confiscated turtles (details below) and placed the turtles in a 1 ha soft-release pen at the selected release site, where they overwintered until the following spring. During March - May 2020, after turtles had emerged from winter dormancy, I searched the pen for turtles that were active on the surface. I placed radio-transmitters (model # R1860; transmitter 3.5g; Advanced Telemetry Systems, MN, USA) on a subset of 20 males and 20 females. At the time of initial transmitter attachments, I performed visual health assessments (details below), weighed turtles to the nearest 1 g (SAUTER EB60; August Sauter; Ebingen, Germany) and measured shell dimensions (MCL, shell width, shell height) to the nearest 1 mm. Using these data, I calculated a pre-release body condition index (BCI) for each confiscated turtle based on morphometric data (weight, carapace width

[CW]) following the formula developed by de Persio et al. (2019) to estimate fat reserves in eastern box turtles:

Fat Volume (BCI) = Exp(Ln(Weight)* 0.400336 + Ln(CW/10)*0.985831 - 2.26447)

On 15 May 2020, I removed sections of the pen wall to allow confiscated turtles to move freely. If any transmittered confiscated turtles died before the pen was opened, I substituted other confiscated turtles into the study, collecting the same morphometric data as described above. I radio-tracked turtles until December 2021 to monitor their post-release fate. During the first year following release, I located and recorded the GPS location of all turtles twice per week from 15 May – 25 Aug 2020, and subsequently recorded animal locations weekly until turtles initiated winter dormancy in December 2020. During winter dormancy, I recorded turtle locations every two weeks. Once turtles emerged from winter dormancy in Spring 2021, I replaced radio-transmitters to allow radio-tracking to continue for another year (until winter dormancy 2021). During Year 2, I recorded turtle locations once weekly from transmitter replacements until December 2021.

Resident Turtles

While monitoring released confiscated turtles during Year 1, I incidentally encountered 36 resident eastern box turtles at the release site. I handled residents similarly to confiscated turtles in terms of marking, performing visual health assessments, collecting morphometric data, and obtaining biological samples at initial capture. Thirty-six individuals were used to characterize the health of the resident box turtle population. I added the first 10 residents (5 females, 5 males; captured 24 May – 29 June 2020) to the radio-telemetry study and radio-

tracked them at the same location frequency as confiscated turtles until December 2021 to follow their fate.

Visual Health Assessments

As part of an overall assessment of their baseline health, I visually inspected each turtle (confiscated and resident) for injuries (wounds, missing limbs, shell damage), parasites, and overt signs of illness, such as lethargic behavior, emaciation, and weakness. Infections with pathogens such as *Ranavirus spp*. and *Mycoplasma spp*. can result in respiratory infections that produce similar clinical signs like nasal discharge, ocular swelling and discharge, eroded nares, and conjunctivitis (Adamovicz et al. 2018). I examined each individual's eyes, nares, tympanum, respiration, musculature, carapace, skin, plastron, cloaca, and oral cavity for any abnormalities or clinical signs of disease.

Pathogen Screening

Using separate sterile Copan Diagnostic Flocked Swabs (Copan Diagnostics Inc, Murrieta, CA, USA), I obtained oral swabs from transmittered confiscated turtles and residents captured during 2020. Due to shy individuals who remained enclosed in their shells, I could not collect samples from one confiscated turtle and 11 resident turtles. I used swabs to test sampled turtles for a total of 23 pathogens that have been recorded in wild populations and captive collections of chelonians (Table 3.1). I included pathogens that have not been recorded in eastern box turtles because confiscated turtles could potentially have been exposed to other turtle species prior to being seized by SCDNR. All swabs were stored at -70 °C for subsequent analysis.

I extracted DNA from each oral swab sample using Qiagen DNA blood mini kits (QIAGEN Inc., Redwood City, CA, USA), and assessed the DNA's concentration and purity using a NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA), and then stored samples at -20°C prior to screening for 23 pathogens using quantitative polymerase chain reaction (qPCR) in a multiplex format using published or in-house primer-probe assays (Table 1). I used pooled pathogen Taqman assays and a preamp mastermix (Thermo-Fisher, Waltham, MA 02454 USA) to perform specific target amplifications. I performed each reaction under the following cycling protocol: 70°C for 30 minutes, 25°C for 10 minutes, 95°C for 1 min, and then 35 cycles at 96°C for 5 seconds and 60°C for 20 seconds. I used sequential dilutions (10^7 to 10^1 copies per reaction) of FV3-like ranavirus (FV3), pan-ranavirus, *Emydomyces testavorans*, box turtle *Mycoplasma* sp., *Mycoplasma agassizii*, *Mycoplasma testudineum*, *Terrapene* herpesvirus 1, *Terrapene* herpesvirus 2, Emydid herpesvirus 1, and *Terrapene* adenovirus 1 as positive controls for each PCR plate and added a non-template control as a negative control onto each plate. I analyzed all reactions using fluidigm real time PCR analysis software (Fluidigm, South San Francisco, CA 94080 USA) and used a simplex reaction to confirm all positive samples. I performed qPCR in triplicate on a QuantStudio3 real time thermal cycler. I deemed samples as positive if all three duplicates had a lower cycle threshold value than the lowest detected standard dilution.

Hemogram Assessment

I drew a maximum of 1 mL of whole blood from the subcarapacial sinus of each turtle (confiscated turtles in October 2019, residents at initial capture in 2020) using a 2.5 cm 25-gauge needle (Hernandez-Divers et al 2002). I placed ~75 μL of whole blood in capillary tubes, which I

subsequently centrifuged (LW Scientific, Zipocrit; 3636 x g) to quantify packed cell volume (PCV). I also used whole blood to prepare 2-3 blood smears, which I stained with Wright-Giemsa (Harleco EMD Millipore, Billerica, MA, USA). A clinical pathologist evaluated a single blood film from each turtle to obtain a white blood cell estimate (WBC), conduct a differential screen for cell morphological abnormalities, and classify heterophils as mature or immature (Stacy et al. 2022). I calculated the proportion of heterophils that were immature by dividing the absolute number of immature heterophils by the total number of heterophils. Total heterophils and immature heterophils could not be quantified in some individuals due to poor quality of samples, resulting in the exclusion of six resident turtles from analysis. PCV could not be accurately determined due to lymph dilution for seven confiscated turtles and 10 residents (two radio-tracked, eight incidentally captured), resulting in their exclusion from analyses where PCV was the response variable.

Statistical Analysis

Baseline Health Metrics

I compared baseline health of transmittered confiscated box turtles with health of all residents encountered during 2020. I excluded one immature confiscated turtle that was too small to determine its sex. I calculated prevalence of each pathogen by sex (male, female) and turtle origin (confiscated, resident) as the percent of individuals testing positive for the pathogen. I report morphometric data (MCL, mass, BCI) and hematology data as means ± 1 standard error for each turtle origin and sex separately.

I compared BCI and PCV between turtles origin using basic linear models in R (R Core Team 2021). I constructed a set of five candidate linear models with BCI and PCV as the

response variable, and Turtle Origin (confiscated, resident) and Sex (male, female) as the categorical predictor variables. Candidate models included a null model, two single predictor models, one additive model, and one interactive model. For both response variables, I compared different candidate models within a set using Akaike's Information Criterion corrected for small sample sizes (△AICc, Burnham and Anderson 2004) in R using the "AICcmodavg" package (Mazerolle et al. 2020). I report the results for all candidate models with △AIC < 2 of the top model.

