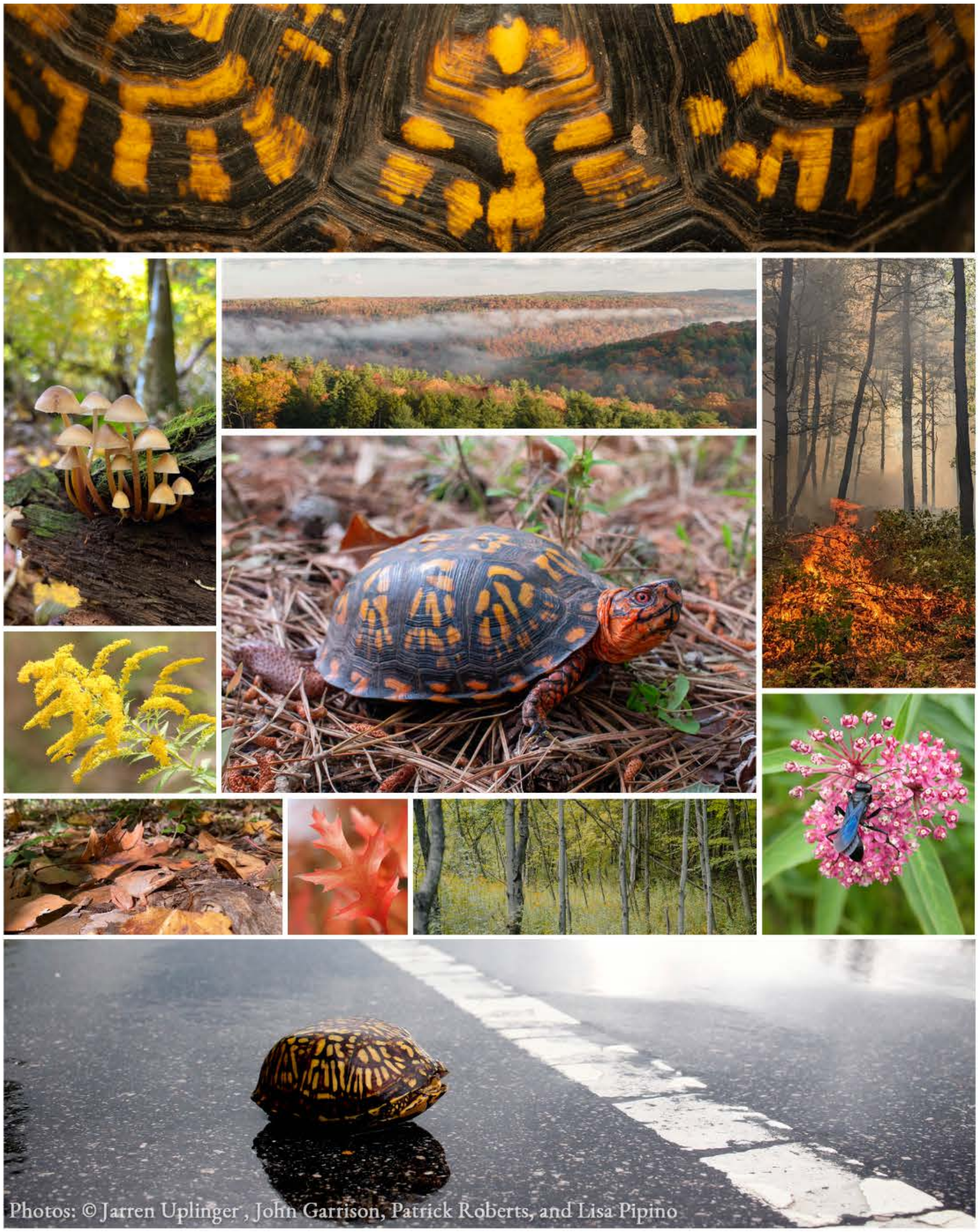
STATUS ASSESSMENT *for the* **EASTERN BOX TURTLE** *in the* NORTHEASTERN UNITED STATES

Maine • New Hampshire • Vermont • Massachusetts • Connecticut • Rhode Island • New York • New Jersey • Pennsylvania • Delaware • Maryland • DC • Virginia • West Virginia



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Status Assessment for the Eastern Box Turtle in the Northeastern United States

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Executive Summary

The *Status Assessment for the Eastern Box Turtle in the Northeastern United States* provides information that will allow the reader to build a solid understanding of the ecology of the eastern box turtle (subspecies woodland box turtle, *Terrapene carolina carolina*) in the northeast, understand the threats to the species, and relevant research conducted to date. This is meant to be a complimentary document to the *Conservation Plan for the Eastern Box Turtles in the Northeastern United States,* which provides recommendations to address and reduce the threats and a framework to increase the potential for the long-term persistence of the eastern box turtle.

In Chapter 1 we summarize the literature on the species' geographic distribution, habitat use and phenology, movements, longevity, population ecology and trends, genetics, ecosystem role, and jurisdictional status. The highlights are as follows. The eastern box turtle is a wide-ranging species that uses several habitat types, with forest habitat being essential for overwintering and open-canopy early successional habitats of great importance for reproduction. They are a long-lived species which reach maturation relatively late in life, have low reproduction, and experience high mortality of the young. This combination of life history characteristics makes them particularly vulnerable to stressors on the population. Population declines have been reported and are likely happening across the range.

In Chapter 2 we provide information on threats including development and fragmentation, loss of nesting habitat, roads, agricultural machinery, fire, predation and parasitism, illegal collection, disease and health, recreational activities, and climate change. We also summarize results from an expert poll, where participants ranked the threats. Many of these threats can have substantial negative impacts on populations. Most populations are likely affected by at least one of the threats, and more likely the synergistic and accumulative effects from multiple stressors.

In Chapters 3-7 we present a regional species population assessment protocol, a sampling summary, an overview of the current understanding of the specie's genetics, results from a landscape impairment evaluation, and list known existing conservation efforts. All except chapter 7 are products from this Regional Conservation Need Grant. The population assessment protocol was developed and tested across the northeast range. Data collected were used to evaluate the methodology and recommendations stated in the protocol to inform refinements to the protocol. The results from a genetic evaluation indicate differentiation by distance but very little genetic population structure throughout the northeast. In addition, a landscape assessment found that approximately 51% of the eastern box turtles' habitat in the northeast has been negatively altered.

Chapter 1. Ecology of the Eastern Box Turtle

Lori Erb, John Garrison, and H. Patrick Roberts

Overview.— Eastern box turtles (subspecies woodland box turtle; *Terrapene carolina carolina*) are a predominantly terrestrial freshwater turtle species identifiable by its highly domed, yellow, orange, or brown patterned carapace and hinged plastron, which can close shut. This species occurs in the eastern United States (US) at its easternmost extent from northern Florida to southern Maine and its westernmost extent from Illinois to Louisiana. In the Northeast, eastern box turtles are generally associated with mature closed-canopy forest, adjacent early-successional communities, and forest ecotones, but can be found within a range of natural and anthropogenic features including pastures, shrublands, residential lawns, gravel pits, powerline rights-of-way, and wetlands. Early successional habitats and forest ecotones with moderate to total sun exposure often serve as nesting areas for this species. In the Northeast, overwintering typically occurs in forests where individuals burrow into the soil or remain in leaf litter packs, mammal burrows, and below fallen root masses. Juveniles and hatchlings inhabit areas of high vegetation density and fields, though few studies have investigated juvenile habitat use. Daily activity patterns depend on temperature, but individuals generally remain active throughout the day. The spatial ecology of this species varies, with straight line home range size ranging between 40–2,145 m and population density varying between 0.22–12.4 turtles per hectare. Eastern box turtles are long-lived organisms, with some authors reporting individuals living over 100 years in the wild, though most individuals are thought to live between 50 and 80 years. Females tend to reach sexual maturity at approximately five to 14 years, whereas males reach maturity at around seven to eight years, with longer time periods in the more northern regions of the species' range. Nesting occurs in late May through July in the Northeast, and females typically only produce one clutch of eggs per year, with clutch sizes ranging from one to eleven eggs per clutch. Eastern box turtles are thought to play an important role within ecosystems, contributing significantly to biomass and nutrient cycling, seed dispersal, seed germination, and trophic balance. Populations do not seem to have high genetic differentiation unless separated by a considerable distance, such as 300–500 km. There is evidence that populations have declined dramatically throughout the species range, including a large population documented to be growing until a recent catastrophic (>95%) decline.

Geographic Distribution

The eastern box turtle has an extensive geographic range throughout the eastern U.S. The southernmost extent is in northern Florida, where it occurs throughout the Atlantic Coast until it reaches the northeastern most extent in southern Maine (Ernst and Lovich 2009; Kimble et al. 2014; Fig. 1). The range continues west from Maine and reaches its northwesternmost extent in central Michigan and Illinois, then continues east of the Mississippi River and reaches its southwestern extent in northern Louisiana and central Alabama (Ernst and Lovich 2009; Kiester and Willey 2015). Within this vast range, populations typically exist below 2000 m in elevation (Dodd 2001; Erb 2012; Chan et al. 2016; Diggins et al. 2016). A hybrid zone between woodland (*Terrapene c. carolina*), three-toed (*Terrapene c. triunguis*), gulf coast (*Terrapene c. major*), and Florida (*Terrapene c. bauri*) box turtles occurs between the eastern panhandle of Florida, southwestern Georgia, east-central Alabama, and northeastern Louisiana (Kimble et al. 2014; Kiester and Willey, 2015). Hybridization also occurs with ornate box turtles in western Louisiana (Blaney 1968; Cureton et al. 2011).

a) b)

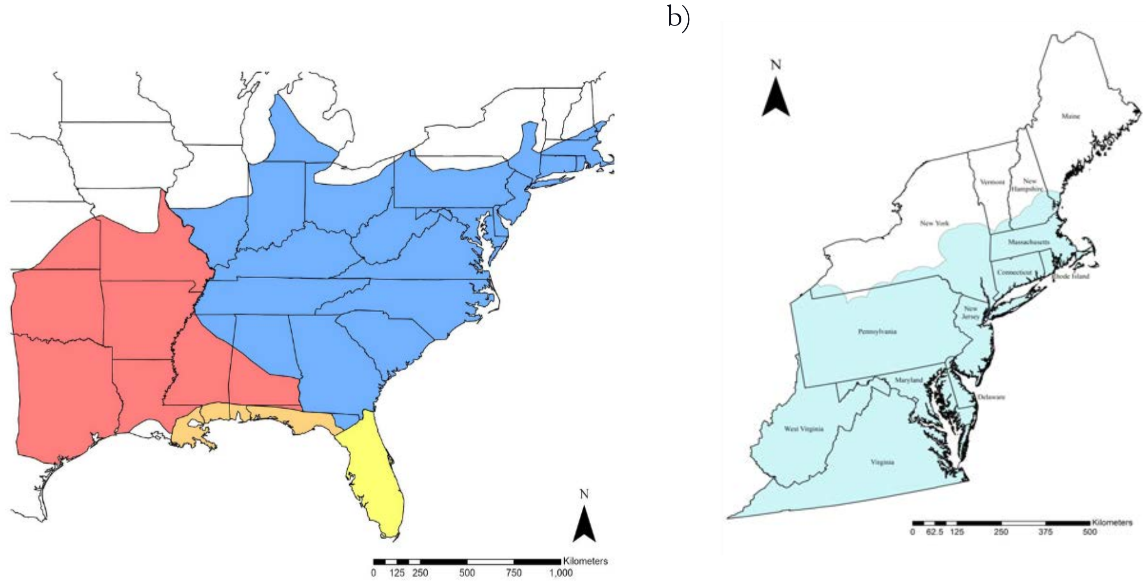

Figure 1. Distribution of a) the four *Terrapene carolina* subspecies throughout the US: *Terrapene c. carolina* displayed in blue, *Terrapene c. triunguis* displayed in red, *Terrapene c. major* displayed in orange, and *Terrapene c. bauri* shown in yellow and b) map of the northeastern United States with a simplified delineation of the expected eastern box turtle distribution in blue.

Habitat

The eastern box turtle is a terrestrial species that inhabits deciduous and mixed forests and has a strong affinity for ecotones (intersections of two habitat types such as forest-field edges) and early successional habitats (open canopy areas) (Dodd 2001; Ernst and Lovich 2009). In addition to forests and ecotones, they inhabit various anthropogenically disturbed habitats such as pastures, shrublands, residential lawns, gravel pits, and powerline rights-of-way (Madden 1975; Strass et al. 1982; Klemens 1993; Cook 2004; Quinn 2008; Erb 2012; Fredericksen 2014). This species also frequents aquatic systems such as shallow emergent wetlands, vegetated edges of large ponds, cranberry bogs, ephemeral wetlands, and slow-moving streams; generally avoiding deep water (Kaye et al. 2001; Donaldson and Echternacht 2005; Erb 2012; Fredericksen 2014; Henriquez et al. 2017). Habitat use and preference varies between populations, sex, and age class (Stickel 1978; 1989; Hagood 2009; Willey 2010). In the northeastern U.S.(Stickel 1950; Dodd 2001; Willey 2010), this species occurs in or near forests dominated by oaks (*Quercus* spp.), maple (*Acer* spp.), American beech (*Fagus grandifolia*), American sycamore (*Platanus occidentalis*), birch (*Betula* spp.), and pines (*Pinus* spp.). Understory vegetation varies between sites and consists of blueberries (*Vaccinium* spp.), raspberries (*Rubus* spp.), Viburnum (*Viburnum* spp.), poison ivy (*Toxicodendron radicans*), mayapples (*Podophyllum peltatum*), and laurels (*Lauraceae* spp.) (Stickel 1950; McKnight 2011). Several invasive species, such as multiflora rose (*Rosa multiflora*), Japanese barberry (*Berberis thunbergii*), autumn olive (*Elaeagnus umbellata*), and Japanese stiltgrass (*Microstegium vimineum*) can also frequently be found in eastern box turtle habitat (Mcknight 2011; Nicholson et al. 2020).

Overwintering Habitat.— Eastern box turtles typically overwinter in loose soils within deciduous or mixed forests (Kaye et al. 2001; Nazdrowicz et al. 2008; Willey 2010; Fredericksen 2014; Kiester and Willey 2015; Koester 2016; Nicholson et al. 2020). Many individuals overwinter under leaf litter packs, which provide important shelter and insulation, helping to retain moisture during brumation (similar to hibernation), and return to the same site each winter (Savva et al. 2010, Willey 2010). The depth in which eastern box turtles overwinter varies among individuals, with some brumating just below the surface of the soil and others burrowing up to 6.5 inches below the surface (Madden 1975, Willey 2010, Woodley 2013). Individuals may burrow deeper into the soil as the winter progresses (Woodley 2013). Overwintering microhabitats may include minor depressions, tree stump holes, under root masses, and mammal burrows (Ernst and Lovich 2009, Willey 2010). The overwintering process is fundamental and forested habitat with leaf litter duff is critical to the survival of eastern box turtles (Fig. 2).

Figure 2. Overwintering forest habitat examples.

Juvenile Habitat Use.— Few studies in the northeastern U.S. have investigated the habitat use of juvenile eastern box turtles. However, using radio telemetry, Nicholson et al. (2020) studied a population of recently hatched juveniles before and after hibernation. Hatchlings dispersed from hibernacula toward the north-northeast direction and moved an average of 3.8 m per day. Before hibernation, hatchlings selected habitats of high shrub, sapling, and tree cover, whereas after winter, they occupied fields with partial sun exposure (Nicholson et al. 2020).

Nesting Habitat.— Early successional habitats and forest ecotones with moderate to total sun exposure and exposed soils are important nesting areas for this species (Madden 1975; Quinn 2008; Hughes et al. 2017; Fig. 3). Access to high-quality upland early successional habitats not edged or bisected by roads or human development is fundamental to successful nesting (Nazdrowicz et al. 2008, Willey 2010; Fredericksen 2014). Eastern box turtles prefer nesting areas with well-drained loose soils, though they can use various soil types and substrates (Quinn 2008, Willey and Sievert

2012; Fig. 4.). Forest gaps may also be used for nesting, particularly in southern U.S. populations (Kiester and Willey 2015). Nest sites often have a south or east-facing slope with moderate to total sun exposure throughout the day, which promotes successful incubation (Congello 1978, Willey and sievert 2010). Females may prefer nest sites free of downed vegetation; however, woody debris, shrubs, and herbaceous vegetation are often found nearby and may provide essential cover for females before and after nesting (Erb 2012; Willey and Sievert 2012; Fig. 3).

Figure 3. Three different nesting habitat examples. Sand-dominated powerline access (left) Sand-loam access road with mounds (top right) Overgrown sand-gravel access road (bottom right)

Figure 4. Eastern box turtle nesting in sand.

Reproduction

The eastern box turtle has delayed maturity and low fecundity making them vulnerable to population declines. This species has been reported to reach sexual maturity around five to ten years of age in some regions (Ernst and Lovich 2009). However, females in the coldest climates within the species' range, including much of the Northeast, may lay their first clutch at 14 years (Willey 2010). Females may not lay eggs every year and eggs have a relatively high natural failure rate (Dodd 2001, Willey 2010). Examples of reproductively active individuals >50 years highlights the importance of adult contributions to recruitment and overall population dynamics (Henry 2003). However, it is unclear how often this occurs and unclear how parent age affects nest success and clutch size (Willey and Sievert 2012).

Mating And Courtship.— Eastern box turtles mate opportunistically, when they run into an individual of the opposite sex, and have been observed mating in various habitats on land and in shallow water (Evans 1953; Evans 1968; Ernst 1981; Belzer 1997; Ernst and Lovich 2009; Kiester and Willey 2015) (Fig. 5). Females can store sperm for up to four years, though they may be able to retain it longer (Ewing 1943). Sperm retention in female eastern box turtles may allow for the persistence of populations at low densities (Hattan and Gist 1975; Gist and Jones 1987). Mating and courtship occur throughout the active season, peaking in spring and fall, and may occur in any habitat type (Ernst 1981; Ernst et al. 1997; Dodd 2001). Eastern box turtles may locate mates visually, though chemosensory cues are also suspected to play a part (Ernst and Lovich 2009; Dodd 2001).

Figure 5. Examples of eastern box turtles mating.

Nesting Ecology.— The nesting season in the Northeast occurs from late May through early July (Fig. 6). Nesting is typically associated with precipitation, which is thought to loosen soils for ease of nest construction and may mask the scent of nests from predators (Stickel 1950, Congello 1978; Kipp 2003; Baker 2009; Ernst and Lovich 2009; Kiester and Willey 2015). Nesting can occur at any time throughout the day, though most of the nesting occurs in the evening (Allard 1948; Ernst and Lovich 2009). Nest depth is variable, and few studies examine this parameter; however, Hughes et al. (2017) report nest depth ranging from 60–70 mm below the surface. Nest depredation can be a limiting factor to recruitment, with some studies reporting all of nests being depredated (Willey and Sievert 2012). Eastern box turtles exhibit temperature-dependent sex determination (TSD), with females being produced at higher temperatures and males being produced at lower temperatures during incubation (Ewert and Nelson 1991).

Average clutch size ranges from 3.15 to 5.8 eggs per clutch, which varies latitudinally, with females in the north laying a single clutch with more eggs per clutch and females in the south laying multiple clutches per year with fewer eggs per clutch (Allard 1935; Ewing 1935; Mitchell 1994; Belzer 2002; Kipp 2003; Cook 2004; Capitano 2005; Wilson and Ernst 2005; Burke and Capitano 2011; Willey and Sievert 2012; Hughes et al. 2017; Nicholson et al. 2020). When comparing the nesting ecology of northern and southern populations, the large clutch size of northern populations is diminished by the high mortality rates, infertility, and single clutching (Willey 2010). Various studies report a correlation between body size to clutch size though these relationships are not statistically significant (Cook 2004; Burke and Capitano 2011; Wilson and Ernst 2005; Willey and Sievert 2012).

Hatchling Ecology.— The hatchling incubation period typically lasts between 50–105 days, taking longer in the cooler climates of the Northeast (Fig. 6). Hatchlings emerge during the fall, typically September and October in the Northeast (Allard 1948; Wilson and Ernst 2005; Willey and Sievert 2012; Nicholson et al. 2020). Hatchlings occasionally overwinter in the nesting chamber (Madden 1975; Palmer and Braswell 1995; Hughes et al. 2017; Nicholson et al. 2020). Nest success is variable and may be very low if nest predators are prolific, which may result in 100% of the nests being depredated (Burke and Capitano 2011; Willey and Sievert 2012; Hughes et al. 2017). Egg infertility may be another reason eggs do not hatch, ranging from 24–100% per clutch (Ewing 1943, Congello 1978, Stuart and Miller 1987).

Seasonal Activity Patterns

Habitat use varies daily and seasonally based on environmental temperatures and thermoregulatory behavior (Donaldson and Echternacht 2005; Iglay et al. 2007). Eastern box turtles typically emerge from their overwintering sites in March and April (Willey 2010; Erb 2012; Fredericksen et al. 2014), are active throughout the warmer months, and prepare for overwintering during the late fall (Ernst and Lovich 2009; Kiester and Willey 2015; Nicholson et al. 2020; Fig. 6). Some variation in the exact timing of the active season is based on annual temperature variations and latitude. As ectotherms, eastern box turtles rely on the external environment to regulate their internal temperature (Adams et al. 1989; Ernst and Lovich 2009; Fredericksen 2014).

Spring.— As temperatures rise in the spring, individuals emerge from brumation and move into areas of high sun exposure, such as field edges, pastures, early successional habitats, and ecotones (Willey 2010; Boucher et al. 2017). In the spring, this species tends to inhabit areas of high structural diversity, with varied vegetation cover, which allows them to easily thermoregulate by moving between thermal gradients as needed (Ernst et al. 1994; Dodd 2001). Emergence from brumation may be correlated with warming soil temperatures (Woodley 2013). Individuals stay near their overwintering site for the first couple of weeks and may return to the hibernacula during the night (Willey et al. 2010). Eastern box turtles typically spend most of the spring months in early successional and ecotone habitats where they forage, mate, and thermoregulate (Willey 2010; Boucher et al. 2017). Nesting occurs late May through July (Wilson and Ernst 2005; Willey and Sievert 2012; Hughes et al. 2017; Nicholson et al. 2020; Fig. 6).

Summer.— During the summer, eastern box turtles typically retreat to forested habitat or shallow wetlands and may estivate (a state of dormancy) while in a form (settled into a slight depression in vegetation or leaves; Fig. 7). Occupying forms likely allow individuals to cool off and retain moisture while remaining hidden from potential predators (Dodd 2001; Rossell et al. 2006). They are often constructed in loose soils, leaf litter, and may be next to rock piles, vegetation, and fallen logs (Stickel 1950; Strass et al. 1982; Fig. 7).

Fall and Winter.— In the fall when the daytime temperature drops, individuals often move back into warmer habitats such as early-successional habitats or forest gaps before moving back into the forests for brumation (Walden and Karraker 2018; Table 1). Winter's first frost usually represents the final days of the active season for eastern box turtles (Ernst and Lovich 2009; Savva et al. 2010). Eastern box turtles typically brumate just below the soil surface, sometimes just under cover of leaf litter, and may even expose the top of the carapace (Madden 1975, Willey 2010; Fig. 8). Individuals may become active during the winter if temperatures are conducive for movement (Boucher et al. 2017).

