

PRESCRIBED FIRE EFFECTS ON EASTERN BOX TURTLES IN SOUTHWESTERN
MICHIGAN

By

Tracy Ann Melvin

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ABSTRACT

PRESCRIBED FIRE EFFECTS ON EASTERN BOX TURTLES IN SOUTHWESTERN MICHIGAN

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Limited information exists on eastern box turtles (*Terrepenne carolina carolina*) and prescribed fire. My thesis focuses on the effects of prescribed fire, nesting ecology, hatchling movements, and efficacy of post-burn surveys for eastern box turtles in a 1226 ha recreation area in Michigan. Using a combination of tracking techniques, I monitored 34 female and 6 male adult eastern box turtles, and 58 hatchlings between 2013 and 2015. In Chapter 1, I summarized the nesting behavior of females, resultant clutch sizes and success, and hatchling movements using summary statistics and general linear mixed models to find that average clutch size was 6 (SE = 0.47), total clutch success ranged from 45.5% to 53.6% of the total clutch emerging, hatchling movements ranged from 3.3 to 123.7 m within the first two weeks of emerging, and that vegetation cover type had a significant effect on clutch success. In Chapter 2, I tested the efficacy of post fire surveys for box turtles and found that average detection probability 48 hrs after a fire was low (0.11) (SE = 0.05) and highly variable among surveyors (range = 0.00 – 0.50). I estimated that 26 hour-long surveys per ha would be required to reliably (95% confidence) detect turtles that were present in burned areas. In Chapter 3, I observed the direct behavioral effects of a growing season prescribed fire on radio tagged box turtles (n = 4). Behaviors included burying and actively negotiating flame fronts. I documented 1 post fire mortality. My results suggest prescribed fire should not be applied annually in grassland areas to minimize hatchling mortality and slow moving, patchy growing season fires should be considered in dry-mesic southern forests to minimize adult box turtle mortality.

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INTRODUCTION

The Eastern Box Turtle, *Terrapene carolina carolina*, is a small terrestrial emydid turtle that ranges from New England to the southern Great Lakes region south to the Carolinas and Georgia; traditionally it is considered to intergrade with additional subspecies to the west and south. A recent genetic study (Martin et al. 2013) suggested elevating the subspecies of *Terrapene carolina* to full species, a scheme adapted by Powell et al. 2016. Although relatively few long-term population studies have been completed, it is generally accepted that eastern box turtle populations are in decline (Williams and Parker 1987; Stickel and Bunck 1989; Schwartz and Schwartz 1991; Hall et al. 1999; Nickerson and Pitt 2012). Substantial declines are most likely the result of anthropogenic stressors including habitat loss and fragmentation, road mortality, collection for the pet trade, nest predation, and disease (Stickel 1978; Williams and Parker 1987; Warwick 1993; Belzer 1997; Hall et al. 1999; Gibbons et al. 2000; Dodd 2001). These anthropogenic stressors are exacerbated by delayed sexual maturity, relatively low clutch sizes, and potentially high hatchling mortality rates (Hall et al. 1999). As with many long-lived ectotherms, eastern box turtles exhibit delayed sexual maturity and extended longevity, with individuals becoming sexually mature at 5-10 years and living beyond 50 years in the wild (Congdon et al. 1993; Dodd 2001). Hence, eastern box turtles are susceptible to continuous population declines if subjected to any short- or long-term disturbances that negatively affect breeding adult survival, nest success, or hatchling survival; changes that can go unnoticed due to their longevity (Madden 1975; Klemens 1989; Doroff and Keith 1990; Dodd 2001, Ernst and Lovich 2009).

Prescribed fire can be an effective and inexpensive tool for vegetation management (Knapp et al. 2009), but the direct (including injury and mortality) and indirect effects on wildlife (including changes in prey, body condition and animal movements or interactions) can be difficult to quantify. Fire effects on reptiles and amphibians have been studied and summarized (e.g., Keyser et al. 2004; Russell et al. 2009; Roloff and Hmielowski 2014; O'Donnell et al. 2015), and the literature generally suggests that prescribed fire has few significant direct effects. However, most of these historical studies relied on animal counts or mark-recapture data that were uncorrected for detection probability (Mazerolle et al. 2007). Furthermore, reptiles and amphibians are often grouped into herpetofauna for fire studies (e.g., Means and Campbell 1981; Smith 2000; Floyd et al. 2002; Renken 2005), potentially biasing results to those species that are readily observable and abundant. For cryptic, mobile, or rare species like eastern box turtles, the error associated with failure to detect when present may have substantial conservation ramifications (Gu and Swihart 2004; Refsnider et al. 2011). Research suggests that losing even small numbers of breeding adults from k-selected, long-lived populations like box turtles can result in irreparable harm to population viability (Congdon et al. 1993; Dodd et al. 2015). Although eastern box turtles may have evolved with fire, current turtle population sizes, fire regimes, and habitat configurations differ from historical conditions and frequently repeated prescribed fires may result in long-term population declines. The direct effects of prescribed fire have been described in past studies with observations of high rates of mortality or injury from exposure to fire (Allard 1949; Babbitt and Babbitt 1951; Bigham et al. 1964; Dolbeer 1969, Gibson 2009).

Indirect effects of prescribed fire may include effects on prey abundance, vegetation used for thermoregulation, camouflage, and insulating duff layers during overwintering, and potential

disruption in behavior or range (Gibson 2009). Box turtles are particularly sensitive to environmental variables that affect ground cover (including vegetation, litter, and subsurface soils) because their entire life history depends on ground conditions (Dodd 2001).

My thesis focuses on the direct and indirect effects of prescribed fire, nesting ecology and hatchling movements, and the efficacy of post-burn surveys on eastern box turtles in a 1226 ha recreation area in Michigan. Using a combination of tracking techniques, I monitored 34 female adult eastern box turtles, 6 males, and 58 hatchlings between the fall of 2013 and spring of 2015. In Chapter 1, I summarized the nesting behavior of females, resultant clutch sizes and success, and hatchling movements using summary statistics and general linear mixed models. In Chapter 2, I tested the efficacy of post fire surveys for box turtles. In Chapter 3, I observed the direct behavioral effects of a prescribed fire on box turtles. Behaviors included burying and negotiating flame fronts. I also documented the short-term effects of growing season fires on woody vegetation and litter. This study is one of the first to test the efficacy of growing season prescribed fires on both vegetation and eastern box turtle behavior and survival. Results of this research provide insight on eastern box turtle behavior when using prescribed fire and can inform management practices.

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CHAPTER 1

NESTING ECOLOGY, HATCHLING BEHAVIOR AND REPRODUCTIVE SUCCESS
OF EASTERN BOX TURTLES IN SOUTHWESTERN MICHIGAN

Abstract

Limited information exists on local productivity and recruitment of eastern box turtles (*Terrapene carolina carolina*), a species of special concern, in landscapes heavily impacted by humans. I documented the nesting behavior, nesting success, hatchling behavior, and known sources of mortality for box turtles at Fort Custer State Recreation Area in Augusta, MI, from 2013 – 2015. I used radio telemetry on adult females (n=34), trailing string and transmitters on hatchlings (n=58), and field surveys of nests that were protected from predation (n=25). I modeled the relationships between: 1) clutch size and physical characteristics of the corresponding female turtle, 2) number of eggs that developed into hatchlings as a function of year and habitat that contained the nest, and 3) number of hatchlings that emerged from the nest by year and habitat. I found that females staged for nesting in late May, and I observed nesting between 2 to 14 June. Turtles nested in a wide variety of habitats, including an abandoned gravel pit, grassland restoration sites, an active disk golf course, and agricultural fields. At the time of nesting, all of these habitats provided suitable nesting substrate with an open vegetation canopy. By the time that hatchlings started to emerge in the fall, some of these habitats (e.g., agricultural fields) differed substantially in vegetation cover, likely affecting hatchling success. Female turtles tended to demonstrate fidelity to nesting habitat patches, but distances to nest locations within a patch varied annually (average = 215.4 m, SE = 61.0, range = 11.7 - 411.4 m). Average clutch size was 6.0 (SE = 0.47), and I found that ≥ 1 hatchling emerged from the nest cavity for

15 of 22 nests (68%). Hatching success (i.e., hatchlings emerged/clutch size) for nests protected from predation ranged from 46% to 54%. After fall emergence from the nest cavity and removal of predator exclosures, hatchlings moved an average of 1.22 (SE = 0.15) m/day, with minimum and maximum straight line distances from the nest cavity of 3.3 and 123.7 m, respectively, to their overwintering sites. I failed to find a significant effect of female physical characteristics on clutch size, and I found no effect of year or habitat on the number of eggs that developed into hatchlings. I found a significant habitat effect on the number of hatchlings that emerged from the nest cavity, with a gravel pit producing significantly more emergent hatchlings than agricultural fields ($\beta = 6.208$, $t = 2.76$, $p = 0.03$). Although female box turtles used agricultural fields for nesting, those nests failed to produce any emergent hatchlings suggesting that these fields serve as population sinks. My modeling results indicated that female physical characteristics were not reliable predictors of clutch size, and that habitat factors were the primary determinant of hatchling recruitment to the turtle population. Results from my study indicated that high quality nesting sites are an important recruitment factor that managers can directly address. High quality nest sites provide low mortality risk to adult females, conditions (e.g., soils, vegetation, solar radiation) that allow eggs to develop into hatchlings, and hiding cover for emerged hatchlings. Furthermore, after fall emergence from nests, my results indicated that hatchlings were vulnerable to land management practices that affect open nesting areas into the following spring.

1.1. Introduction

Eastern box turtles (*Terrapene carolina carolina*) were historically common throughout the eastern United States but may now be locally extirpated (Dodd 2001). Although few long-term population studies have been completed, it is generally accepted that eastern box turtle populations are in decline range-wide (Williams and Parker 1987; Stickel and Bunck 1989;

Schwartz and Schwartz 1991; Hall et al. 1999; Nickerson and Pitt 2012). Substantial declines are most likely the result of anthropogenic stressors including habitat loss and fragmentation, road mortality, collection for the pet trade, nest predation, and disease (Stickel 1978; Williams and Parker 1987; Warwick 1993; Belzer 1997; Hall et al. 1999; Gibbons et al. 2000; Dodd 2001). Effects of these anthropogenic stressors on box turtle populations are further exacerbated by delayed sexual maturity, relatively low clutch sizes, and potentially high hatchling mortality rates (Hall et al. 1999).

As with many long-lived ectotherms, eastern box turtles exhibit delayed sexual maturity and extended longevity, with individuals becoming sexually mature at 5-10 years and living beyond 50 years in the wild (Congdon et al. 1993; Dodd 2001). Hence, eastern box turtles are susceptible to continuous population declines if subjected to disturbances that negatively affect breeding adult survival, as nest success or hatchling survival is generally considered low (Madden 1975; Klemens 1989; Doroff and Keith 1990; Dodd 2001). During the incubation stage, low reproductive success is often linked to predation by skunks (*Mephitis mephitis*), foxes (*Urocyon cinereoargenteus*, *Vulpes vulpes*), raccoons (*Procyon lotor*), crows (*Corvus* spp.), snakes (*Heterodon* spp., *Lampropeltis* spp.), and ants (Ernst et al. 1994; Dodd 2001; Flitz and Mullin 2006). Changes in weather can also result in nest failures (Hallgren-Scaffidi 1986; Brooks et al. 1991; Ernst et al. 1994; Belzer 2002). From hatchling emergence to shell ossification, eastern box turtles are subjected to predation by a variety of insects, mammals, amphibians and birds (Madden 1975; Ernst et al. 1994; Dodd 2001; Belzer 2002), including crows, vultures (*Cathartes aura*), barn owls (*Tyto alba*), shrews (*Blarina* spp.), ground squirrels (*Ictidomys* spp.) and eastern chipmunks (*Tamias striatus*) (Ernst et al. 1994; Harding 1997; Belzer et al. 2002). Studies suggest that to maintain stable populations, eastern box turtles rely on

high survivorship of reproductive-aged adults (Klemens 1989, 2000; Congdon and Gibbons 1990; Doroff and Keith 1990; Congdon et al. 1993; Hall et al. 1999; Dodd 2001), assuming that at least some of the hatchlings will periodically recruit into the breeding population. However, with exceptionally high predation rates on eggs in nests, and land management practices that potentially result in hatchling mortality, concerns over recruitment of young are justified.

Although landscape alteration is arguably the most critical factor in turtle population declines across North America (Mitchell and Klemens 2000; Dodd 2001), information is lacking on the recruitment of eastern box turtles in areas subjected to intense human activity (Doroff and Keith 1990; Kipp 2003; Budischak et al. 2006), like that found in parks or recreational areas. Human activity can exacerbate negative stressors on local turtle populations by increasing road or trail mortality, turtle collection, exposure to pollution, the presence of subsidized predators, and mortality from household pets (Wilcox and Murphy 1985; Williams and Parker 1987; Dodd et al. 1989; Belzer and Steisslinger 1999; Mitchell and Klemens 2000). Furthermore, the maintenance and ecological restoration activities of certain parks and recreational areas may pose threats to extant box turtle populations through mowing, use of industrial equipment, herbicide application and prescribed fire.

Although box turtle nesting ecology has been studied across North America (e.g., Allard 1935; Kipp 2003; Wilson and Ernst 2005; Flitz and Mullin 2006; Burke and Capitano 2011a; Willey and Sievert 2012), limited information exists on nest success and hatchling movements even though that information is critical to conservation (Burke et al. 2000; Kipp 2003; Rosenburg and Swift 2013). Nesting ecology studies have mainly focused on substrates, clutch sizes and nesting times (Allard 1935; Kipp 2003; Wilson and Ernst 2005; Flitz and Mullin 2006; Burke and Capitano 2011a). Studies on turtle hatchlings have generally been limited to

describing sizes, physiological factors (Dinkelacker et al. 2005), and aspects of overwintering (Breitenbach et al. 1984; Ultsch et al. 2007; Burke and Capitano 2011a, b). These studies have improved our understanding of terrestrial or semi-terrestrial turtle neonate ecology and movements (Butler and Graham 1995; Keller et al. 1997; Forsythe et al. 2004), yet multi-year studies that directly link nesting ecology or hatchling movements and behavior to habitat management are rare.