Predictors of First Year Survival of Confiscated Turtles

To better understand the factors that influence survival of confiscated turtles, I ran a survival analysis using Cox proportional hazard models with "survival" and "survminer" packages in R. I considered only first year survival. Because confiscated turtles that were transmittered in April – May 2020 were known to be alive on 1 January 2020, I evaluated survival over the period of 1 January – 31 December 2020. The continuous predictor variables included pre-release BCI, PCV, Total Heterophils, and Proportion of heterophils that were immature. The categorical predictor variables were Sex and Coinfection Status. The coinfection status of an individual turtle was set to 1 if only a single pathogen was detected; 2 if a turtle was infected with only two pathogens, and 2+ if it was infected with more than two pathogens. I was careful in the selection of models to avoid overfitting and also built models that made the most biological sense when looking at my predictor variables, resulting in 23 candidate models: a global model, a null model, six single parameter models, and 15 additive models that considered two or more predictors.

For the survival analysis, I compared different candidate models within a set using Akaike's Information Criterion corrected small sample sizes (△AICc, Burnham and Anderson 2004) in R using the "AICcmodavg" package (Mazerolle et al. 2020) and report the results for all candidate models within △AICc < 2.

Results

Of the 40 confiscated turtles I radio-tracked, females (n=20) ranged in size from 110-141 MCL and 207-508 g and males (n=20) ranged from 121-150 MCL and 272-567 g (Table 3.2). For comparing baseline health metrics between confiscated and resident turtles, I included both radio-tracked residents (n=10) as well as residents incidentally encountered during the 2020 field season (n=26). Among the 36 residents included, females (n=16) ranged from 118-138 MCL and 334-549 g and males (n=20) ranged from 112-149 MCL and 297-574 g.

Body Condition

Pre-release body condition of confiscated turtles ranged from 7.57 – 14.48 (average BCI = 11.18 ± 0.23), compared to 9.05 – 14.44 (average 11.92 ± 0.21) for residents at initial capture (Table 3.2). The top model for body condition was the additive model that included both Turtle Origin and Sex, but the model garnered only 0.38 of the AICc weight (Table 3). The second best model was the interactive model, followed by the model that included Turtle Origin alone. Collectively the top three models gathered 0.86 of AICc weight. Turtle Origin appeared in all three models and was the only significant predictor. As predicted, confiscated turtles—particularly females—tended to have lower body condition than resident turtles (Figure 3.1).

Hematology

Across all individuals, PCV ranged from 9 – 33, with females tending to have higher PCV than males (Figure 3.2) in both confiscated and resident turtles. Although the top model for PCV included Sex as the sole predictor variable, it gathered only 0.43 of the AICc weight and Sex was not a significant predictor (Table 3.4). The null model was the second best model with a △AICc of 0.86. None of the factors I examined significantly influenced PCV. Other hematology parameters, including total heterophils and number of immature heterophils, are summarized by sex and turtle origin (Table 3.2). Two confiscated female turtles had extreme values (≥ 0.50) for proportion of immature heterophils. I did not note any evidence of lymph contamination at the time samples were collected nor did I note any behavioral or other abnormalities during visual health assessments that would explain the extreme values.

Pathogen Prevalence and Coinfection Status

I screened a total of 39 confiscated turtles and 25 (8 transmittered, 17 incidentally encountered) resident turtles for 23 pathogens that have previously been documented in captive collections and wild populations of chelonians (Table 3.1). As predicted, confiscated turtles harbored a higher diversity of pathogens than did residents, with seven pathogens detected in confiscated turtles and four detected in residents. For the four pathogens detected in both turtle origin, prevalence was higher in confiscated than in resident turtles: box turtle *Mycoplasma* sp. (41.0% in confiscated vs. 8.0% in resident turtles), Emydid herpesvirus 1 (5.13% vs. 4.0%), *Terrapene* herpesvirus 1 (35.9% vs. 20.0%), and Chlamydiaceae family (51.2% vs. 12.0%). Of the three pathogens that were only detected in confiscated turtles, FV3-like ranavirus (FV3) was the most prevalent, occurring in 87.1% of individuals (Table 3.1). In addition, I detected

Terrapene herpesvirus 2 in 23.0% of confiscated turtles and *Terrapene* adenovirus in 41.0% of individuals.

All confiscated turtles tested positive for at least one pathogen, compared to 40.0% of residents. In addition, coinfection with more than one pathogen was more common and complex in confiscated than resident turtles (84.6% vs. 8.0%, respectively). Of the 39 confiscated turtles for which oral swabs were available, 15.3% tested positive for only a single pathogen, 30.7% for two pathogens, 28.2% for three pathogens, 12.8% for four pathogens, 7.6% for five pathogens, and 5.2% for six pathogens (Appendix 1). Among the 25 resident turtles screened for pathogens, 32.0% tested positive for one pathogen and 8.0% had coinfections involving two pathogens. The most common coinfection in confiscated turtles included FV3 and box turtle *Mycoplasma* sp. and the most common coinfection for residents was *Terrapene* herpesvirus 1 and Chlamydia.

Survival and Its Predictors

Of the 40 radio-tracked confiscated turtles, two were lost in Year 1 and one in Year 2 due to radio transmitter failure; in addition, 14 (7 females, 7 males) died from 7 May – 21 September 2020. Year 1 survival ranged from 60.0% - 65.0% depending on whether turtles lost from the study are presumed alive or presumed dead (Figure 3.3). Of the 24 confiscated turtles accounted for that survived Year 1, 95.8% - 100% survived the subsequent year. All 10 transmittered resident turtles survived the two year tracking period. Thus, as predicted, survival of confiscated turtles was lower than resident turtles during Year 1, but survival was similar between the two turtle origins in Year 2.

In models relating survival to health metrics (Table 3.5), four models fell within ΔAICc < 2 and included either total heterophils or proportion of immature heterophils as single predictor

models or in additive models with PCV. These 4 models account for 0.76 of the cumulative AIC weight. Although there is uncertainty among these specific models, the results collectively suggest that PCV, total heterophils and proportion of heterophils that are immature play a potential role in survival. The top ranking model was the additive model including PCV and proportion of immature heterophils, garnering 0.30 of AICc weight (Table 3.5). PCV had a negative coefficient (-0.06) with a hazard ratio of 0.94, indicating that for every 1 unit increase in PCV, risk of mortality decreased by 6%. Similarly, immature heterophils had a negative coefficient (-2.95) with a hazard ratio of 0.05, indicating that an increase in the proportion of immature heterophils from 0.0 to 1.0 is associated with a 95% decrease in mortality risk of mortality. However, neither PCV nor proportion immature heterophils were significant predictors of survival.

Because I suspected that results of survival analysis could be strongly influenced by inclusion of the two individuals with extreme values for proportion of immature heterophils, I refit the survival models after excluding these individuals. The resulting four models fell within $\Delta AICc < 2$ and again included either total heterophils or immature heterophils as single predictor models or in additive models with PCV. However, when the outliers were excluded, the top model was the additive model that included PCV and total heterophils, gathering 0.33 of the AICc weight (Table 3.6). PCV had a negative coefficient (-0.07) and a hazard ratio of 0.94, indicating that for every 1 unit increase in PCV risk of mortality decreases by 7%-- similar to the previous analysis including the outliers. However, total heterophils had a positive coefficient (0.17) and a hazard ratio of 1.17, indicating that for every 1 unit increase in total heterophils the risk of mortality increased by 17%.