Eastern box turtles are cold tolerant and exhibit a physiological response to freezing, where glucose is produced by the liver and used for cryoprotection (Costanzo and Claussen 1990; Costanzo et al. 1993; Storey et al. 1993; Costanzo et al. 1995). The eastern box turtle is the largest known vertebrate to exhibit this physiological adaptation to cold, similar to several amphibian species (Costanzo and Claussen 1990; Dodd 2001). Further, eastern box turtles do not store fat before overwintering (Brisbin 1972). Despite this cryoprotection adaptation, some individuals still succumb to cold temperatures during the winter (Cook 2004; Nazdrowicz et al. 2008). Eastern box turtles overwintering sites in low elevation and flood plain locations have also experienced mortality events, likely due to flood events (Stickel 1978; Hallgren-Scaffidi 1986). Therefore, selecting a suitable overwintering site may determine an individual's survival during brumation and might explain why this species exhibits overwintering site fidelity (Willey 2010). Eastern box turtles may return to the same area for brumation each year (Hall et al. 1999; Savva et al. 2010; Moon 2011).

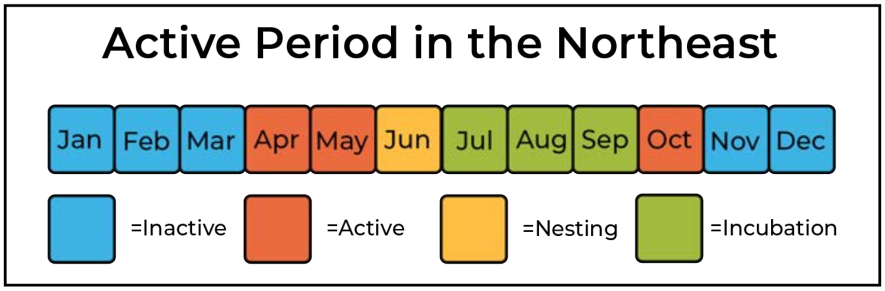

Figure 6. Active period of eastern box turtle in the northeastern United States.

Figure 7. Eastern box turtle in a partial form under leaf litter in forest (left). Eastern box turtle in a partial form under shallow leaf litter adjacent to a fallen log (right).

Figure 8. Overwintering eastern box turtle in Massachusetts.

Table 1. Reference chart for the activity and habitat type, for eastern box turtles in the northeastern United States.

Activity	Habitat Type
Foraging, mating, thermoregulation	• Deciduous or mixed forests • Early-successional habitat • Fields • Shallow wetlands
Nesting and incubation	• Open canopy settings • Early-successional habitat • Well-drained soils • Fields • Gravel pits • Powerline rights-of-way
Overwinter	• Deciduous or mixed forests • Buried in soil • Under leaf litter • In stump holes • Mammal burrows • Pits and depressions

Daily Activity Patterns

Eastern box turtles are primarily active diurnally, which has been observed in field studies and laboratory experiments (do Amaral et al. 2002a; do Amaral et al. 2002b; Kiester and Willey 2015). Daily patterns include eastern box turtles foraging and basking in early successional habitat in the cooler hours of the day, then retreating into forms (Fig. 6) in the shade of the forest or herbaceous cover during the warmest parts of the day (Stickel 1950; Kiester and Willey 2015). Generally, box turtles prefer warm, but not hot, areas with high humidity (Stickel 1950; Madden 1975; Dodd 2001; Erb et al. 2015). Wetlands also appear to play an important role in thermoregulation at some sites as eastern box turtles frequently use these areas during the hottest and driest months of the year (Kaye et al. 2001; Donaldson and Echternacht 2005; Erb 2012). Additionally, movements over large areas are correlated with precipitation (Madden 1975; Strang 1983; Donaldson and Echternacht 2005).

Spatial Ecology

The spatial ecology of eastern box turtles is highly variable between individuals and populations. Many studies have investigated eastern box turtle home range (Nichols 1939; Stickel 1950; Madden 1975; Davis 1981; Strang 1983; Bayless 1984, Stickel 1989; Weatherby 1996; Dodd 2001; Cook 2004; Lentz 2005; Ciaranca and Kelly 2007; Willey 2010; Kapfer et al. 2013; Habeck et al. 2019). Some individuals may stay confined throughout the year, while others may move long distances. The home ranges of individuals may overlap, and box turtles may occasionally exhibit aggressive behavior, though this may be uncommon behavior (Grace 2000; Stickel 1989). Eastern box turtles also exhibit strong fidelity to home ranges that may shift slightly from year to year (Willey 2010).

Home range size varies by individual, between sexes, with latitude, and due to landscape characteristics. Home ranges are variable by individual with the smallest observed straight line home range being 40 m and the largest being 2,145 m (Stickel 1950; Nazdrowicz et al. 2008; Ernst and Lovich 2009; Willey 2010). Females tend to have more extensive home ranges than males, and older, larger individuals tend to have more extensive home ranges than smaller, younger turtles (Stickel 1950; Cook 2004; Baker 2009; Ernst and Lovich 2009; Aall 2011; Habeck et al. 2019). Straight line home range has been found to be much larger in Massachusetts compared with populations in Pennsylvania, Maryland, Virginia, Tennessee, and Indiana (Willey 2010; Table 2). Home range might be larger in Massachusetts due to low population density, large distances between resources, or low forest productivity (Willey 2010). Home range sizes tend to be larger in higher latitudes and areas of less habitat diversity, such as large tracts of forest (Kipp 2003; Iglay et al. 2007; Willey 2010; Kiester and Willey 2015). Urbanization may also affect home range size, with eastern box turtles in heavily fragmented and urban landscapes exhibiting smaller home ranges (Iglay et al. 2007).

Table 2. A comparison of reported minimum convex polygons (MCP) by state indicates a latitudinal gradient in home range size.

State	Authors	MCP (hectares)
West Virginia	Aall 2011	2
Maryland	Lentz 2005	4.7
New York	Madden 1975; Capitano 2005	4–8
Connecticut	Quinn 2008	5
Massachusetts	Willey 2010	0.5–135.9

Barriers to movement.— Large bodies of water and mountain ridges may be barriers to movement. For example, lakes and rivers are typically barriers to movement between populations, although several studies report observations of box turtles swimming and occasionally using wetlands and streams (McCauley 1945; Tyler 1979; Donaldson and Echternacht 2005; Frederickson 2014). Large ridges, such as in the Appalachian Mountains, may also generally be barriers to the movement of individuals, with only a few box turtles having been reported to scale steep terrain and moderate elevations (Wilbern 1982; Chan et al. 2016; Diggins et al. 2016).

Longevity

Eastern box turtles are long-lived organisms, with some reports of individuals living over 100 years in the wild (Graham and Hutchison 1969, Mitchell 1994, Nelson 2003). Numerous reports of individuals with dates carved into their shells have been used to estimate longevity to over 100 years (Townsend 1926; Babcock 1927; Deck 1927; Edney and Allen 1951; Price 1951; Oliver 1953; Dodd 2001; Henry 2003; Belzer 2008). Record longevity for this species is 138 years; however, this is likely an uncommon occurrence, with most wild individuals living in intact habitat being between 50 and 80 years of age (Oliver 1955; Stickel 1978; Hall et al. 1999). Not all individuals are likely to live this long, particularly in more anthropogenically altered habitats (Stickel 1978; Henry 2003; Ernst and Lovich 2009).

Population Ecology

Limited research exists regarding the population ecology and life history characteristics of the eastern box turtle. Population densities reported vary widely from one population to another, even within the same general locality (Madden 1975; Wilson and Ernst 2005; Nazdrowicz et al. 2008; Willey 2010). Juvenile box turtles are difficult to detect and therefore it is challenging to draw conclusions about age class distribution of populations. Multi-decade studies focusing on the survival of eastern box turtles are needed to better understand survival rates for this long-lived species.

Population dynamics.— Despite the broad distribution of the eastern box turtle, research is needed to better understand its life history characteristics. Population densities vary by population with densities ranging from less than one turtle per hectare to over ten turtles per hectare (Table 3; Habeck et al. 2019). Populations may reach higher densities in early successional habitats (Nazdrowicz et al. 2008). A certain proportion of males may become transient and play a crucial role in gene flow (Dodd 2001; Kiester et al. 1982).

Table 3. Population densities of eastern box turtles from several studies in the northeast United States.

State	Authors	Turtles per hectare
Virginia	Wilson and Ernst 2005	16
Maryland	Stickel 1950; Hallgren-Scaffidi 1986; Hagood 2009	53.4–12.4
Delaware	Nazdrowicz et al. 2008	0.22–3.62
New York	Madden 1975	3.71
Massachusetts	Willey 2010	0.3–3.8

Population structure.— Juveniles are not detected as frequently as adults in field studies, which may be due to their small size and ability to hide well in low vegetation (Nicholson et al. 2020). However, two studies found that juveniles represented 32% of one population in Maryland (Hall et al. 1999) and 31% of a population in Delaware (Nazdrowicz et al. 2008). Sex ratios vary between sites with some studies reporting more males than females and some populations with a 50:50 sex ratio (Hall et al. 1999; Nazdrowicz et al. 2008; Kemp 2022).

Survivorship.— The life history of eastern box turtles makes them especially susceptible to increases in adult and juvenile mortality (Erb 2011). This species exhibits delayed sexual maturity and high adult survival with some individuals living over 100 years (Dodd 2001; Ernst and Lovich 2009). If adult mortality rates are high, it is difficult for populations to sustain themselves, and populations may take several decades to recover (Congdon et al. 1994; Hall et al. 1999). Survivorship for long-lived turtle species is relatively low for nests, hatchlings, and young juveniles (Congdon 1983; Williams and Parker 1987; Duchak and Burke 2022; Geller and Parker 2022). Eastern box turtles are no exception with reported low nest success rates (Willey and Sievert 2012) and low juvenile survival

(Yahner 1974; Madden 1975). Conversely, annual adult survival rates are relatively high in undisturbed or moderately disturbed systems (Yahner 1974; Schwartz et al. 1984; Doroff and Keith 1990; Nazdrowicz et al. 2008).

Population Trends

Several long-term studies have investigated population trends of eastern box turtles over time. One population in Maryland has been studied from 1942 to the present (Stickel 1950; Stickel 1978; Hallgren-Scaffidi 1986; Stickel 1989; Stickel and Bunck 1989; Hall et al. 1999; Henry 2003; Hagood 2009; Royle and Turner 2022). This population is reported to have declined in population size by >70% since initial estimates and is becoming increasingly male dominated (Stickel 1950; Hall et al. 1999). Another population of eastern box turtles in Delaware declined substantially from 91 individuals in 1968 to 22 individuals in 2002 (Niederriter and Roth 2004; Nazdrowicz et al. 2008). A long-term study in Pennsylvania revealed a decline of over 70% in population size over 40 years (Kemp et al. 2022). However, despite recorded and anecdotal declines, some populations persist at high densities where the landscape can support populations (Kiester and Willey 2015). Population declines have been attributed to increased adult mortality on roads, by agricultural equipment, and to a lesser extent, prescribed fire in various studies (Nazdrowicz et al. 2008; Ernst and Lovich 2009; Hagood 2009; Kiester and Willey 2015). Anecdotes, scientific studies, and examples of local extinctions all point toward a general population decline in eastern box turtles (Stevens 1994; Dodd 2001; Nazdrowicz et al. 2008; Ernst and Lovich 2009; Hagood 2009; van Dijk 2011; Erb 2012; Kiester and Willey 2015). A population viability analysis for populations in Massachusetts indicated that a protected area must be capable of supporting 300 individuals to ensure population stability over 200 years and is likely applicable to populations in other states in the northeast (Erb 2012).

Genetics

The genetics of the eastern box turtle are under ongoing investigation. Currently there are four species of box turtle (Martin 2012; Martin et al. 2013; Kiester and Willey 2015; Lovich and Gibbons 2021), the coahuilan box turtle (*Terrapene coahuila*) which has no recognized subspecies, spotted box turtle (*Terrapene nelsoni*) which has two recognized subspecies (northern spotted (*Terrapene n. nelsoni*) and southern spotted (*Terrapene n. klauberi*)), ornate box turtle (*terrapene ornata*) which has two recognized subspecies (ornate (*Terrapene o. ornata*) and desert (*Terrapene o. luteola*)), and the eastern box turtle (*Terrapene carolina*) which has six recognized subspecies (woodland (*Terrapene c. carolina*), three-toed (*Terrapene c. triunguis*), gulf coast (*Terrapene c. major*), Florida (*Terrapene c. bauri*), Yucatan (*Terrapene c. yucatana*), and Mexican (*Terrapene c. mexicana*)). Further, populations do not seem to have high genetic differentiation unless separated by a considerable distance, such as 300–500 km (Hagood 2009; Kimble et al. 2014). Less than one percent of individuals in a sample population were siblings, with siblings on average 11.6 km apart, indicating that dispersal may be prolific (Kimble 2012). This low genetic differentiation may be due to the historical connectivity of populations or due to these transient individuals who help disperse genes across the landscape (Kimble et al. 2014). Genetic isolation and inbreeding do not appear to be significant threats to eastern box turtle populations, perhaps due to their longevity (Kiester and Willey 2015). Nest site fidelity in females and sex-based dispersal may result in fine scale spatial genetic structure (Moore et al. 2020). For more information see chapter 5.

Ecosystem Role

Eastern box turtles consume various plants, playing a significant role in the dispersal and germination of seeds (Dodd 2001; Jones et al. 2006). Mayapple (*Podophyllum peltatum*) seeds that are consumed by eastern box turtles germinate faster and have higher success rates than seeds that are not consumed (Rust and Roth 1981). In addition to plants, this species eats a variety of fungi and aids in dispersing spores (Braun and Brooks 1987; Ernst and Lovich 2009). Freshwater turtles are significant contributors to biomass and typically have higher biomasses than endothermic species (Iverson 1982; Lovich et al. 2018). Eastern box turtles are reported to contribute 3.1–3.9 kg of biomass per hectare (Stickel 1950; Iverson 1982). Additionally, eastern box turtles play a crucial role in energy and nutrient cycling when they consume other species, breaking down organic matter in the process, and when other animals prey upon them and their eggs, rich in protein and lipids (Lovich et al. 2018).

Jurisdictional Status

Eastern box turtles have been listed under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2013). As of 2014, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) considers eastern box turtles to be extirpated in Canada (COSEWIC 2014). At the range-wide level, eastern box turtles are considered "Vulnerable" by the International Union for the Conservation of Nature (van Dijk 2011) and "Secure" by NatureServe (NatureServe 2016). NatureServe reasoning states:

> "The species has a very large range in eastern North America. It can be locally abundant in many areas though has almost certainly declined in some as a result of habitat loss and fragmentation as well as over collecting for the pet trade. Overall, the species is secure because of the large number of viable occurrences, but nevertheless it is of conservation concern in some regions."

At the regional-level, eastern box turtles are considered a Regional Species of Conservation Concern in the Northeast and Southeast (Terwilliger Consulting, Inc. and the Northeast Fish and Wildlife Diversity Technical Committee 2013; eastern box turtles are grouped with gulf coast box turtles and three-toed box turtles). Some of the northeastern states provide protection for eastern box turtles under their endangered species acts (Table 4).

Table 4. Legal status of the eastern (woodland) box turtle. State Rank: E=Endangered, SC=Special Concern. SGCN = Species of Greatest Conservation Need: Y = yes, N = no, YP = yes with permit or permission from the state's commissioner.

	ME	NH	MA	RI	CT	NY	NJ	PA	DE	MD	VA	WV
NatureServe Rank	S1	S1	S3	S4	S3	S3	S3	S3S4	S5	S5	S4	S5
State Rank	E	E	SC	-	SC	SC	SC	-	-	-	-	-
SGCN	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Possession Legal	N	N	N	N	N	N	N	N	Y	Y	Y	N
Collection Limit	0	0	0	0	0	0	0	0	1	1	0	0
Commercial Trade Legal	N	N	N	N	N	N	N	N	N	N	N	N
Captive Breeding Allowed	N	N	N	N	YP	N	YP	N	YP	YP	N	N

Chapter 2. Threats to Population Persistence

Lori Erb, John Garrison, and H. Patrick Roberts

Overview.— Eastern box turtles exhibit delayed sexual maturity, low reproductive output, and low nest and juvenile survival, making it difficult for populations to remain stable if adult mortality rates are high. Many eastern box turtle populations face numerous anthropogenic and natural pressures that decrease survival rates of individuals across age classes. Habitat loss and alteration, including development and roads, reduce the connectivity of and resources available to a population and likely exacerbate other threats from cars, incidental collect, and predation to this species. Forest management practices such as prescribed fire, timber harvesting, brush removal, and other uses of heavy machinery may substantially increase mortality rates. Agricultural machinery associated with tilling soil, harvesting crops, and haying can result in mortality. Roads also represent a major source of mortality via vehicle collisions. Predators may influence populations via nest and juvenile predation. Human recreation such as ATV-use may threaten this species by degrading nesting habitats and killing individuals. There are pervasive diseases (e.g., Ranavirus, Herpesvirus) infecting eastern box turtles that have caused mass mortality events and can greatly affect the persistence of a population. Illegal collection represents one of the most significant threats to the eastern box turtle in the Northeast and throughout its geographic distribution. The eastern box turtle likely represents the most illegally traded turtle species in the U.S. Non-commercial incidental collection of individuals for pets also likely represents a significant threat. Although the implications of climate change for this species remain unclear, warming trends may negatively affect eastern box turtles.

Habitat Loss

Maintaining intact natural systems is crucial to conserving eastern box turtles in the northeastern U.S. and throughout the range. Habitat loss and fragmentation are primarily due to residential, commercial, and industrial development as well as roads. All of which contribute to the reduction of habitat as well as increase the threats to eastern box turtle populations. For example, development can affect the local hydraulic flow and may impact populations by flooding out habitats that eastern box turtles use. Winter flooding, due to hydrologic alteration, can negatively affect overwintering conditions for turtles and is suspected in one population decline in Maryland (Hall et al. 1999).

Development and Fragmentation.— The development of natural areas for human settlements is a persistent threat to eastern box turtle populations. It is estimated that between 1973 and 2000, 3.7 million hectares of forests in the eastern U.S. were converted into other types of land (Drummond and Loveland 2010). Residential development is implicated in causing populations to decline and, in some cases, become locally extinct (Stickel 1978; Williams and Parker 1987; Graham et al. 2022). Land use change and habitat fragmentation are linked to low abundance in eastern box turtle populations (Niederriter 2000; Lieberman 1994; Willey et al. 2022). One concern related to increased development is the corresponding reduction in connectivity among populations and resources (Dodd 2001; Iglay et al. 2007; Hagood 2009; Willey 2010; Martin and Root 2020). These barriers may force individuals to nest in lower quality habitats, lead to inbreeding, and result in an overall decline in the health of individuals and the population at large (Hagood 2009; Erb 2015). While genetic isolation does not appear to be a threat to eastern box turtle populations currently, it could emerge as a concern in the coming decades (Hagood 2009; Kimble et al. 2014).

Loss of Nesting Habitat.— Availability of suitable nesting habitat is an essential requirement of functional populations (Steen et al 2012). A decline or extended absence of nesting habitat will eventually lead to lower recruitment but may also increase adult mortality rates if nesting females must travel farther distances in search of suitable nesting habitat, potentially having to cross roads to get there; this has been implicated in the decline of a population in Delaware (Neiderriter and Roth 2004; Kiester and Willey 2015). Nesting habitat may decline due to development (e.g., early successional habitat converted into a gravel lot; Erb 2012). Nesting habitat may also be degraded due to more natural changes in the landscape, such as a preferred nesting habitat undergoing natural succession and invasive species overtaking and shading out an area (Williams and Parker 1987). It is crucial that eastern box turtle nesting habitat is identified and protected to ensure populations have the recruitment needed to persist (Willey and Seivert 2012).

Roads

Various studies have investigated the impact of roads on eastern box turtle populations and conclude that roads contribute to population declines (e.g., Gibbs and Shriver 2002). The mortality of eastern box turtles on roads is likely a significant threat to the persistence of populations as it contributes to additive mortality and likely affects metapopulation dynamics by reducing movement of individuals between populations (Kiester and Willey 2015). In fact, automobile collision was noted as the top reason for intake by nine wildlife rehabilitation facilities in Pennsylvania from 2017-2019, including 43% (130 of 305) of eastern box turtles treated. While road mortality is certainly associated with smaller movements related to seasonal activity habitat selection, larger movements associated with nesting and dispersal may make individuals especially susceptible to being hit by cars (Fahrig and Rytwinski 2009; Compton 2010; Erb 2012; Kiester and Willey 2015). Population fragmentation may eventually lead to negative genetic impacts, with a decrease in genetic diversity (Shepard et al. 2008; Hagood 2009; Marsack and Swanson 2009).

Road mortalities are higher during the spring nesting season, when turtles are more active and make larger movements. Some studies report male skewed sex ratios in populations adjacent to roads, likely due to the increased vulnerability of females during nesting movements (Steen et al. 2006; Stickel 1978; Niederriter and Roth 2004; Nazdrowicz et al. 2008). A wildlife clinic in North Carolina analyzed ten years of data of wild turtles (including eastern box turtle) injuries caused by cars and lawn mowers and reported that injuries peaked in May and June (Sack et al. 2017).

Individual turtles behave differently to threats such as road traffic. Some will cross the road without pausing, others will avoid the roads, and some will approach the road but retract into their shell when a vehicle approaches. Shepard et al. (2008) report road avoidance in eastern box turtles at a population in Illinois and speculate that this may be due to the individuals who do not avoid roads having already died. Roadside habitat can be attractive to eastern box turtles due to the presence of suitable nesting habitat. Roads could also affect populations by increasing the risk of predation and collection. Predators sometimes use roads as a travel corridor and roadside turtles are more likely to encounter humans (Steen and Gibbs 2004; Erb 2012).

Gibbs and Shriver (2002) created statistical models investigating the effects of road density and traffic volume on turtle populations. They reported that for terrestrial turtles such as eastern box turtles, daily traffic columns for most major highways would cause a mortality rate of >5%, which would result in population decline (Gibbs and Shriver 2002). The detrimental effects of roads on eastern box turtle populations will likely increase as urban sprawl and associated construction of new roads continues. Large multi-lane roads and highways act as barriers to the dispersal of turtles, which may result in a population becoming isolated physically and genetically (Gibbs and Shriver 2002). Additionally, small to medium-sized roads with moderate traffic still affect the persistence of turtle populations and contribute to additive mortality (Erb 2012; Fig. 9).

Figure 9. Eastern box turtle crossing a rural road with low volumes of traffic.

Anthropogenic Land Use

Anthropogenic changes in land use are one of the leading factors threatening turtle populations, likely exacerbating other threats such as development, forestry activities, and disease (Willey and Sievert 2012; Kiester and Willey 2015). Agricultural machinery and lawn mowers can injure or kill box turtles (Applegate et al. 2016; Sack et al. 2017). Forestry activities such as prescribed fire and timber harvesting may cause injuries or mortality (e.g., Harris et al. 2020; Buchanan et al. 2021).

Agriculture.— Industrial monocropping and haying procedures have become commonplace in the northeastern U.S. and have a tremendous impact on native wildlife, including eastern box turtles (Labisky 1957; McLaughlin and Mineau 1995; Herkert 1997; Saumure and Bider 1998; Nazdrowicz et al. 2008). The use of large industrial machinery causes additive mortality in eastern box turtle populations and can cause population declines (Nazdrowicz et al. 2008; Hester et al. 2008; Kiester and Willey 2015). In example, mower injuries were the third most common injury reported by nine wildlife rehabilitation facilities in Pennsylvania from 2017-2019, including 6% (19 of 305) of eastern box turtles treated. Field edges attract female turtles for nesting and can be hit by machinery during field preparation and harvests and has resulted in male skewed sex ratios (Nazdrowicz 2008).