My goal was to better understand potential recruitment vulnerabilities of eastern box turtles in a managed recreation area of southwest Michigan. I used a combination of radio telemetry on adult female box turtles, trailing string and telemetry on hatchlings, and field surveys of predator protected nests to determine nesting dates, nest locations, clutch sizes and success rates, and hatchling movements during summers of 2013 - 2015. My objectives were to: 1) document when and where adult female eastern box turtles nested, 2) evaluate nest success, 3) quantify short-term hatchling movements, and 4) identify factors influencing productivity and recruitment. In the absence of nest predation, my results provide insight into the factors that potentially limit eastern box turtle populations in recreation areas that are intensively managed for restoration and used heavily by the public.

1.2. Methods

1.2.1. Study Area

This study was conducted within the boundaries of Fort Custer State Recreation Area (FCSRA; 1,226 ha), located in the southern lower peninsula of Michigan (Kalamazoo and Calhoun Counties), USA. This area is characterized by heterogeneous vegetation patterns from topographic, climate, and human influences including high levels of agricultural and urban development (Albert 1995; Eagle et al. 2005). Most of the soils are calcareous and loamy,

derived from underlying limestone, shale, and sandstone bedrock (Eagle et al. 2005). Glacial till deposits are primarily loams, silt loams and clay loams (Eagle et al. 2005). The climate is considered humid continental, although FCSRA is strongly influence by the Maritime Tropical air mass and proximity of Lake Michigan, resulting in a warmer climate, moderated inland temperature fluctuations, and induced lake-effect snow (Eichenlaub 1979; Denton 1985; Albert et al. 1986; Eichenlaub et al. 1990). The average length of the growing season was 154 days (Albert et al. 1986). Mean monthly (May to October) minimum and maximum temperatures during the study ranged from $-1.7 - 7.2^{\circ}\text{C}$ and $22.2 - 34.4^{\circ}\text{C}$, respectively.

Southwestern Michigan was historically dominated by fire-dependent oak (*Quercus* spp.) savanna and prairie (Albert 1995), and contained the only extensive areas of mesic prairie found in Michigan (Kost 2004). In the early 1800's, plant communities included dry and dry-mesic southern forest (oak-hickory (*Carya* spp.)), oak barrens (mixed oak savanna), emergent marsh, and southern (mixed hardwood) swamp (Palmgren 2004). Currently, intensive agriculture is concentrated in this region because of the relatively mild climate and productive soils (Eagle et al. 2005). Natural vegetation in this region is broadly classified as black oak-white oak (*Q. velutina*-*Q. alba*) savannas and forests, and beech-sugar maple (*Fagus grandifolia*-*Acer saccharum*) forests (Palmgren 2004). Currently, degraded patches of oak barrens and prairie openings, scattered oak-hickory forests, and large blocks of non-native black locust (*Robinia pseudoacacia*) patches occur on FCSRA (Palmgren 2004).

I chose FCSRA for this project because it has been the focus of ecological restoration activities since 1997. FCSRA is located within the most northerly range of the eastern box turtle. My study area was centered on Management Units 2, 4, 9, 14, 17 and 18 (Figure 1.1). The dominant restoration management regime in grassland areas was medium-scale (< 111 ha) spring

or fall prescribed burning (2-year intervals), followed by herbicide treatments in some areas. A large area (52 ha) of mature black locust experienced windthrow in 2001 (Figure 1.1, Palmgren 2004). This area was in active restoration to open barrens and prairie during my study.

1.2.2. Radio Telemetry of Adult Females

I used visual encounter surveys and turtle detector dogs to find adult eastern box turtles in May of 2012 and June of 2013. Surveys were conducted during daylight hours, when box turtles are known to be active. Captured adult box turtles found during these surveys (and others found fortuitously during routine radio telemetry) were radio-tagged and tracked using Holohil R1-2B 14.5g Transmitters (Holohil Systems Ltd., Carp, Ontario, Canada) attached to the right or left anterior pleural carapacial scutes using multi-purpose 5-minute set epoxy putty (Loctite®, Henkel 289 Corporation, Cary, NC, USA). Minimum age was estimated by counting annual rings on the carapace (Legler 1960). Turtles were also individually marked using the Cagle method (Cagle 1939). Radio-tagged adult female box turtles were located 1-3 times per week during the active seasons (April through October) from May 2012 to August of 2015 using portable telemetry receivers (Advanced Telemetry Systems, Isanti, MN, USA). During active nesting periods (mid-May through June), females were located nightly between the hours of 1800 and 2200 to determine nesting status. If a female was found to be alert after 1900, she was subsequently checked for digging behavior until 2200. Once a female was found to be digging, her location was marked using a GPS. The site was left alone until morning when sites were then checked for egg deposition by gently digging into the soil until the surface of at least one egg was observed. Turtle capture and handling was conducted by Michigan Department of Natural Resources (MDNR) employees or volunteers and followed MDNR protocols for animal welfare.

Additionally, all animals were handled in compliance with Michigan State University's Institutional Animal Care and Use Committee guidelines (IACUC Approval # 04/14-077-00).

1.2.3. Nest Protection and Monitoring

Within 24-72 hrs of initiation of female nesting activity, box turtle nests were covered with 0.61 x 0.61 x 0.31 m bottomless wooden-frames wrapped in 0.64 cm wire mesh (Figure 1.2). These exclosures were preserved and camouflaged with an acrylic-based solid stain in olive drab green and featured a removable lid attached with 4 - 3.2 cm deck screws. Exclosures were dug 5 cm into the soil surface around the nest. Nests were checked daily starting August 1st when 152 x 91 x 22 mm sponges were placed in the corner farthest from the nest cavity and wetted daily to provide moisture for the hatchlings upon emergence. Emergence was determined by identification of an emergence hole near the nest cavity. After emergence in 2013 and 2014, nests were excavated to count eggs through reconstruction of eggshell evidence. Evidence of predation included egg shells scattered outside of the cavity, cavities that were disturbed by digging, or an abundance of ants on the eggs. I calculated the number of developed hatchings and emergence success and, if feasible, determined causes of hatching and emergence failure. If nests did not emerge in the fall, they were left caged and excavated in June of the following year. Emergence monitoring was paused from November – April in these cases.

1.2.4. Hatchling Movements

After emergence, hatchlings were weighed using a Micro-Line 10 g x 0.1 g spring scale (Pesola®, Baar, Switzerland), and individually marked with a notch on a marginal scute with a nail clipper (Cagle 1939). Each hatchling was fitted with 13 cm long trailing string of orange fly line backing (RIO® Products, Idaho Falls, ID) by threading it through the left or right 11th marginal scute with a sterilized hand needle and tying with an improved clinch knot (Breder

1927; Stickel 1950). A subset ($n = 7$) of hatchlings found in 2014 was also fitted with 0.52 g BD-2X transmitters (Holohil Systems Ltd., Carp, Ontario, Canada) attached to the carapace with silicone aquarium sealant (Marineland®, Spectrum Brands, Blacksburg, VA). We released hatchlings within 0.5 m of each nest cavity after they were weighed and measured. Hatchlings were relocated after dusk using black lights (for the string) and telemetry, every 24-48 hrs. Hatchling locations were recorded using a handheld GPS. Burying behavior into mineral soil was recorded, along with vegetation type, percent concealment, and type of concealment used.

1.2.5. Data Analysis

I calculated summary statistics for nest site fidelity, clutch sizes, incubation period, number of emerged hatchlings per nest, and hatchling movements. I used a nonparametric Kruskal-Wallis test to determine if nest initiation date, clutch sizes, clutch success (emerged hatchling/egg), or incubation days varied by year (Corder and Foreman 2009), and a Dunn test for multiple comparisons (Benjamini-Hochberg 1995; adjustment to the p-value) to identify which years differed. Zar (2010) noted that the Dunn test is appropriate for groups with unequal numbers of observations.

I used generalized linear mixed effects models (GLM) with a Poisson distribution in program R 3.0.2 (R Development Core Team 2014) to estimate the effects of independent covariates on clutch size, the number of developed eggs, and the number of emerged hatchlings. Independent covariates for the clutch size model included female mass (g) taken after she laid eggs in all but two cases (β_1), minimum female age (β_2), whether the female showed signs of fire scarring (β_3), and year (β_4). Independent covariates for the number of developed eggs model included year (β_1) and vegetation type that contained the nest (β_2). Vegetation types were modeled as factors and included tallgrass prairie, forb-dominated prairie, corn fields, and an

abandoned gravel pit (Figure 1.3). Independent covariates for the number of emerged hatchlings model included year (β_1), vegetation type (β_2), and clutch size (β_3). Individual female turtles were treated as random effects in all models.

1.3. Results

1.3.1. Nesting Behavior

I monitored 34 female eastern box turtles from 2013 – 2015 (Table 1.1). Fifteen of those were monitored for 3 years, and 18 for 2 years (Table 1.1). In 2013, 8 of 31 (26%) turtles were tracked to their nesting site and produced a clutch. I found nests and documented clutches for 10 of 19 (53%) and 5 of 17 (29%) females in 2014 and 2015, respectively (Table 1.1). I caution that these results should not be viewed as a measure of clutches per female in any given year because there were two possible outcomes for females with unknown nesting status: 1) they truly did not nest, or 2) they nested but the activity went undetected by the research team.

Radio-tagged female box turtles were not observed staging earlier than 29 May in 2014 and 2015. Staging is the process of moving to an area with open vegetation and exposed mineral soil to begin searching for and subsequently digging a nest cavity to lay eggs. Nesting follows staging, and I observed nesting between 2 to 14 June. The initiation of nesting varied by year (Kruskal-Wallis test, $\chi^2 = 13.02$, $df = 2$, $p = 0.001$), with nesting occurring earlier in 2013 compared to other years (Dunn test, 2013 -2014, $z = 3.39$, $p = 0.001$; 2013 – 2015, $z = 2.51$, $p = 0.009$; 2014 – 2015, $z = -0.262$, $p = 0.40$). All nesting behaviors (e.g., digging, laying eggs, covering eggs) were observed between 1830 and 0700. Females would finish laying eggs in a single night.

In addition to the 23 nests with clutches that I found using telemetry (Table 1.2), I also encountered 2 untagged females while they were nesting. These opportunistic females were not

measured or marked but I placed an exclosure over their nests. Hence, I monitored 25 nests from 2013-2015. I lost 3 of those nests to predation. The predator exclosure protecting one nest was removed (likely by a human) and this nest was subsequently depredated. An additional nest was partially depredated by a predator that tunneled under the exclosure, and another nest was predated before the exclosure was deployed (<24 hrs after the female finished). Another nest was lost when the female left before covering her eggs, all of which were inside an open nest cavity when checked 24 hrs later, and presumed non-viable. By deploying predator exclosures 24 to 36 hrs after females completed nesting, I was able to monitor 84% of the potential nests that I found for hatchling development and emergence.

1.3.2. Reproductive Female Mortality

I documented mortality for 2 of 34 (6%) radio-tagged adult females during the 3 years of my study. One adult female may have died as a result of long-term infection during the course of this study. She was staging to nest in a tallgrass prairie during an herbicide application during May 2014. For the remainder of the active season, I noted varying levels of swollen eyes and eye discharge. I found this female dead in early March 2015; she had failed to bury into mineral soil during the winter. The other adult female appeared to die as a result of injuries sustained from a prescribed fire.

1.3.3. Nest Sites

Nest sites (n=25) were dispersed throughout FCSRA in 7 areas representing different vegetation cover types. Four nests were located in tallgrass prairie dominated by big bluestem (*Andropogon gerardii*) that was currently undergoing active restoration (Table 1.2). One of these nests was in an area of recent herbicide application with no live vegetation. Four nests occurred in a corn field, 2 in a forb-lichen dominated radio-antenna field, 5 in a grass and forb dominated

abandoned gravel pit, 2 in mowed areas of a disc golf course, and 8 in a different active prairie restoration site that was co-dominated by big bluestem (*Andropogon gerardii*), spotted knapweed (*Centaurea maculosa*) and common mullen (*Verbascum thapsus*) with autumn olive (*Elaeagnus umbellata*) and staghorn sumac (*Rhus typhina*) dispersed throughout. All nests were in loose sandy soil.

1.3.4. Nest Site Fidelity

One turtle was successfully tracked to her nesting sites in all 3 years of my study (Turtle 3-8,9,10; Table 1.1). For this female, the average distance between yearly consecutive nest sites was 388.3 m (SE = 23.2). All three of these sites were in the same open active prairie restoration area that was co-dominated by big bluestem, spotted knapweed and common mullen, with autumn olive and staghorn sumac dispersed throughout (92 ha). I collected data on nest site fidelity for 6 additional turtles for 2 years (Table 1.1). Average distance between yearly consecutive nests was 215.4 m (SE = 61.0). No nest locations were <11.7 m from a prior nest location. Radio-tagged females were never observed nesting or staging in patches of vegetation different from previous years. My results indicated that female turtles exhibited high fidelity to patches of vegetation and not specific nest locations, if those patches contain areas with sparse vegetation cover and exposed mineral soil.

1.3.5. Clutch Size, Incubation Period, and Clutch Success

Average clutch size was 6.0 (SE = 0.47), and clutch sizes did not differ by year (Kruskal-Wallis Test, $\chi^2 = 0.58$, df = 2, p = 0.75). Mean incubation period for hatchlings (counted from nest date to emergence date in the fall) was 113.8 days (SE = 5.02, range = 89-141), and there was no difference among years (Kruskal-Wallis Test, $\chi^2 = 0.41$, df = 2, p = 0.81).