Discussion

My study is among the first to follow the fate of soft-released eastern box turtles that were seized from the illegal wildlife trade and repatriated back into the wild. I evaluated their pre-release body condition, hematology and pathogen prevalence and compared them to resident box turtles that occurred sympatrically at the release site. I also attempted to identify which pre-release health metrics best predicted post-release survival of confiscated box turtles. Body condition was lower in confiscated turtles than in residents but did not influence their post-release survival. Confiscated turtles harbored a greater diversity of pathogens, exhibited higher pathogen prevalence, and had more complex coinfection patterns compared to resident turtles. Confiscated turtles also exhibited lower survival in the first year than did residents, and lower PCV and higher total heterophils were associated with greater risk of mortality. In the second year following release, both confiscated and resident turtles experienced 100% survival.

As predicted, confiscated turtles –particularly females—had a lower average body condition than resident turtles. Often animals are held in questionable conditions with poor husbandry during their time in the illegal wildlife trade (IUCN, SSC 2002, IUCN, SSC 2019). When SCDNR seized the turtles that were included in the study, they reported that turtles were being held outdoors during the hot summer months in open metal cattle tanks without access to food or water, explaining the lower body condition of confiscated turtles compared to the wild turtles found at the study site at the end of winter dormancy. Surprisingly, body condition was not associated with survival of telemetered confiscated turtles when hematological variables were also considered. The body condition index I used had been developed specifically for estimating fat reserves in eastern box turtles and was validated using computed tomography – a medical imaging technique (dePersio et al. 2019). Body condition can have important

consequences for turtles, with animals having higher fat stores exhibiting greater probability of reproduction (Congdon and Tinkle 1982), having higher reproductive output (Litzgus et al. 2008), and being better able to survive periods of dormancy (Price 2017). It is important to note that the body condition values for confiscated turtles was based on morphometric data collected at the time of initial transmitter attachment in April 2020 – after individuals had eight months to recover either in the temporary holding facility or in the soft release pen time. Thus, only individuals that had survived through their first winter dormancy were telemetered and included in the survival analysis. Body condition may have been important in determining which confiscated turtles survived earlier phases of the study (i.e., placement in soft-release pen or winter dormancy). Providing some level of supportive care —such as fluids or nutritional support—to confiscated turtles may have positively influenced survival and improved release outcome (Otten et al. 2022).

I detected seven pathogens in confiscated turtles but only four in wild residents, including two pathogens (Emydid herpesvirus 1 and Chlamydia) not previously reported for box turtles in the literature but found in both confiscated and residents in the study. For pathogens that were found in both groups, prevalence tended to be higher in confiscated than resident turtles, and all confiscated turtles were infected with at least one pathogen. Prior to confiscation, turtles were held in overcrowded tanks and under stressful conditions, increasing potential contact rates and exacerbating the propensity for exposed individuals to develop clinical signs of disease (Fischer and Romero 2019). Ranavirus (FV3) was by far the most prevalent pathogen, infecting 87.1% of the confiscated turtles, and was the pathogen of greatest concern. Ranaviruses reportedly have high morbidity and mortality rates in eastern box turtles (71 - 100%) and typically there is a short time (~30 days) between infection and death (Adamovicz et al. 2020). Interestingly, 21 of the

confiscated turtles that were infected with Ranavirus survived the two year study period. However, surviving animals can become reinfected, shedding the virus for up to 55 days after infection even when they exhibit only mild or even no clinical signs (Adamovicz et al. 2020).

While I did not detect ranavirus in any residents encountered at the release site, a resident box turtle captured in 2019 elsewhere on the SRS did test positive for ranavirus (Tuberville, unpublished data). Ranavirus was detected and was the suspected cause of mortality of an individual eastern mud turtle (*Kinosternon subrubrum*) captured on the SRS in 2014 (Winzeler et al. 2015). In addition, the pathogen has been detected in salamanders and anurans captured at several isolated wetlands on the SRS (Love et al. 2016). Given the prevalence of ranavirus in confiscated box turtles released as part of this study, continued pathogen surveillance is warranted to characterize post-release disease dynamics, including the potential for horizontal transfer of ranavirus to the resident population.

The complex coinfections in confiscated box turtles can likely be attributed to other species that they came in contact with prior to their seizure. Although box turtles constituted the majority of animals seized, SCDNR also recorded several species of aquatic turtles as well as native amphibians. The risks of pathogen transmission and coinfection are high in confiscations, as turtles are kept in poor conditions prior to their seizure and exposure to other species is inevitable (IUCN, SSC 2002, IUCN, SSC 2019). However, there are few confiscations that are actually screened for pathogens, thus making it hard to compare presence, prevalence, and coinfections in the study with other turtle confiscations (Rosen and Smith 2010). The most common coinfections observed was ranavirus and box turtle *Mycoplasma* sp. Box turtle *Mycoplasma* sp., detected in both confiscated and resident turtles, is normally coupled with mild symptoms and low mortality rates (Ossiboff et al. 2015b, Sim et al. 2016, Adamovicz et al.

2018). Prior to this study, SREL has not routinely collect swabs from resident eastern box turtles for pathogen screenings, thus understanding pathogen prevalence in the resident box turtle population will necessitate surveillance at locations on the SRS where they are unlikely to have been in contact with released confiscated turtles. My results underscore the importance of knowing the pathogen status of both resident and confiscated turtles to make informed decisions regarding the disposition of confiscated turtles.

As I predicted, the survival rate for radio-tracked confiscated turtles was lower than residents (60.0% – 65.0% vs 100%, respectively) during the first year but similar (95.8% - 100%) to residents in the second year. This is not surprising as confiscated animals face a broader array of inescapable stressors than do wild turtles (Ashley 2014). In addition to having poorer body condition, confiscated animals are often stressed due to crowded conditions and poor husbandry (IUCN SSC 2002, IUCN SSC 2019). There is also a "release cost" associated with most translocations in which survival of released animals might be expected to be less – particularly in the first year following release (Sarrazin and Legendre 2000).

Collectively PCV, total heterophils, and proportion of heterophils that are immature seem to play a role in survival. Risk of mortality tended to decrease with increasing PCV and decreasing number of total heterophils. PCV can be indicative of certain health issues such as anemia and dehydration (Saggese 2009, Stacy et al. 2011). Hematology estimates in eastern box turtles can vary widely among seasons and populations and with age and sex (Adamovicz 2019). Packed cell volume of resident box turtles fell within reference values reported for healthy wild eastern box turtles in Illinois (Adamovicz and Allender 2022) and only a single confiscated turtle had a PCV (9%) that fell below the range, indicating possible anemia (Stacy et al 2011). Surprisingly, we observed no significant difference in PCV between confiscated and resident

turtles. However, PCV for confiscated turtles was based on samples collected in October 2019 after turtles had a period of recovery in the temporary holding facilities, in which they had access to food and water. Thus, our study likely underestimates the role of these hematological parameters in predicting post-release survival. A pattern might have been detected if sample collection had taken place on the day that the turtles were acquired from SCDNR, but I elected to prioritize improving their holding conditions while minimizing animal handling time.

Total heterophils and proportion of heterophils that were immature were also represented in the best supported models, although there was high model uncertainty. Increased number of total heterophils was associated with greater risk of mortality in confiscated turtles. Heterophils respond to inflammatory stimuli, thus individuals with an infection or inflammation are more likely have higher values of heterophils (Stacy et al. 2011). For example, elevated heterophils in brown water snakes (*Nerodia taxispilota*) were associated with greater probability of detecting the pathogen associated with snake fungal disease (*Ophidiomyces ophidiicola*) and a higher probability of skin lesions (Haskins et al., *in prep*.). Surprisingly, however, pathogen coinfection status was not a significant predictor of survival but may have been due to the fact that no confiscated turtles were pathogen-free and only 15.3% tested positive for a single pathogen, while 84.7% had two or more pathogens.