Fire.— Some populations persist in forests that are managed with fire. The ecological relationship of box turtles to fire has been examined in various studies throughout the range (Howey and Roosenburg 2013; Fredericksen et al. 2015; Harris et al. 2020). Burns sometimes lead to substantial mortality events and can influence the demographics of populations (Hunsinger 2001; Buchanan et al. 2021). Even if turtles do not succumb to direct mortality during a burn, they may suffer injuries (internal and/or external) that cause physiological stress and/or a weakened immune system, leaving them more susceptible to disease, desiccation, and other stressors. Fires that occur during the eastern box turtles' active season and with high intensity or high severity are more likely to result in turtle injuries and mortalities (Greenberg et al. 2018; Harris et al. 2020; Buchanan et al. 2021).

Timber Harvest.— Maintaining high-quality forests is fundamental to the persistence of eastern box turtles; therefore, protecting and properly managing these areas is integral to conserving populations (Erb 2012). Forest thinning and gaps can provide varied habitat structure providing a range of thermal conditions ideal for box turtles to thermoregulate. Forest gaps can serve as important juvenile habitat (Felix at al. 2008). The most pressing issue that forestry presents to eastern box turtle survival is direct morality by crushing individuals with heavy machinery and logging trucks (NHESP 2006). Forestry activities could result in the mortality of individuals at any point throughout the year, including the overwintering season, as eastern box turtle brumate in forested upland, just below the surface of the soil, or in leaf packs (Savva et al. 2010; Erb 2012). Removing fallen trees and snags (dead or dying trees) can harm populations, as eastern box turtles use these as refuge for overwintering and estivation (Erb 2012; Woodley 2013). These structures may also function as refugia during and after fire events (e.g., Buchanan 2021; Harris et al. 2020). Practices intended to change the forest from mixed deciduous to more coniferous may negatively affect the turtle population by changing the dynamics of overwintering and other aspects of their ecology (Erb 2012). Eastern box turtles in Massachusetts chose overwintering sites that did not have an abundance of coniferous tree species (Willey 2010).

Predation and Parasitism

Various predators consume eastern box turtles and their nests, which influences population demographics and recruitment rates. Predators such as northern racoons (*Procyon lotor*), Virginia opossum (*Didelphis virginiana*), coyote (*Canis latrans*), and striped skunk (*Mephitis mephitis*) have increased in abundance in the eastern U.S. due to the removal of apex predators, land use changes, and a surplus of food (e.g., human garbage or pet food left outside; Berger 1999; Bozek et al. 2007; Guiden et al. 2019). Few studies examine the relationships of predators and eastern box turtles; however, various studies report metrics of nest depredation rates and observations of predation of adults (Ernst and Lovich 2009; Tetzlaff et al. 2020a). Flitz and Mullin (2006) report that 88% of nests (21/24) were depredated and noted that northern raccoons were the likely culprit. Willey and Sievert (2012) report nest depredation rates of up to 100% at sites surrounded by anthropogenic land cover and that nest depredation rates varied by location.

Juveniles may be preyed upon by birds of prey, corvids, gulls, squirrels, chipmunks, rats, snakes, and mesopredators (Ernst and Lovich 2009). Adults are less likely than juveniles to be preyed upon but may be consumed by mesopredators, bobcats, mustelids, and domestic dogs (Ernst and Lovich 2009). Tetzlaff et al. (2020a) monitored 3D-printed juvenile eastern box turtle decoys with camera traps in Illinois and found that northern raccoons attacked models most frequently, followed by Virginia opossums and squirrels (Sciuridae). Kashon and Carlson (2018) conducted a behavioral study of wild eastern box turtles in Indiana and concluded that bolder individuals are more likely to have shell injuries attributed to predation attempts than reclusive individuals. Kashon and Carlson (2018) observed northern raccoons, coyotes, and Virginia opossum at the study location where they captured eastern box turtles with injuries attributed to predation. Tetzlaff et al. (2020b) monitored acclimation pens of eastern box turtles, being repatriated or translocated, with camera traps and observed potential predators (northern raccoon and squirrels) inspecting the pens. However, predators lost interest in 34 days, suggesting researchers may want to wait until predators have lost interest in acclimation pens before releasing the turtles. Feral cats opportunistically kill wildlife and are responsible for the decline of several species globally, though it is unknown how they might be affecting eastern box turtle populations (Hester et al. 2008; Loss et al. 2013).

Insects such as fire ants (*Solenopsis invicta*), flesh flies (e.g., *Cistudinomyia cistudinis*), and mosquitoes also feed on eastern box turtles (Ernst and Lovich 2009). Fire ants are a threat in the South, but their current range only extends North into one small corner of Virginia. However, the range is predicted to extend well into the Northeast, possibly up to New York, over time due to climate change (Korzukhin et al. 2001). This species also is the host for several parasites such as species of fly and helminths, which may affect the health and survival of individuals (Peters 1948; Moraga et al. 2012; Doke et al. 2022). Recent cases of myiasis have been reported at several locations in Massachusetts. *Cistudimonyia cistudinis* are suspected to be the parasite, but other parasites may cause myiasis. Studies are underway to better understand the distribution of infected box turtles. Eastern box turtles infected by this ectoparasite exhibit reduced mobility and swelling (Fig. 10). The swelling can restrict the turtle's ability to completely retract into its shell, making it more vulnerable to predators. It is yet unclear if the infection itself can cause mortality.

Figure 10. Examples of myiasis parasitism of eastern box turtles.

Collection from Wild Populations

Commercial Collection.— The illegal wildlife trade is a growing criminal enterprise that threatens the traded species and their environments, promotes the spread of invasive species and disease, and funds other organized criminal activities (Ferrier 2009; Cardoso et al. 2021; Uhm et al. 2021). Collection for the pet trade is a persistent threat to eastern box turtles, as individuals removed from the wild are no longer contributing to the gene pool, reproduction, and population demographics (Erb 2012; Dodd et al. 2015; Kiester and Willey 2015; Fig. 11). Hundreds and sometimes thousands of eastern box turtles are traded each year, and this species is the most illegally traded turtle globally (Easter et al. 2023) Individuals are collected en masse for sale in the domestic and international pet trade (Easter et al. 2023). Over 100,000 eastern box turtles were removed from the wild and exported to Europe, Japan, and China in the early 1990s (Thorbjarnarson et al. 2000). Removing this volume of individuals from the wild likely resulted in population declines and possibly even extirpations. Eastern box turtles became listed under appendix 10.2 of the Convention on International Trade in Endangered Species (CITES) treaty in 1994, which protects them from commercial sales, though trade of this species has only increased since then (Lieberman 1994; Easter et al. 2023). Despite halting the international trade of this species, black market sales persist, an ongoing threat to wild eastern box turtle populations.

Figure 11. Confiscated eastern box turtle wrapped in a sock (left) and confiscated eastern box turtles in a storage bin (right).

Incidental Collection.— Incidental collection is likely more prevalent in areas where human and turtle populations are closely juxtaposed such as human recreation areas. Several studies have documented the impacts of human disturbance on turtle populations and raised concerns about the collection in areas of high turtle density (Garber and Burger 1995; Heppard and Buckholz, 2019; Selman et al. 2013; Moore and Seigel 2006). At a site in Massachusetts with intermediate levels of human recreation, Willey (2010) reports four instances of people attempting to collect individuals as pets. If these four individuals had been successfully collected, it would have represented a 2.3% increase in the mortality rate of this population, as removed individuals are essentially "ecologically dead" (Willey 2010). Erb (2012) reported that several eastern box turtles are confiscated by the state of Massachusetts each year after learning of the incidental collection of individuals for pets, and this is commonly reported in other states. Many of the turtles collected as pets are females searching for nesting habitat (Willey 2010; Erb 2012). Removing any number of adult females from a population can threaten the persistence of populations, especially if the populations are of low density (Willey 2010).

Turtle Races.— In addition to the illegal trade and incidental collection, turtle races threaten the eastern box turtle. Turtle races, also known as turtle derbies, are public events where people bring a turtle to a "racetrack" and are released en masse to see which person brought the fastest turtle (Dodd 2001). After the turtle race has concluded, the turtle may be taken home as a pet, eaten, or released into the wild (Dodd 2001). Many individuals at these events have their shells painted, glittered or stickered, some with materials very toxic to turtles. Turtle races have been banned in many northeastern states, for example turtle races began in Maryland in 1941 until they were banned by the Maryland Department of Natural Resources in 2016 (Giese 2013; McDaniel 2016). Despite efforts to ban these events, they persist in some areas of the US, especially in the Midwest (Alex Heeb pers. comm.; Self 2022). Turtle races threaten the survival of individuals and are likely to influence the dynamics of populations by removing or translocating individuals and spreading disease for turtles returned to the wild.

Disease and Health

Wildlife diseases can detrimentally affect the health of an individual eastern box turtle and the population at large. Disease is one of three major threats noted by nine wildlife rehabilitation facilities in Pennsylvania from 2017-2019, with disease being the second most common reason for intake, including 12% (.3 of 305) of eastern box turtles treated. Two of the most known diseases affecting populations of eastern box turtles are from the herpesvirus and Ranavirus family (*Iridoviridae*; Allender et al. 2011; Sim et al. 2015). A third lesser-known disease, Adenovirus, is now being studied. Ranavirus is the most virulent and threatening to populations and has affected reptiles, amphibians, and fish (Langdon and Humphrey 1987, Daszak et al. 1999, Green et al. 2002, Johnson et al. 2008). Unfortunately, Ranavirus is present in many species used as bait and pets, increasing the chance of transmission into the wild (Picco and Collins 2008).

Ranavirus infections have been reported in wild and captive populations of eastern box turtles (De Voe et al. 2004; Allender et al. 2006; Kane et al. 2016). Cases of Ranavirus in eastern box turtles have been documented in Massachusetts, North Carlina, Tennessee, and Virgina (Allender et al. 2011; Erb 2012) and may have caused population declines in Pennsylvania, Texas, and Georgia (Johnson et al. 2008; Belzer and Seibert 2011). In a study in Maryland, 22% (23/103 turtles) of the individuals being radio-tracked died due to a suspected disease and when necropsied tested positive for Ranavirus (Farnsworth, pers. Comm.; Erb 2012). Fifty individuals were found dead in a population in Illinois, five surviving individuals were diagnosed with polymicrobial necrotizing bacterial infection (Adamovicz et al. 2018). Ranavirus has also been the cause of a mass mortality event in a captive population, turtles that were confiscated from an illegal collector and likely originated from the wild (Sim et al. 2015). Mortality events of this magnitude could result in a population decline (Erb 2011).

The transmission mode in Ranavirus is unknown; however, mosquitoes may act as intermediate hosts (Johnson et al. 2008, Belzer and Seibert 2011). Adamovicz et al. (2015) analyzed health data for 40 individual eastern box turtles and found that sick individuals had lower levels of potassium, phosphorus, sodium, calcium, albumin, globulin, and total protein than healthy individuals. Ranavirus can be detected in the bone marrow of eastern box turtle skeletons; therefore, skeletons found after mortality events could be tested (Butkus et al. 2017). Eastern box turtles with bacterial infections thermoregulate more than usual to increase their body temperature, which helps them fight off their infection (Monagas and Gatten 1983). Do Amaral et al. (2002b) also observed this fever induction through thermoregulation in eastern box turtles in laboratory settings.

Herpesvirus and Adenovirus appear to be less virulent than Ranavirus but still pose a serious threat to eastern box turtles. Herpesvirus appears to be relatively widespread. Prevalence in populations tested to date range from 31.3%-40% (Sim et al. 2015, Yonkers et al. 2015; Kane et al. 2016; Lane et al. 2017; Engel et al. 2020). Lane et al. (2017) also found that herpesvirus is more prevalent during the summer months, when temperatures are higher. Adenovirus has also been found in wild eastern box turtles. In Virginia, 55.7% of 106 individuals tested positive for box turtle Adenovirus (Franzen-Klein et al. 2019). Modes of transmission of these diseases are still unknown, but herpesvirus is likely to spread through nasal discharge (Lane et al. 2017).

Multiple stressors increase the chance of negative disease outcomes whether that be infection of multiple diseases or other stressors. For example, turtles have been found to be more susceptible to disease infections when stressed by other disturbances such as a forest fire (Howey and Roosenberg 2013; Cross et al. 2020; Albery et al. 2021). Turtles can also be infected with multiple pathogens at the same time. In one such case, eastern box turtles tested positive for Ranavirus, Herpesvirus, mycoplasma, and Adenovirus in Illinois and Tennessee. Archer et al. (2017) suggested that conservation efforts should attempt to detect multiple pathogens to properly characterize health screenings. This species is also more susceptible to disease infections when environmental temperature drops below the seasonal average temperature (Agha et al. 2017).

Pesticides, herbicides, and other chemicals may influence the health of individuals and turtles may be an important indicator of environmental contaminants (Meyers-Schone and Walton 1994; Holliday et al. 2001; Sleeman et al. 2008). Exposure to pesticides likely decreases the general health of an individual and has been associated with listlessness, ocular, nasal, and otic infections in this species (Tangredi and Evans 1997). The bioaccumulation of chemicals (e.g., Mercury, Lead, polychlorinated biphenyls (PCBs)) is reported in several species closely related to eastern box turtles and can result in immunosuppression and calcium deficiency (Pagano et al. 1999; Bishop et al. 2010; Aplasca et al. 2019).

Recreation

Human recreation is known to influence the behavior of individuals, has been implicated in the decline of populations of closely related freshwater turtle species and likely impacts eastern box turtle populations (Garber and Parker 1995; Heppard and Buckholz, 2019; Selman et al. 2013; Moore and Seigel 2006). However, few studies have examined the relationships between recreation in relation to this species. Extensive trails likely result in greater human-turtle interactions and increase the risk of incidental collection. All-terrain vehicles (ATV) use in areas of high turtle density such as powerline rights-of-way, gravel pits, and early successional habitats where nesting may be concentrated, threatens the survival of individuals and their nests. Shell fragments of eastern box turtles were found in the tracks of an ATV or truck in a powerline right-of-way (Erb 2012).

Climate Change

Climate change has long been predicted to impact the survival, life history, and distribution of freshwater turtles including the eastern box turtle (McCallum et al. 2009). This species prefers an internal body temperature of 20°C–30°C, which may be affected by increasing air temperatures (do

Amaral et al. 2002a; do Amaral et al. 2002b; Martin and Root 2020). Precise impacts are complex and difficult to predict but climate change may result in an increase in nest failures, loss of shoreline habitat, skewed sex ratios, and disease outbreaks. Climate change may also benefit the eastern box turtle by reducing overwintering mortality, and longer warm seasons may allow for increased nest success, especially in northern portions of the range (Erb 2012). Saava et al. (2010) predict that climate change may influence when eastern box turtles begin overwintering and emerge from their overwintering sites.

Nesting is greatly influenced by climate, precipitation, and temperature. In a particularly unusual cold and wet year, researchers in Massachusetts reported delayed nesting, low volumes of nesting females, and high rates of nest failure (Erb 2012). Climate change is predicted to increase the frequency and intensity of tropical storms and increase annual precipitation in the northeastern U.S. (Weisse and Storch 2010). Increased precipitation and tropical storms could impact populations negatively by inundating nests and drowning hatchlings with flood waters, which has already been observed in several species of freshwater turtle (Standing et al. 1999; Duchak and Burke 2022).

Rising temperatures and potential droughts are also predicted with climate change. Increased annual air temperature could result in increased disease prevalence among populations, thus further proliferating diseases such as Ranavirus (Harvell et al. 2002; Rohr and Raffel 2010; Allender et al. 2011; Buttke et al. 2021). Climate change is expected to increase the frequency and intensity of droughts in certain portions of the Northeast, which could impact the survival of individuals as well as the health of the forests upon which eastern box turtles rely (Hanson and Weltzin 2000; Strzepek et al., 2010).

Many eastern box turtle populations occur along the Atlantic Coastal Plain; these areas are highly susceptible to habitat loss due to rising sea levels (Davis 1987; Najjar et al. 2000; Martin and Root 2020). Anthropogenic development is also prevalent in these areas, potentially restricting successful emigration if core habitats become inundated by rising tides. Martin and Root (2020) developed a climate change projection model for this species and report moderate changes in suitable climate throughout the range. They predict that distribution changes may be most likely along the edges of the current range.

Climate change may result in skewed sex ratios in species that exhibit TSD such as the eastern box turtle as air temperatures rise (Ewert and Nelson 1991). Eastern box turtles produce more females at higher temperatures, therefore this species may benefit in this way by increased air temperatures, as females are generally considered more ecologically valuable. Turtle species that exhibit TSD may be able to adapt to increasing air temperatures by changing their nest site preferences and the phenology of nesting (Refsnider and Jansen 2015).

Relative Severity of Threats According to Expert Opinion

In 2019, state, federal, nonprofit, and academic biologists with expert knowledge of the eastern box turtles responded to a poll regarding potential threats to the species (Fig. 12). Respondents were asked to score a series of threats from 1 (very high threat) to 5 (no threat). In this provisional assessment roads were the top current threat with development, habitat loss and alteration also ranking high. Conversely, invasive species and climate change were of lesser concern at this time. Many experts noted a lack of information on the threats and the need for more research to better understand which currently has the greatest impact on eastern box turtles and how these may change over time. Periodically (every 5-10 years) conducting a threats assessment, as more information becomes available, would be useful to adaptively direct conservation actions to address the top threats.

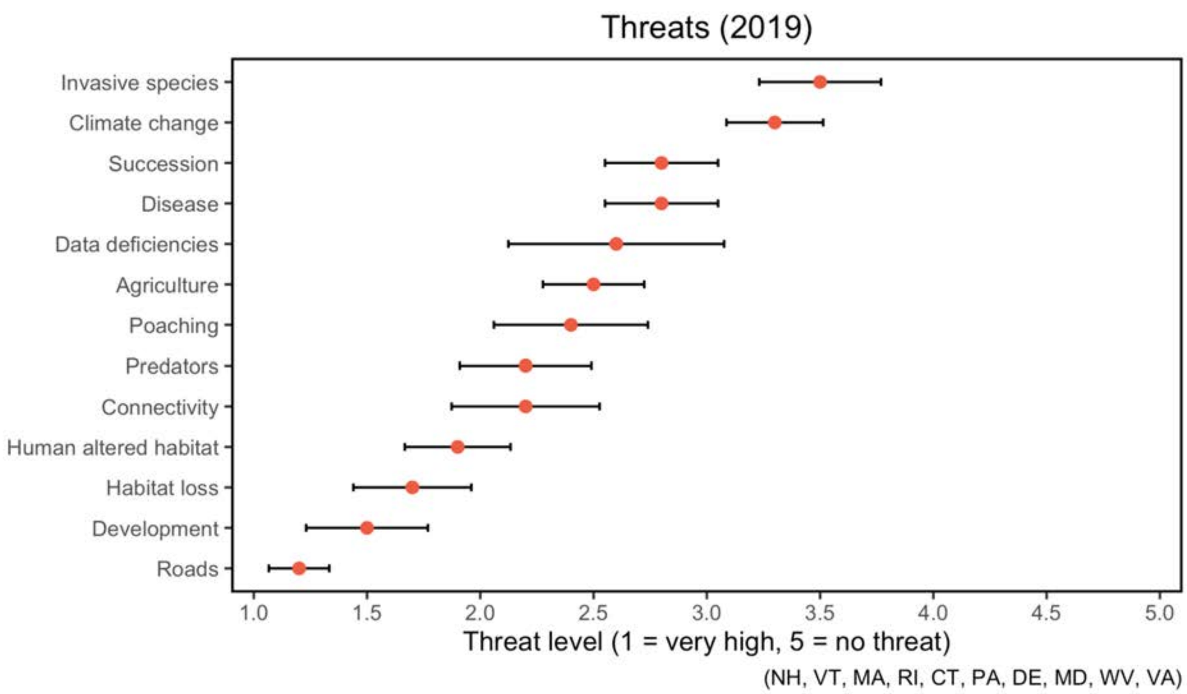

Figure 12. Results of a poll that asked eastern box turtle researchers, conservationists, and other experts to hierarchically rank their perception of the threats to this species by order of threat level.

Chapter 3. Eastern Box Turtle Population Assessment Protocol

Lori Erb, H. Patrick Roberts, and the
Northeast Eastern Box Turtle Working Group

Introduction

An Eastern Box Turtle (woodland box turtle, *Terrapene carolina carolina*) Population Monitoring Protocol developed by the Northeast Eastern Box Turtle Working Group. This standardized sampling protocol is intended to provide a framework for assessing Eastern Box Turtle populations throughout the northeastern United States. The basic elements of the protocol include Rapid Assessments (RA) and Demographic Assessments (DA). RAs are intended to allow for the efficient assessments of relative abundance at a given site, while DAs, which require more intensive sampling using the same protocol, provide a means for estimating population size and other demographic parameters. Two sampling options are described: (Option 1) circular-plot based sampling (strongly encouraged), (Option 2) feature polygon-based sampling. Early sampling will primarily be focused on a visual survey approach, but we also recommend the evaluation and potential future inclusion of two additional approaches (i.e., trap-assisted and dog-assisted surveys). This standardized population monitoring protocol is designed to be flexible, and to allow use in a variety of habitat and project types throughout the northeastern United States and elsewhere throughout the species range.

Goal

Provide a flexible and efficient framework for detecting and monitoring Eastern Box Turtle populations that will facilitate the assessment of distributional trends, patterns of occupancy and abundance, long-term population trends, and effects of habitat management throughout the northeastern United States.

Objectives

1. Assess Eastern Box Turtle occupancy and relative abundance throughout the northeastern United States.
2. Provide a framework for tracking trends in occupancy over time.
3. Quantify population densities for a subset of sampled populations.
4. Provide a framework for tracking trends in population density over time.
5. Assist in the evaluation of the effects of habitat management actions on Eastern Box Turtle populations.
6. Provide a flexible, yet standardized monitoring framework that is compatible with monitoring efforts throughout the range, including citizen science efforts.

Guidelines

Site Selection

A survey site may be any area containing habitat that could potentially support Eastern Box Turtles (e.g., early successional, forest, or ecotone conditions). Ideally, survey sites should be located >1,200 m apart (approx. twice the average annual movement distance in Massachusetts [Willey 2010]). Alternatively, sites can be chosen <1,200 m apart, but should be separated by a clear barrier to movement (e.g., lake or 4 lane highway). For sites that are unfamiliar to surveyors, performing a reconnaissance site visit is advisable to assess site access and current ground conditions of survey areas. To stratify sampling efforts ecologically and geographically across the region, provisionary sampling targets for physiographic areas and states are provided at the end of this document (Appendix B). It is also recommended that, where possible, surveyors select survey sites along a gradient of rural-urban conditions, habitat patch sizes, and habitat types.

Below we describe methods for defining your specific survey area within survey sites for three different survey methods. Option 1 (circular plot survey) is the recommended method currently. Option 2 (feature polygon survey) is provided for surveyors that do not have access to specialized mapping programs (beyond Google Earth) and/or GPS units.

<u>Option 1: Circular plot survey</u>

This option is strongly preferred for the Rapid Assessment method. Within your site, place four 28-m radius (1/4 ha) **circular plots** centered within suitable habitat or potentially suitable Eastern Box Turtle habitat (Fig 13). For example, suitable habitat may be a field-forest ecotone, section of a power line corridor, old gravel pit, or a patch of forest. The Northeast Eastern Box Turtle Working Group (NEEBTWG) recommends that surveyors target **early successional habitat adjacent to mature forest**, but surveyors may also consider other areas frequently used by Eastern Box Turtles during the spring months in your region (e.g., forested habitats). The four paired circular sampling plots should be non-overlapping and no more than 350 m from each other (approximately ½ the average distance between overwintering location and early successional habitat in Massachusetts [Willey 2010]). Circular plots are strongly preferred, however, square plots ¼ in size could also be used.