For nests protected from predation, ≥ 1 egg hatched in 15 of 22 (68%) nests (Table 1.2). Of the 7 nests that did not produce emergent hatchlings, 2 (29%) had part of the clutch not break out of their eggs, and the other part break free of the eggs but die within the cavity. Three of the 6 nests that failed to emerge from eggs occurred in a corn field. At the time of nest initiation in June, the corn was approximately 0.30 m tall.

The average number of hatchlings that emerged from protected nests was 3.0 (SE = 0.62) and did not differ by year (Kruskal-Wallis Test, $\chi^2 = 0.32$, df = 2, $p = 0.85$). I found that mean clutch success, defined as the number of hatchlings successfully emerged from the nest cavity divided by clutch size, was 0.49 hatchlings/egg (SE = 0.09). Clutch success did not differ by year (Kruskal-Wallis Test, $\chi^2 = 0.28$, df = 2, $p = 0.87$). For nests that contained eggs that were not predated but still did not emerge ($n=16$; Table 1.2), I found that 36-47% of the eggs were undeveloped or had hatchlings that died in the egg, and 1-4% that hatched but died in the nest cavity. My results indicated that most clutches (83%), if predation loss is excluded, experienced egg or hatchling loss.

I failed to identify significant parameters in the clutch size and count of developed eggs models (Table 1.3). Although the Kruskal-Wallis test indicated no year effect on clutch sizes, the generalized linear model suggested some support for a year effect ($p = 0.06$), with higher clutch sizes later in the study (Table 1.3). Additionally, weak support for fire scarring ($p = 0.10$) was identified, with higher clutch sizes from fire scarred females (Table 1.3). The number of emerged hatchlings varied by cover type, with nests in the gravel pit producing significantly more emerged hatchlings compared to nests in the corn field (Table 1.3). There was also some support ($p = 0.11$) for nests in Eagle Prairie producing more hatchlings than nests in the cornfield (Table 1.3). Year and clutch size did not influence the number of emerged hatchlings (Table 1.3).

1.3.6. Hatchling Movements, Behavior, and Known Sources of Mortality

For clutches laid in 2013, I found that hatchlings ($n = 26$ from 4 nests) emerged between September 7th and October 26th, 2013, and into the following spring ($n = 5$ from 3 nests) between April 30th and May 30th, 2014. One nest emerged as an entire clutch in the spring after overwintering in the nest cavity ($n = 2$) on April 30th, 2014, whereas 1 nest had a portion of the clutch emerge in the fall ($n = 4$), and the remainder in the spring ($n = 3$, emerged between May 15-30th, 2014). For clutches laid in 2014, I observed hatchlings emerge in the fall ($n = 28$ from 5 nests) between September 12-30th. For clutches laid in 2015, I observed hatchlings emerge in the fall ($n = 7$ from 2 nests) between September 7th and October 15th, 2015. Two nests emerged as an entire clutch between May 6-15th, 2016, after overwintering in the nest cavity ($n = 8$ hatchlings).

Of the 75 hatchlings I encountered during this study (Table 1.2), I successfully relocated 38 individuals at least once. I experienced a wide range of success in relocating hatchlings, ranging between <1 to 231 days after emergence for individuals. I followed 10 individuals (from 6 nests) to their overwintering locations. Six of these 10 individuals were radio-tagged. The individuals that I tracked to overwintering locations were subsequently located the following spring in approximately the same location (± 1 m). Hatchlings remained in overwintering locations and dispersed between May 9th and May 15th ($n = 2$) in 2013 and between May 1st and May 9th ($n = 7$) in 2014.

I tracked hatchlings using the monofilament-UV light method for an average of 12.8 days (SE = 11.2) for an average total distance of 12.8 m (SE = 11.9, range = 0.76 – 55.86). I tracked hatchlings with both the UV monofilament and radio tags for an average of 37.7 days (SE = 2.8), for a total distance of 37.9 m (SE = 15.7, range = 3.26 - 123.7). From both methods combined,

hatchlings moved an average of 1.2 m/day (SE = 0.2) in the fall. I observed a wide range of hatchling movement patterns; one hatchling overwintered 3.3 m from the nest cavity while another moved at least 123.7 m away within 3 days.

I documented movement by hatchlings consistently away from the nest site without returning. I found that telemetered hatchlings that emerged in the fall in prairie areas and that were successfully tracked to their overwintering sites ($n = 9$) remained in this vegetation type throughout the winter and into the spring. Hatchlings were highly concealed (greater than 75% concealed) in 268 of 320 relocations (84% of relocations). Concealment materials included dead and live vegetation, fallen leaves, root base thickets of big bluestem, and mineral soil. Hatchlings seemed to only use mineral soil as concealment when beginning to overwinter. I observed hatchlings beginning to overwinter as early as October 19th, 2013 and October 5th, 2014, which I defined as burying completely into the mineral soil and remaining in the same location for more than 1 week. Hatchlings overwintered between 1.5 and 3 cm below the soil surface. In the process of overwintering, hatchlings would usually turn 180 degrees into the soil to bury.

I was unable to determine the fates for 51 of 58 emerged hatchlings, attesting to their cryptic life history strategy and difficulties in tracking individuals for long periods of time. Known sources of mortality on hatchlings included a vehicle ($n = 1$), prescribed fire applied in the spring ($n = 1$), mammalian predation (burrowing rodent, $n = 1$), and avian predation ($n = 4$).

1.4. Discussion

I determined nesting dates, clutch sizes and clutch success, and hatchling behaviors of eastern box turtles over 3 seasons in a recreation area in southwestern Michigan. Collectively, results from this study provide insights into recruitment vulnerabilities of this species in recreational landscapes that are being restored with active management that included prescribed fire. Similar

to other box turtle studies at more northerly latitudes, I found that females consistently staged for nesting in late May, and nested in early June. For example, Doroff and Keith (1990) found that ornate box turtles (*Terrapene ornata ornata*) laid eggs between 29 May and 26 June and hatched after 79-84 days in south-central Wisconsin. Kipp (2003) documented nest (n = 39) excavation by eastern box turtles between 27 May and 11 July in Delaware, and Willey and Sievert (2012) documented nests (n = 34) in Massachusetts between 27 May and 10 July, with peak nesting in early June. At northerly latitudes, eastern box turtles seem to consistently nest from the end of May into early July.

My observed clutch sizes were similar to clutch sizes documented by others studying box turtles. Doroff and Keith (1990) documented average clutch sizes for ornate box turtles ranging between 4.1 (nests = 8) and 2.8 (nests = 8) in southern Wisconsin. Kipp (2003) found that clutch sizes ranged between 1 and 9 (mean = 4.6; nests = 53) in Delaware, and Willey and Sievert (2012) found 3-10 eggs/clutch (mean = 5.8; nests = 31) in Massachusetts. In my study of nests protected from predation, the majority (68%) of nests successfully hatched at least 1 egg, and between 45 and 55% of the clutch emerged from the nest cavity on any given year. Others have also found that <70% of clutches produce emerged hatchlings, ranging from 24 to 36% (Kipp 2003; not all nests protected from predation), 55% (Willey and Sievert 2012; all nests protected from predation), and up to 69% (Doroff and Keith 1990; not all nests protected from predation)

Adult female eastern box turtles demonstrated site fidelity to patches of open nesting areas, but the specific location of nest sites within each patch varied considerably. This result is consistent with Kipp (2003), who found that female box turtles generally nested within 295 m of previous nest locations. Fidelity to patches of nesting habitat may be a result of limited habitat availability. For example, adult females in a similar study northwest of FCSRA consistently

staged in the same open area from year to year, migrating from many locations (P. Laarman, pers. comm.). This patch was believed to be the only suitable nesting habitat reasonably available. Females at FCSRA seemed to migrate to the nearest open area, and were never seen staging in other open areas during subsequent nesting seasons. As nesting habitat becomes increasingly fragmented from vegetation succession and human activities (like roadways), box turtles may increasingly use less than ideal habitat for nesting, like agricultural fields (Kipp 2003; this study). The fidelity of eastern box turtles to suitable nesting habitat and the tendency to use agricultural fields (that appear to act as population sinks) highlight the importance of focusing management on providing open nesting areas.

Once emerged, hatchling box turtles moved approximately 1.2 m/day away from the nest cavity, but individual daily movement varied substantially. Most hatchlings remained in the vegetation patch containing the nest cavity, and burrowed into vegetation or soil (also observed by Burke and Capitano 2011b). Hatchlings entered overwinter sites in early to mid-October and emerged in early to mid-May. Hatchlings overwintering and subsequently emerging in grasslands adjacent to oak, or dry-mesic southern forests may be particularly vulnerable to spring prescribed fires through these grasslands and woodlands, primarily because they tend to hide in herbaceous vegetation and leaf litter, and when buried are close (<2 cm) to the soil surface (Ernst et al. 1995). This is particularly problematic for ecosystem restoration programs that require annual prescribed burning as recruitment classes may never get a chance to enter the population.

As a k-selected species, the conservation of eastern box turtles depends on the protection of breeding adults (Congdon et al. 1993). Anthropogenic activities having negative effects on breeding adults can result in local population extirpation, albeit gradual and perhaps imperceptible (Doroff and Keith 1990). Although I documented an apparently low mortality rate

for adult females (6% over 3 years), even low levels of adult female mortality in eastern box turtles can have significant detrimental effects on population persistence (Williams and Parker 1987; Congdon et al. 1993). One source of confirmed mortality in my study occurred as a result of prescribed fire where the turtle failed to evade an advancing fire front (even though other turtles successfully negotiated the fire; Melvin 2017:Chapter 3). Prior to the burn, this female did not exhibit any outward signs of impairment or stress, but she was slow in responding to the flame front compared to other adult turtles. The cause of the other documented adult mortality could not be unequivocally confirmed.

Environmental factors such as surface temperature, ground temperature, and vegetation cover can be correlated with nest success of box turtles (Morjan and Valenzuela 2001; Kipp 2003). Incubation period can depend on soil characteristics such as temperature, soil type and moisture (Allard 1948). Although I did not measure soil characteristics, I observed that nest location and the associated vegetation cover type was the most significant factor in determining hatchling emergence. For example, I observed that females nested in agricultural fields when the cover crop (corn) was short and sunlight directly reached the soil. These nests all contained eggs that showed some level of developed neonates, but all of these clutches failed to produce emerged hatchlings. It seems reasonable to presume that agricultural fields can act as sinks to local box turtle populations. The threats include exposing adult females to heavy equipment or chemical use, but perhaps more importantly the lack of recruitment presumably caused by cover crops shading nests to the point of incubation failure.

Similar to other studies (e.g., Kipp 2003; Willey and Sievert 2012), I observed direct predation effects on eastern box turtle recruitment. Predator exclosures played a critical role in the clutch successes that I documented. Although I had a limited sample of unprotected nests, all

experienced depredation and resulted in complete loss of the clutch. Similar to other landscapes that support box turtles, FCSRA supports a diversity of reptilian egg predators including raccoons, skunks, and opossums (*Didelphis virginiana*). It is important to note that my results on nesting and hatchling emergence success represent potential eastern box turtle recruitment as the data were collected from protected nests. Anecdotally, my data suggested that the presence of predators in the study area likely has a strong negative effect on box turtle recruitment.

Managing box turtle habitat can be challenging. Too much sunlight can desiccate the soil and reduce foraging opportunities (Saumure and Bider 1998; Kipp 2003), whereas too little sunlight may prevent eggs from developing. The effects of changing environmental conditions may negatively influence reproductive potential and cause the decline of local populations (Kipp 2003; Currylow et al. 2012). If sufficient nesting habitat does not exist within the home ranges of box turtles, nesting may occur in locations where eggs or hatchlings may not survive (Kipp 2003). This may also be true for tallgrass prairie restoration that utilizes mowing, prescribed fire, and herbicide application to create thick, homogenous communities of native grasses, such as big bluestem. I found that sparsely vegetated areas within these types of communities are important to successful nesting.

Here, I observed potential stressors to recruitment of eastern box turtles that included predation, habitats that appeared to act as population sinks, and hatchling vulnerabilities to prescribed fire. Understanding the nesting ecology and subsequent hatchling box turtle survival, movements and behavior in altered or fragmented landscapes is a necessary precursor to developing management strategies for box turtle conservation. I suggest that future research on recruitment and nest success focus on the role of agricultural fields of any cover crop, better understanding micro-scale differences in soil temperature and moisture in restored tallgrass

prairies, and potential manipulation of site fidelity exhibited by females with man-made nesting areas.

1.5. Management Implications

Land managers using prescribed fire should carefully consider the frequency of burns in nesting habitat, as hatchling turtles tended to remain in close proximity to these habitats through the fall and into the following spring. Recognizing that prescribed fire is an important component of box turtle habitat conservation, I recommend that annual burns be avoided, preferentially timing burns every 3 to 4 years and with consideration for the minimum amount of fire necessary to maintain grasslands. Managers should also recognize that portions of the adult female population may be nesting in agricultural fields, potentially requiring a shift in field locations or change in agricultural practices. For example, suitable nesting habitat in buffers along agricultural fields may intercept females from entering the area of the cover crop. Furthermore, I suggest targeted control of predator populations in areas known to support nesting box turtles.

1.6. Acknowledgements

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APPENDIX

Table 1.1. Radio-tagged female eastern box turtles at Fort Custer State Recreation Area, southern Michigan, and years they were successfully tracked to a nesting site where eggs were laid, 2013-2015. Within a year, 1 = tracked to nesting site, 0 = not tracked to nesting site, and “.” = unknown nesting status.