Overall, the results highlight the importance of screening confiscated turtles for pathogens prior to their release into the wild. Making the decision to add residents as a control to the study was essential in order to have a baseline to compare data from the confiscated turtles. Resident animals are important for providing reference conditions with which to compare released confiscated turtles. The metrics we assessed health parameters can be essential in understanding the health of seized individuals. Monitoring PCV levels, which had a slight

influence on the survival of the confiscated turtles, is a cost effective way of determining important aspects of health. Only a small amount of blood is needed, and it can be done inhouse. While screening for pathogens can be costly and time consuming, it is a necessary step before reintroductions to eliminate the possibility of pathogen exposure to native populations. Screening the native population for pathogens prior to the reintroduction of confiscated individuals would provide important information for evaluating the relative risks associated with planned releases. Allowing a recovery period and supportive care in the first stages of a confiscation seemed to be a beneficial strategy, although collecting blood samples and body condition at the initial confiscation and pre-release could have provided solid evidence for this assumption. However, it does allow individuals to recuperate and provides time to screen for pathogens in confiscated animals before management decisions are made. This study is the first to follow the fate and assess the survival of soft-released confiscated eastern box turtles potentially providing critical information that can be used as guidance in future confiscations.

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Table 3.1. Twenty-three pathogens that were screened for based on oral swabs from radio-tracked confiscated eastern box turtles and resident eastern box turtles found on the Savannah River Site, Aiken County, SC, USA. Oral swabs were submitted to the University of Illinois Veterinary Diagnostic Laboratory. One confiscated turtle and eleven residents could not be screened for pathogens as samples could not be collected due to shy individuals. These pathogens have been detected in both captive collections and wild populations of chelonians. Indicated below are the literature source for PCR primers, literature source documenting the pathogen's occurrence (if applicable) in *T. carolina*, and prevalence confiscated and resident turtles.

Pathogen	**Previously documented in *T. carolina?***	**Literature documenting *T. carolina* infections**	**Source for qPCR primers**	**Confiscated ♀ (n=20)**	**Confiscated ♂ (n= 19)**	**Resident ♀ (n= 10)**	**Resident ♂ (n=15)**
Frog Virus 3 - Ranavirus	Yes	Johnson et al. 2008	Allender et al. 2013	85.0%	89.4%	0%	0%
Box turtle *Mycoplasma sp.*	Yes	Ossiboff et al. 2015b	Adamovicz 2018	55.0%	26.3%	10.0%	6.6%
***Terrapene* herpesvirus 1**	Yes	Kane et al. 2017	Kane et al. 2016	45.0%	26.3%	10.0%	26.6%
***Terrapene* herpesvirus 2**	Yes	Sim et al. 2016	Engel et al., 2020	15.0%	31.5%	0%	0%
***Terrapene* adenovirus**	Yes	Franzen-Klein et al 2020	Franzen-Klein et al 2020	35.0%	47.3%	0%	0%
Emydid herpesvirus 1	No		M. Allender unpublished	5.0%	5.2%	10.0%	0%
Chlamydiaceae family	No		M. Allender unpublished	55.0%	47.3%	0%	20.0%
Mycoplasma agassizii	No		Braun et al. 2014	0%	0%	0%	0%
Mycoplasma testudineum	No		Braun et al. 2014	0%	0%	0%	0%
***Ambystoma tigrinum* virus**	No		Pallister et al. 2007	0%	0%	0%	0%
Bohle iridovirus	No		Pallister et al. 2007	0%	0%	0%	0%
Epizootic hemorrhagic necrosis virus	No		Pallister et al. 2007	0%	0%	0%	0%
Salmonella tymphimurium	No		Levin et al. 2009	0%	0%	0%	0%
Salmonella enteritidis	No		Levin et al. 2009	0%	0%	0%	0%
Testudinid herpesvirus 2	No		Braun et al. 2014	0%	0%	0%	0%
Anaplasma phagocytophilum	No		Vargas-Hernandez et al. 2016	0%	0%	0%	0%
Intranuclear coccidiosis	No		Alvarez et al. 2013	0%	0%	0%	0%
Tortoise *Helicobactor* sp.	No		M. Allender unpublished	0%	0%	0%	0%
Pathogenic *Leptospira* spp.	No		Smythe et al., 2002	0%	0%	0%	0%
***Coxiella* spp. IS1111 gene**	No		Klee et al. 2006	0%	0%	0%	0%
***Coxiella* spp. ICD gene**	No		Klee et al. 2006	0%	0%	0%	0%
Emydomyces testavorans	No		Woodburn et al., 2019	0%	0%	0%	0%
***Emydiodea blandingii* herpesvirus 1**	No		Lindemann et al., 2018	0%	0%	0%	0%

Table 3.2. Morphometric measurements and hemogram data from confiscated and resident eastern box turtles on the Savannah River Site, Aiken, SC, summarized by turtle origin and sex and presented as mean ± 1 SE (and range). Morphometric data were collected in Spring 2020 at time of transmitter replacement for confiscated turtles and initial capture for resident turtles. Hematology data were based on samples collected from confiscated turtles in October 2019 prior to being placed in soft-release pens and from residents at time of initial capture in 2020 The morphometric parameters include mean carapace length (MCL), mass, and body condition index (BCI) based on the formula presented in dePersio et al. (2019). The hematology parameters include packed cell volume (PCV), white blood cell (WBC) counts, total heterophils, absolute number of immature heterophils, lymphocytes, monocytes, eosinophils, and basophils. Hematology information could not be obtained for 3 confiscated turtles and 2 residents.

	Confiscated females		**Confiscated males**		**Resident females**		**Resident males**	
Parameter	**n**	**Mean±1 SE (range)**	**n**	**Mean±1 SE (range)**	**n**	**Mean±1 SE (range)**	**n**	**Mean±1 SE (range)**
Morphometrics								
MCL (mm)	20	126.7±1.9 (110– 141)	20	133.9±1.6 (121-150)	16	128.9±1.4 (118-138)	20	136.1±2.5 (112-149)
Mass (g)	20	377.2±16.3 (207 – 508)	20	407.2±16.4 (272 – 567)	16	458.5±11.8 (334 – 549)	20	438.2±17.5 (297 – 574)
BCI (Fat volume, mL)	20	10.67±0.33 (7.57-13.38)	20	11.69±0.31 (8.83-14.48)	16	11.8±0.33 (9.48-13.80)	20	12.0±0.35 (9.05-14.44)
Hematology								
PCV (%)	17	18.5±1.0 (9.00-24.00)	16	16.8±1.12 (9.00-26.00)	14	19.5±1.46 (14.00-33.00)	12	16.8±1.35 (10.00-26.00)
WBC count (K/µl)	17	19.51±1.7 (11.00-41.20)	20	17.53±0.78 (9.80-23.20)	15	16.24±1.34 (7.90-25.80)	19	13.65±0.61 (9.30-19.20)
Total heterophils (K/µl)	17	9.26±1.0 (4.20-22.00)	20	8.73±0.50 (3.80-14.00)	14	7.42±0.61 (3.30-11.00)	16	5.43±0.47 (2.60-10.00)
Immature heterophils (K/µl)	17	1.29±0.49 (0.00-0.51)	20	0.78±0.13 (0.01-2.30)	14	0.36±0.08 (0-00.98)	16	0.30±0.06 (0.00-0.77)
Lymphocytes (K/µl)	17	0.46±0.08 (1.60-6.70)	20	0.54±0.09 (2.30-7.5)	15	0.35±0.09 (1.60-5.30)	19	0.35±0.07 (1.40-5.60)
Monocytes (K/µl)	17	1.24±0.22 (0.26-3.90)	20	0.88±0.14 (0.17–2.30)	15	0.39±0.08 (0.00-0.96)	19	0.41±0.07 (0.09-1.30)
Eosinophils (K/µl)	17	1.68±0.22 (0.19-3.30)	20	1.63±0.17 (0.19-2.90)	15	2.86±0.38 (0.90-5.80)	19	2.57±0.22 (0.52-4.20)
Basophils (K/µl)	17	1.85±0.28 (0.44-4.40)	20	1.46±0.21 (0.29-3.60)	15	2.35±0.31 (0.16-3.80)	19	1.80±0.18 (0.56-3.00)