<u>Option 2: Feature polygon survey</u>

This option is best for Demographic Assessment sites. Within a selected site, delineate a polygon encompassing a **feature polygon** that will be surveyed (Fig 14). A "feature" is defined as any component/aspect of the landscape consisting of suitable or potentially suitable Eastern Box Turtle habitat. For example, suitable habitat may be a field-forest ecotone, section of a power line corridor, old gravel pit, or a patch of forest. The NEEBTWG recommends that surveyors target **early successional habitat adjacent to mature forest**, but surveyors may also consider other areas frequently used by Eastern Box Turtles during the spring months in your region (e.g., forested habitats). Delineated feature polygons should be 2–4 ha in size and take on any shape. Multiple features may be designated at a single large site (e.g., state park with multiple patches of field/forest ecotone habitat patches) if they are separated by >1,200 m OR a barrier to movement (i.e., 4-lane highway, lake, larger river).

Visual Encounter Surveys

Rapid Assessment

Conditions for Surveys

- *Sampling period*: mid-April – June (recommended). In more southern locations (mid-Atlantic region and south), mid-April may work for habitat with thin and/or low growing vegetation and June surveys may also be effective. Optimal survey dates may vary by geographic location and yearly variation in spring weather conditions.
- *Time of day*: 7:00 AM to 3:00 PM.
- *Weather conditions*: Surveys can take place under most weather conditions, but avoid extended (>3 days) cold (< 60 degrees) and hot (> 85 degrees) periods.

Options 1: Circular Plot Surveys (strongly preferred)

- *Sampling area (see Fig 13):* Sampling plots should be searched as evenly and thoroughly as possible. Surveyors may find it useful to create and upload plot boundary points or plot center points into a GPS unit or use Google Earth on their cell phone (if cell reception is available) to help guide them during the survey. If the center points in a GPS, surveyors can use the "go to" feature to stay within 28 m of the center point.
- *Number of surveys:* Each set of plots should be surveyed **3 times within a single season**.
- *Survey effort* (see Table 5): Each ¼ ha sampling plot should be searched for 11 min with one surveyor (approximately 0.75 person hrs./ha and a total of 45 min of active search time for 4 plots). This excludes time spent processing turtles. If two surveyors were surveying the plot, they would search for 5.5 minutes each. No more than 2 surveyors should be used at a given plot. It is recommended that no more than 2 surveyors sample a site during each survey. However, when >2 surveyors are used they should survey different plots so that no more than 2 surveyors search a single plot. All sampling plots within the same site should be surveyed during the same day and at least 48 hours should separate any two sampling events at a given site.
- *Survey effort for* ***thickly vegetated sites*** *(see Fig 15 and 16)*: The time to survey each plot can be extended up to 22 min per plot in instances where the vegetation is very thick, and it is difficult to see the ground. This may be the case at sites throughout the sampling period (shrubby locations), only during surveys at the tail end of the sampling period when the grasses and forbes are taller and thicker OR may not be necessary during any of your surveys depending on the site.

- *Data*: It is highly recommended to record tracks during surveys. GPS unit or app can be used to save an independent set of tracks for each survey. Please see the data forms for track naming convention. Please see the data forms for track file naming convention. The start time, end time, weather conditions, and habitat features will be noted. Survey field forms can be found in Attachment B and at northeastturtles.org.
- See Appendix A for step-by-step Survey Instructions

Option 2: Feature Survey

- *Sampling area*: The entire feature (Fig. 14) should be surveyed as evenly and thoroughly as possible. Surveyors may find it useful to create and upload feature boundary points into a GPS unit or use Google Earth on their cell phone to help guide them during the survey.
- *Number of surveys:* Each feature should be surveyed **3 times within a single season,** and at least 48 hours should separate any two sampling events at a given feature.
- *Survey effort:* During each survey, surveyors should spend 0.75 person hours per hectare searching for turtles. This excludes time spent processing turtles. It is recommended that no more than 2 surveyors be used during a single survey, however if additional surveyors are used the survey time should be modified accordingly (see Table 5).
- *Survey effort for* ***thickly vegetated sites*** *(see Fig 15 and 16)*: The time to survey each plot should be doubled to 1.5 person hours per hectare. Double the times in Table 5.
- *Data*: It is highly recommended to record tracks during surveys. GPS units or apps (e.g., GAIA, Avenza) can be used to save an independent set of tracks for each survey. Please see the data forms for track naming convention. Please see the data forms for track file naming convention. Survey start time, end time, weather conditions, turtles observed, and habitat features will be noted. Survey field forms will be provided.
- See Appendix A for step-by-step survey instructions.

Demographic Assessment

Visual Surveys (preferred)

For demographic assessments, features will be delineated as described above, and the Rapid Assessment methodology will be followed. A minimum of four to six additional survey events will also be required for a total of ≥7-9 independent surveys (dependent on the number of recapture events) at demographic sites within a two-year time frame.

Data Management

Data should be entered into the regional database using the Data Entry Excel spreadsheet. For GPS track data collected please label each track with the following convention: SiteID_YYMMDD. The turtle photos should be labeled as follows: State Code_Site ID_Turtle ID_YYMMDD_C or P. Photos of the carapace should end with a C and photos of the plastron should end with a P.

Alternative Methodologies Under Evaluation

Trap-Based Surveys

Where time and resources allow it would be valuable to evaluate trapping with use of drift fences and passive unbaited box traps with adjustable wings (Fig 17) as a potential alternative method for a demographic assessment. We recommend use of 2-4 drift fences of 56 m in length (equivalent to the diameter of a ¼ ha circular plot) (Fig 18). Silt fencing material would work well for the drift fence. Trap density should be 12 traps/plot with traps placed on either end of the drift fence and on both sides of the fencing (Fig 19) as well as approximately every 10 m along the drift fence on both sides. Traps should be deployed for X trap nights and checked daily.

Dog-Assisted Surveys

Dog-assisted surveys should be evaluated as an additional optional survey method for both RA and DA population assessments. The protocol would follow the same survey conditions and sampling methods as the visual encounter surveys with one exception. Surveyors should perform at least four surveys per site. We recommend a comparison study between the visual encounter surveys and dog-assisted surveys. This would be done by alternating human versus dog-assisted survey at each site. For example, you would conduct a dog-assisted survey during your first and third site visit and a human survey during your second and forth visit. Handlers should follow behind the dog and any turtles missed by the dog and found by the handler should be counted as turtles found off the clock and recorded on the survey form under "#Off-clock".

Since dogs may search the entire plot or feature more quickly than humans, we recommend noting in the comments field how much time you think it took for the dog to adequately search the survey area. However, the dog should continue to search the area for the full recommended time (11 minutes for a plot or 0.75 person hrs./ha). This data collected during year 1 (trial) will be used to determine if we need to adjust the recommended survey time for the dog-assisted surveys.

Other Survey Requirements

All participants must have permits from their state wildlife agency, IACUC protocol if necessary (for University associated research), and follow the NEPARC disinfection protocol. http://www.northeastparc.org/products/pdfs/NEPARC_Pub_2014-02_Disinfection_Protocol.pdf.

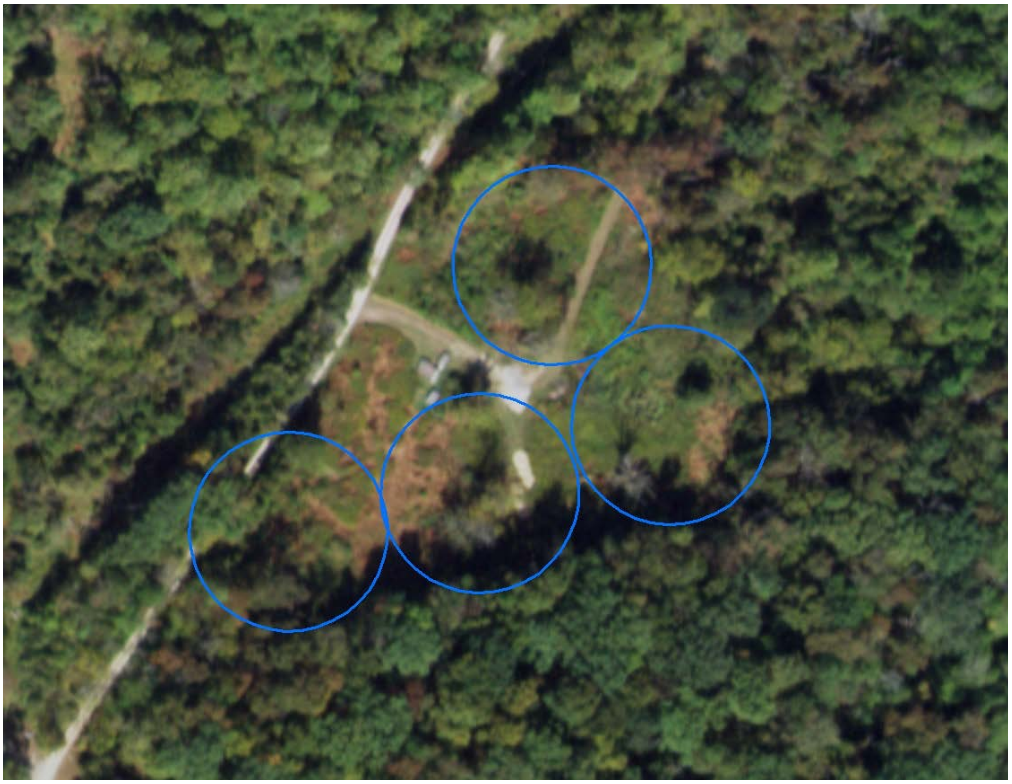

Figure 13. Four ¼ ha sampling plots (blue) within suitable Eastern Box Turtle Habitat.

Figure 14. A feature polygon (blue) within a site.

Figure 15. Image of a thinly or regularly vegetated habitat on the left and thickly vegetated habitat on the right.

Figure 16. Graphics of thinly vegetated habitats on the left and thickly vegetated habitats on the Right.

Figure 17. A photo of a passive box trap with adjustable wings.

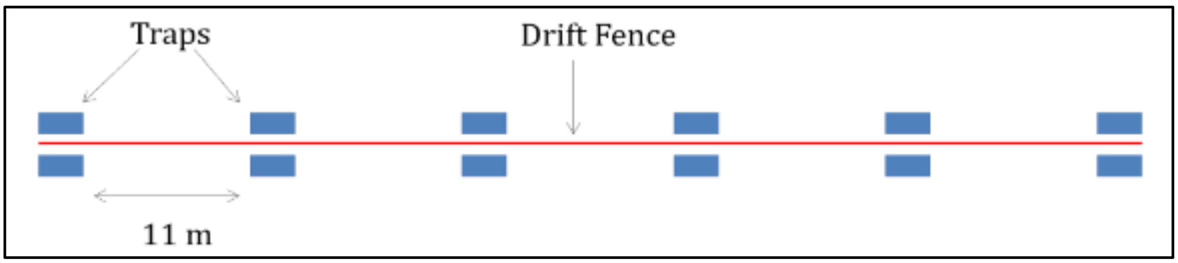

Figure 18. A diagram of one drift fence and 12 traps set up.

Figure 19. Four 56 m long drift fences were set up at a site.

Table 5. Survey time chart to calculate the number of minutes needed to reach a 0.75 person hours/ha of effort given the number of surveyors and area to be surveyed.

Size of Site (ha)	**Number of Surveyors**				
	1	***2***	***3***	***4***	***5***
1	45	23	15	11*	9*
1.25	56	29	19	14*	11*
1.5	68	35	23	17	14*
1.75	79	40	26	19	16
2	90	46	30	22	18
2.25	101	52	34	25	20
2.5	113	58	38	28	23
2.75	124	63	41	30	25
3	135	69	45	33	27
3.25	146	75	49	36	29
3.5	158	81	53	39	32
3.75	169	86	56	41	34
4	180	92	60	44	36

*Surveys should not be less than 15 min in length.

Chapter 4. Sampling Summary

Kelley Flaherty and Lori Erb

Introduction

We evaluated a 3-year (2020–2022) dataset collected using the Eastern Box Turtle Population Assessment Protocol to determine whether covariates such as survey method (plots vs features), sampling effort, and weather covariates affected the detection probabilities. A variety of climate and weather variables have been shown to affect the activity of box turtles making them more or less apparent to potential observers. These include time of year, time of day, temperature, precipitation, and humidity (Erb et al. 2015; Foster 2021; Parlin et al. 2015). Similarly, sampling protocol has the potential to influence the probability of detection. Increased sampling effort and increased plot size may increase the chance of encountering turtles present at or near a sampling point. The purpose of this analysis was to evaluate which of the weather or observer variables affected detection probabilities across a broader species range to inform further monitoring protocols.

Methods

We used data collected from sites across the larger study area including Rhode Island, Massachusetts, New York, Virginia, West Virginia, Pennsylvania, Maryland, New Jersey, Delaware, and the District of Columbia during 2020–2022, to evaluate the effectiveness and efficiency of two survey methods, plot vs feature-based surveys. We then use the plot data to investigate covariate effects on the probability of detection. We fit closed-population models using the "unmarked package" (Fiske and Chandler, 2011) in R. All covariates evaluated were selected *a priori*.

For feature surveys, we compared the null (.) model for both occupancy and detection between plot sites and feature sites. To do this we pooled the plots by site (4 plots/site) and compared those data to the feature data, resulting in somewhat more equal survey effort/area. We also used the features to calculate the effort as the area searched per person hour and modeled the results as a covariate of detection to determine the optimum search area.

Using the plot data, we selected surveys from sites that were surveyed ≥ 3 times in a given year. We assumed no difference in the effect of covariates on detection probabilities between years, so sites were pooled from all years into a single analysis. We modeled each of the covariates separately to determine the effect of each on the probability of detection. Where missing values were present, we omitted those records from analysis for that model only. We then evaluated the effect of covariates on the probability of detection. For covariates significantly affecting detection probabilities, we identified the value at which detection fell below 0.20 as higher detection probabilities would allow for the ability to detect changes in occupancy levels in future monitoring (Beaudrot et al. 2019). We evaluated observation level covariates including the Julian date of the survey, the start time of the survey, the duration of the survey, air temperature recorded during the survey, the relative humidity, the time since rain, the cloud cover, and the accumulated growing degree unit for the plot data. For cloud cover, we used the midpoint of each cover category and performed an arcsine square root transformation on midpoint data.

Results

We evaluated 476 plots and found naive occupancy and detection rates of 57% and 28% respectively. When plots were pooled by site, for 119 sites, naive occupancy and detection rates were 76% and 54% respectively. In comparison, we evaluated 30 feature sites and found naive occupancy and detection rates of 54% and 57% respectively. We found similar naïve occupancy estimates between the plot and feature data. However, the feature data had a higher naïve detection probability than observed in the plot data (Fig. 20). We also examined the search effort as a combination of feature area and observer time. This measure of effort would be like the time searched variable assessed for the plot data. For the feature data, we did not find that search time significantly affected detection probabilities although probability did increase with increasing search time (Fig. 29, $p < 0.40$).

For plot data, we found detection probability was higher during surveys that were conducted earlier in the season (Fig. 21, $p < 0.022$) with the Julian date of 159 (June 8th) as the date in which detection probability fell below 0.2. The accumulated growing degree units was also a significant covariate of detection in the single variable model with 1095 as the point in which detection probability fell below 0.2 (Fig. 22, $p < 0.034$). Detection probabilities were higher with increased search time with 11 minutes being the point at which detection probability rose above 0.2 (Fig. 23, $p < 0.001$) but the time of day at which the survey began did not influence detection (Fig. 24, $p < 0.73$). Detection probabilities decreased with time since a rain event with 4 days as the point at which detection probability fell below 0.2 (Fig. 25, $p < 0.012$). However, other observation level weather variables including temperature ($p < 0.25$), percent humidity ($p < 0.62$) and cloud cover ($p < 0.79$) did not affect detection (Fig. 26-28).

Discussion

We found similar occupancy estimates between plot (57%) and feature (54%) datasets with fewer sample sites (30 vs 476) used in the feature sites recorded. The detection probabilities for the feature dataset compared with the plots was much higher suggesting sampling features may be more efficient. However, when pooling the plots by site, the occupancy estimates are considerably higher than feature surveys (76% vs 54%) and the detection estimates are similar (54% vs 57%). Our feature survey sample size was quite small. Additional feature surveys are needed to increase our sample size to allow us to better determine if one method is more efficient than the other.

The average effort for feature sampling was 63 ± 42 minutes/ ha compared to the suggested 44 minutes/ha suggested in the plot protocol with some features being searched much more intensively. The average feature area searched was 2.9 ± 1.2 ha compared with the 1 ha area searched with the plot protocol. Extra effort combined with larger search areas may have affected the detection probabilities in the feature dataset. Although not significant, the detection probabilities in the feature dataset tended to decline with increased effort. It could be that without strict time constraints, surveys with lower inherent detection probability might be searched more intensively.

Many of the covariates examined with a significant effect on detection probabilities agree with the current recommended sampling protocols already in place for monitoring box turtle populations including sampling dates and plot time searched. The effect of accumulated growing degree units on the detection probability is likely related, in part, to the Julian date. The growing degree units provide a proxy for changes in vegetation on a plot over time and between sites. However, because they are not constant from year to year in a location and can vary significantly even within state boundaries, the measure is less informative for future sampling. Although sampling features takes longer

We did not find a significant effect of humidity on turtle detection probabilities although previous studies have (Erb et al. 2015). This could be due to the method of determination as no specific protocol for recording humidity in the field was suggested in this study. We did find that there was a significant decrease in detection probability with an increase in the number of consecutive days following a rainfall event with detection probabilities falling below 0.20 after 4 days. This suggests that for optimum detection, future sampling should be limited to 4 days following a rain event and that extended periods without rain may hamper detection.

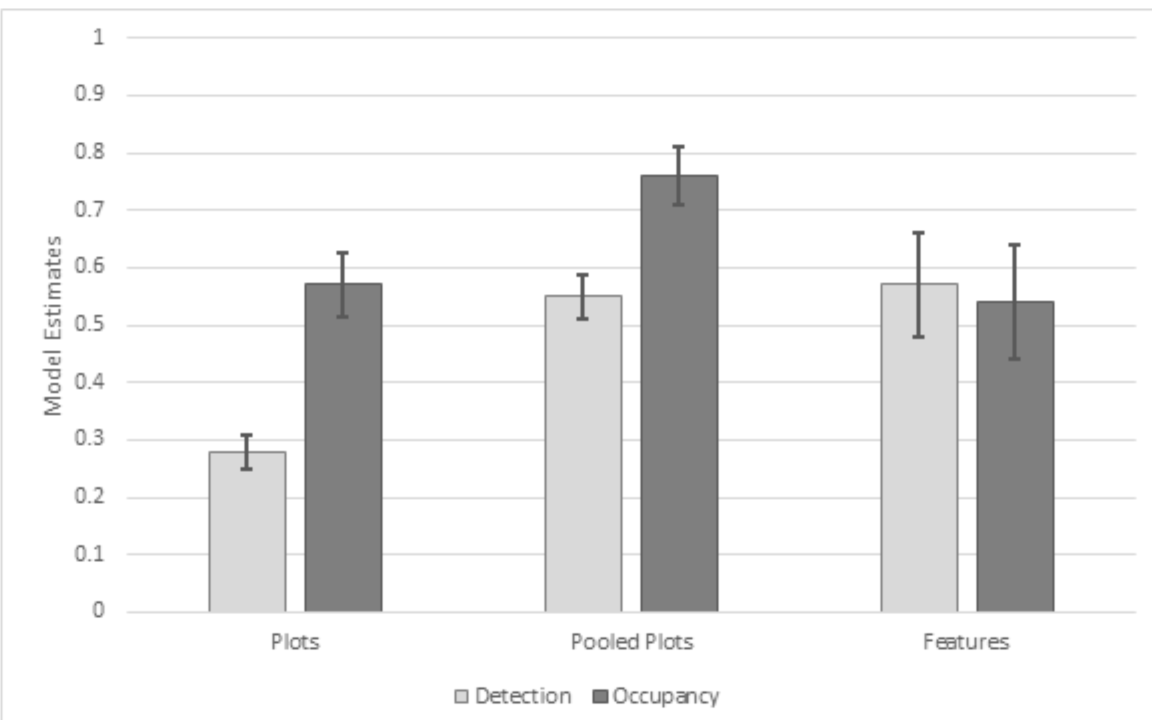

Figure 20. Comparison of the naïve occupancy and detection estimates for plot and feature data. Bars represent 95% confidence intervals on the estimates.

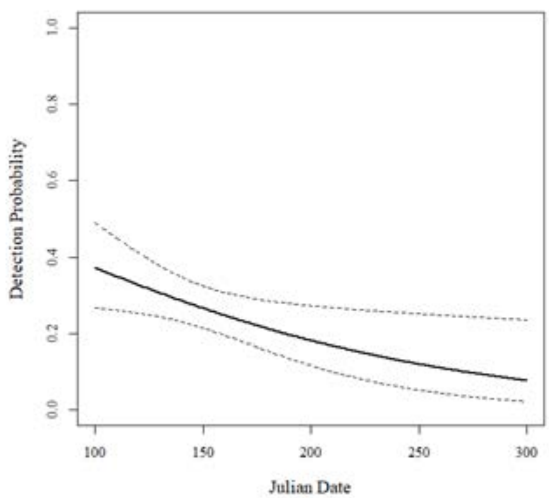

Figure 21. The detection probability plotted against the Julian date in which plots were sampled. Dotted lines represent 95% confidence intervals on predictions.

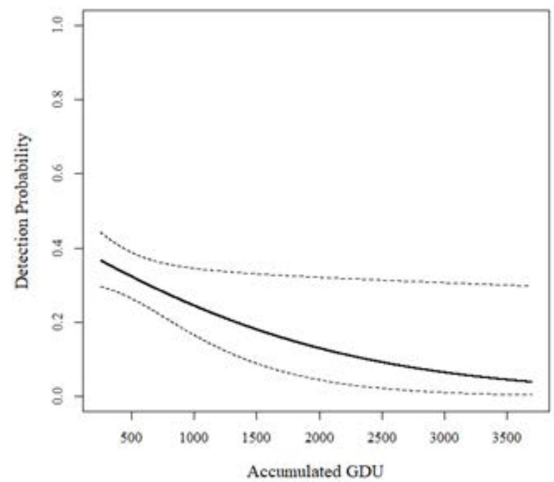

Figure 22. The detection probability plotted against the Accumulated Growing Degree Units for the sampling point and date. Dotted lines represent 95% confidence intervals on predictions.

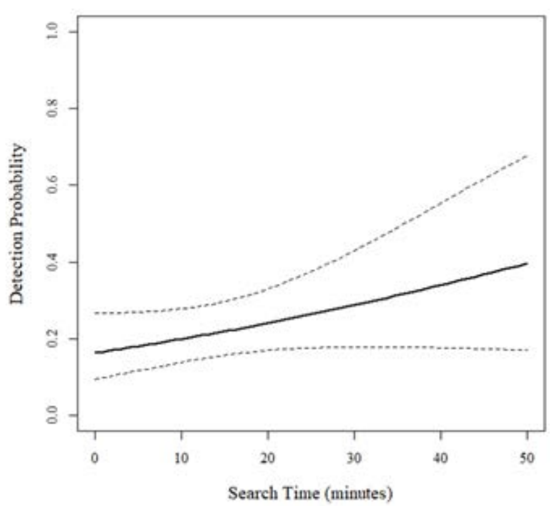

Figure 23. The detection probability plotted against the search time for the plot. Dotted lines represent 95% confidence intervals on predictions.