Turtle ID	Monitoring Year		
	2013	2014	2015
3-8,9,10	1	1	1
2,3,8-0	1	1	0
2-9,10	1	1	0
1,2-3	0	1	1
1,9-9,11	0	1	1
2,9-3	0	1	1
1,2,11-1	0	1	0
2,8,9-0	0	1	0
2,9-9	0	1	0
1,2-2	0	1	0
1,2,10-9	0	0	0
1,3,8-12	0	0	0
1,3,9-1	0	0	0
1,3,9-2	0	0	0
8--0	0	0	0
1-1,2	1	0	.
1,11-2	0	0	.
1,2,10-3	0	0	.
1,2,10-1	1	.	.
1,2,3-1	1	.	.
1,2,3-8	1	.	.
1,3-1	1	.	.
1,11-1	0	.	.
1,2,10-2	0	.	.
1,2,3-2	0	.	.
1,3,8-1	0	.	.
1,3,8-2	0	.	.
1,3-1	0	.	.
2,10,11-0	0	.	.
2,9,11-0	0	.	.
8,9-0	0	.	.
2,3-1	.	0	.
0-2,3,10	.	.	1
0-2,8,9	.	.	0
Total Monitored (Total Nests)	31 (8)	19 (10)	17 (5)

Table 1.2. Eastern box turtle nests monitored in the Fort Custer State Recreation Area, southwestern Michigan, 2013-2015, including vegetation type containing the nest, date of nest initiation, clutch size, number of emerged hatchlings, and information on hatchling movements.

Nest ID	Vegetation Type ^a	Date Nested	Clutch Size	Emerged Hatchlings	Hatchling Movements	
					Number Recorded	Average Daily (m/day) (SE)
1	Disk Golf Course	6/4/2013	2	1	. ^d	.
2	Prairie Restoration 1	6/9/2013	9	8 ^b	10	1.91 (0.52)
3	Prairie Restoration 1	6/11/2013	6	6	19	0.80 (0.12)
4	Gravel Pit	6/10/2013	5	5	20	1.06 (0.08)
5	Gravel Pit	6/14/2013	9	8	38	1.47 (0.25)
6	Gravel Pit	6/10/2013	3	0	.	.
7	Corn Field ^{**}	6/6/2013	2	2 ^b	.	.
8	Corn Field	6/13/2013	6	0	.	.
9	Corn Field	6/10/2013	6	0	.	.
10	Corn Field	6/8/2013	8	0	.	.
11	Prairie Restoration 2	6/2/2014	9	0	.	.
12	Prairie Restoration 2	6/7/2014	2	2	.	.
13	Gravel Pit	6/2/2014	3	0 ^c	.	.
14	Prairie Restoration 1	6/2/2014	10	6	60	1.07 (0.08)
15	Prairie Restoration 1	6/3/2014	8	8	47	1.20 (0.12)
16	Prairie Restoration 1	6/8/2014	6	6	61	0.25 (0.03)
17	Prairie Restoration 1	6/7/2014	7	6	61	6.25 (1.52)
18	Prairie Restoration 1	6/6/2014	5	0	.	.
19	Antenna Field	6/7/2014	5	0	.	.
20	Disk Golf Course	6/2/2014	5	0 ^c	.	.
21	Prairie Restoration 2	6/3/2015	4	2	.	.
22	Prairie Restoration 2	6/4/2015	7	0 ^c	.	.
23	Gravel Pit	6/8/2015	7	6	.	.
24	Antenna Field	6/5/2015	7	3	.	.
25	Prairie Restoration 1	6/5/2015	8	6	.	.

^a Disk Golf Course = mowed portion of disk golf course; Prairie restoration 1 = active prairie restoration site that was co-dominated by big bluestem (*Andropogon gerardii*), spotted knapweed (*Centaurea maculosa*) and common mullen (*Verbascum thapsus*) with autumn olive (*Elaeagnus umbellata*) and staghorn sumac (*Rhus typhina*) dispersed throughout; Prairie restoration 2 = big bluestem dominated in active restoration; Gravel Pit = grass and forb dominated abandoned gravel pit; Corn field = actively farmed corn field (** denotes a nest in a 3 m wide agricultural

Table 1.2. (cont'd).

buffer area adjacent to the corn field); Antenna field = forb and lichen dominated radio-antenna field; Herbicide Area = herbicided area in a big bluestem dominated tallgrass prairie in active restoration.

^b Nest contained at least one hatchling that overwintered in the nest cavity and emerged the following May.

^c Clutch lost to predation.

^d No data.

Table 1.3. Generalized linear mixed effects model results for yearly clutch size, number of developed eggs, and number of emerged hatchlings of eastern box turtles at Fort Custer State Recreation Area, southwestern Michigan, 2013-2015.

Model	Parameter	Estimate	SE	<i>T</i>	p-value
Clutch Size	Female mass (β_1)	-0.006	0.009	-0.585	0.58
	Female age (β_2)	0.190	0.234	0.0811	0.44
	Fire scarring (β_3)	2.464	1.28	1.92	0.10
	Year (β_4)	1.676	0.754	2.22	0.06
Count of Developed Eggs	Year (β_1)	-0.240	0.911	-0.264	0.80
	Vegetation Type (β_2)				
	Corn				
	Eagle Prairie	0.445	1.965	0.226	0.83
	Gravel Pit	3.045	2.284	1.333	0.22
Count of Emerged Hatchlings	Tallgrass Prairie	-1.626	2.513	-0.647	0.54
	Year (β_1)	-0.666	0.915	-0.728	0.49
	Vegetation Type (β_2)				
	Corn				
	Eagle Prairie	4.110	2.279	1.803	0.11
	Gravel Pit	6.208	2.247	2.762	0.03
	Tallgrass Prairie	1.501	2.795	0.537	0.67
	Clutch Size (β_3)	0.376	0.268	1.405	0.20

Figure 1.1. Management unit boundaries used for studying nesting ecology of eastern box turtles in of Fort Custer State Recreation Area, southwestern Michigan. Boundaries are portrayed in bold.

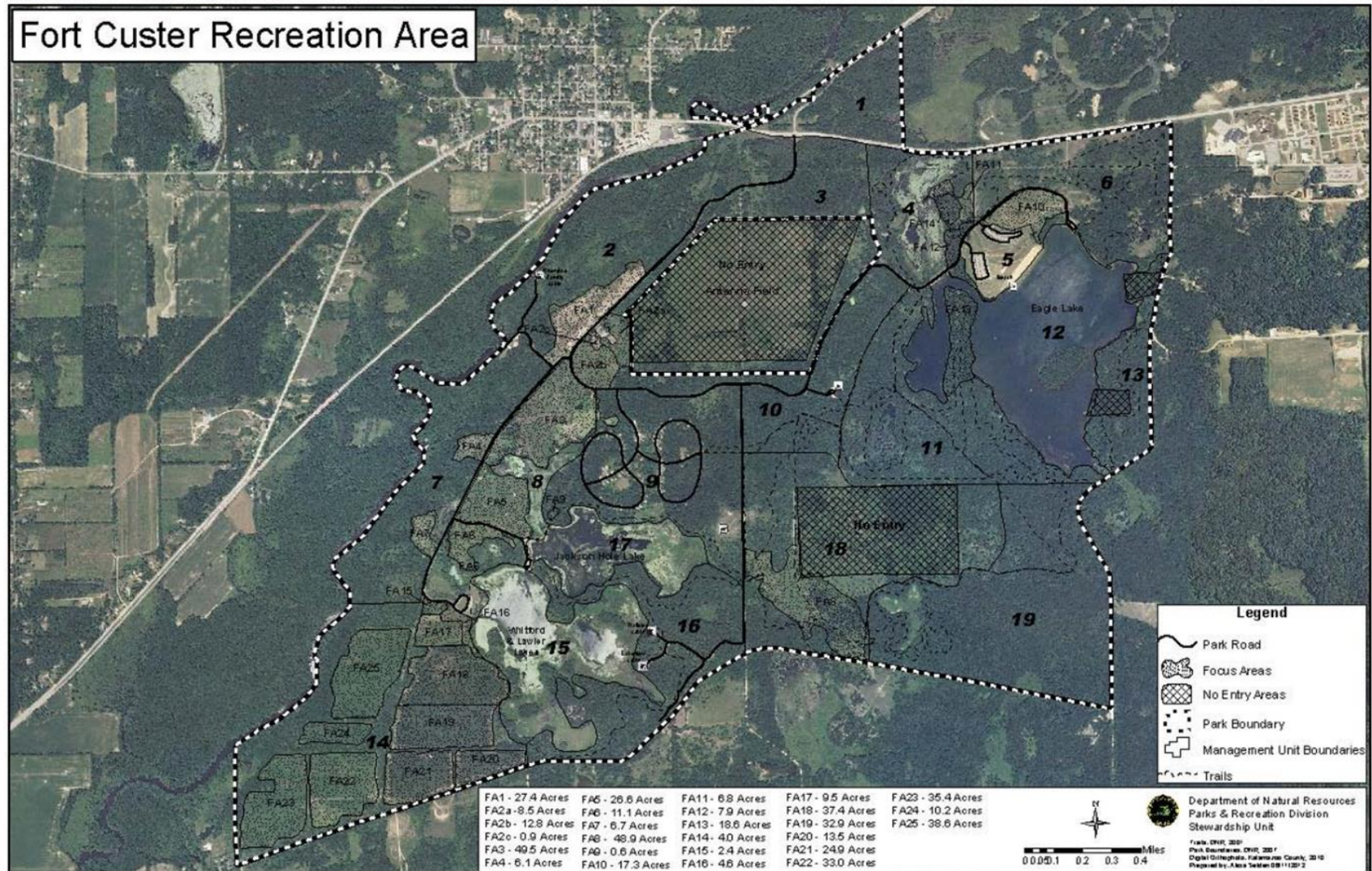
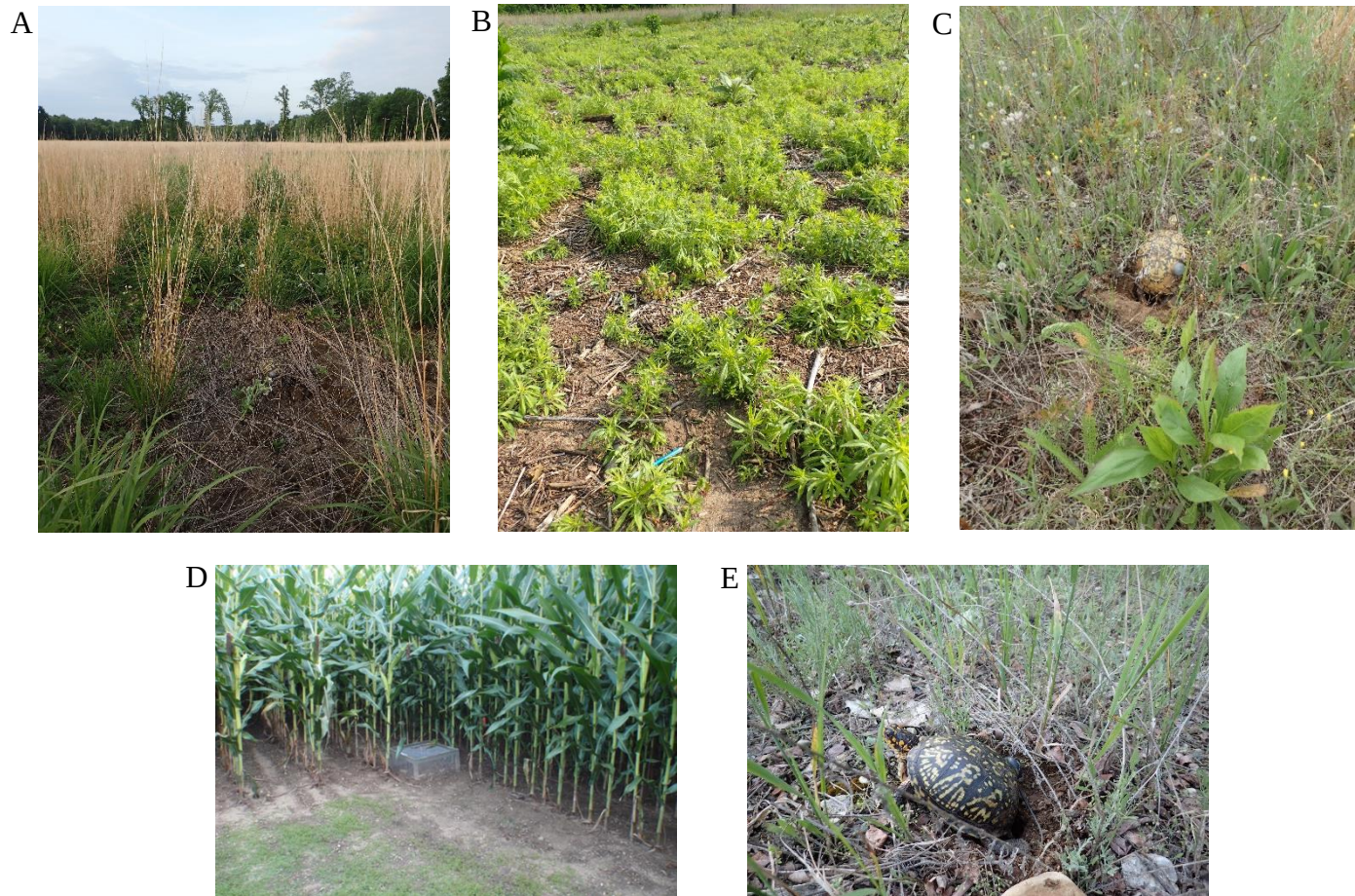


Figure 1.2. Predator exclosures used at Fort Custer State Recreation Area to protect eastern box turtle nests. Exclosures were 0.61 x 0.61 x 0.31 m bottomless wooden frames wrapped in 0.6 cm wire mesh, preserved and camouflaged with an acrylic-based solid stain in olive drab green. Exclosures featured a removable lid attached with (4) 3 cm deck screws. Exclosures were dug 5 cm into the soil surface around the nest.