Table 3.3. Candidate models used to detect predictors that had an influence on pre-release body condition index (BCI) in confiscated eastern box turtles and initial BCI in resident turtles on the Savannah River Site in Aiken, County, SC, USA. Both predictors were categorical variables corresponding to turtle origin (Origin) and sex. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	△ AICc	Cum. Wt.	Origin	Sex	Sex*Origin
Origin+Sex	10.87	4	270.92	0	0.38	**0.70**	0.6	
Sex*Origin	10.6	5	271.33	0.41	0.7			-0.85
Origin	11.18	3	272.59	1.66	0.86	**0.74**		
Sex	11.18	3	273.63	2.7	0.96		0.6	
Null	11.53	2	275.59	4.66	1			

Table 3.4. Candidate models used to detect predictors that had an influence on PCV in confiscated and resident eastern box turtles on the Savannah River Site in Aiken, County, SC, USA. Both predictors were categorical variables corresponding to turtle origin (Origin) and sex. The coefficient estimate for each predictor in a model is given under the column with the corresponding name; significant predictors are listed in bold for model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) – models are ordered by cumulative weight (Cum. Wt.). Models above the line show models that fell within the criterion for the △AICc value (<2).

Model	Intercept	K	AICc	Delta	Cum. Wt.	Origin	Sex	Sex*Origin
Sex	18.96	3	352.7	0	0.43		-2.1	
Null	17.96	2	353.56	0.86	0.7			
Origin+Sex	18.75	4	354.84	2.13	0.85	0.49	-2.1	
Origin	17.72	3	355.59	2.89	0.95	0.54		
Sex*Origin	18.52	5	357.05	4.35	1			-1.01

Table 3.5 – All candidate models used to evaluate role of predictors on the first year post-release survival of 40 radio-tracked confiscated turtles released on the Savannah River Site in Aiken, County, SC, USA. Categorical variables include sex (S – male, female) and coinfection status I, with the latter corresponding to the number of pathogens detected in individuals (1, 2, 2+). Continuous variables included packed cell volume (P), proportion of immature heterophils that were immature (I), total heterophils (T), and pre-release body condition (B). Each predictor variable's coefficient and hazard ratio (coef | HR) are listed under the corresponding column. Coinfection status has two coefficients and hazard ratios – one value represents the coinfection by two pathogens (C=2), and the other represents the coinfection by more than two pathogens (C=2+). For model selection, the Akaike Information Criterion was corrected for small sample sizes (AICc) and models are ordered by cumulative weight (Cum. Wt.). Models above the line show are those that fell within the criterion for delta AICc value (<2)

Model	K	AICc	Delta	Cum. Wt.	S	B	P	T	I	C=2	C=2+
P+I	2	67.53	0	0.3			-0.06 | 0.94		-2.95 | 0.05		
I	1	68.12	0.6	0.52					-2.52 | 0.08		
P+T	2	69.24	1.71	0.65			-0.05 | 0.94	0.02 | 1.02			
T	1	69.51	1.99	0.76				0.02 | 1.01			
S+I	2	70.37	2.85	0.84	-0.22 | 0.79				-2.4| .008		
B+I	2	70.39	2.86	0.91		-0.001 |		-2.52 | 0.08		
S+T	2	71.55	4.02	0.95	-0.31 | 0.73			0.02| 1.03			
B+T	2	71.77	4.25	0.99		0.0006 | 1.0		0.01| 1.02			
S+B+P+C+I	6	74.28	6.76	1	-0.74 | 0.47	-0.05 | 0.95	-0.08 | 0.91		-3.67 | 0.02	-0.68 | 1.97	0.96 | 0.37
All	7	77.2	9.67	1	-0.73 | 0.47	-0.05 | 0.95	-0.08 | 0.91	0.007 | 1.0	-3.74 | 0.02	-0.69 | 1.98	-0.95 | 0.38
P	1	79.67	12.15	1			-0.09 | 0.91				
P+C	3	80.8	13.27	1			-0.11 | 0.89			-0.59 | 1.8	-0.48 | 0.61
B+P	2	81.22	13.69	1		-0.17 | 0.84	-0.10 | 0.89				
S+P	2	81.83	14.31	1	0.20 | 1.22		-0.09 | 0.91				
S+B+P	3	83.62	16.1	1	0.09 | 1.10	-0.16 | 0.85	-0.11 | 0.89				
S+B+P+C	5	84.99	17.46	1	-0.50 | 0.61	-0.23 | 0.80	-0.13 | 0.88			0.63 | 1.87	-0.72 | 0.49
C	2	97.86	30.34	1						0.74 | 2.10	-0.36 | 0.69
Null	0	97.96	30.44	1							
B	1	99.86	32.33	1		-0.08 | 0.92					
S+C	3	99.88	32.36	1	-0.32| 0.72					-0.74 | 2.11	-0.47 | 0.62
S	1	100.06	32.53	1	0.06 | 1.06						
B+C	3	100.1	32.57	1		-0.06 | 0.94				0.74 | 2.11	-0.35 | 0.69
S+B	2	102.07	34.55	1	-0.03 | 0.97	-0.09 | 0.92					

Table. 3.6 – All candidate models used to evaluate the role of predictors on survival of radio-tracked confiscated turtles on the Savannah River Site in Aiken, County, SC, USA. This particular analysis excludes two individuals (B036, B199) that had extreme values for percent of heterophils that were immature (I). Categorical variables include sex (S -male, female) and coinfection status (C). Continuous variables included packed cell volume (P), proportion of immature heterophils that were immature (I), total heterophils (T), and pre-release body condition (B). Each predictor variables coefficient and hazard ratio (coef | HR) are listed under each predictor variable. Coinfection status has two coefficients and hazard ratios – one value represents the coinfection by two pathogens (C=2), and the other represents the coinfection of two plus pathogens (C=2+). For model selection, the Akaike Information Criterion was corrected for small sample sizes and models are ordered by cumulative weight (Cum. Wt.). Models above the line are those that fell within the criterion for delta AICc value (<2)