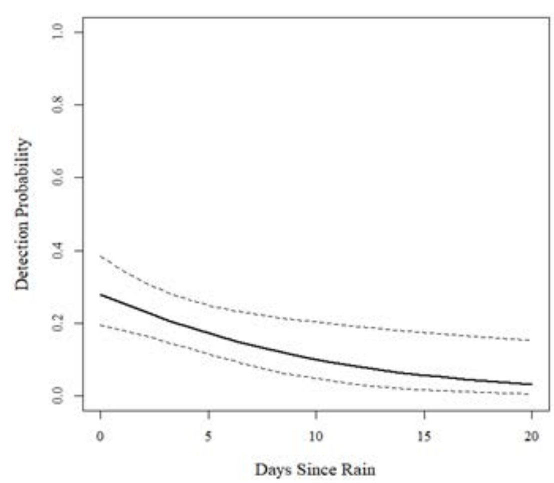

Figure 25. The detection probability plotted against the number of days since a rain event. Dotted lines represent 95% confidence intervals on predictions.

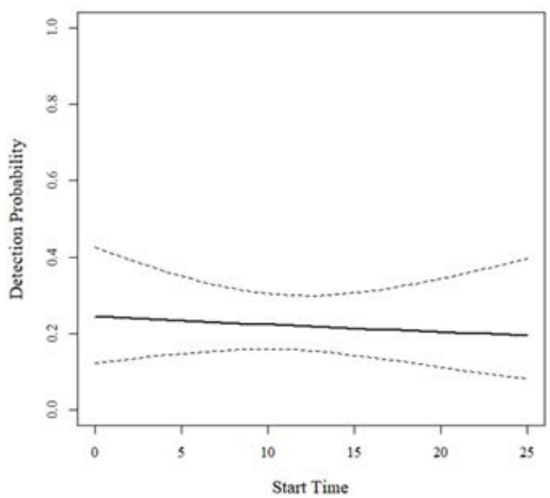

Figure 24. The detection probability plotted against the time of day (decimal hours) plots were searched. Dotted lines represent 95% confidence intervals on predictions.

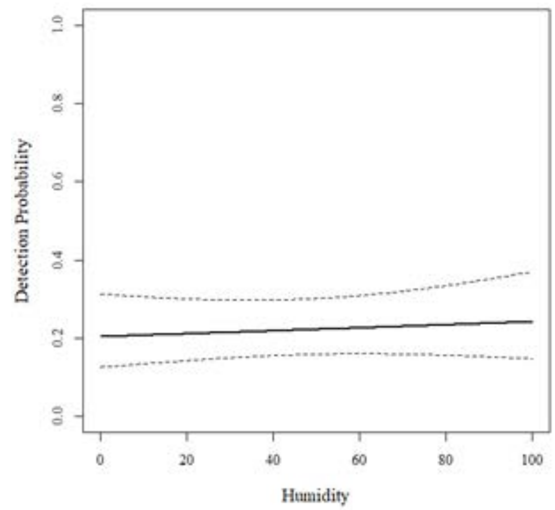

Figure 27. The detection probability plotted against the % humidity. Dotted lines represent 95% confidence intervals on predictions.

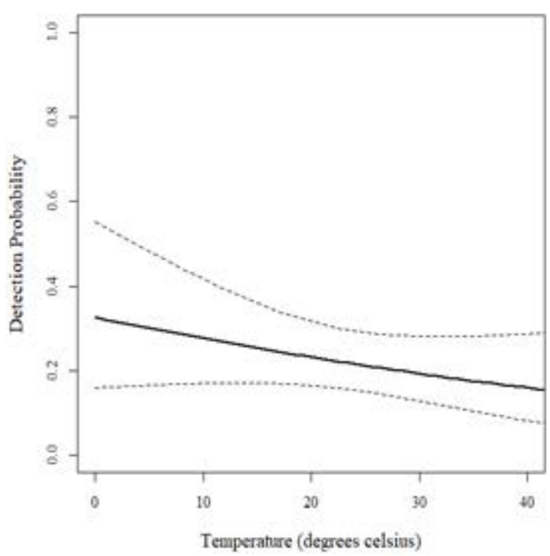

Figure 26. The detection probability plotted against the temperature recorded during plot sampling. Dotted lines represent 95% confidence intervals on predictions.

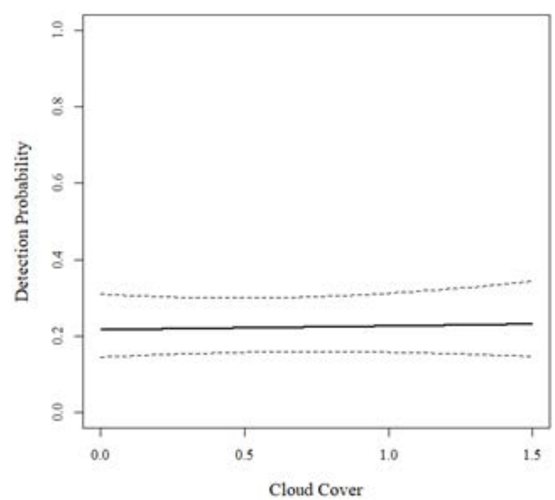

Figure 28. The detection probability plotted against the % cloud cover recorded during plot sampling. Dotted lines represent 95% confidence intervals on predictions.

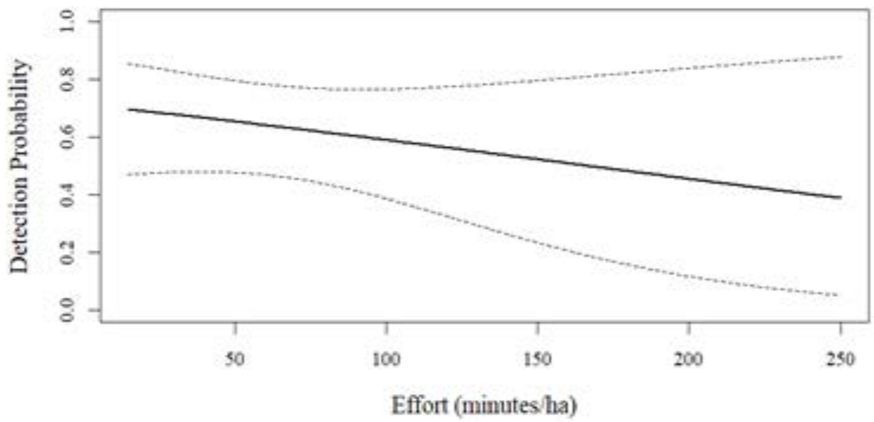

Figure 29. The detection probability plotted against the effort with which features were sampled. Dotted lines represent 95% confidence intervals on predictions.

Chapter 5. Genetics of the Eastern Box Turtle in the Northeastern United States

Alexander Krohn, PhD and JJ Apodaca, Ph.D.
Tangled Bank Conservation

Overview.— Genomics can be a powerful tool to understand how populations are related in any species. Eastern box turtles are a species of conservation concern, so a thorough understanding of levels of migration, barriers to migration and population dynamics is crucial to better protect the species. If significant differentiation exists within the range of a species, it may be necessary to designate different conservation units that require unique conservation actions for the species. We sequenced 127 eastern box turtles across the Northeast to better understand population dynamics, and to determine if significant conservation units existed. For each of those turtles, we sequenced tens of thousands of genetic loci using RADseq. Overall, we found little population structure, but a strong pattern of Isolation by Distance. Isolation by Distance indicates a gradual change in genetic variation as geographic distance increases. We found little differentiation across more than 1000 km. This genetic pattern may reflect either historical connectivity through once-contiguous habitat, or via anthropogenic movement. Even though there are not strong breaks in genetic connectivity, we find that spatial autocorrelation of genetic distance stops decreasing after approximately 150 km and encourage managers to treat Box Turtles within 150 km as a single historical population.

Introduction

Population genomics is a crucial tool for conservation. Genomics can help determine, for example, population sizes, population connectivity, barriers to migration, and help prioritize conservation actions (Hohenlohe and Rajora 2021). Importantly, for species that are rare, or that move infrequently, by using thousands of loci per individual and individual-based analyses, population genomics can infer this information from just a few samples per population (Felsenstein 2006; Prunier et al. 2013; Barley et al. 2015; Krohn et al. 2019).

The eastern box turtle (*Terrapene carolina carolina*) was once common across its range but is now of conservation concern in many of the states where it resides in the northeastern United States (Dodd 2002). As a long-lived species, assessing movement and population ecology can be difficult, and can result in small sample sizes (Iglay et al. 2007; Howeth et al. 2008; Marsack and Swanson 2009). Previous genetic work with this species, and other box turtle species, has shown little differentiation across large areas (Howeth et al. 2008; Kimble et al. 2014), but no study had used thousands of loci to determine finer-scale and genome-wide patterns of differentiation.

As part of an ongoing conservation assessment for the species, we used genomics to assess population connectivity, and to determine if any populations required classification as distinct conservation units (Funk et al. 2012; Coates et al. 2018). We amassed eastern box turtle samples from across the northeastern United States and used genome-scale methods to measure genetic diversity and connectivity across the sampled area.

Methods and Results

We successfully sequenced 1.1 billion reads from 218 Eastern Box Turtles from WV, VA, DE, MD, PA, NJ, NY, RI, CT, and MA using a 3RAD approach (Bayona-Vásquez et al. 2019). The quality of samples varied significantly, so we removed any samples that had fewer than 1 million raw reads, fewer than 2,000 loci successfully sequenced, or individuals that were missing data at more than 75% of their SNPs. We found that swabs had the most variable quality, and often resulted in high proportions of missing data (Fig. 30). Filtering the dataset left 127 individuals, including individuals from each of the above states. For these 127 individuals, we assembled the reads into clusters representing loci, aligned raw reads to each locus, and called SNPs on each locus for each individual using ipyrad (Eaton and Overcast 2020). The final dataset contained 1,739,485 SNPs. Not all of those SNPs were ever shared among all individuals, so different subsets of SNPs were used for each analysis.

We ran four preliminary analyses to quantify population structure. First, because previous work had shown a lack of population structure across the northeastern US, but a strong pattern of Isolation by Distance, we tested for Isolation by Distance (Wright 1943; Kimble et al. 2014). From the 127 individuals, we included biallelic SNPs present in at least 75% of individuals, then randomly selected one SNP per RAD locus to remove the effect of linkage disequilibrium. The final dataset contained 127 individuals and 23,219 unlinked SNPs. We used the program SNPRelate (Zheng et al. 2012) to calculate the proportion of genetic differences (Nei 1987) between each individual, then plotted this pairwise genetic difference against pairwise geographic distance for each individual. We used a partial Mantel test to see if pairwise genetic distance increased significantly with pairwise geographic distance, as one would expect under Isolation by Distance. We found that pairwise genetic distance increased with geographic distance, indicating Isolation by Distance plays a role in this dataset (Fig. 31; Mantel $r = 0.211$, $P = 0.001$, linear $R^2 = 0.046$).

This pattern of Isolation by Distance can also be a form of spatial autocorrelation, where nearby individuals are more closely related to each other than individuals further apart (Smouse and Peakall 1999). By using a permutation test on the autocorrelation coefficient between genetic and geographic distance, we can test whether there is a stronger correlation than we would expect by chance, and if that correlation stops at a certain distance (Smouse and Peakall 1999). We thus binned our data into bins according to Sturges' rule (Sturges 1926; Apodaca et al. 2013) and ran a permutation test of the autocorrelation coefficient at those bins with 1,000 permutations using the same dataset as above. We found that the correlation between geographic and genetic distance is higher than expected by chance until approximately 150 km (Figure 32; autocorrelation coefficient at 75 km = 0.0004, $P < 0.001$; autocorrelation coefficient at 150 km = -0.008, $P = 0.17$). This indicates that although there are no strong breaks in the pattern of Isolation by Distance, individuals less than 150 km apart are more closely related than one would expect by chance.

Second, we decomposed all the genetic variation into axes that contained the most variation in the dataset using a Principal Component Analysis (PCA). We used a hierarchical approach with the PCA, removing the most divergent groups at each subsequent hierarchical level (Janes et al. 2017;

Lawson et al. 2018), to better visualize all genetic structuring, and best identify a confiscated individual to its state of origin. We calculated the PCA using the built-in PCA tool in ipyrad (Eaton and Overcast 2020), which imputes missing data based on the mean allele frequency in a population. We filtered SNPs to those present in more than 50% of individuals, and at least one individual per state.

The first PCA used all 127 individuals and 35,952 unlinked SNPs. The overall PCA showed WV, RI, and all other states as distinct clusters (Fig. 33a). Next, we removed individuals from RI and WV. This second PCA contained 79 individuals and 58,430 unlinked SNPs. Overall, it showed CT, NY and NJ as distinct, with the other states forming a single cluster (Fig. 33b). We next removed individuals from CT, NY, and NJ from the analysis to separate the remaining states in PC-space. This third PCA included 67 individuals and 48,517 unlinked SNPs. This third PCA showed PA, VA, and MD as distinct, but grouped MA and DE together (Fig. 33c). Finally, to separate individuals between MA and DE, we ran a final PCA with just individuals from DE and MA. This final PCA included 43 individuals and 64,854 unliked SNPs. This final PCA showed the two states as distinct (Fig. 33d). Thus, by using this hierarchical clustering approach, we can differentiate individuals from different states.

Third, we ran a Bayesian clustering analysis to assign individuals a percentage of admixture from the number of populations that best fit the data. We used the program fastSTRUCTURE (Raj et al. 2014) to delimit populations, both with the logistical prior for fine-scale population structure, and without. We tested the fit of models with $K = 1$ populations to $K = 10$. We used the same dataset for the Isolation by Distance analysis. fastSTRUCTURE found that $K = 1$ best described the data, regardless of priority. This shows that while states can be differentiated by the PCA, they are not so differentiated as to be considered separate populations. This lack of structure was also found with mitochondrial data over larger geographic scales (Kimble et al. 2014).

Fourth, and finally, to better understand patterns for state managers, and to better quantify patterns of differentiation across the range, we broke our dataset down by state. For each state, we calculated a variety of population genetic statistics to quantify levels of genetic diversity in the state, and levels of differentiation among states. We used the same dataset as the Isolation by Distance analyses. To estimate genetic diversity, we calculated the observed heterozygosity (H_O; Nei 1987) and within population gene diversity (i.e., expected heterozygosity, H_S; Nei 1987, Goudet 2005) using the package 'hierfstat' (Goudet 2005) in R. To quantify levels of inbreeding, we calculated within-population subdivision (F_{IS}; also known as the inbreeding coefficient Nei 1987) using the package hierfstat (Goudet 2005). Finally, as a measure of population connectedness or differentiation, we calculated pairwise F_{ST} (Weir and Goudet 2017), again using the 'hierfstat' (Goudet 2005). As background, H_O and H_S vary from 0 to 1 and represent the proportion of heterozygous sites observed (H_O) and expected under "neutral" conditions (H_S). F_{IS} is $1/H_O$-H_S, and varies from -1 to 1, with populations with an excess of heterozygous sites having negative F_{IS} values, and a paucity of heterozygous sites having positive values. F_{ST} values vary from 0 to 1 with 0 being a panmictic population, and 1 being a completely subdivided population. We found that states had similar levels of genetic diversity (Table 6). Some states showed higher levels of F_{IS} ($F_{IS} > 0$), indicating a scarcity of heterozygotes that may indicate inbreeding. F_{ST} values were low ($F_{ST} < 0.1$) even from VA and MA (Table 7), further corroborating that there is little population structure across the northeastern US.

We caution against strong interpretations of these statistics given that there is no significant population structuring across the range that corresponds to state boundaries, and we have small sample sizes for state-wide analyses. However, there is some utility in quantifying levels of differentiation using F_{ST}, and levels of genetic diversity at the sample-site level using H_O and H_S. For example, we find that states show similar levels of genetic diversity. We find that all states except NJ and NY have fewer heterozygotes than expected. While it is hard to draw inferences about an entire state from such few samples, it is interesting to note this excess occurs across the sampling sites, and may be indicative of problems that reduce heterozygosity, like population bottlenecks and inbreeding.

Discussion

Our dataset agrees with previous studies showing that there is low genetic differentiation across large areas of the NortheastU.S.in eastern box turtles (Marsack and Swanson 2009; Kimble et al. 2014). While we were able to distinguish among the various states (Fig. 31), these do not represent different genetic populations. Overall, the major pattern from Virginia to Massachusetts is one of gradual genetic change via Isolation by Distance, rather than discrete populations.

It is likely that eastern box turtles historically formed one contiguous population across the eastern U.S. While some eastern box turtles do not stray far from their original capture location (Currylow et al. 2012), mother-offspring pairs have been documented as far as 27 km apart (Kimble et al. 2014). Thus, it is possible that migration across contiguous habitat throughout the range may diminish population genetic structure. Alternatively, this lack of genetic structure could be an artifact of eastern box turtle's long generation times. Even if wide scale habitat alterations with European settlement caused barriers to migration, this alteration could have occurred in as few as three box turtle generations (Marsack and Swanson 2009; Kimble et al. 2014). It is possible that the barriers may not be easily observable in genetic data after so few generations. Finally, eastern box turtles are important for spiritual, ceremonial, and nutritional reasons to many Native American groups across the U.S. (Gillreath-Brown and Peres 2018). It is also possible that eastern box turtles have been translocated by humans over the past 20,000 years, causing the observed lack of population structure. Regardless, given the long generation time of turtles, and as is the case with other turtles (Kuo and Janzen 2004; Schwartz and Karl 2006; Shaffer et al. 2017; Dutcher et al. 2020), the observed genetic pattern likely reflects historical connectivity, rather than contemporary migration. To preserve this historical pattern of Isolation by Distance, our data suggest that managers should prioritize connecting existing eastern box turtle populations to nearby populations with high quality habitat corridors.

We calculated the level at which the correlation between geographic and genetic distance significantly decreases, to give managers an idea of the geographic scales at which eastern box turtles once interacted. Around 150 km the correlation is no larger than we would expect by chance, indicating that eastern box turtles may have interacted genetically over large distances, at least in the past. This is a larger distance than one would expect from movement studies (Dodd 2002; Iglay et al. 2007), but on the same order of magnitude as other genetic studies (Kimble et al. 2014). It is possible that our dataset with thousands of loci picked up finer scale patterns than previous work using fewer than 20 loci.

In conclusion, our study suggests that eastern box turtles form a single population characterized by Isolation by Distance across the Northeast. This pattern of low range-wide genetic differentiation is likely historical in nature and indicates that managers should prioritize maintaining the historical connectivity across large scales.

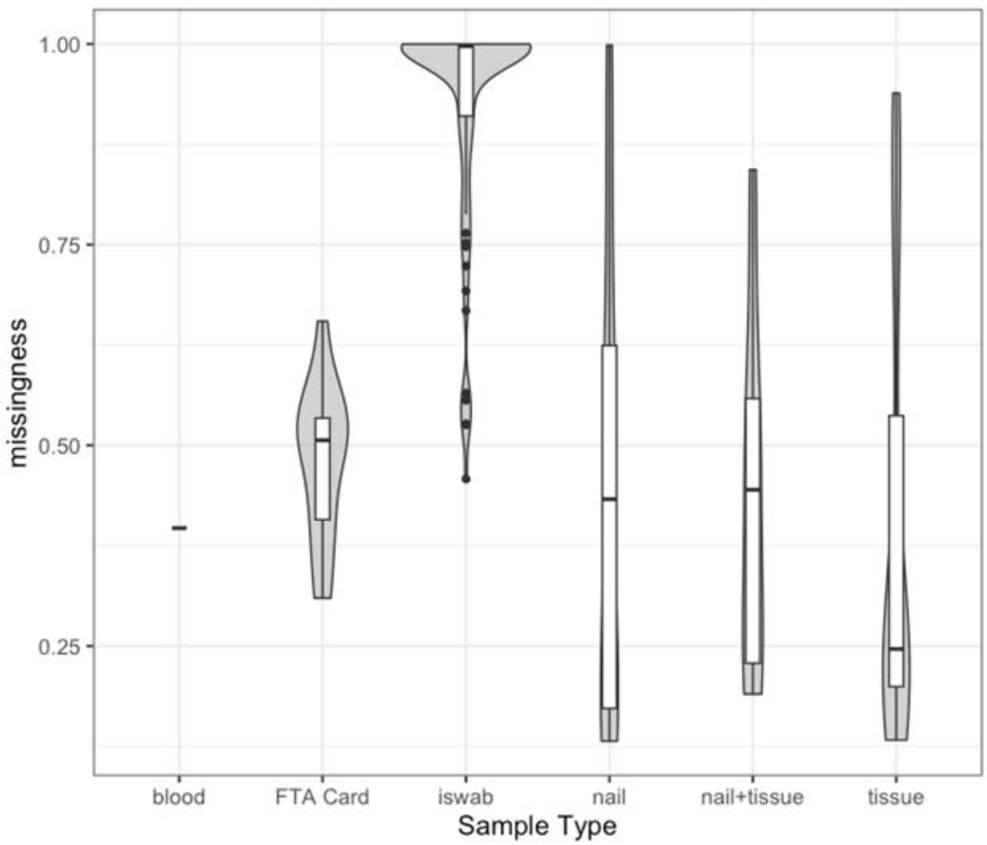

Figure 30: Proportion of per-individual missing data by sample type for 205 sequenced Box Turtles that had more than 1 million reads sequenced, and more than 2,000 loci returned. Missingness is averaged over 8,556 SNPs in this plot. Box plots (white) show the mean, first and third quartiles, 1.5*interquartile range, and outliers. Violin plots (gray) show a density distribution of samples.

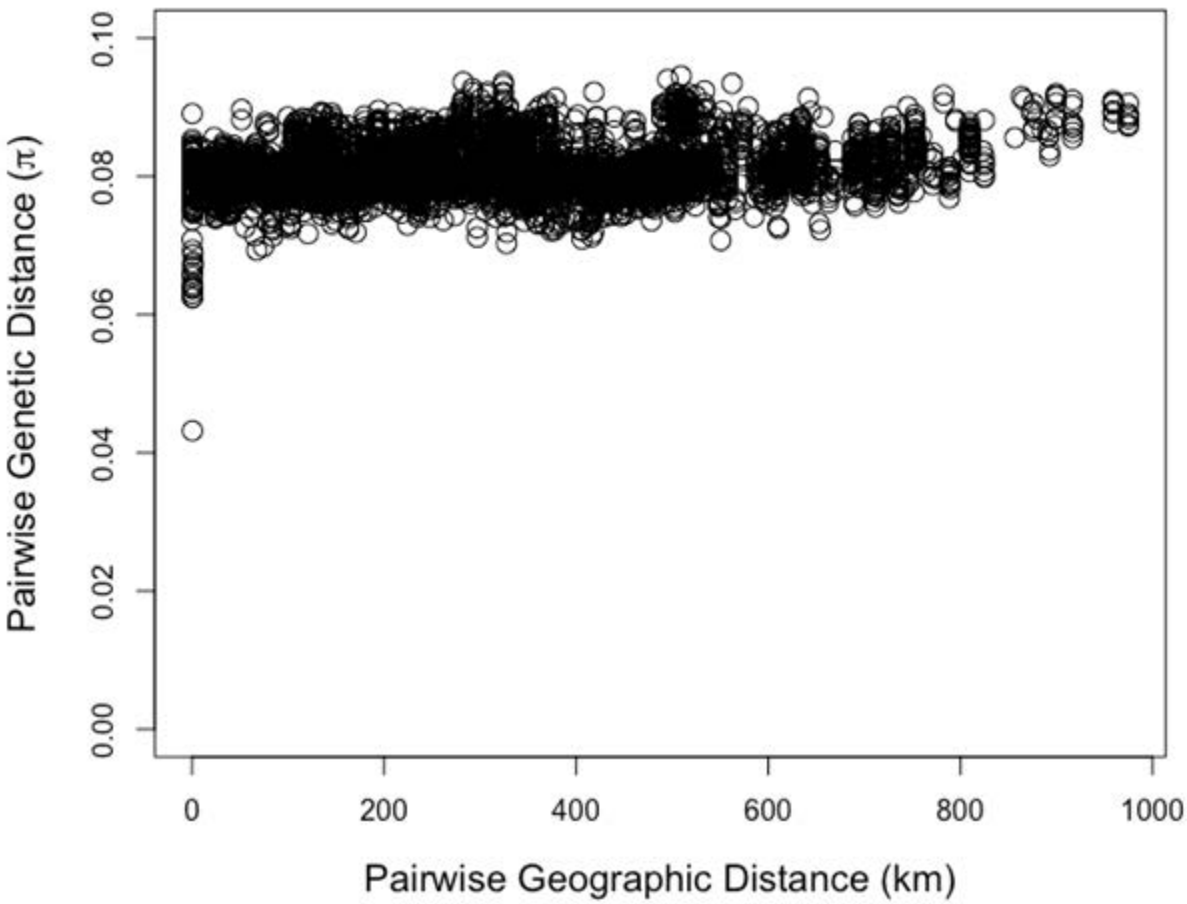

Figure 31: Pairwise genetic distance versus pairwise geographic distance for 127 Box Turtles (5,050 pairwise comparisons). Genetic distance increases significantly with geographic distance (Mantel r = 0.211, P = 0.001, linear R^2 = 0.046), indicating that Isolation by Distance plays a significant role in this dataset.