Figure 1.3. Five nesting sites by vegetation type in Fort Custer State Recreation Area, Michigan, USA. A = big bluestem (*Andropogon gerardii*) dominated tallgrass prairie in active restoration, B = herbicide applied area of big bluestem (*Andropogon gerardii*) dominated tallgrass prairie in active restoration, C = forb and lichen dominated field, D = agricultural field (corn), E = grass and forb dominated abandoned gravel pit.



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CHAPTER 2

RELIABILITY OF POST-FIRE SURVEYS FOR EASTERN BOX TURTLES

Abstract

Natural resource managers may rely on post-fire surveys to understand the direct effects of prescribed fires on wildlife. It is generally assumed that post-fire surveys are effective at quantifying fire-related mortality or injury, as individuals should be readily observed within burned areas. I estimated detection probability during post-fire surveys for eastern box turtles (*Terrapene carolina carolina*) in southwestern Michigan. Immediately prior to a May (growing season) fire, I confirmed that 7 adult box turtles fitted with radio transmitters occupied the proposed burn area. Two days after the burn I reconfirmed turtle locations and subsequently conducted 6 independent visual encounter surveys through 2 burned areas (0.75 and 1.0 ha) that contained telemetered turtles. For these 12 surveys, I found that average detection probability per survey was low (0.11) (SE = 0.05) and highly variable among surveyors (range = 0.00 – 0.50). Based on this average detection probability, I estimated that 26 hour-long surveys per ha would be required to reliably (95% confidence) detect turtles that were present in burned areas. I also found that individual turtles directly exposed to fire remained buried for up to 12 hrs after the fire was extinguished and were thus unavailable for detection immediately after the burn. Further confounding post-fire survey results, buried turtles rapidly moved to unburned areas after emerging from their subterranean refugia. My results suggested that typical visual encounter surveys conducted for eastern box turtles after prescribed burning do not accurately reflect occupancy status or fire caused mortality.

2.1. Introduction

Prescribed fire can be an effective and inexpensive tool for vegetation management (Knapp et al. 2009), but the direct (including injury and mortality) and indirect effects on wildlife (including changes in body condition and animal movements or interactions) can be difficult to quantify. Fire effects on reptiles and amphibians have been studied and summarized (e.g., Keyser et al. 2004, Russell et al. 2009, Roloff and Hmielowski 2014, O'Donnell et al. 2015), and the literature generally suggests that prescribed fire has few significant direct effects. However, most of these studies relied on animal counts or mark-recapture data that were uncorrected for detection probability (Mazerolle et al. 2007). Furthermore, reptiles and amphibians are often grouped into herpetofauna for fire studies (e.g., Means and Campbell 1981; Smith 2000; Floyd et al. 2002; Renken 2005), potentially biasing results to those species that are readily observable and abundant. For cryptic, mobile, or rare species like eastern box turtles (*Terrapene carolina carolina*), the error associated with failure to detect when present may have substantial conservation ramifications (Gu and Swihart 2004, Refsnider et al. 2011).

Eastern box turtles tend to occur in fire maintained ecosystems (Russell et al. 1999) and, although once common (Ernst and Lovich 2009), are now recognized as a species of conservation concern throughout much of their geographic range (van Dijk 2011). Post-fire visual encounter surveys for eastern box turtles are used by some land management agencies and researchers to estimate fire impacts on turtle populations (e.g., Platt et al. 2010). Results from these surveys are also used to inform implementation of burn programs. Most burn plans with the potential to impact species of conservation concern make reference to minimizing fire effects on these organisms. To substantiate this claim, agency personnel sometimes conduct post-fire surveys that typically involve walking through burned areas looking for animals or animal

remains. However, box turtles are difficult to locate and observe as they are well camouflaged, exhibit cryptic behavior such as remaining buried under leaf litter or in dense thickets for long periods of time (Dodd 2001), and can remain motionless when predators or researchers are near (T.A. Melvin, pers. obs.). Additionally, box turtles are generally regarded as crepuscular, with most activity occurring in early morning or evening (Dodd 2001), including nesting, which generally takes place after dark (Flitz and Mullin 2006).

My goal was to better understand the effectiveness of post-fire surveys for eastern box turtles. I quantified detection probability shortly after a prescribed fire using visual encounter surveys in areas that were knowingly occupied by radio-tagged turtles. I also estimated the number of post-fire surveys needed to reliably detect box turtles, and described how post-fire behaviors of turtles influenced their availability for detection. My results can be used to improve the post-fire survey process for eastern box turtles.

2.2. Methods

2.2.1. Study Area

My study was conducted at Fort Custer State Recreation Area (FCSRA; 1,226 ha), located in the southern lower peninsula of Michigan (Kalamazoo and Calhoun Counties), USA. This area was characterized by heterogeneous vegetation patterns resulting from varied topography, climate, and human influence including high levels of agricultural and urban development (Albert 1995, Eagle et al. 2005). Most of the soils are calcareous and loamy, derived from underlying limestone, shale, and sandstone bedrock (Eagle et al. 2005). Glacial till deposits are primarily loams, silt loams and clay loams (Eagle et al. 2005). The climate is considered humid continental, although FCSRA is strongly influence by the Maritime Tropical air mass and proximity to Lake Michigan, resulting in a warmer climate, moderated inland temperature

fluctuations, and induced lake-effect snows (Eichenlaub 1979, Denton 1985, Albert et al. 1986, Eichenlaub et al. 1990). The average length of the growing season was 154 days (Albert et al. 1986). Mean monthly (May to October) minimum and maximum temperatures during the study ranged from -1.7 – 7.2° C and 22.2 – 34.4° C, respectively.

The region containing FCSRA was historically dominated by fire-dependent savanna and prairie (Albert 1995), and contained the only extensive areas of mesic prairie found in Michigan (Kost 2004). In the early 1800's, plant communities included dry and dry-mesic southern forest (oak-hickory; *Quercus* spp. – *Carya* spp.), oak barrens (mixed oak savanna), emergent marsh, and southern (mixed hardwood) swamp (Palmgren 2004). Currently, FCSRA supports degraded patches of oak barrens and prairie openings, scattered oak-hickory forests, and large blocks of non-native black locust (*Robinia pseudoacacia*), surrounded primarily by agriculture (Palmgren 2004, Eagle et al. 2005). Restoration activities on FCSRA included use of prescribed fire to maintain and improve savanna and prairie ecosystems. Prior to my study, prescribed fires were generally small to medium-scale (1.6 to 414.4 ha), conducted in spring (before leaf out, typically during the months of March and April), and occurred in 1 to 4-year intervals followed by herbicide treatments in some areas.

My research focused on a 31.6 ha management unit in FCSRA (Figure 2.1). Within the unit, I delineated 2 (0.75 and 1 ha) visual encounter survey areas that overlapped transmitter box turtle home ranges (Figure 2.1). Survey area A consisted of a sloping hill (slope 19%, aspect 270-330) of mixed oak-hickory forest with a pre-burn understory consisting of red-osier dogwood (*Cornus sericea*), sassafras (*Sassafras albidum*), wild black raspberry (*Rubus occidentalis*) and multiflora rose (*Rosa multiflora*) with duff layers 4-7 cm deep. Survey Area B consisted of dense stands of black locust and mixed oak-hickory forest, with multiflora rose in

the understory, and open grassland dominated by spotted knapweed (*Centaurea stoebe*) and sweetclover (*Melilotus officinalis*) with little bluestem (*Schizachyrium scoparium*), goldenrod (*Solidago* spp.) and milkweed (*Asclepias* spp.) throughout.

2.2.2 Research Burn and Survey Area Descriptions

I used a grid of semi-permanent 2 m radius circular plots spaced 40 m apart (n = 80) to describe fire characteristics throughout the eastern half of the management unit. At the center of each plot, I placed a 1.2 m untreated wooden stake to estimate flame heights above the soil surface. I also placed a set of 3 Omega TL-10 adhesive, non-reversible fire temperature labels (OMEGA Engineering, INC., Stamford, Connecticut) that recorded temperatures between 87 – 260° C. Temperatures were recorded to the nearest 6° C. Labels were affixed to 11 x 20 cm piece of aluminum roof flashing that was bent to a 90° angle and anchored into the soil so that the side with the temperature labels ran parallel to and 4 cm above the soil surface. I recorded the times that the flame front reached different plot centers to estimate rate of spread, and estimated flame heights by observing how high flames reached when they first contacted the wooden stakes.

2.2.3. Radio-telemetry and Fire Observation of Eastern Box Turtles

Turtles were initially captured in May 2012 and June 2013 using visual encounter surveys and turtle detector dogs. Adult box turtles were radio-tagged with a Holohil R1-2B 14.5g transmitter (Holohil Systems Ltd., Carp, Ontario, Canada) attached to the right or left anterior pleural carapacial scutes using multi-purpose 5-minute set epoxy putty (Loctite®, Henkel 289 Corporation, Cary, NC, USA). I estimated minimum age by counting annual rings on the carapace (see Wilson et al. 2003). I also marked turtles by notching the shell (Cagle 1939). Radio-tagged adult box turtles were monitored during the active seasons (April through October) from May 2012 to August 2015 using portable telemetry receivers (Advanced Telemetry

Systems, Isanti, MN, USA), which allowed me to delineate core use areas within the management unit. I located telemetered turtles 24 hrs before the research burn at which point transmitters were spray-painted with RUST-OLEUM™ High-Heat Spray to prevent fire damage. The carapace was covered with a spray guard to prevent heat resistant paint from adhering to the turtle. All animals were handled in compliance with Michigan State University's Institutional Animal Care and Use Committee guidelines (IACUC Approval # 04/14-077-00).

After ignition of the prescribed fire, I relocated radio-tagged turtles from behind the flame front to observe fire-related behaviors. I stayed 5-10 m away from the turtles to minimize my influence on behaviors. I visually inspected telemetered turtles for direct injury and mortality after the fire was extinguished and for 12, 24 and 48 hrs thereafter. After 48 hrs, I checked the turtles 1-3 times per week.

2.2.4. Detection Probability Experimental Design

After the fire was completely extinguished (~48 hrs after ignition), I spaced parallel transects 10 m apart in the 2 detection survey areas (Figure 2.1). Transects were marked using spray paint applied to tree stems at eye-level. Surveyors slowly walked the transects and scanned for box turtles within 5 m of their position. Each surveyor (n=6) made one pass on all transects in each survey area at roughly 1-hour intervals, between 1:30 and 7:00 p.m. Surveyors had similar amounts of experience in negotiating the study site and had comparable training in visually identifying eastern box turtles. Surveyors had no knowledge of the number of transmitted turtles in the survey areas and did not disclose the results of their survey to other surveyors.

I located turtles using radio telemetry prior to each new hourly survey to confirm availability of individuals for detection. Each time I relocated one of the marked turtles, I noted the position and level of concealment. During surveys, each observer also recorded the locations

of detected box turtles using a handheld GPS unit, and recorded behavior and percent of the carapace (top shell) obscured by vegetation, soils, or other natural objects when viewed from straight above the turtle.

2.2.5. Data Analysis

I quantified post-fire detection probability of box turtles using the marked subsample method (Lancia et al. 2005, Refsnider et al. 2011). I calculated box turtle detection probability, β_i , for each of the 12 survey periods using equation 12 in Lancia et al. (2005:121):

$$\beta_i = \frac{m_i}{n_i}$$

where m_i was the number of transmitted box turtles observed during the i visual-encounter survey and n_i was the number of transmitted box turtles available to be observed during the i survey period. I used the average detection probability over 6 surveys to characterize within survey area detection probability, and averaged across all 12 surveys to portray overall detection probability, $\hat{\beta}$. I estimated the number of surveys required to reliably (with 95% confidence) detect box turtles using equation (4) in Kéry (2002:331):

$$N \text{ min} = \frac{\log \alpha}{\log(1 - \rho)}$$

where $\alpha = 0.05$ and ρ was equivalent to my overall detection probability, $\hat{\beta}$.

2.3. Results

2.3.1. Fire Characteristics and Turtle Behavior

Average flame lengths of the fire were approximately 8 cm (SE = 0.63), average rate of spread was approximately 1.5 m/minute (SE = 0.19), and average maximum temperatures were 149.3° C (SE = 7.9). Approximately 51% of the management unit burned resulting in a mosaic of

burned and unburned upland habitats and an unburned emergent marsh. Within the survey areas, 90% and 65% burned in A and B, respectively.

Prior to fire ignition 11 telemetered turtles occupied the management unit but 6 were in a wetland that did not burn. An additional marked turtle was within a dense black locust stand that did not burn. The other 4 turtles were directly subjected to the flame front. These turtles exhibited varying behaviors in response to the fire. One began to bury into mineral soil and stopped once the flame front slowed and self-extinguished before reaching her location (roughly 3 m away), 2 turtles completely buried into mineral soil (approximately 13 cm down), and one remained above ground and moved 1-2 m from her original location. This individual was overtaken by the flame front, burning 90-100% of the carapace. Subsequently, this turtle moved to an unburned black locust patch within 12 hrs and then to a wetland where she remained until dying two weeks later.

I monitored these marked turtles for 2 years, and before the prescribed fire I only observed burying behavior into mineral soil when individuals were entering or exiting brumation in the late fall (October – November) or early spring (March-April). The 2 turtles that I observed burying into mineral soil in advance of the flame front remained buried for 12 hrs following fire extinguishment and subsequently moved to an adjacent wetland. Regardless of their exposure to the flame front, marked turtles remained soaking in the adjacent wetland, or within 3-5 m of the wetland edge, for 1-3 weeks following the fire. When moving back to burned areas from the wetland, turtles seemed to remain within 1-2 m of unburned habitats that included decaying logs, patches of leaf litter, or other areas unaffected by fire. They remained in these areas until post-burn vegetation growth provided cover.

2.3.2. Detection Probability

During the post-burn visual-encounter surveys, 7 transmittered turtles (5 in area A, 2 in B; Figure 2.1) were confirmed to be within the survey areas. Observers detected 2 of the 7 turtles. One observer detected 2 individuals, three observers detected one individual, and the remaining two observers failed to detect turtles (Table 2.1). There were no unmarked turtles detected during the surveys. The detection probability for individual surveys ranged between 0.00 and 0.50 (Table 2.1). Average detection probability for 12 surveys was 0.11 (SE = 0.05), and I estimated that 26 hour-long surveys per ha would be required to have 95% confidence that all available turtles were detected.