	K	AICc	Delta	Cum. Wt.	S	B	P	T	I	C=2	C=2+
P+T	2	66.18	0	0.33			-0.07 | 0.93	0.17 | 1.18			
T	1	67.45	1.27	0.5				0.11 | 1.12			
P+I	2	67.53	1.35	0.67			-0.06 | 0.94		0.04 | 1.04		
I	1	68.12	1.95	0.8					0.13 | 1.14		
S+T	2	69.67	3.5	0.85	-0.16 | 0.86			0.12 | 1.12			
B+T	2	69.72	3.54	0.91		-0.02 | 0.97		0.11 | 1.12			
S+I	2	70.37	4.2	0.95	-0.12 | 0.89				0.01 | 1.01		
B+I	2	70.39	4.21	0.99		-0.03 | 0.97			0.25 | 1.30		
S+B+P+C+I	6	74.28	8.11	1	-0.60 | 0.55	-0.09 | 0.91	-0.01 | 0.9		-0.95 | 0.38	0.68 | 1.97	-0.92 | 0.39
All	7	77.2	11.02	1	-0.39 | 0.68	-0.11 | 0.89	-0.11 | 0.89	0.14 | 1.15	0.16 | 1.17	0.81 | 2.24	-0.65 | 0.51
P	1	77.49	11.31	1			-0.09 | 0.91				
P+C	3	78.06	11.89	1			-0.12 | 0.88			0.78 | 2.19	-0.39 | 0.67
B+P	2	78.57	12.39	1		-0.21 | 0.81	-0.12 | 0.89				
S+P	2	79.21	13.03	1	0.46 | 1.59		-0.11 | 0.89				
S+B+P	3	80.65	14.47	1	0.39 | 1.47	-0.20 | 0.82	-0.13 | 0.88				
S+B+P+C	5	82.45	16.28	1	-0.23 | 0.79	-0.23 | 0.79	-0.15 | 0.86			0.75 | 2.11	-0.56 | 0.56
C	2	95.55	29.37	1						0.86 | 2.38	-0.31 | 0.73
Null	0	96.2	30.03	1							
B+C	3	97.73	31.56	1		-0.08 | 0.92				0.87 | 2.40	-0.29 | 0.74
S+C	3	97.83	31.65	1	-0.17| 0.84					0.86 | 2.37	-0.37 | 0.68
B	1	97.96	31.78	1		-0.10 | 0.90					
S	1	98.18	32	1	0.20 | 1.22						
S+B	2	100.15	33.98	1	0.10 | 1.11	-0.09 | 0.91					

Figure 3.1. Body condition of released confiscated eastern box turtles (n=40) and resident eastern box turtles (n=36) on the Savannah River Site in Aiken, County, SC, USA. Body condition was calculated using the formula developed by dePersio et al. (2019) based on carapace width and weight collected at time of transmitter placement for confiscated turtles (April 2020) and at initial capture for residents (May--Oct 2020).

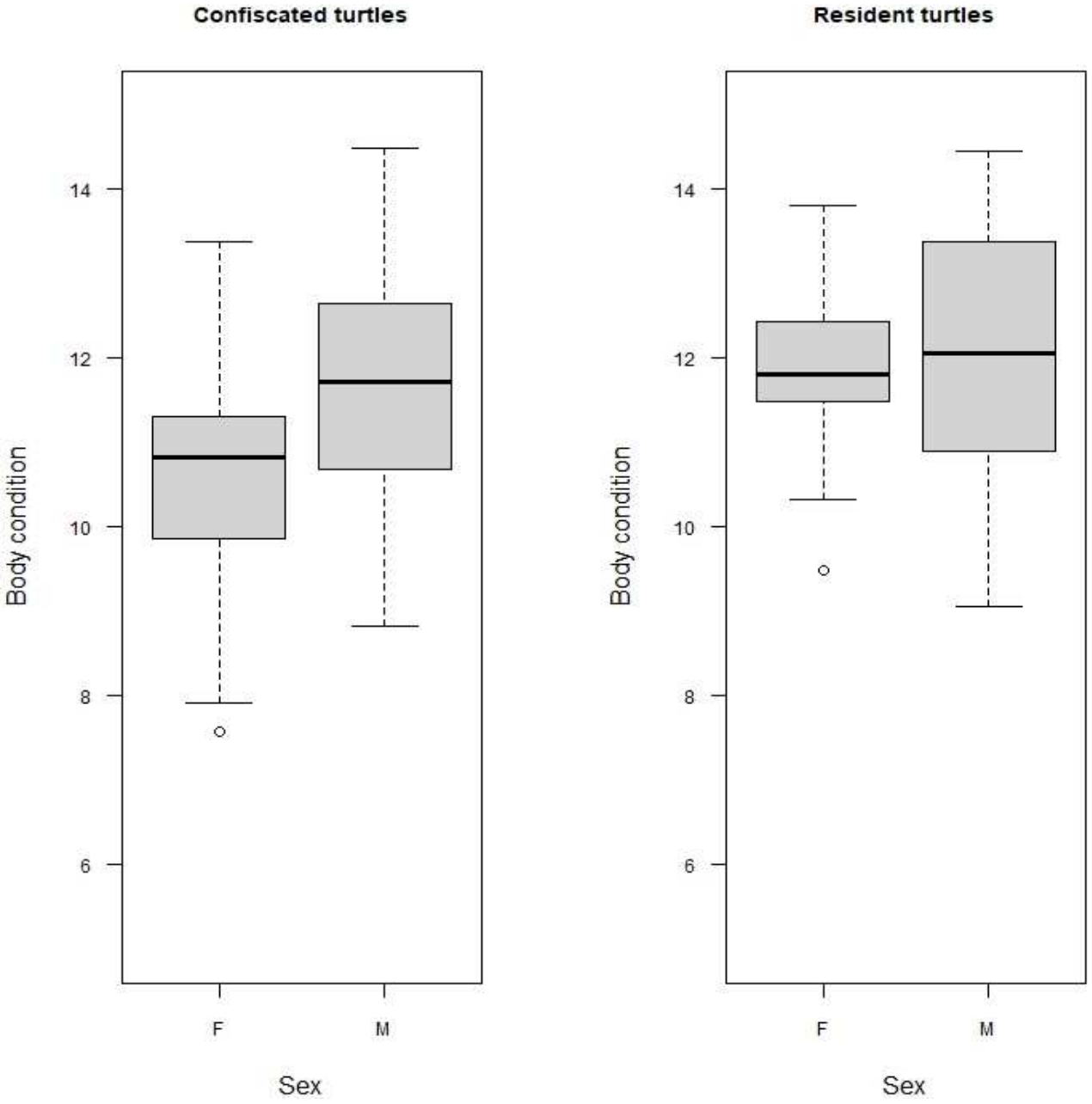


Figure 3.2. Packed cell volume (PCV) levels in released confiscated eastern box turtles (n=33) and resident turtles (n=26) located on the Savannah River Site in Aiken County, SC, USA. PCV was based on samples collected from confiscated turtles prior to placement in the soft-release pen (October 2019) and from resident turtles at their initial capture (May-Oct 2020). Blood samples could not be obtained from all individuals and samples with overt lymph contamination were excluded.