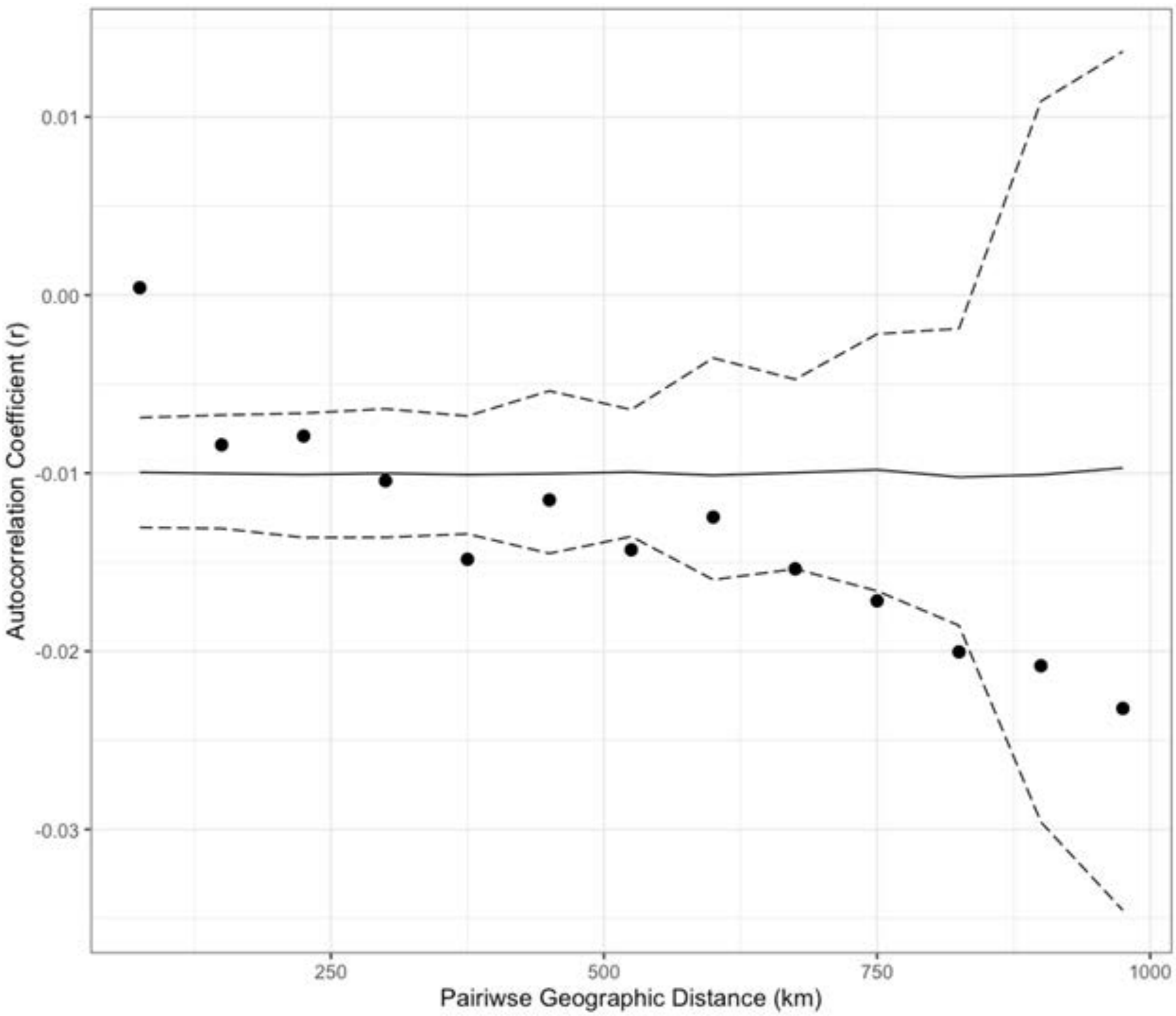

Figure 32: Autocorrelation between genetic and geographic distance. Pairwise values (n = 5,100) are binned into 13 bins according to Stuge's rule. Autocorrelation coefficients (r) for the actual bins are shown as solid circles. We used a permutation test of each bin to calculate a null distribution of autocorrelation coefficients (see methods and Smouse and Peakall (1999)). The mean of the permutation test is shown with the solid line, and the 95% empirical confidence interval is shown with the dashed line. Thus, solid dots outside of the confidence interval are significant at the α = 0.05 level.

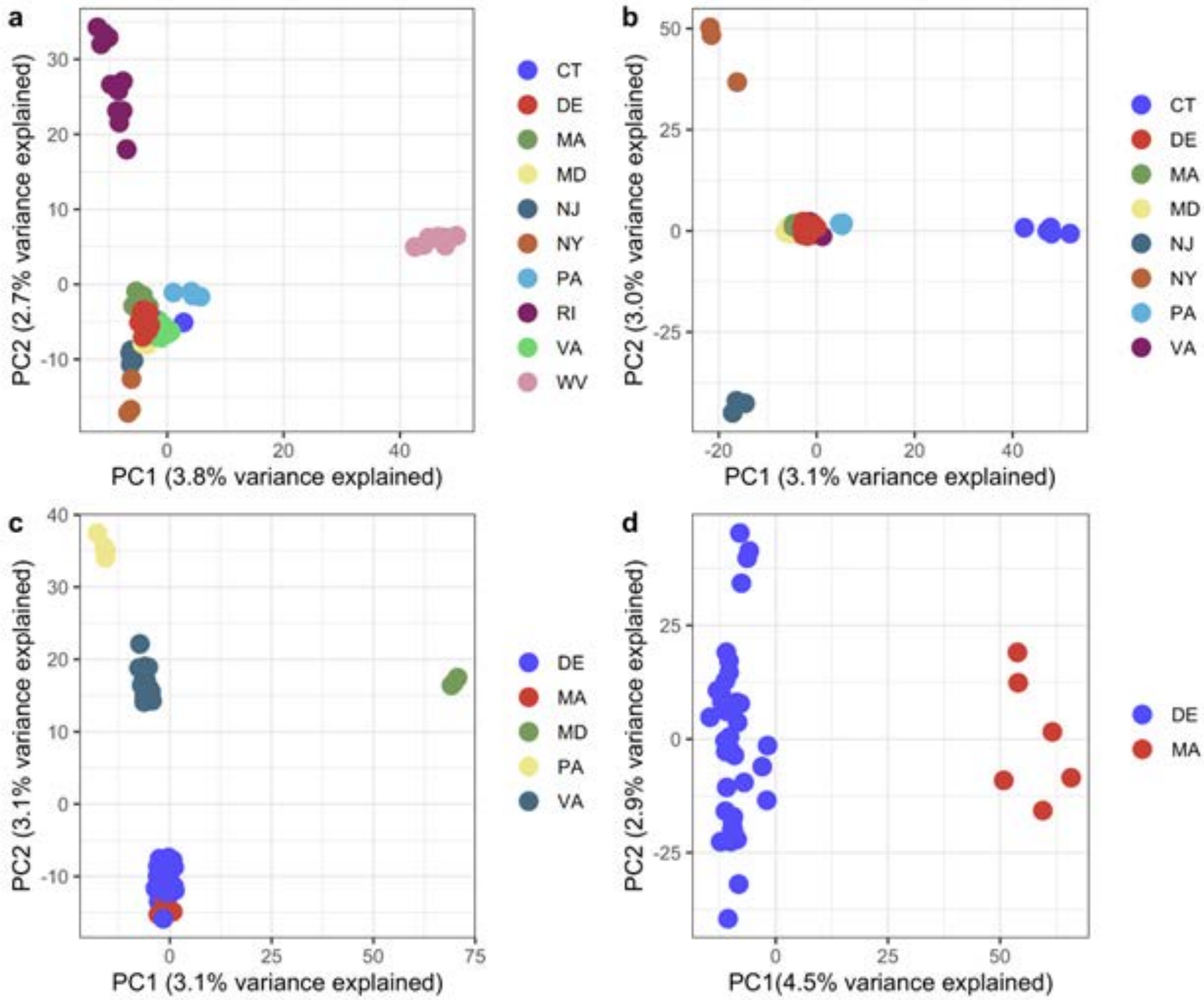

Figure 33: Principal Component Analyses of four subsets of Box Turtles. All 127 Box Turtles sampled (35,952 SNPs; a), 79 individuals excluding those from RI and WV (58,430 SNPs; b), 67 individuals excluding those from RI, WV, CT, NY, and NJ (48,517 SNPs; c), and finally 43 individuals from just DE and MA (64,854 SNPs; d).

Table 6: Observed heterozygosity (H_O), within population gene diversity (H_S; i.e., expected heterozygosity (Goudet 2005)), within-population subdivision (F_{IS}; i.e. inbreeding coefficient), and sample sizes for each state.

State	n	H_O	H_S	F_{IS}
CT	5	0.0804	0.0835	0.0377
DE	37	0.0789	0.0853	0.0748
MA	6	0.0879	0.1016	0.1352
MD	3	0.085	0.0919	0.0748
NJ	4	0.0798	0.0829	0.0365
NY	3	0.0803	0.067	-0.1986
PA	4	0.0854	0.0916	0.0678
RI	14	0.0732	0.0802	0.0874
VA	17	0.0809	0.0871	0.0715
WV	8	0.0837	0.0905	0.075

Table 7: Pairwise population subdivision (F_{ST}) for each state. Note that comparisons involving fewer than four individuals should be interpreted with caution.

	CT	DE	MA	MD	NJ	NY	PA	RI	VA	WV
CT	NA									
DE	0.0043	NA								
MA	0.0142	0.0141	NA							
MD	0.0044	0	0.0141	NA						
NJ	0	0.0006	0.0097	0	NA					
NY	0.0906	0.0884	0.1004	0.0972	0.0921	NA				
PA	0.0039	0.0091	0.0227	0.0076	0.0055	0.0917	NA			
RI	0.0193	0.0138	0.0239	0.015	0.0119	0.1072	0.0207	NA		
VA	0.0074	0.0037	0.0194	0	0.0022	0.0906	0.0099	0.0178	NA	
WV	0.0197	0.0323	0.0452	0.0253	0.0307	0.1147	0.0114	0.0473	0.0286	NA

Chapter 6. Landscape Impairment of the Eastern Box Turtle Across the Northeast

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Overview.— The goal of this assessment was to contribute to the understanding of the conservation status of the eastern box turtle, a wide-ranging terrestrial generalist, in the northeastern United States (Maine to Virginia) by (1) characterizing relationships with anthropogenic land use and (2) estimating the extent of land-use driven habitat impairment for the region. We used a regional dataset of occurrence records combined with pseudo-absences to develop occupancy models to first estimate the potential distribution in the northeastern U.S. and then predict habitat suitability within that distribution. We observed a strong positive relationship between probability of occurrence and canopy cover (within 180 m) and a strong negative relationship with hay/pasture fields (360 m), cultivated crops (180 m), imperviousness (360 m), and forest loss primarily from timber harvesting (since 2000; 1,440 m). We estimate that approximately 51% of eastern box turtle habitat in the northeastern U.S. is impaired by land use. While our results indicate impairment throughout the region, the majority of habitat loss is predicted from Pennsylvania and Delaware to Virginia. The apparent negative relationship with timber harvesting could have implications for contextualizing the global status of the species, considering both the vast historical and contemporary extent of logging throughout its distribution. This study, in combination with previous long-term studies documenting population declines, provides compelling evidence of widespread population decline, and suggests that greater attention to the conservation status of the eastern box turtle is warranted, particularly within the northeastern U.S.

Introduction

The goal of this assessment was to contribute to the understanding of the conservation status of the eastern box turtle by (1) characterizing relationships with anthropogenic land use and (2) estimating the extent of land-use driven habitat impairment for the region. We expected that, as suggested in previous literature, the eastern box turtle would be negatively associated with agricultural cover (Erb and Jones 2011) and development (Roe et al. 2021, Graham et al. 2022), and the combined effect of these variables would predict extensive habitat impairment throughout the region.

Methods

Occurrence Records.— We primarily collected records from state agencies, natural heritage programs, reptile atlas projects, and personal datasets of the authors. In cases where descriptive location information was available, we compared coordinates to location descriptions to confirm accuracy and removed records that were inaccurate. We excluded all records with accuracy >200 m. We excluded records from Maine because the widely scattered points may represent escaped pets rather than natural occurrences (Derek Yorks, pers. comm.). Like previous studies (e.g., Willey et al. 2022), we excluded records >30 years prior to the start of this study (i.e., before 1990) from consideration in analyses. To reduce sampling bias, we randomly selected remaining records that maintained a minimum of two km separation distance.

Pseudo-Absences.— We did not have information about where eastern box turtles do not occur (i.e., "true" absences) and thus relied upon randomly generated locations, hereafter referred to as "pseudo-absences" (Chefaoui and Lobo 2008, Barbet-Massin et al. 2012). Through visual inspection, it was clear that the occurrence dataset was spatially biased toward roads (records are often reported by drivers that find turtles crossing roads). Therefore, we generated random points that were equally biased toward roads using the "Create Spatially Balanced Points" tool in ArcMap 10.5 (Environmental Systems Research Institute, Inc., Redlands, CA). We generated approximately 10 pseudo-absences for every occurrence record (Barbet-Massin et al. 2012, Guisan et al. 2017) within three different subregions corresponding to New England states (Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, and Maine), "Mid-Atlantic" states (New York, New Jersey, and Pennsylvania), and southern states (Maryland, Delaware, District of Columbia, West Virginia, and Virginia). We use this subregional approach because we wanted pseudo-absences to reflect intraregional variation in both landscape context (e.g., road density) and sampling intensity (there tended to be a higher density of records in states where the species is considered more threatened and therefore tracked more extensively). We generated pseudo-absences within 100 km of occurrence records with the intention of capturing relevant climatic gradients determining the northern extent of the range.

Environmental Variables.— Variables reflecting climate included minimum January temperature, mean July temperature, mean July precipitation, mean April precipitation, mean annual precipitation, growing season degree days (GDD), and elevation. Temperature and precipitation data represent 30-year normals (1981–2010; 800 m resolution rasters) acquired from the PRISM Climate Group (PRISM 2021). We acquired growing season degree days, which represents the sum of daily mean temperatures above 10˚C, from Designing Sustainable Landscapes (Plunkett et al. 2022). We derived elevation from the National Elevation Dataset (USGS 2009).

Land-use variables included percent canopy cover, proportion cultivated crops, proportion hay/pasture fields, mean imperviousness, proportion developed, road density, and proportion forest loss. We acquired all land-use data, except forest loss, from the Multi-Resolution Land Characteristics Consortium 2016 National Land Cover Database (NLCD; Coulston et al. 2012, Yang et al. 2018). We acquired forest loss data from the Global Forest Change dataset (Hansen et al. 2013). This dataset (30-m resolution) estimates forest loss of any kind for each year since 2000. We were interested in the extent to which this variable represented timber harvesting or natural disturbances, which, in contrast to urbanization and agricultural conversion, allow subsequent succession of natural communities to occur. Thus, using ArcMap, we estimated the proportion of areas that experienced forest loss that were developed, cultivated crops, hay/pasture, or barren land (as defined by NLCD). We resampled each variable to a 90-m resolution for computational purposes and measured each variable at six arbitrarily chosen spatial scales: the individual cell, 90 m, 180 m, 360 m, 720 m, and 1,440 m. We extracted all raster values from presence/pseudo-absence locations using the "raster" package (Hijmans 2019) in R statistical software (R Core Team 2022).

Statistical Analyses We followed a two-stage process for estimating regional habitat impairment. First, we estimated the "potential" distribution by modeling the eastern box turtle climate niche in the study area and removing all open water habitats (as defined by NLCD). While this is a liberal approach, the highly generalist nature of the eastern box turtle within terrestrial communities suggests that they were likely primarily limited by wetlands and climate (Bleakney 1958, Adler 1968) prior to European colonization, although relatively little is known about how Native American populations influenced the distribution (Adler 1970). We chose to not exclude woody emergent wetlands from the predicted potential distribution because, while many of these wetlands are clearly not suitable, populations can at least seasonally occupy portions of woody and emergent wetlands that either dry out or are very shallow (Donaldson et al. 2005). We used generalized linear models with a binomial distribution to relate presence/pseudo-absence data to climate. First, three simple models were run for each variable that included either a linear term, a quadratic term, or a linear interaction with elevation. The variable(s) that performed best with respect to Akaike's Information Criterion (AIC; Burnham and Anderson 2002) were selected for inclusion in the comparison of more complex models. While the driving ecological mechanism remains unclear, we considered an interaction between climate variables and elevation because the maximum suitable elevation threshold appears to vary throughout the range, perhaps in relation to climate. Previous distribution modeling efforts (e.g., Designing Sustainable Landscapes) have not considered elevation and presumably as a result, predicted substantial amounts of suitable habitat at high elevations where they do not occur. We examined correlations among variables and when correlations were high ($r > 0.7$), we kept the variables that performed best with respect to AIC. Next, models with all variable subsets were compared (where both variables in interactions had to be considered together) using the "MuMIN" package in R (Barton 2016). We then used the top model to predict the potential distribution throughout the region using the "dismo" package (Hijmans et al. 2022) in R. To ensure a liberal estimate of potential distribution that emphasizes known occurrence locations, we chose a binary threshold for suitability that achieved sensitivity (true positive prediction rate) = 0.99. We assessed predictive ability by performing a five-fold cross-validation procedure. To avoid over-predicting the potential distribution at the northern edge of the range, we only considered areas within 50 km of a known record.

Next, we modeled habitat impairment via land use using only records and pseudo-absences that fell within the predicted potential distribution. Similar to modeling climate, we used generalized linear models with a binomial distribution to compare (using AIC) three simple models for each land cover-scale combination that included either a linear term, quadratic term, or a linear interaction with GDD. We examined an interaction with GDD days because, like most ectotherms, eastern box turtle life history is strongly influenced by climate, and we expected that increased growth rates and (possibly) reproductive output in warmer areas could mediate population relationships with land cover. We chose the best performing scale and term(s) for each land cover type and, after removing highly correlated variables, compared all variable subsets to find a "top" model that included multiple land cover types. Last, we performed a five-fold cross-validation procedure and then predicted suitability throughout the potential distribution. We selected a suitability threshold by taking the value that maximized the combined true positive and true negative rates (Liu et al. 2005, Hallfors et al. 2016). We assessed model performance using the Area Under the Curve metric (Guisan et al. 2017).

We provided further context for areas predicted to be suitable within the potential distribution by summarizing the percentage of suitable habitat that is characterized as developed (NLCD 2016) or woody or emergent wetland (NLCD 2016). We summarized habitat impairment and these metrics by state and EPA Level 3 Ecoregion.

Results

Our occurrence database included 21,386 records after erroneous and inaccurate records were removed, spanning 12 states including New Hampshire (122), Massachusetts (3,842), Rhode Island (106), Connecticut (737), New York (438), New Jersey (942), Pennsylvania (3,304), Maryland (614), Delaware (390), District of Columbia (23), West Virginia (6,889), and Virginia (3979). The occurrence dataset was reduced to 3,990 records when we applied the 2-km minimum separation distance. The top performing model for potential distribution included interactions between elevation and both minimum January temperature and annual precipitation. Cross-validation yielded mean AUC = 0.63 (range 0.62–0.64). The full potential distribution model (i.e., using all data) predicted 299,210 km^2 of habitat across the study area (Table 8, Table 9, Fig. 35, 36, 37, 38).

Simple land cover models indicated strong positive relationships between probability of box turtle occurrence and percent canopy cover within (180 m, influenced by GDD), and strong negative relationships with cultivated crops (180 m, quadratic), hay/pasture (360 m, influenced by GDD), imperviousness (360 m, quadratic), development (360 m, quadratic), road density (360 m, quadratic), and forest loss (1440 m, linear; Fig. 34). Our assessment of the forest loss variable revealed that developed, cultivated crops, hay/pasture, or barren land corresponded to only 11% of the area that experienced forest loss. Imperviousness, development, and road density were highly correlated ($r > 0.7$) and thus only imperviousness, which performed best with respect to AIC, was considered in more complex models. The top land cover model included cultivated crops, an interaction between hay/pasture and growing degree days, imperviousness, and forest loss, but not canopy cover. Cross-validation yielded mean AUC = 0.66 (range 0.65–0.67). The full model (i.e., using all data) predicted that 51% of the potential distribution is currently impaired (Fig. 35A). Within suitable habitat, 11% (5% of potential distribution) was classified as woody/emergent wetlands and 10% (5% of potential distribution) was classified as some form of development.

Discussion

Our findings suggest that the eastern box turtle has experienced extensive habitat impairment in the northeastern U.S., with approximately 51% of the potential distribution altered or influenced by anthropogenic land use. This estimate is on par with other turtle species considered more threatened in the region, such as the wood turtle (*Glyptemys insculpta*; 58%, Willey et al. 2022). When considered with the body of literature demonstrating individual population declines throughout the range (Hall

1999, Williams and Parker 1987, Nazdrowicz et al. 2008, Jones et al. 2021), this study suggests the species has experienced widespread population decline.

These results indicate that the highest proportional levels of habitat impairment have occurred in Virginia (64%), Delaware (60%), Pennsylvania (58%), and the District of Columbia (84%, although DC represents <0.001% of potential habitat). When low-intensity development (e.g., roads) and woody/emergent wetlands (many of which are likely not suitable for eastern box turtles) were considered (i.e., removed), Delaware (13%), New Jersey (27%), Maryland (29%) had the lowest levels of unimpaired upland, apart from DC (7%). West Virginia appears to represent a stronghold for the species in the Northeast, with not only the lowest proportional impairment and highest area of suitable habitat (regardless of whether additional urbanization and wetland habitat are considered), but also some of the least fragmented landscapes (Fig. 35A, 37). There also appear to be substantial disparities in impairment among ecological contexts, with ecoregions at the core of the regional distribution, such as the Piedmont and Southeastern Plains, experiencing severe habitat impairment (83% and 60% respectively), and others such as the Central Appalachians experiencing relatively low impairment (24%). Notably, when considering wetlands and low-intensity development, the Middle Atlantic Coastal Plain and Atlantic Coastal Pine Barren ecoregions displayed extensive declines in unimpaired habitat, with only 15% and 28% of the potential distribution remaining unimpaired, suggesting that these ecoregions are likely more vulnerable than our basic predictions indicate.