Of the 7 turtles that occupied areas used for post-fire detection surveys, 3 were directly subjected to the flame front and 4 were within the wetland that did not burn. These 7 turtles did not move from their initial pre-survey locations for the duration of the 6 surveys in each area. Although the 7 turtles were above ground 48 hrs after the fire was extinguished, they appeared to seek concealment under live and dead herbaceous vegetation or downed wood (e.g., Figure 2.2). Overhead concealment ranged from 5 to 80% among turtles (Figure 2.2). The two detected individuals were concealed by woody debris. One was found in a fire blackened area (5% concealed), and another in a patch of unburned vegetation (20% concealed). Concealment for the 5 undetected individuals ranged from 10 to 80%. These turtles were in unburned live vegetation.

2.4. Discussion

A typical post-fire survey for eastern box turtles involves walking the burned area and visually identifying animals or their remains, usually 24-48 hrs after the fire is extinguished. Using visual detection surveys, I found that average detection probability for adult eastern box turtles 48 hrs after a prescribed fire was low. Detection probability by individual surveyor varied considerably but never exceeded 0.50. I found that turtle behaviors following the fire contributed to low

detectability. These behaviors included burying for up to 12 hrs after the fire, and movements off the burned area within 4 hrs if turtles did not bury, or within 4 hrs after surfacing from burying. Given average surveyor capability, a growing season fire that resulted in a mosaic of burned and unburned patches, highly variable concealment of turtles, and post-fire turtle behavior, I estimated that 26 hour-long surveys would be needed to have 95% confidence that turtles in the area were detected. My results indicated that visual encounter surveys of burned areas underestimate direct and indirect fire effects on box turtles.

Although several terrestrial turtle species are of conservation concern worldwide, little is known about survey efficacy. For 8 visual encounter surveys, detection probability was 0.03 for telemetered ornate box turtles (*Terrapene ornata*) in a sand prairie in northwestern Illinois (Refsnider et al. 2011). Detection probability can apparently be improved by using dogs. For example, Kapher et al. (2012) found that detection varied between 0.33 and 0.67 for eastern box turtles using detector dogs in lowland and upland deciduous forest in North Carolina. My results are consistent with Refsnider et al. (2011) that found low visual detection probability for terrestrial turtle species.

Following prescribed fire, I found that turtles moved into or remained within unburned wetland areas and tended to remain in or near these areas until herbaceous vegetation started to recover in burned areas. I purposefully expanded my survey plots to include these unburned areas but none of the turtles were detected during the visual encounter surveys. I only documented direct flame contact for one of the telemetered turtles during the fire, and this turtle moved off the burned area and soaked in an adjacent wetland prior to death. Turtles severely injured by fire may be more prone to hide, seek shelter, or soak (Dodd 2001). My results suggested that shortly after prescribed fire, box turtles 1) move away from the burned areas, 2)

seek refugia that can make detection difficult 3) remain hidden in refugia for hours after the fire has passed, and 4) can appear healthy only to later succumb to fire-related injuries. Collectively, these behaviors can lead natural resource managers to conclude that box turtles were not present in the burned area, or that turtles temporarily left the burned area, and therefore the fire had little effect on movement, injury, or health.

My study is one of few that quantitatively evaluated post-fire behaviors and detection probability for marked terrestrial turtles. Eastern box turtles tend to occupy ecosystems that were historically maintained by fire, thus fire is often prescribed as a restoration tool. For species of conservation concern, like box turtles, managers are often tasked with acknowledging fire effects but tend to lack empirically based guidance on appropriate survey techniques. Although my study provides that guidance, I caution that my results are based on a single prescribed fire in one management unit during the early growing season. The efficacy of post-fire surveys for eastern box turtles likely varies by fire intensity and the amount and spatial distribution of vegetation and debris so I encourage additional detection studies in other fire and vegetation conditions.

2.5. Management Implications

Failure to account for detection probability and post-fire behaviors can result in erroneous management decisions for eastern box turtles with respect to fire frequency, season, intensity, and ignition type. During the growing season (defined here as after leaf-out) when box turtles are active, I suggest that visual surveys can be more effective by watching for turtles as they move to refugia during prescribed fires, and by focusing post-fire searches on refugia like large downed logs, areas of unburned vegetation, and adjacent unburned areas (particularly wetlands). When planning a growing season fire, I encourage managers to purposefully retain unburned refugia within the burn area and ensure that turtles can negotiate the flame front to this refugia. Fires

should be slow and creeping backfires, lit in a way that maintains a single linear flame front. I further suggest that growing season fires should be prevented from spreading into grasslands, where faster fire spread rates may prohibit turtles from evading the flame front.

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APPENDIX

Table 2.1. Number of post fire marked eastern box turtles (*Terrapene carolina carolina*) available for detection (n_i), visually detected by observers (m_i), and detection probability (β_i) by survey and survey area, Fort Custer State Recreation Area, southwestern Michigan, USA, 2015. Detection probability (β_i) was calculated using the marked subsample method (Lancia et al. 2005:121).

Survey	Survey area A			Survey area B		
	Marked turtles	Detected	Detection probability	Marked turtles	Detected	Detection probability
	(n_i)	(m_i)	(β_i)	(n_i)	(m_i)	(β_i)
1	5	1	0.20	2	0	0
2	5	1	0.20	2	1	0.50
3	5	0	0	2	0	0
4	5	1	0.20	2	0	0
5	5	0	0	2	0	0
6	5	1	0.20	2	0	0
Mean (SE)			0.13 (0.04)			0.08 (0.08)

Figure 2.1. Management unit (yellow polygon) at Fort Custer State Recreation Area in southwestern Michigan, USA, 2015. Post fire visual-encounter survey areas A (0.75 ha) and B (1.0 ha) delineated by white polygons.



Figure 2.2. Post fire concealment of eastern box turtles (*Terrapene carolina carolina*) monitored using radio telemetry at Fort Custer State Recreation Area in southwestern Michigan, USA, 2015. A = 20% concealed under logs in burned area, B = 80% concealed, 20 m into unburned wetland, C = 10% concealed at the edge of unburned wetland, D = 5% concealed leaf litter and live vegetation in unburned patch of oak and black cherry (*Prunus serotina*).



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CHAPTER 3

DIRECT AND INDIRECT EFFECTS OF A GROWING SEASON PRESCRIBED FIRE ON EASTERN BOX TURTLES IN SOUTHWESTERN MICHIGAN

Abstract

I examined the effects of a slow moving, growing season fire on the direct behavior and subsequent survival of adult eastern box turtles in southwestern Michigan, 2015. The fire occurred during the active season for box turtles (May), when adults have been active and above soil for at least two weeks. I used plot-based pre- and post-burn measurements to quantify fire characteristics and resulting effects on woody vegetation. I also directly observed behaviors of telemetered turtles during the fire and documented mortality and habitat use for the remainder of the active season. My results indicated that a growing season prescribed fire significantly reduced small (≤ 1 cm diameter) woody stems, and resulted in cover of leaf litter $< 10\%$ in burned areas. The significant reduction of woody stems was consistent across all groups of vegetation, including non-native, native fire-adapted, and native species that were not adapted to fire. Greatest losses were observed for native, non-fire adapted (76% loss) and non-native (70% loss) species. Fire adapted species were less impacted (53% loss). The growing season fire did not result in a significant reduction of vegetation > 1 cm diameter, although some individual species declined (e.g., autumn olive, black locust, glossy buckthorn, hickory, black cherry, and sassafras). Box turtles exhibited varying behaviors in response to the fire including burying and actively evading and negotiating the flame front. One turtle died from injuries sustained in the fire and females within the management unit where prescribed fire was applied were not observed nesting that year.

3.1. Introduction

Prescribed fire is used to delay vegetation succession, restore historical disturbance processes, recycle nutrients, manage wildlife habitat, and control exotic invasive vegetation (Knapp et al. 2009). The severity, uniformity, and spatiotemporal extents of prescribed fire influences vegetation pattern, productivity, and corresponding behavioral responses of fauna located within treated areas (Smith 2000). Although prescribed fire can be an effective and inexpensive tool for vegetation management, direct (including injury and mortality) and indirect (including changes in body condition and animal movements or interactions) effects on herpetofauna are poorly understood (Russell et al. 1999; Keyser et al. 2004). Studies to date have generally focused on short-term pre- and post-fire abundance and species richness (Griffiths and Christian 1996; Cole et al. 1997; Moseley et al. 2003; Keyser et al. 2004; Greenburg and Waldrop 2008). Some studies indicated that fires have few direct effects on local herpetofauna (Means and Campbell 1981; Russell et al. 1999; Floyd et al. 2002), and that species historically occurring in fire dependent ecosystems have adaptations that reduce negative fire impacts (Erwin and Stasiak 1979; Means and Campbell 1981; Driscoll and Henderson 2008; Smith 2000). In drawing these conclusions, researchers have frequently grouped reptiles and amphibians into herpetofauna (Means and Campbell 1981; Smith 2000; Floyd et al. 2002; Renken 2005; Platt et al. 2010). This grouping tends to ignore differences in behavioral responses by individual species that may affect survivorship. More importantly, this grouping fails to acknowledge the ramifications of losing individuals from populations of long-lived species with delayed reproductive maturity and potentially biases the analysis towards the responses (often abundance) of r-selected species.

Eastern box turtles (*Terrapene carolina. carolina*) historically occupied habitats subjected to fire throughout eastern North America (Russell et al. 1999), including grasslands,

oak (*Quercus* spp.) dominated woodlands, and more mesic ecosystems (Dodd 2001; Spencer and Thompson 2003). Although once common (Ernst and Lovich 2009), eastern box turtles are now recognized as a species of conservation concern throughout much of their geographic range (Swarth and Hagood 2004; van Dijk 2011). In Michigan, eastern box turtles are documented to occur in 36 counties, with last confirmed occurrence dating to 1933 for some observations (MNFI 2017). In some of these counties, areas are managed with prescribed fire to restore fire-dependent ecosystems, control invasive vegetation, and hinder woody encroachment.

Research suggests that losing even small numbers of breeding adults from k-selected, long-lived populations like box turtles can result in irreparable harm to population viability (Congdon et al. 1993; Dodd et al. 2015). Although eastern box turtles may have evolved with fire, current turtle population sizes, fire regimes, and habitat configurations differ from historical conditions. To accomplish ecosystem restoration goals, managers often use prescribed fire regimes that differ from historical regimes in frequency, time of year, and spatial extent. These fire regimes have direct effects on eastern box turtles including high rates of mortality and injury from exposure to fire (Allard 1949; Babbitt and Babbitt 1951; Bigham et al. 1964; Dolbeer 1969; Gibson 2009). Indirect effects may include changes in prey abundance, vegetation used for thermoregulation, camouflage, and insulating duff layers during overwintering, and potential disruption in behavior or range (Gibson 2009). Box turtles are particularly sensitive to environmental variables that affect ground cover (including vegetation, litter, and subsurface soils) because their entire life history depends on ground conditions (Dodd 2001). For box turtles, thermoregulation determines individual responses to habitat changes (Currylow et al. 2012). For example, Currylow et al. (2012) evaluated box turtle movement and thermal ecology

for two years following timber harvest and found that movements were shorter and more frequent after timber harvest.

Because prescribed fire is a popular management technique in Michigan, understanding both the efficacy of fires in achieving vegetation goals and in minimizing box turtle injury, mortality, and survivorship is fundamental to conservation of this species. The general consensus regarding fire and eastern box turtle conservation is to restrict burning to the early spring months, before turtles have emerged from overwinter hibernacula (see Woodley 2013), or to late fall once box turtles have entered subterranean hibernacula. As early spring is ambiguous, and annual weather conditions vary, spring prescribed fires in Michigan can potentially coincide with box turtle emergence. When emerging from hibernacula, box turtles are lethargic and have low energy reserves (Dodd 2001; Woodley 2013). Woodley (2013:23) found that box turtles in Michigan “often stayed within 1 to 5 meters of their burrow for 1 to 2 weeks after emerging – seeming to indicate that turtles need a substantial interval of time to recover full mobility after emerging in the spring”, and that they would be “especially vulnerable during this time to any surface disturbance”. Early spring prescribed fires conducted in landscapes where box turtles may overwinter, such as upland oak ecosystems with heavy duff layers, can be fast moving and intense. Thus, early spring fires, if timed to box turtle emergence, can have substantial negative impacts on turtle populations. Fires later in spring or into the growing season, after box turtles have become fully active, may allow turtles to evade slow moving flame fronts.

I examined the effects of a slow moving, growing season fire on the behavior and subsequent survival of adult eastern box turtles. This fire occurred during the active season for box turtles, when adults have been active and above soil, outside hibernacula for at least two weeks. I used pre- and post-burn behavioral observations from telemetered turtles, and fire and

vegetation plots to quantify fire effects. My research objectives were to: 1) quantify the efficacy of the growing season burn in reducing woody succession and controlling invasive plants, 2) describe behaviors of individual turtles as the flame front advanced, 3) assess direct mortality of turtles from the burn, and 4) quantify short-term indirect fire effects, such as changes in behavior and survivorship. My results represent one of the only known studies on eastern box turtle behaviors during a growing season prescribed fire, and offer insights into post-fire habitat use and survivorship.