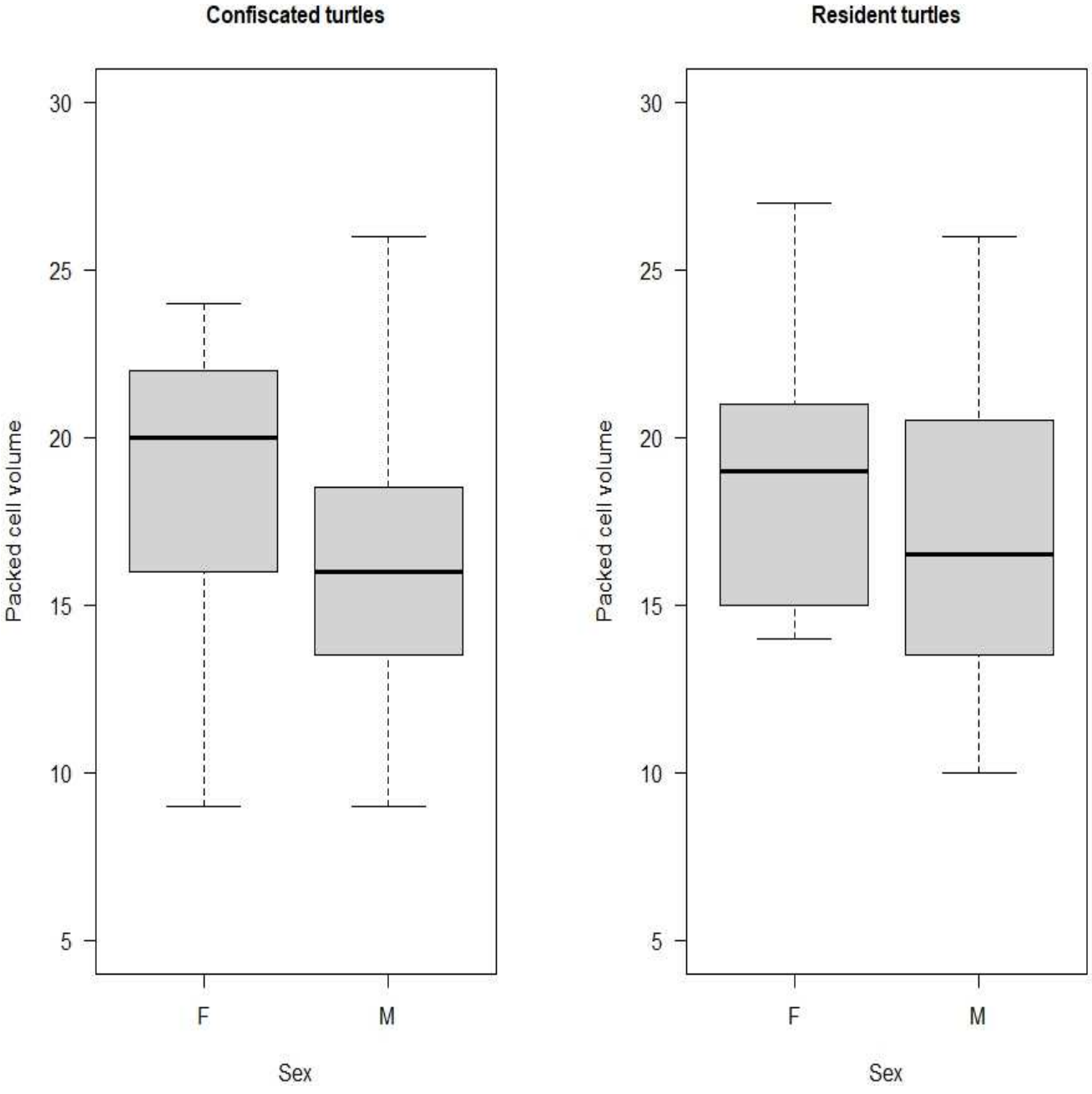
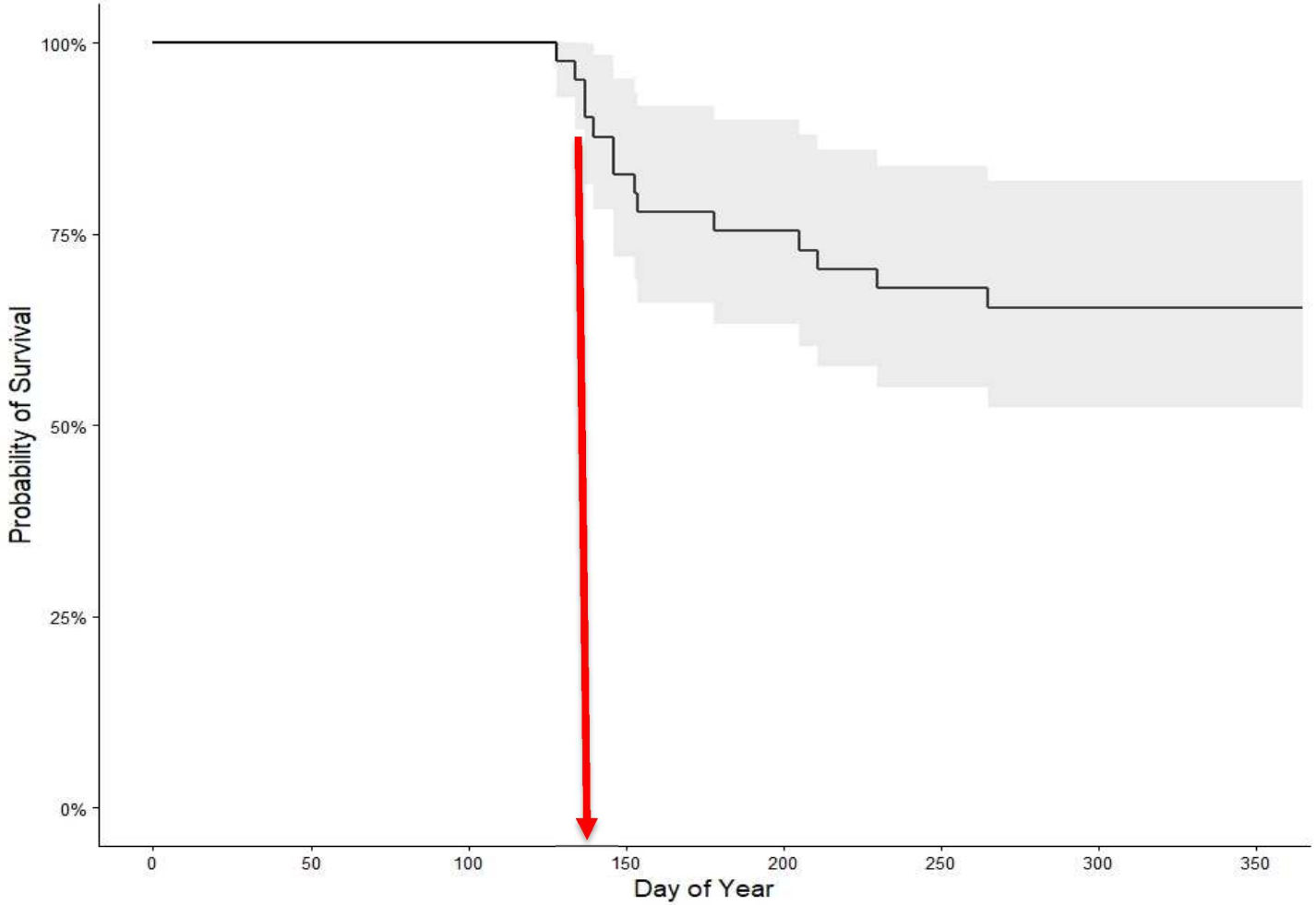


Figure 3.3. Cox survival curve of 40- radio-tracked confiscated eastern box turtles during the year in which they were released on the Savannah River Site, Aiken County, SC, USA. Turtles were initially held in a soft-release pen but the pen walls were opened on 15 May 2020 (day 136, indicated by vertical arrow), allowing turtles to move freely.