The observed negative associations with urbanization and agriculture lend support to the notion that these land uses are major threats to the eastern box turtle. Urban environments may lead to lower adult and juvenile survival through factors such road mortality, incidental collection, and human-subsidized predators (Gibbs and Shriver 2002, Garber and Burger 1995, Marchand and Litvaitis 2004). While there is limited research on the subject, a few studies have documented negative relationships between density and urbanization (Roe et al. 2021, Graham et al. 2022). Frequently mowed hay fields may represent a particularly significant threat because turtles are often drawn to fields (Erb and Jones 2011) from adjacent forest for increased solar exposure and foraging opportunities. Although studies have suggested mortality associated with hay fields may negatively affect populations (Nazdrowicz et al. 2008, Erb and Jones 2011), we are unaware of studies demonstrating negative relationships with agriculture across populations. Conversion to cultivated crop cover results in the loss of habitat, but in contrast to hay fields, may not have the same negative effect on surrounding areas because crop fields are likely less attractive for thermoregulation and foraging — this may explain why the relationship was characterized by a smaller spatial scale (180 m for crops compared to 360 m for hay/pasture). Future research should consider examining the varying effects of agriculture on population demographics both within and across populations.

Although the forest loss variable (Fig. 39) reflects a range of causes of deforestation, our assessment indicated that development and agriculture made up a small proportion of areas with forest loss, thus suggesting that most of the forest loss (Hensen et al. 2013) was likely the result of forest management. Relatively few studies have examined this species' relationship with timber harvesting (Currylow et al. 2012, Agha et al. 2018) and we are unaware of any studies regarding population-level effects. Low levels of timber harvesting may benefit populations by providing a gradient in microclimate, direct and diffuse solar radiation, forage availability (Griffiths and Christian 1996), and protective cover (Greenberg 2001, Agha et al. 2018). However, modern timber harvesting requires heavy machinery that, when used intensively (e.g., large clearcuts), has the potential to cause mass-mortality, regardless of whether turtles are active or overwintering underground. Closed-canopy forests serve as both overwintering habitat and thermal refugia during summer when temperatures can approach lethal temperatures (>41.5℃) in large canopy openings (Hutchison et al. 1966, Currylow et al. 2012, Roe et al. 2017). Therefore, large clearcuts that eliminate entire patches of forest, may trigger a dispersal response (Dodd et al. 2006) that increases the chances of mortality, even if individuals are not killed during machine use. This could be particularly consequential for populations situated within highly fragmented landscapes such as those in southern Virginia. Timber harvesting affects millions of hectares of forest each year in the United States (Masek et al. 2011), much of which occurs within the eastern box turtle distribution (Hensen et al. 2013). Thus, developing an understanding of the effects of intensive forest management on populations and individual behavior should represent an important research priority.

When considered within the context of historic land use, the potential negative relationship with timber harvesting suggests the regional population has possibly experienced even greater declines than our assessment indicates. By the mid-19th century much of the northeastern U.S. was deforested primarily from timber harvesting and agriculture (Thompson et al. 2013) and therefore many local populations may have been extirpated or severely reduced in size prior to reforestation during the 20th century. Despite widespread restoration of suitable conditions, the window of time since maximum deforestation (approximately 1850–present) only represents six or fewer generations and thus many populations could still be recovering, even if additional anthropogenic threats to adult, juvenile, and nest survival are minimal. Historical land-use patterns have been shown to shape contemporary herpetofaunal communities in other ecosystems. For example, legacy land use better predicts ornate box turtle (*Terrapene ornata*) occurrence than current vegetation conditions in North American prairies (Royal et al. 2022). However, it is possible that past logging was less detrimental for populations than contemporary operations given that heavy machinery was not available. Nevertheless, the vast extent of historical forest loss could certainly have led to declines that are observable today.

Our findings indicate that land cover relationships may vary climatically. Both hay/pasture and canopy cover displayed interactions with growing season degree days (GDD), but the effect was relatively minimal. However, ad hoc examinations of top interaction models (that did not perform as well as the top univariate model) for other variables showed patterns of lessening negative relationships with both cultivated crops and imperviousness as GDDs increased. It is possible our data (i.e., presence/pseudo-absences) was not sufficient for examining GDD-dependent land-use relationships. Since individual turtles can often occupy areas long after severe population decline and functional extinction, it is possible that other response variables that are more sensitive to land use, such as abundance, may display a stronger interactive relationship with GDD.

We recommend not using these results to draw conclusions about relatively small extents (e.g., individual populations). We used a relatively large raster resolution (90m), which means that these predictions are unable to reflect fine-scale habitat patterns that might be important for populations. Misclassifications are also known to be present within the National Land Cover Database (Brown et al. 2016) and therefore we cannot ensure that all locations are without error. These analyses are also imperfect in that occupancy analyses that use true absence data instead of pseudo-absences could yield different relationships. For example, the random nature of pseudo-absence placement means that some were placed within suitable contexts, which could result in weaker predicted relationships with land use than true presence/absence data might produce. The longevity of the species also means that unsuitable contexts that still harbor turtles long after population collapse, such as suburban areas and small forest fragments within highly developed areas, likely had inflated suitability predictions. Last, while eastern box turtles are forest-associated, populations are highly dependent upon open habitats, particularly for nesting, and therefore the deep interior large forest blocks may not actually represent suitable habitat as our results suggest. However, further research is needed on this topic.

Conclusion

This study suggests that habitat across a large proportion of the eastern box turtle range is impaired, and to the extent that survival and reproduction are negatively affected by habitat impairment, these findings support the contention (Dodd 2001, Roe et al. 2021; Table 8) that this species has likely undergone widespread population decline. While the area of potentially suitable remaining habitat is greater than those of aquatic or semi-aquatic species that are considered more threatened (e.g., spotted turtle [*Clemmy guttata*], Blanding's turtle [*Emydoidea blandingii*], wood turtle [*Glyptemys insculpta*], wetlands currently receive far greater direct and/or indirect protection under federal and state legislation in the United States (Table 9). Moreover, due to the perceived commonness of the species, there have been few standardized efforts to document populations and demographic trends (although see Roe et al. 2021) and thus, there is often very little understanding of whether populations even occur within seemingly suitable contexts, let alone their conservation status. An important immediate next step is to begin to address data-deficiencies by establishing a collaborative effort to implement both rapid and long-term population monitoring efforts across not only the northeastern U.S., but also the broader subspecies distribution.

Table 8. Summary, by state, of predicted habitat impairment and percent of unimpaired habitat categorized the National Land Cover Database as wetland or developed.

State	Area (km²)	Area impaired (km²)	% impaired	Within unimpaired habitat	
				% wetland	% developed
Connecticut (CT)	11462	3348	29	11	14
Delaware (DE)	5000	2989	60	51	16
District of Columbia (DC)	160	134	84	6	48
Maine (ME)	983	559	57	25	8
Maryland (MD)	23266	11988	52	26	14
Massachusetts (MA)	14627	6163	42	20	16
New Hampshire (NH)	4787	2564	54	17	11
New Jersey (NJ)	18823	8238	44	34	17
New York (NY)	16131	7987	50	1	16
Pennsylvania (PA)	56451	32564	58	2	11
Rhode Island (RI)	2693	1032	38	17	14
Vermont (VT)	207	105	51	3	9
Virginia (VA)	97107	61983	64	9	7
West Virginia (WV)	47514	11807	25	0	5
Total	299210	151462	51	11	10

Table 9. Summary, by Environmental Protection Agency Level 3 Ecoregion, of predicted habitat impairment and percent of unimpaired habitat categorized as wetland or developed by the National Land Cover Database.

EPA Ecoregion Level 3	Area (km²)	Area impaired (km²)	% impaired	Within suitable habitat	
				% wetland	% developed
Atlantic Coastal Pine Barrens	13869	5841	0.42	0.32	0.20
Blue Ridge	9555	3264	0.34	0.00	0.04
Central Appalachians	24815	6040	0.24	0.00	0.05
Eastern Great Lakes Lowlands	784	519	0.66	0.20	0.15
Erie Drift Plain	2532	1542	0.61	0.17	0.09
Middle Atlantic Coastal Plain	22542	11892	0.53	0.57	0.12
North Central Appalachians	1063	159	0.15	0.03	0.12
Northeastern Coastal Zone	36803	16054	0.44	0.16	0.16
Northern Allegheny Plateau	990	164	0.17	0.09	0.08
Northern Appalachian and Atlantic Maritime Highlands	8733	2734	0.31	0.08	0.09
Northern Piedmont	30882	21398	0.69	0.06	0.20
Piedmont	33170	27478	0.83	0.04	0.09
Ridge and Valley	58603	31446	0.54	0.01	0.06
Southeastern Plains	19511	11658	0.60	0.18	0.13
Western Allegheny Plateau	34992	11255	0.32	0.00	0.07

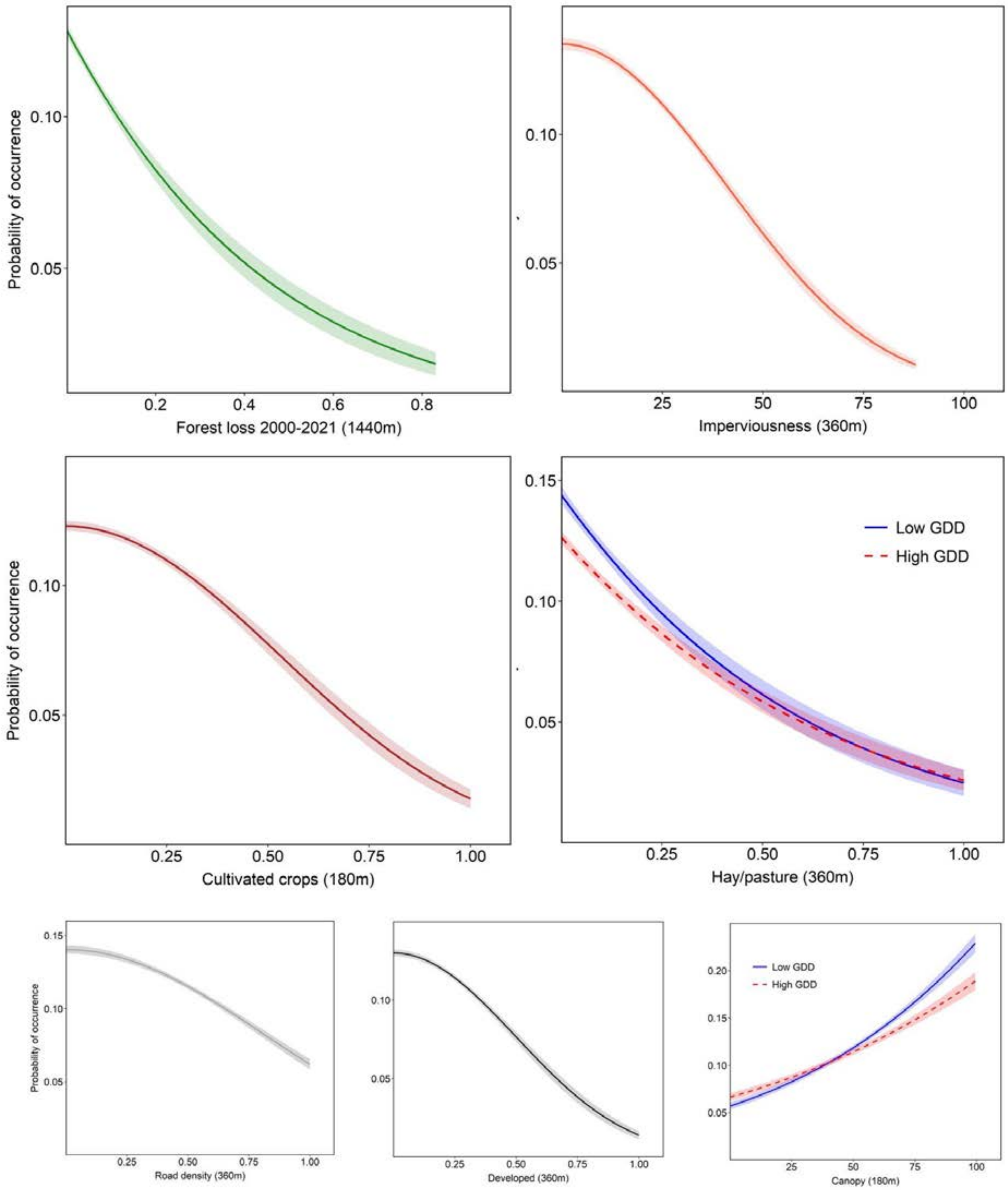

Figure 34. Probability of occurrence of eastern box turtles in relation to land use. Plots depict predictions for simple models only containing a single variable or an interaction with growing season degree days (GDD). Developed land, road density, and canopy cover were not included in the top model used to predict habitat impairment.

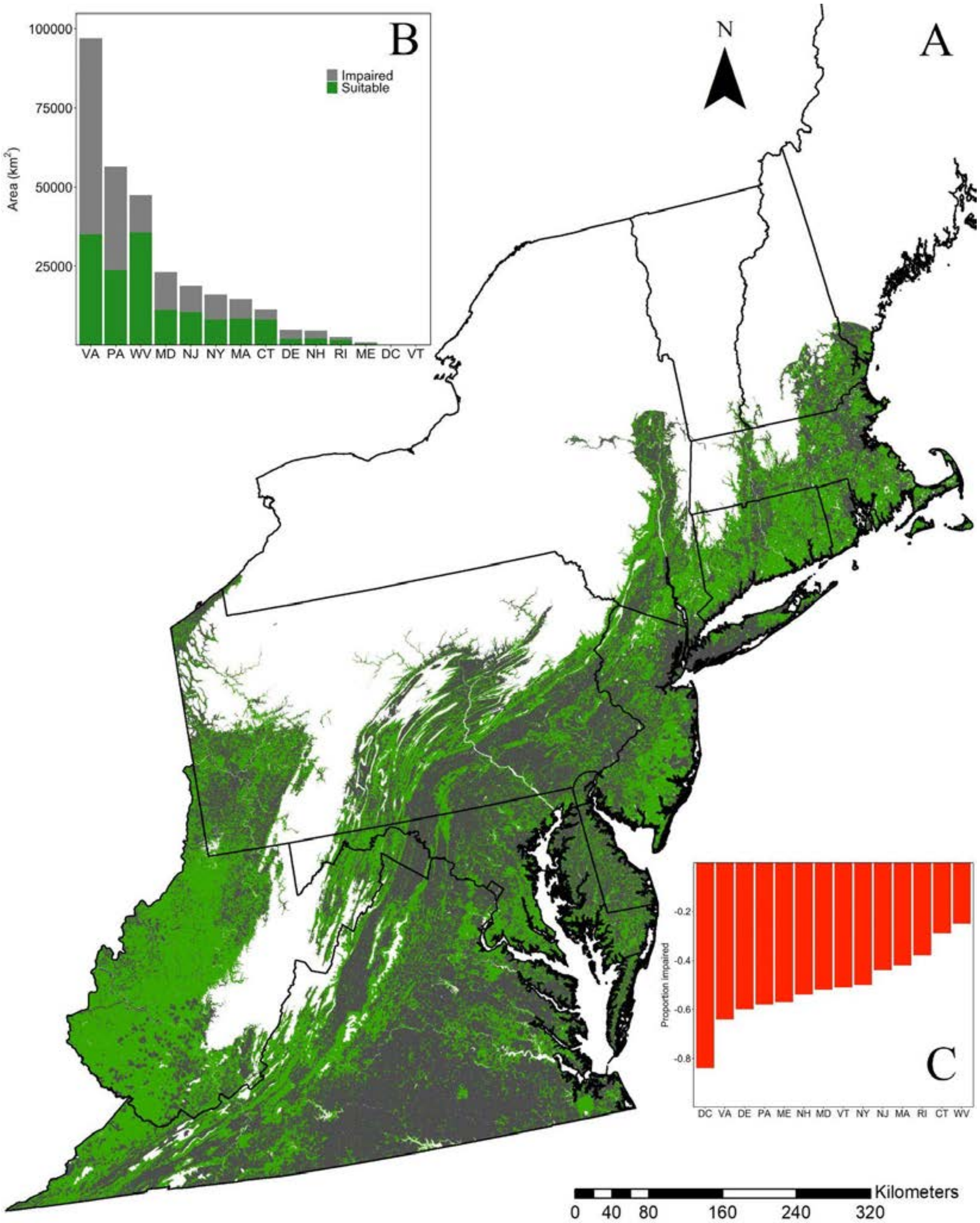

Figure 35. Predicted habitat impairment (green = suitable, gray = impaired) within the potential eastern box turtle distribution in the northeastern U.S. (A) as well as area (B) and proportion (C) of impaired and suitable habitat within each state in the region. See figure 3 for state labels.

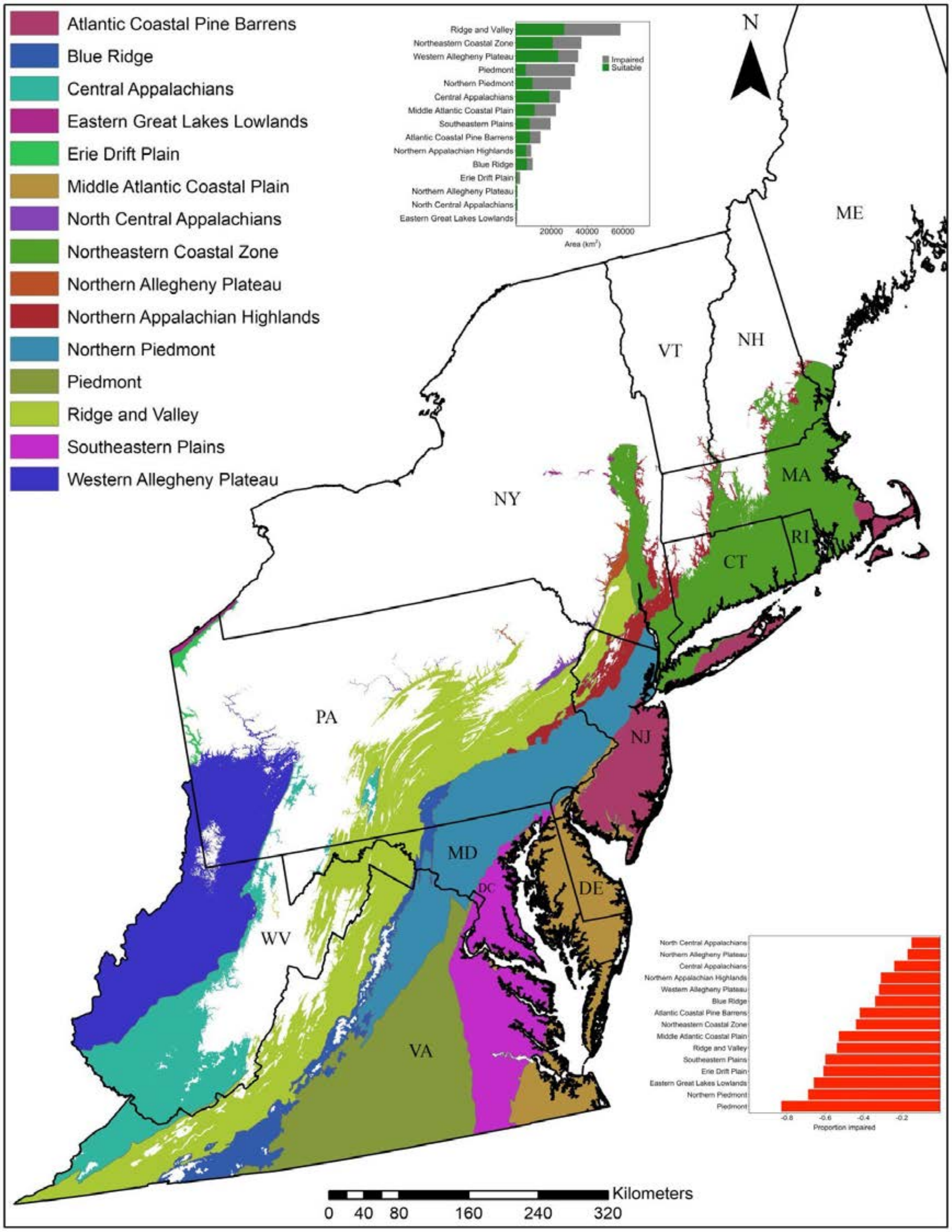

Figure 36. Map of Environmental Protection Agency Level 3 Ecoregions within the potential eastern box turtle distribution in the northeastern United States (A) as well as the area (B) and proportion (C) of impaired and suitable habitat within each ecoregion.

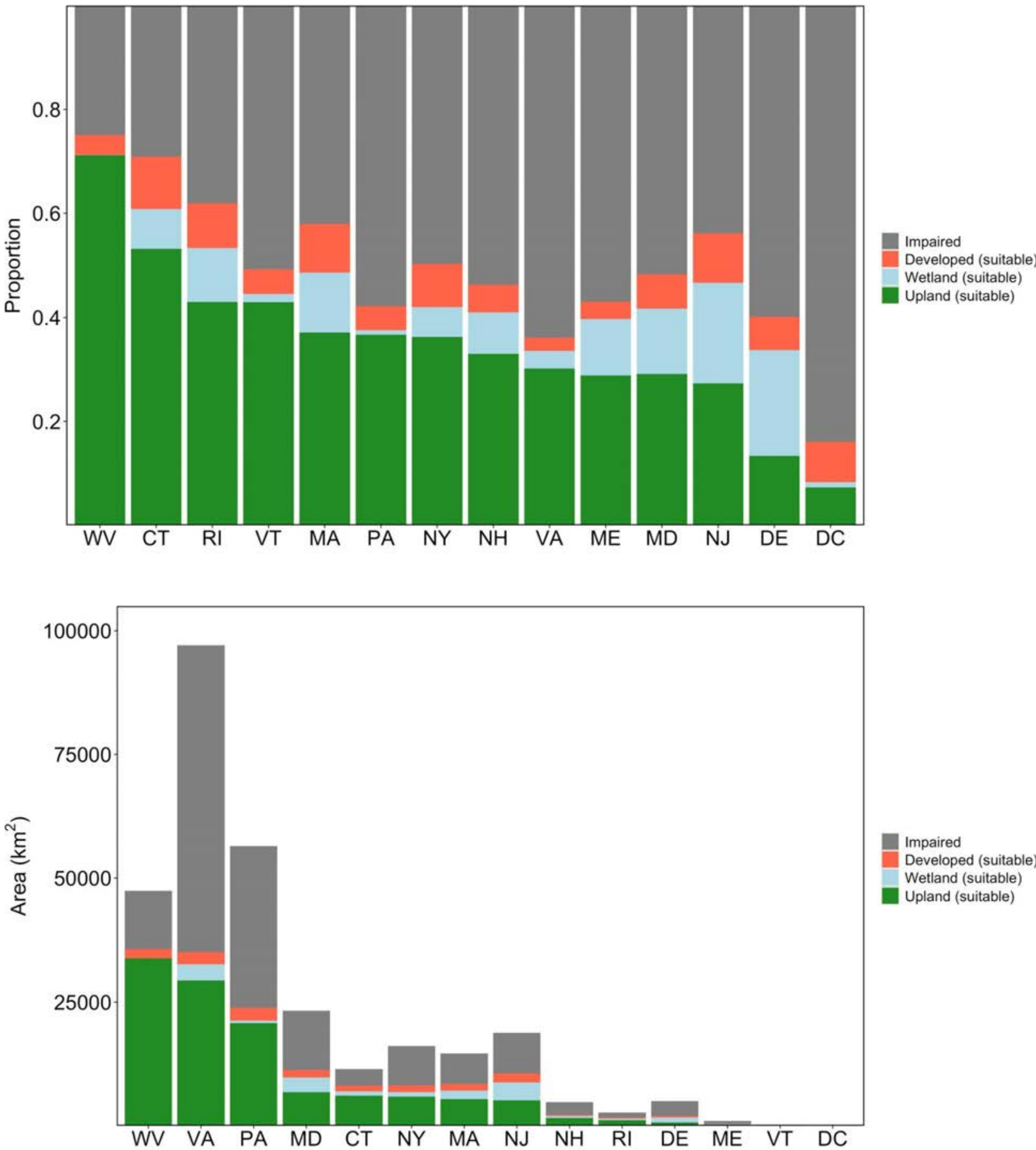

Figure 37. Proportion (top) and area (bottom) of impaired habitat, suitable upland habitat, developed areas (within suitable habitat), and woody/emergent wetlands (within suitable habitat) within each state.