3.2. Methods

3.2.1. Study Area

This study was conducted at Fort Custer State Recreation Area (FCSRA; 1,226 ha), located in the southern lower peninsula of Michigan between the cities of Battle Creek and Kalamazoo, USA. The FCSRA had heterogeneous vegetation caused by differences in topography, climate, and human influence including high levels of agricultural and urban development (Eagle et al. 2005; Albert 1995). Most of the soils are calcareous and loamy, derived from underlying limestone, shale, and sandstone bedrock (Eagle et al. 2005). Glacial till deposits are primarily loams, silt loams and clay loams (Eagle et al. 2005). The climate is considered humid continental, although FCSRA is strongly influenced by the Maritime Tropical air mass and proximity to Lake Michigan, resulting in warmer temperatures, moderated inland temperature fluctuations, and induced lake-effect snow (Eichenlaub 1979; Denton 1985; Albert et al. 1986; Eichenlaub et al. 1990). The average length of the growing season was 154 days (Albert et al. 1986). The mean minimum and maximum temperatures during the study (May to October, 2013 - 2015) ranged from -1.1 – 7.2°C and 22.2 – 34.4°C, respectively. FCSRA and the adjacent Fort

Custer Training Center (a 3,063 ha federally owned military reservation) represent one of the largest contiguous blocks of public land in southwest Michigan (Cohen et al. 2009).

The FCSRA was historically dominated by fire-dependent oak (*Quercus* spp) savanna and prairie (Albert 1995), and contained the only extensive areas of mesic prairie found in Michigan (Kost 2004). In the early 1800's, plant communities included dry and dry-mesic southern forest (oak-hickory (*Carya* spp)), oak barrens (mixed oak savanna), emergent marsh, and southern (mixed hardwood) swamp (Palmgren 2004). Currently, intensive agriculture is concentrated around FCSRA because of the relatively mild climate and productive soils (Eagle et al. 2005). Natural vegetation in this region is broadly classified as black oak-white oak (*Q. velutina*-*Q. alba*) savannas and forests on droughty soils, and beech-sugar maple (*Fagus grandifolia*-*Acer saccharum*) forests on more mesic sites (Palmgren 2004). Currently, FCSRA supports degraded patches of oak barrens and prairie openings, scattered oak-hickory forests, and large blocks of non-native black locust (*Robinia pseudoacacia*), surrounded primarily by agriculture (Palmgren 2004; Eagle et al. 2005). Restoration activities on FCSRA included use of prescribed fire to maintain and improve savanna and prairie ecosystems. Prescribed fires were generally small to medium-sized (1.6 to 414.4 ha), conducted in spring (before leaf out, typically during the months of March and April), and occurred in 1 to 4-year intervals followed by herbicide treatments in some areas.

The FCSRA is located within the most northerly range of the eastern box turtle. I chose FCSRA for this project because it has been the focus of ecological restoration activities using prescribed fire since 1997. My research focused on a 31.6 ha management unit in FCSRA (Figure 3.1). The unit included dense patches of invasive black locust, emergent marsh, dry-mesic southern (oak-hickory) forest, open grassland, red pine (*Pinus resinosa*) plantation, and

mixed lowland hardwoods (mesic southern forest, maple-beech dominated). This unit included a 12.5-hectare emergent marsh with open water (Figure 3.1). The unit was previously burned during early spring (typically the month of March) in 2001, 2003, 2006, and 2008. It was also partially burned in 2007.

3.2.2. Vegetation and Fire Data Collection

Pre- and post-fire vegetation information was collected using a grid of semi-permanent 2 m radius circular plots spaced 40 m apart ($n = 80$). I marked the center of each plot using a wooden stake and recorded the location with a handheld GPS. I recorded woody stem count per species and growth class; growth classes included ≤ 1 cm, 1 to < 3 cm, and ≥ 3 cm. I also recorded litter depth. At the center of each vegetation plot, I placed a set of 3 Omega TL-10 adhesive, non-reversible labels that recorded fire temperatures (OMEGA Engineering, INC., Stamford, Connecticut) ranging from 87 – 260° C (Figure 3.2). Temperature labels were affixed to a rectangle (11 x 20 cm) of aluminum roof flashing. The rectangle was bent to a 90-degree angle and fixed into the soil so that the side with the temperature labels ran parallel to and 4 cm above the soil surface. I recorded rate of fire spread by observing the time at which the flame front reached different plot centers. Average char height for each wooden stake was recorded in centimeters and flame height was estimated using direct observation during the fire.

Completeness of burn was recorded as percentage of charred vegetation at each vegetation plot 48 hrs after the burn. Pre-fire vegetation sampling was completed in June 2014, and post-fire vegetation sampling was conducted in September 2015.

3.2.3. Radio-telemetry and Observation of Eastern Box Turtles During the Burn

I used meandering visual encounters and wildlife detector dogs to find adult eastern box turtles during daylight hours in May of 2012 and June of 2013. Captured adult box turtles were radio-

tagged with a Holohil R1-2B 14.5g Transmitter (Holohil Systems Ltd., Carp, Ontario, Canada) attached to the right or left anterior pleural carapacial scutes using multi-purpose 5-minute set epoxy putty (Loctite®, Henkel 289 Corporation, Cary, NC, USA). I estimated minimum age by counting annual rings on the carapace (Legler 1960), and permanently marked turtles by notching the shell (Cagle 1939). I visually located radio-tagged turtles 1-3 times per week during the active seasons (April through October) from May 2012 to August 2015 using portable receivers (Advanced Telemetry Systems, Isanti, MN, USA).

I located telemetered turtles 24 hrs before the research burn at which point transmitters were spray-painted with RUST-OLEUM™ High-Heat Spray to prevent fire damage. I covered the carapace with a spray guard to prevent heat resistant paint from adhering to the turtle. Upon fire ignition, I relocated research animals from behind the flame front using radio-telemetry and subsequently observed fire-related behaviors. I observed turtles from 5-10 m away to minimize influence on behavior. I subsequently relocated the turtles 12, 24 and 48 hours after the burn and assessed each for direct injury and mortality; thereafter I checked turtles 1-3 times per week. All animals were handled in compliance with Michigan State University's Institutional Animal Care and Use Committee guidelines (IACUC Approval # 04/14-077-00).

3.2.4. Data Analysis

I summarized fire characteristics using descriptive statistics. I also reported on average stem counts by species for 3 size classes; ≤ 1 cm, > 1 and < 3 cm, and ≥ 3 cm for pre- and post-fire conditions. To test for stem count differences between pre- and post-fire, I used a Hotelling's T^2 that tests for the differences in a multivariate vector (in this case species counts) across treatment types (Mardia et al. 1979).

3.3. Results

3.3.1. Fire Characteristics

The MDNR implemented the research burn on May 20, 2015, corresponding to leaf out and the early part of the growing season. Average flame lengths were 8 cm (SE = 0.63), average rate of spread was 1.5 m/min (SE = 0.19), and average maximum temperature was 149.3° C (SE = 7.9). Approximately 16.2 ha of the 31.6 ha management unit burned, resulting in a mosaic of burned and unburned upland habitats and an unburned emergent marsh. Additionally, fire failed to carry into patches of dense locust and mixed lowland hardwoods where little fine fuels were available.

3.3.2 Direct Effects of Fire on Woody Vegetation and Leaf Litter

Pre-fire woody vegetation (based on 52 plots) was a mixture of non-native (Table 3.1), native fire-adapted (Table 3.2), and other native species found in dry mesic southern forests (Table 3.3). On average, the most prevalent small diameter (≤ 1 cm) stems included native species; red osier dogwood, black cherry, *Viburnum* spp., black raspberry, and hickory (Table 3.2). Medium-sized ($1 < \text{diameter} < 3$ cm) stems were mostly dominated by non-native species including autumn olive, black locust, honeysuckle, hickory, and glossy buckthorn (Table 3.1). The most prevalent largest woody stems (≥ 3 cm diameter) were autumn olive, black locust, black cherry, hickory, and white oak (Tables 3.1, 3.2, 3.3). Leaf litter cover in pre-fire plots that eventually carried fire was 84.5% (SE = 3.3, range = 5-100).

Only 52 of 82 (63%) vegetation plots burned during the May 2015 prescribed fire. The fire reduced average leaf litter cover by 77.3%, resulting in average post-fire leaf litter cover of 7.3% (SE = 1.6, range 0-50%). Non-native stems ≤ 1 cm were reduced by 59% (Table 3.1), and this was a significant reduction (Hotelling's T^2 , $F = 2.25$, $P = 0.01$). Counts of larger non-native

stems (1-3 cm and ≥ 3 cm diameter) were reduced (e.g., 24% reduction for 1-3 cm stems), but this was not a significant decline (Hotelling's T^2 , $F < 0.43$, $P > 0.91$).

Stem counts of native, fire adapted species ≤ 1 cm diameter were significantly reduced (Table 3.2; Hotelling's T^2 , $F = 3.64$, $P < 0.001$). No differences in stem counts of native, fire-adapted species were identified for medium or large stems (Hotelling's T^2 , $F < 0.39$, $P > 0.79$). Lastly, stems counts of native, non-fire adapted species ≤ 1 cm were significantly reduced (Table 3.3; Hotelling's T^2 , $F = 3.64$, $P < 0.001$), whereas 1-3 cm and ≥ 3 cm were not significantly reduced (Hotelling's T^2 , $F < 0.11$, $P > 0.97$). Total counts of small stems (≤ 1 cm diameter) were reduced by 67%. Total counts of medium stems (1-3 cm diameter) were reduced by 27%. Total counts of larger stems (≥ 3 cm diameter) were reduced by 17%. I did not measure the effects of vegetative decreases on prey abundance or thermoregulation directly.

3.3.3. Direct Fire Effects on Box Turtle Behavior

Prior to fire ignition, 11 turtles occupied the management unit but 6 of those were in a 12.5-ha emergent marsh and unaffected by the flame front. An additional radio-tagged turtle was within a dense black locust patch that did not burn. The other 4 turtles were directly subjected to the flame front. These turtles exhibited varying behaviors in response to the fire. One began to bury into mineral soil and stopped once the flame front slowed and self-extinguished before reaching her location (roughly 3 m away), 2 turtles completely buried into mineral soil (approximately 13 cm down), and one remained above ground and moved 1-2 m from her original location. This individual was overtaken by the flame front, burning 90-100% of her carapace. Subsequently, this turtle moved to an unburned black locust patch within 12 hrs and then to the emergent marsh where she remained until dying two weeks later.

The 3 box turtles that were observed digging into mineral soil reacted to the advancing flame front when it was 10-20 m away. Two of these turtles were in grassland within the management unit, and one was on the edge of a southern mesic forest and grassland. These turtles actively negotiated the flame front by moving away quickly and then began to dig into mineral soil. These behaviors resulted in one female outrunning the flame front to an area of low fuels and partially burying into mineral soil in a mesic forest, and one male completely burying into mineral soil in a grassland area. A female that actively negotiated the flames was eventually overtaken by two flame fronts that converged on her from fires that were lit separately in a grassland area. A firefighter picked up this female and placed her in an area that had already burned to prevent her from being completely overtaken by flames. Once in the burned area she immediately buried herself in mineral soil under an unburned decaying log. The fourth female remained in form, or within its shell, until the flame front was within 5 m and was overtaken by the flame front in a grassland area.

I failed to observe any turtle mortalities on the day of the burn. My observations indicated that when flame fronts were slow moving (1.5 m/minute), adult eastern box turtles detected oncoming flame fronts 10-20 m away. Whether they detected oncoming flame fronts by sight, sound, or smell is unclear, although, I observed the individual in the mesic forest becoming alert and arching her head out of her shell when it seemed she could only detect the fire by its crackling sound, or smell, as it was 20 m away and had short (8 cm) flame lengths. I observed adult turtles successfully negotiating and evading this slow-moving fire, and I also observed one adult turtle completely fail to react to the fire, resulting in death. If burn refugia (soft mineral soil, or stumps and logs that will not burn) are accessible, converging flame fronts do not restrict

movements (fires are not lit using a strip method), and fires remain creeping (not grassland fires), some box turtles can safely negotiate prescribed fires during the active season.

3.3.4. Indirect Fire Effects on Box Turtles

I defined indirect effects of prescribed fire on eastern box turtles as potential changes in behavior or space use not associated directly with exposure to flames. Disruptions in behavior or space use could be indirectly related to fire effects on prey abundance and vegetation used for thermoregulation or concealment (Gibson 2009), for example. I observed behavioral changes in adult box turtles that included long-term burying, potential long-term avoidance of burned areas, and failure to stage or nest. Adult box turtles that exhibited a burying strategy to cope with the fire (n=3) remained buried for 12 hrs following fire extinguishment. These adults subsequently began migrating to the emergent marsh, which occurred within the home ranges of these turtles. Adults traveled between 0 to 160.6 m to reach this marsh. Regardless of their exposure to the flame front, marked turtles remained soaking in the emergent marsh, or within 3-5 m of its edge, for 1-3 weeks following the fire.

When moving back to burned areas from the emergent marsh, turtles remained within 1-2 m of unburned habitats that included decaying logs, patches of leaf litter, or other areas unaffected by fire. They also seemed to remain in these areas until post-burn vegetation growth provided cover. Soaking in the wetland in late May and early June was not abnormal behavior, as I observed similar behavior in years prior to the burn. The year of the prescribed fire, female turtles that were in the management unit during the fire did not nest. Typical nesting season started in early to mid-June.