CHAPTER 4

CONCLUSION

Reptiles and amphibians are in drastic decline around the world due to habitat loss, invasive species, pollution, disease and parasitism, climate change, and unsustainable use (Gibbons et al 2000). Turtles are one of the most vulnerable major class of vertebrates, even more so than birds, mammals, and fishes (Lovich et al. 2018), and wild individuals are often pursued and collected to serve as food or pets in the illegal wildlife trade (Turtle Conservation Coalition, 2011). Illegal collection has had a destructive impact on the native populations of both aquatic and terrestrial turtles worldwide (Stanford et al. 2020). Although confiscations by law enforcement do occur, often they are not returned to the wild because their original location is unknown, or there is a potential risk of pathogen exposure to established wild populations (D'Cruze and Macdonald 2016). Confiscated turtles are commonly excluded from conservation translocations due to concerns about their unknown origin and disease. Therefore, a majority are held in permanent captivity, or they are euthanized, when holding facilities are unavailable (IUCN, SSC 2002, D'Cruze et al. 2016, IUCN, SSC 2019). There is an urgency to find a potential resolution whereby confiscated turtles can be released into the wild to replenish vanishing populations.

As a frequently trafficked species (Dodd 2002, Farrell et al. 2006) and the focus of several wild-to-wild translocation efforts (Hester et al. 2008, Rittenhouse et al. 2008, Farnsworth and Seigel 2013), the eastern box turtle provides the perfect opportunity to evaluate whether confiscated turtles can be reintroduced back into the wild and contribute to the recovery of wild

turtle populations. Several populations of box turtles across the eastern United States have been the focus of long-term monitoring projects that indicate the species has been declining rapidly for decades, and collection due to the illegal pet trade was one factor that contributed to this decline. (Kemp et al. 2022).

Over 200 eastern box turtles were confiscated from the illegal wildlife trade by SCDNR in August of 2019. After initial data collection and a recovery period, the confiscated turtles were brought to a research set-aside on the Savannah River Site where they were released and monitored over the course of two years. My thesis used this particular case study to provide much needed post-release monitoring data to assess whether confiscated turtles are suitable for release back into the wild. Specifically, I monitored their post-release movement patterns, initial health, pathogen presence, and post-release survival.

In Chapter 2, I evaluated movement patterns in released confiscated turtles and sympatric resident turtles who were added to the study to serve as a control. I collected locality data from all turtles for two years (May 2020 to December 2021) and calculated a suite of metrics to characterize settling behavior, movement patterns, and between-year site fidelity. I found that individual turtles with a lower body condition at release settled earlier than turtles with a higher body condition at release. Confiscated turtles exhibited greater movement than resident turtles in Year 1 – particularly when including the settling period, but their home ranges reduced and became comparable to residents in the subsequent year. This indicates that the large movement in Year 1 is most likely due to exploratory movements by confiscated turtles during their settling phase. Lastly, both turtle origins exhibited high between-year site fidelity.

In Chapter 3, I evaluated the body condition, hematology, and pathogen prevalence and their effects on survival of confiscated and resident box turtles. As predicted, I found that residents had a higher body condition than that of confiscated turtles, particularly in males. A higher prevalence and diversity of pathogens was detected in confiscated turtles compared to resident turtles. Survival in confiscated turtles during the first year of release was lower than that of resident turtles (60% - 65% vs. 100%) but became comparable in the second year at 95.8% - 100%. Although not significant, the Cox proportional hazard survival analysis tied PCV and heterophils (total heterophils and proportion of heterophils that are immature) to survival. PCV was higher and total heterophils were lower in turtles that lived versus died.

Overall, my results provide important information and possible guidelines for law enforcement and conservation agencies when evaluating whether a confiscation can be released into the wild or not. The establishment of home ranges and increased survival rates within their second year of release, suggests that under certain circumstances, confiscated turtles can be reintroduced into the wild. Recommendations that could support a positive outcome would include providing supportive care and evaluating PCV as a simple and inexpensive method of having insight to the health of individuals. Releasing individuals in good quality habitat on a contiguous landscape and a soft-release method of translocation may also increase site fidelity in confiscated individuals and provide the best outcome. However, the high prevalence of pathogens in the confiscated turtles prove that it is important to err on the side of caution. Screening for pathogens can reduce the introduction of disease in established wild populations and providing supportive care as mentioned above, can provide a recovery period that also allows time for pathogen screenings as well as other management decisions. In order for confiscated turtles to effectively contribute to conservation efforts while minimizing risks to

resident populations, it will be essential to secure resources necessary to provide pathogen screening, quarantine facilities and supportive care prior to release.

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and Freshwater Turtle Specialist Group, Turtle Conservation Fund, Turtle Survival Alliance, Turtle Conservancy, Chelonian Research Foundation, Conservation International, Wildlife Conservation Society, and San Diego Zoo Global, 54 pp.

Appendix 1. Identification number (ID), Sex (F = female, M = male), mean carapace length (MCL), and pathogen coinfection presence represented by "X" in confiscated eastern box turtles on the Savannah River Site, Aiken, SC. Seven pathogens were found in 39 confiscated turtles. Emydid herpesvirus 1., *Terrapene* Herpesvirus 1., *Terrapene* Herpesvirus 2., Box Turtle Mycoplasma, *Terrapene* Adenovirus, and Chlamydiaceae family. Fate is listed below as "Dead" or "Alive." All deaths occurred in the first year of release, no turtles died in the second year of data collection.

ID	Sex	MCL	*Frog Virus 3 - Ranavirus*	Emydid herpesvirus 1	*Terrapene* herpesvirus 1	*Terrapene* herpesvirus 2	Box turtle *Mycoplasma sp.*	*Terrapene Adenovirus*	Chlamydiaceae family	Fate
4	F	134	X		X		X	X	X	Alive
7	M	133	X		X			X		Alive
9	F	134							X	Alive
10	F	123	X		X		X			Dead
16	M	139				X		X		Dead
36	F	118	X		X			X	X	Alive
38	M	145	X					X		Dead
45	M	142	X			X			X	Dead
63	M	141	X		X		X			Dead
67	M	125	X			X				Dead
70	F	126	X							Dead
71	F	121	X				X			Alive
73	M	130	X							Dead
77	F	130	X		X		X	X		Alive
78	M	121	X		X			X	X	Alive
84	F	118	X			X				Dead
88	M	128	X							Alive
91	F	110			X	X			X	Alive
94	F	120	X							Alive
95	M	139	X				X	X	X	Alive
98	F	128	X				X			Alive
106	F	132							X	Alive
108	F	111	X		X		X	X	X	Dead
115	F	127	X				X	X		Dead
123	M	134	X					X	X	Alive
145	M	150	X		X				X	Alive
154	M	136	X			X				Dead
158	M	129	X					X		Dead
169	F	137	X				X			Alive

ID	Sex	MCL	*Frog Virus 3 - Ranavirus*	Emydid herpesvirus 1	*Terrapene* herpesvirus 1	*Terrapene* herpesvirus 2	Box turtle *Mycoplasma sp.*	*Terrapene Adenovirus*	Chlamydiaceae family	Fate
177	F	129	X		X	X	X	X	X	Dead
181	F	141					X		X	Dead
191	M	122	X		X		X	X	X	Alive
192	M	132	X			X		X		Alive
194	M	132	X				X		X	Alive
195	F	130	X					X		Alive
198	M	132	X	X		X	X	X	X	Alive
199	F	128	X						X	Alive
200	F	138	X		X		X		X	Alive
206	M	132	X		X				X	Alive