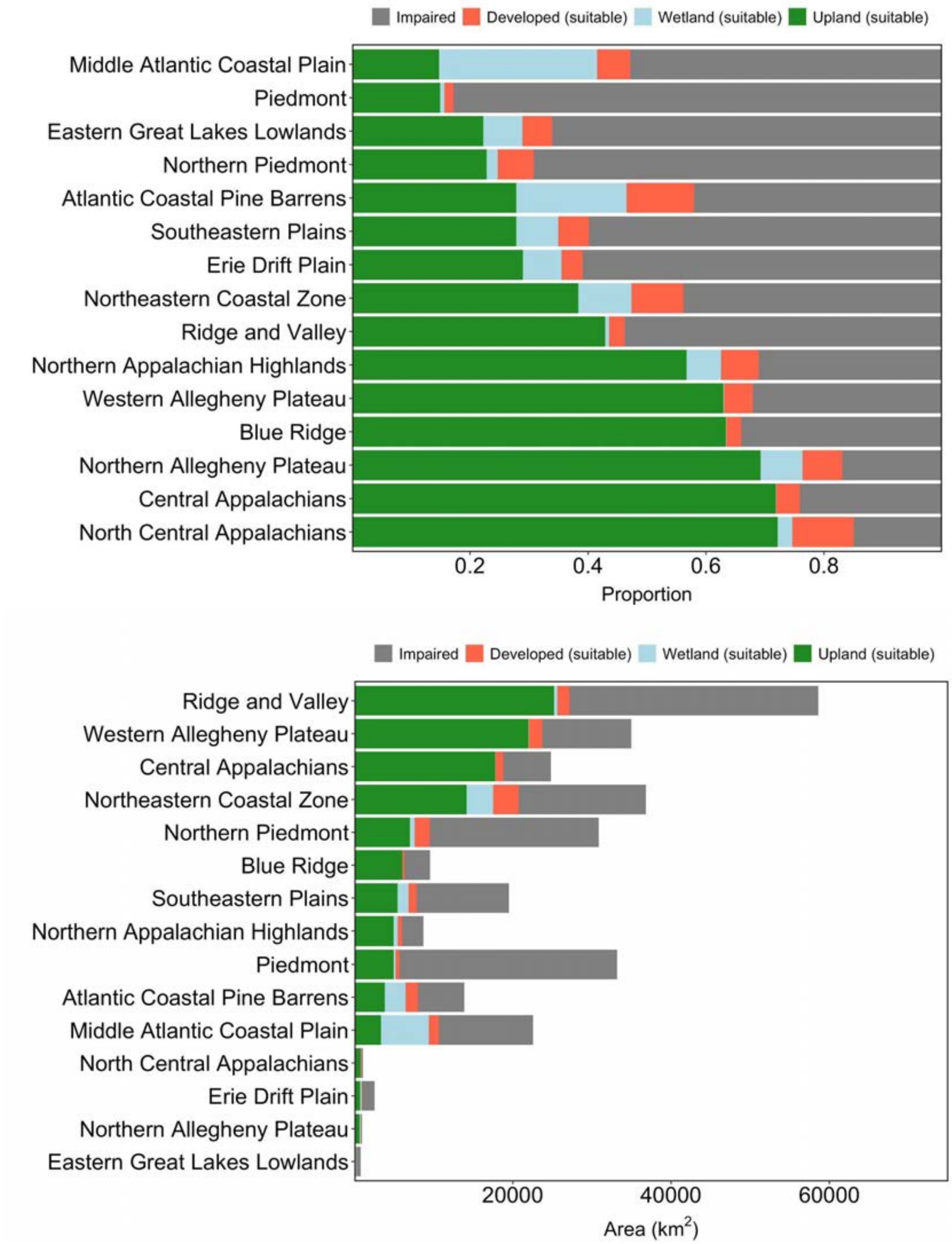

Figure 38. Proportion (top) and area (bottom) of impaired habitat, suitable upland habitat, developed areas (within suitable habitat), and woody/emergent wetlands (within suitable habitat) within Level 3 EPA Ecoregions.

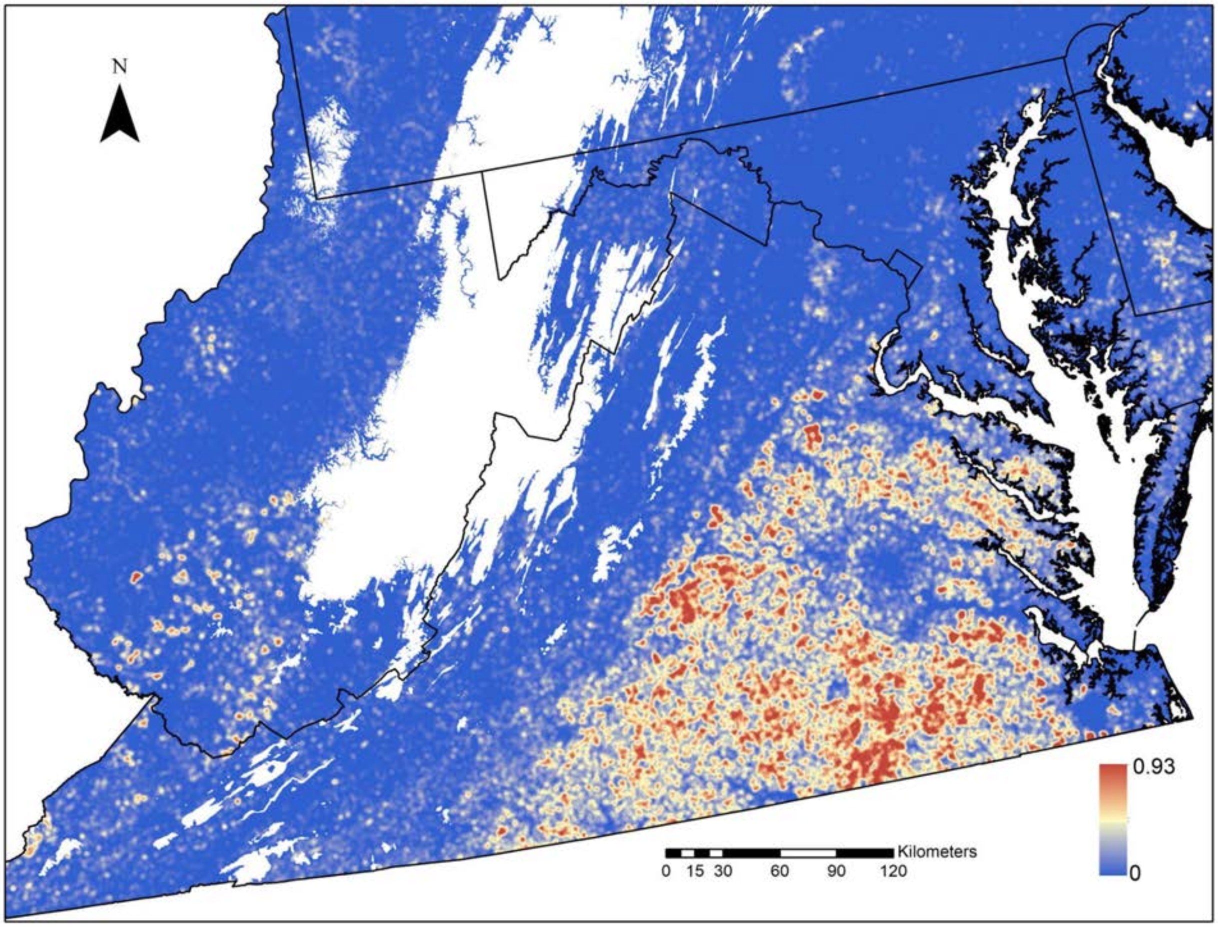

Figure 39. Proportion forest loss from 2000–2021 within 1,440 m in the southern portion of the northeastern U.S. Red indicates high forest loss while blue indicates low forest loss.

Chapter 7. Other Conservation Efforts

John Garrison and Lori Erb

North American Box Turtle Conservation Committee

The North American Box Turtle Conservation Committee contributes to the survival of wild box turtle populations by promoting research and education. This committee funds research for box turtles through the Lucille F. Stickel Research Award. They hold annual box turtle conservation workshops where experts present recent research results and conservation initiatives. This committee is composed of various box turtle specialists throughout North America.

Collaborative to Combat the Illegal Trade in Turtles (CCITT)

The Collaborative to Combat the Illegal Trade in Turtles (CCITT) was formed in 2018 with the goal of advancing the understanding, preventing, and eliminating the illegal trade and collection of turtles native to North America. This collaborative is composed of federal, state, and tribal agency biologists, academic researchers, non-governmental organizations, and members of law enforcement. The CCITT includes several working groups (e.g., confiscation and repatriation, data and research, human dimensions, law enforcement, regulatory and judiciary) which all work towards understanding, preventing, and eliminating the illegal trade and collection of native turtles.

In 2020, the CCITT published a Call to Action Letter which raised awareness for the illegal trade of turtles and explained why this is such an important conservation issue. In this letter the CCITT outlined five key areas that would help understand and prevent illegal turtle trade: 1) Coordinate state regulations to help address current conservation risks to these species; 2) Provide additional resources for wildlife law enforcement to prevent illegal collection and trafficking; 3) Enhance public outreach that communicates the severity and scale of the crisis and works towards eliminating national and international demand for wild-collected turtles; 4) Increase resources for emergency housing and care of confiscated turtles to relieve strain on law enforcement organizations; 5) Implement science-based planning to guide temporary and final disposition of confiscated turtles.

Northeast Partners in Amphibian and Reptile Conservation (NEPARC) box turtle education and conservation information

The Northeast Partners in Amphibian and Reptile Conservation (NEPARC) webpage provides several resources for education and outreach regarding this species. Resources for education and outreach include a box turtle coloring page, a box turtle poster which reads "please don't take me home", a postcard urging people to not take box turtles home, and an informational video of how you can help box turtles to cross roads. Information on life history, status, and identification of box turtles includes an identification guide to the 4 subspecies, state regulations summary, a list of box turtle conservation and education resources and a link that helps you find a wildlife rehabilitation center near you.

Fire Management Studies in the Northeast

Several research projects associated with eastern box turtles and fire are underway in New Jersey, Massachusetts, and Pennsylvania. The New Jersey Division of Fish and Wildlife contracted the Pinelands Commission to evaluate the effects of fire on the eastern box turtle and snakes in the pinelands of New Jersey. Massachusetts Division of Fisheries and Wildlife is partnering with a PhD student to evaluate the effects of prescribed fire on box turtles in Massachusetts. In Pennsylvania, a researcher at East Stroudsburg University is looking at the effects of fire-based management activities on terrestrial vertebrates and the Mid-Atlantic Center for Herpetology and Conservation is tracking eastern box turtles and green snakes at multiple serpentine habitat sites to evaluate the effect of fire and pre-fire management on these species.

Fly Parasitism in Northeastern Turtles

Fly larvae (Myiasis) are an ectoparasite to eastern box turtles where the larvae develop within the skin of turtles. Infected turtles exhibit reduced mobility, swelling, holes in the flesh that contain maggots, and may cause elevated mortality risk. The University of Massachusetts Amherst, USFWS, USGS, and the Massachusetts Army National Guard are currently conducting research on a population of eastern box turtles that is known to have myiasis in Cape Code, MA. Little is known about the distribution of this ectoparasite and how it affects the ecology and survival of hosts. Turtle researchers can submit observations of turtles with myiasis to help understand the distribution of this ectoparasite.

Massachusetts Conservation Plan

In 2012, the Massachusetts Division of Fisheries and Wildlife, Natural Heritage and Endangered Species Program drafted a conservation plan for the eastern box turtle throughout the state. This project reviews the biology, conservation concerns, and threats of this species. It also established habitat conservation goals, identified conservation areas, assessed population viability, and conducted a habitat assessment analysis. The state was divided into four main conservation areas and a strategy was put forth to protect known eastern box turtle sites. This project also reviewed habitat management, threat mitigation techniques and established baseline population estimates for many of the sites throughout the state.

North Carolina Box Turtle Connection

The North Carolina Box Turtle Connection is a state-wide conservation initiative that aims to collect data on populations and inform the public about this species. This organization is composed of representatives from two universities, four state agencies, and the public. Their goals are to collect baseline population data across the state and monitor population trends over time. This organization engages the public by using the citizen science they collect. The North Carolina Box Turtle Connection also plans to study the variance between populations throughout the state, determine the factors threatening populations, and determine the status of this species in the state. Their webpage features a wealth of educational information and graphics that can be used for education and outreach.

West Virginia citizen science initiative

The eastern box turtle is a high priority Species of Greatest Conservation Need in West Virginia, and the West Virginia Department of Natural Resources designed this project to better understand the distribution of this species throughout the state. They created an online reporting system for citizen scientists to report observations of this species. This project received over 6000 verified observations of eastern box turtles which allowed for a better understanding of the distribution throughout the state.

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Appendix A. Step by Step Survey Instructions for Monitoring Populations

Site Selection

- Select your sampling site (e.g., a 4-6 ha patch of field/forest edge habitat within a state park).
- Map the survey area (using 28-m radius circles or a feature polygon) using Google Earth, ArcGIS, or some other mapping app.
- Visit the site to determine if the mapped area is an appropriate habitat for eastern box turtles, feasible to survey and determine if any modifications need to be made to the survey area mapping.
- You may want to flag/mark the survey area boundary while you're there in preparation for surveys at a later time.
- Send an electronic copy (as shp or kmz file) of the final survey area boundary to the state project lead.

Other field Season Preparations

- Obtain appropriate state permits (or letters of authorization) and landowner access permissions.
- Contact your state herpetologist to obtain turtle notch codes and a diagram of that state's marking scheme (only for those with permission to notch turtles).
- Determine field equipment needs (clip board, calipers, pesola scales, etc.) and obtain needed equipment.
- Print site maps and/or upload circular plot center point coordinates (or survey area boundary points) into a GPS unit of mapping app on your phone.
- Print data sheets. Consider using weatherproof paper if you'll be surveying during rain events.
- Clean and disinfect all sampling equipment with 10% bleach solution and rinse well. For sensitive equipment like pesola scales you can wipe down the tool with a paper towel wetted in 10% bleach.
- Review the survey protocol, procedures, and survey sheets.

Survey Procedures

- Use maps, GPS, or a phone app to navigate to the site.
- If needed, determine, and mark the survey area boundary. If doing this immediately prior to the survey, try not to walk through the survey area as little as possible.
- If you plan to use a GPS unit or a mapping app to track your survey path (recommended when possible), turn the GPS unit/app on, clear the previous track and start tracking.
- Fill out the *Box Turtle Visual Rapid Assessment* field form.

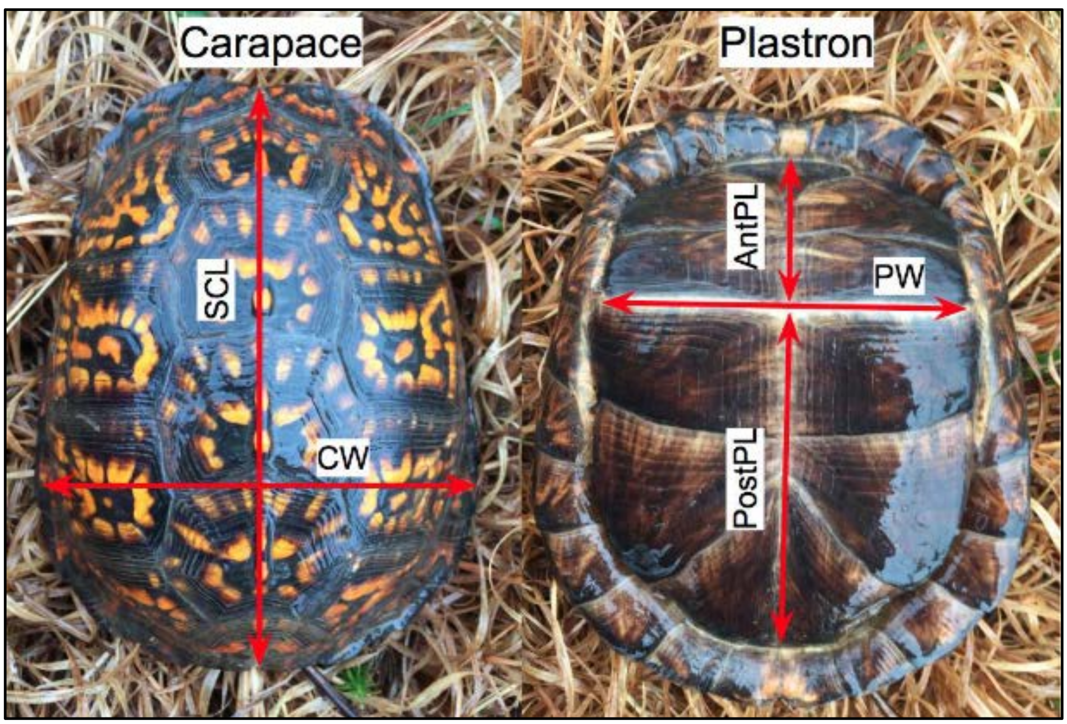
- Review the field form and fill in what you can prior to the survey (e.g., site name, site code, date, annual visit, observers, cloud cover, rain, etc.).
- Survey option descriptions:
  - Non-random sites = survey areas that were non-randomly selected.
  - Random site = survey areas selected using a randomized point generator in ArcGIS or some other mapping app.
  - Full Random = survey areas where the effect of habitat management actions is being monitored and points were randomly placed within the management area or entire park/property.

- When you are ready to start surveying, set a timer or stopwatch.
  - Optional - If you find it helpful, each time you stop the survey for any reason (e.g., process turtles, answer a phone call), record the stop time on the Site Visit Log, and then record the time when you resume the survey. Keep track of total time spent looking for turtles on this form (see example below).
- Walk/survey the entire survey area as evenly as possible but giving slightly more time to thickly vegetated areas and less time to open areas where visibility is good. Your total survey time should equal approximately 0.75-person hrs./ha (e.g., 45 min per circular plot assuming one surveyor). You will be walking at a brisk pace. For larger survey areas (> 2 ha) it helps to visually divide the feature into sections and time your survey of each section. For example, if you have a square 4 ha feature you can divide it into quadrates and time your survey of each quadrate making sure you finish each within 45 minutes (assuming one surveyor).
- At the end of the survey, record the end time and complete the *Box Turtle Visual Rapid Assessment* form. Also record the end time on the Site Visit Log (if used).
- *Save your track and label it* "SiteID_YYYYMMDD

Turtle Processing

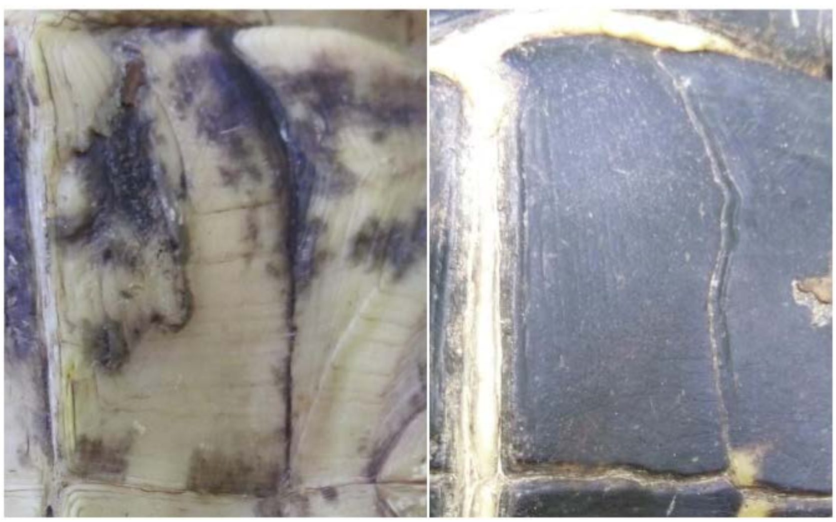
- Complete a *Box Turtle Individual Form* for each turtle found (including recaptures).
- Record the following information.
  - *Site name*.
  - *Site code* (optional).
  - *Survey type* (options are feature or plot). For plot surveys please note which of the four plots you are surveying by checking the appropriate box.
  - *Visit* - note whether this is your first, second, or third visit. An additional option is available if you visit the site more than 3 times.
  - *Observer(s)* that found the turtle (full name).
  - *Date* of the survey (mm/dd/yyyy).
  - *Time* the turtle was found.
  - *Turtle ID#* (coordinate with your state lead for a notch code system).
  - *Sex* (male, female, unknown).
  - *Age* (A=adult, J=juvenile).
  - *Waypoint ID* (where appropriate).

- *Unmarked, marked 1st capture, within yr recap* – unmarked is for turtles that have not been marked to date. Marked 1st capture is for turtles that were marked in previous years, but this is the first time they were captured during the field season. Within yr recap is for any turtles that were captured previously during the same field season.
- *Coordinates* of the location where the turtle was found. Please use decimal degrees for the lat long (dd.dddd).
- *SCLmin (mm)* – straight line carapace length measure down the middle of the carapace. See the diagram below.
- *CW (mm)* – measure of the widest point of the carapace.
- *AntSPL (mm)* – measure down the middle of the anterior portion of the plastron.
- *PostSPL (mm)* – measure down the middle of the posterior portion of the plastron.
- SPLmin (optional) - if the turtle hinge is completely open and you are able to get a straight-line measure of the full plastron length you may use this field opposed to the AntSPL and PostSPL.
- *PW (mm)* – measure the width at the humeral-pectoral seam.
- *SH (mm)* – measure the maximum height of the carapace (typically at the hinge).



- *Mass (g).*
- *Photo file names* (optional) if it helps you organize your photos at the end of the field season. Always take a full frame photo of the carapace and plastron for each capture. Please also take photos of any dead turtles or carcasses that you find and provide any relevant information in the comments field.
- *PIT number* (optional) if you PIT tag the turtle.
- *Wear class* of the plastron scute.

0% wear–distinct deep growth rings and <50% wear–growth rings less distinct but most visible



≥50% wear-many rings lightly visible and ≥90% wear-growth rings not visible or only barely

- *Visible annuli* - the number of annuli that are visible.
- *Gravid* or *not gravid* for females that you are able to palpate.
- *General health* of the turtle including any signs of sickness (lethargy, nasal discharge, swollen eyes, etc.)
- *Injuries* observed including missing limbs or toes, eye wounds, or stub tails.
- *Scute morphology* - note if the turtle has a normal number of marginal, vertebral, and costal scutes. If not normal, please note what irregularity is present.
- Use the shell sketch to note the notches and any injuries.
- *Comments* to provide any additional information that may be important.

- Check with your state lead to determine how to handle notching turtles with an irregular number of marginal scutes (e.g., 11 or 13) on one or both sides of the carapace. Researchers use several ways to count marginal scutes including from the anterior to the posterior, head to the bridge and tail to the bridge, and posterior to anterior.

Equipment List

- Field Forms
- Survey Protocol and Instructions
- Camera
- Transect tape 28 m or longer (to set up circular plots)
- Flagging (optional; to mark survey area boundaries)
- Site maps (optional)
- GPS unit or mapping app (optional)
- Clip board
- Pencil or pen
- Thermometer
- Caliper(s) or ruler (e.g., 200 mm)
- Pesola scale(s) (optional, e.g., 300 g and 1000 g;)
- Small triangle file(s)

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