3.4. Discussion

My results indicated that a growing season prescribed fire significantly reduced small (≤ 1 cm diameter) woody stems, and resulted in cover of leaf litter $< 10\%$. The significant reduction of small woody stems was consistent across all species groupings of vegetation, including non-native, native fire-adapted, and native species that were not adapted to fire. Greatest losses were observed for native, non-fire adapted (76% loss) and non-native (70% loss) species. Fire adapted species were less impacted (53% loss). The growing season fire did not result in a significant reduction of vegetation > 1 cm diameter, although some individual species declined (e.g., autumn olive, black locust, glossy buckthorn, hickory, black cherry, and sassafras). It is generally accepted that prescribed fire is not a reliable tool for killing belowground perennating tissues (Rice and Smith 2008), and that fire frequency integrated with other management techniques such as herbicide or manual removal is the most effective strategy for controlling non-native species. Treatment efficacy, however, is related to both the biology of the individual species, treatment(s) timing, and spatial extent of the invasive species (Richburg and Patterson 2003). Growing season prescribed fires occur when plants are divesting energy into aboveground structures and hence may be more effective at controlling certain species. Growing season fires can help mimic the historical seasonality and range of variability of fire in certain landscapes. As part of an overall burn regime, growing season fires can increase pyrodiversity, or the mix of fire seasons, burn intensity, and frequency. This can ultimately lead to higher biodiversity (Howe 1994; Copeland et al. 2002). Repeated early spring burns in dry-mesic southern forests may fail to set back woody species and even cause rigorous re-sprouting of certain woody species if the fire is conducted before leaf out (Cohen et al. 2015). Managers seeking to manipulate restoration regimes to include growing season fires should carefully consider the heterogeneity of fuels

within management units, as growing season fires that spread to grasslands can cause box turtle mortality (Melvin 2017: Chapter 1).

Although variable within the management area, I observed that growing season burns conducted during leaf-out coincided with some adult eastern box turtles occupying wetlands. Hence, these turtles were not directly impacted by the burn. For those turtles in the burn area, fires conducted when the turtles were fully active allowed responsive behaviors such as burying and seeking refugia in decayed logs and wet areas. I observed successful evasive behavior when flame lengths averaged approximately 8 cm and average rate of spread was approximately 1.5 m/min. My observations indicated that eastern box turtles reacted to fire and could evade fire, but I also observed mortality. Box turtles were unable to evade fast-moving fires, or gain access to fire refugia when converging flame fronts restricted movements.

Because this was a relatively small, single burn in a heterogeneous landscape that directly affected a small number of turtles, further work that determines the indirect and direct effects of fire in different vegetation types is important. Longer term studies on the relationships between eastern box turtles and fire seasonality, ignition type, ignition time of day (box turtles are mainly active during morning and evening hours in hot summer months), and intensity are crucial to conservation in areas subjected to fire. Research relating fire with indirect effects such as changes in disease prevalence, diminishing health, winter refugia site selection, nesting habits and home range are critically needed. One of the main issues with prescribed fire and box turtle conservation is how long-term mortality relates to fire frequency.

Given that the home range of an animal should, at least partially be an expression of fitness (Roloff and Haufler 1997, 2002), it follows that changes in home range space use can be used to measure animal response to environmental perturbations. The habitat quality for box

turtles appears to directly link to the abundance of leaf litter (Dodd 2001; Weiss 2009) and hence, fire removal of leaf litter should lower the quality of box turtle habitat, at least in the short term. If frequent burns maintain low leaf litter throughout a box turtle home range, it seems reasonable to assume a negative fitness consequence. Lack of significant changes in home ranges before and after prescribed fire events might indicate that turtles essentially remained in unburned patches surrounded by temporarily unsuitable burned habitat. Gibson (2009) noted that turtles not directly injured by fire maintained the extent of their home ranges following fire, but these turtles changed their space use within the home range to restricted patches of unburned leaf litter, which I also observed. This behavior could result in lower body index values throughout the growing season after a fire and behavior changes in selecting winter refugia sites, movements the following year, susceptibility to disease and indirect mortality.

Little data exist on the effects of prescribed fire on terrestrial turtle nests or hatchlings; a research topic that is hindered by lack of a cost-effective methodology for detecting terrestrial hatchlings in heavily managed areas. One of the biggest challenges facing box turtle conservation is documenting and understanding the factors that affect hatchling survival. Because hatchling box turtles are cryptic, secretive, and small, little data exists on seasonal movements, detection rates, or prescribed fire mortality. Hatchlings appear to hide under litter, which exposes them to fire, rather than burrowing or creating forms (Ernst et al. 1995), which I also observed. Because box turtles typically nest in open, grassy areas that can be subjected to frequent fire, and because hatchlings tend to stay relatively close to their nest site, further understanding seasonal movements and fire mortality is critical to overall conservation of this species.

My study is one of few that observed direct fire effects, post-fire behaviors, and vegetation effects of a growing season fire on marked terrestrial turtles. Eastern box turtles tend to occupy ecosystems, at least partially during the year, that were historically maintained by fire thus fire is often prescribed as a restoration tool in these ecosystems. Some managers may argue that because box turtles are found in areas once maintained by prescribed fire, they must inherently be a fire-adapted species. Although I observed evasive behavior to oncoming flame fronts, I also observed an individual not react. I caution that this study only observed 4 individuals and their reactions to fire. In this case, 25% of turtles encountering flame fronts did not react. I also caution that fire-adapted species, or exhibiting fire adapted behaviors, does not necessarily constitute eastern box turtles being fire dependent. For example, Willey and Sievert (2012:367) found large ranges of canopy cover (0-65%) within 5 m of eastern box turtle nests (n = 34) in Massachusetts, and noted that turtles “spent more time near the nest area if woody or herbaceous material in which to hide and forage was present”. Eastern box turtles may nest in smaller forest openings when larger grasslands are not available, and making broad overarching consensus that box turtles are fire dependent may actually result in more direct fire-related mortality of breeding adult females.

For species of conservation concern, like box turtles, managers are often tasked with restoring vegetation types to earlier successional stages, but tend to lack empirically based guidance on how these management techniques affect this species. Although my study provides that guidance, I caution that my results are based on a single prescribed fire in one management unit during the early growing season. The effects of growing season fire on eastern box turtles and vegetation likely varies by fire intensity and the amount and spatial distribution of vegetation

and debris. I encourage additional long-term studies on known cohorts of box turtles, in other fire and vegetation conditions, and studies that include effects on body condition.

3.5. Management Implications

Managers should avoid burning grasslands when adult female box turtles are seeking nesting sites, between mid-May and mid-June for southwestern Michigan. I also recommend that managers avoid firing types that result in converging flame fronts or fast-moving fronts when burning dry-mesic southern forests. Managers should also be aware that the seasonality of fire likely plays a large role in minimizing box turtle mortality. Early spring fires, if timed to box turtle emergence, have the potential to cause mortality when box turtles are lethargic after emerging from winter hibernacula. Fires later in spring or into the growing season, after box turtles have become fully active, may allow turtles to evade slow moving flame fronts. When planning for growing season fires, managers should consider plant phenological characteristics, such as the emergence and growth stages of leaves on certain deciduous tree species, the appearance and flowering of specific forest floor species along with specific weeks or days of the month when labeling fire seasonality as “spring” or “growing season”, as the timing of these events can change between years. I also recommend patchy fires that leave refugia such as decaying logs and unburned patches around seeps or wetland areas to accommodate the indirect behavioral effects of seeking long-term refuge.

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APPENDIX

Table 3.1. Pre- and post- fire average stem counts (SE) within 2 m radius circle plots (n=52) by diameter size classes for non-native woody species. These plots carried an early growing season (May 20, 2015) prescribed fire in a 31.6 ha management unit at Fort Custer State Recreation Area, Michigan, USA. Pre-fire vegetation sampling was conducted in June 2014, and plots were visually inspected in May 2015 to verify that major changes to the plant community (e.g., conversion) did not occur. Post-fire vegetation sampling was completed in September, 2015.

Non-native Species		Woody Stem Diameter Class					
		≤ 1 cm		1 < diameter < 3 cm		≥ 3 cm	
		Pre-fire	Post-fire	Pre-fire	Post-fire	Pre-fire	Post-fire
Autumn olive	<i>Elaeagnus umbellata</i>	0.90 (0.40)	0.33 (0.15)	0.69 (0.24)	0.56 (0.20)	0.48 (0.26)	0.42 (0.21)
Black locust	<i>Robinia pseudoacacia</i>	0.41 (0.18)	0.06 (0.04)	0.27 (0.11)	0.21 (0.10)	0.23 (0.15)	0.23 (0.15)
Glossy buckthorn	<i>Frangula alnus</i>	1.57 (0.29)	0.76 (0.21)	0.13 (0.07)	0.04 (0.03)	0.06 (0.04)	0.04 (0.03)
Common buckthorn	<i>Rhamnus cathartica</i>	1.45 (0.83)	0.96 (0.76)	0.02 (0.02)	0.02 (0.02)	0.00	0.00
Bush honeysuckle	<i>Lonicera</i> spp.	0.55 (0.18)	0.08 (0.08)	0.19 (0.14)	0.08 (0.05)	0.00	0.00
Multiflora rose	<i>Rosa multiflora</i>	0.55 (0.23)	0.12 (0.07)	0.04 (0.04)	0.04 (0.04)	0.00	0.00
Bittersweet	<i>Celastrus orbiculatus</i>	0.18 (0.12)	0.02 (0.02)	0.00	0.00	0.00	0.00

Table 3.2. Pre- and post- fire average stem counts (SE) within 2 m radius circle plots (n=52) by diameter size classes for native, fire adapted woody species. These plots carried an early growing season (May 20, 2015) prescribed fire in a 31.6 ha management unit at Fort Custer State Recreation Area, Michigan, USA. Pre-fire vegetation sampling was conducted in June 2014, and plots were visually inspected in May 2015 to verify that major changes to the plant community (e.g., conversion) did not occur. Post-fire vegetation sampling was completed in September, 2015.

Native, Fire Adapted Species		Woody Stem Diameter Class					
		≤ 1 cm		1 < diameter < 3 cm		≥ 3 cm	
		Pre-fire	Post-fire	Pre-fire	Post-fire	Pre-fire	Post-fire
Shagbark hickory	<i>Carya ovata</i>	1.63 (0.63)	1.04 (0.21)	0.17 (0.09)	0.13 (0.07)	0.13 (0.06)	0.12 (0.05)
Red oak	<i>Quercus rubra</i>	0.75 (0.19)	0.33 (0.13)	0.02 (0.02)	0.00	0.12 (0.07)	0.12 (0.07)
White oak	<i>Quercus alba</i>	1.10 (0.41)	0.57 (0.17)	0.08 (0.08)	0.08 (0.08)	0.12 (0.05)	0.12 (0.05)
Red osier dogwood	<i>Cornus sericea</i>	6.49 (1.29)	1.78 (0.48)	0.00	0.00	0.00	0.00

Table 3.3. Pre- and post- fire average stem counts (SE) within 2 m radius circle plots (n=52) by diameter size classes for native, woody species not adapted to fire. These plots carried an early growing season (May 20, 2015) prescribed fire in a 31.6 ha management unit at Fort Custer State Recreation Area, Michigan, USA. Pre-fire vegetation sampling was conducted in June 2014, and plots were visually inspected in May 2015 to verify that major changes to the plant community (e.g., conversion) did not occur. Post-fire vegetation sampling was completed in September, 2015.

Native, Species Not Adapted to Fire		Woody Stem Diameter Class					
		≤ 1 cm		1 < diameter < 3 cm		≥ 3 cm	
		Pre-fire	Post-fire	Pre-fire	Post-fire	Pre-fire	Post-fire
Black cherry	<i>Prunus serotina</i>	2.43 (0.61)	0.82 (0.22)	0.12 (0.06)	0.12 (0.06)	0.15 (0.08)	0.12 (0.06)
Wild black raspberry	<i>Rubus occidentalis</i>	1.69 (0.43)	0.18 (0.10)	0.00	0.00	0.00	0.00
Choke cherry	<i>Prunus virginiana</i>	1.49 (0.37)	0.25 (0.14)	0.00	0.00	0.00	0.00
Cottonwood	<i>Populus deltoides</i>	0.10 (0.07)	0.10 (0.07)	0.06 (0.06)	0.06 (0.06)	0.06 (0.04)	0.06 (0.04)
Black currant	<i>Ribes americanum</i>	0.37 (0.14)	0.04 (0.04)	0.00	0.00	0.00	0.00
Hophornbeam	<i>Ostrya virginiana</i>	0.51 (0.17)	0.20 (0.09)	0.00	0.00	0.00	0.00
Hawthorn	<i>Crataegus</i> spp.	0.00	0.00	0.02 (0.02)	0.02 (0.02)	0.00	0.00
Mulberry	<i>Morus rubra</i>	0.02 (0.02)	0.00	0.00	0.00	0.00	0.00

Table 3.3. (cont'd).

Native, Species Not Adapted to Fire		Woody Stem Diameter Class					
		≤ 1 cm		1 < diameter < 3 cm		≥ 3 cm	
		Pre-fire	Post-fire	Pre-fire	Post-fire	Pre-fire	Post-fire
Sassafras	<i>Sassafras albidum</i>	0.31 (0.13)	0.12 (0.08)	0.10 (0.08)	0.02 (0.02)	0.04 (0.03)	0.02 (0.02)
Serviceberry	<i>Amelanchier arborea</i>	0.29 (0.27)	0.00	0.00	0.00	0.00	0.00
Staghorn sumac	<i>Rhus typhina</i>	0.59 (0.35)	0.24 (0.18)	0.10 (0.10)	0.10 (0.10)	0.02 (0.02)	0.02 (0.02)
Sugar maple	<i>Acer saccharum</i>	0.69 (0.22)	0.02 (0.02)	0.06 (0.06)	0.06 (0.06)	0.00	0.00
Viburnum	<i>Viburnum</i> spp.	2.33 (0.75)	0.94 (0.55)	0.02 (0.02)	0.02 (0.02)	0.00	0.00
American elm	<i>Ulmus americana</i>	1.10 (0.41)	0.00	0.04 (0.03)	0.02 (0.02)	0.02 (0.02)	0.02 (0.02)
Wild grape	<i>Vitis</i> spp.	0.71 (0.27)	0.00	0.00	0.00	0.00	0.00

Figure 3.1. A 31.6 ha management unit consisting of a dense patch of invasive black locust (A), a 12.5 ha emergent marsh (B), dry-mesic southern (oak-hickory) forest (C), open grassland (D), red pine plantation (E), and mixed lowland hardwoods (mesic southern forest, maple-beech dominated) (F) at Fort Custer State Recreation Area, Michigan, USA.

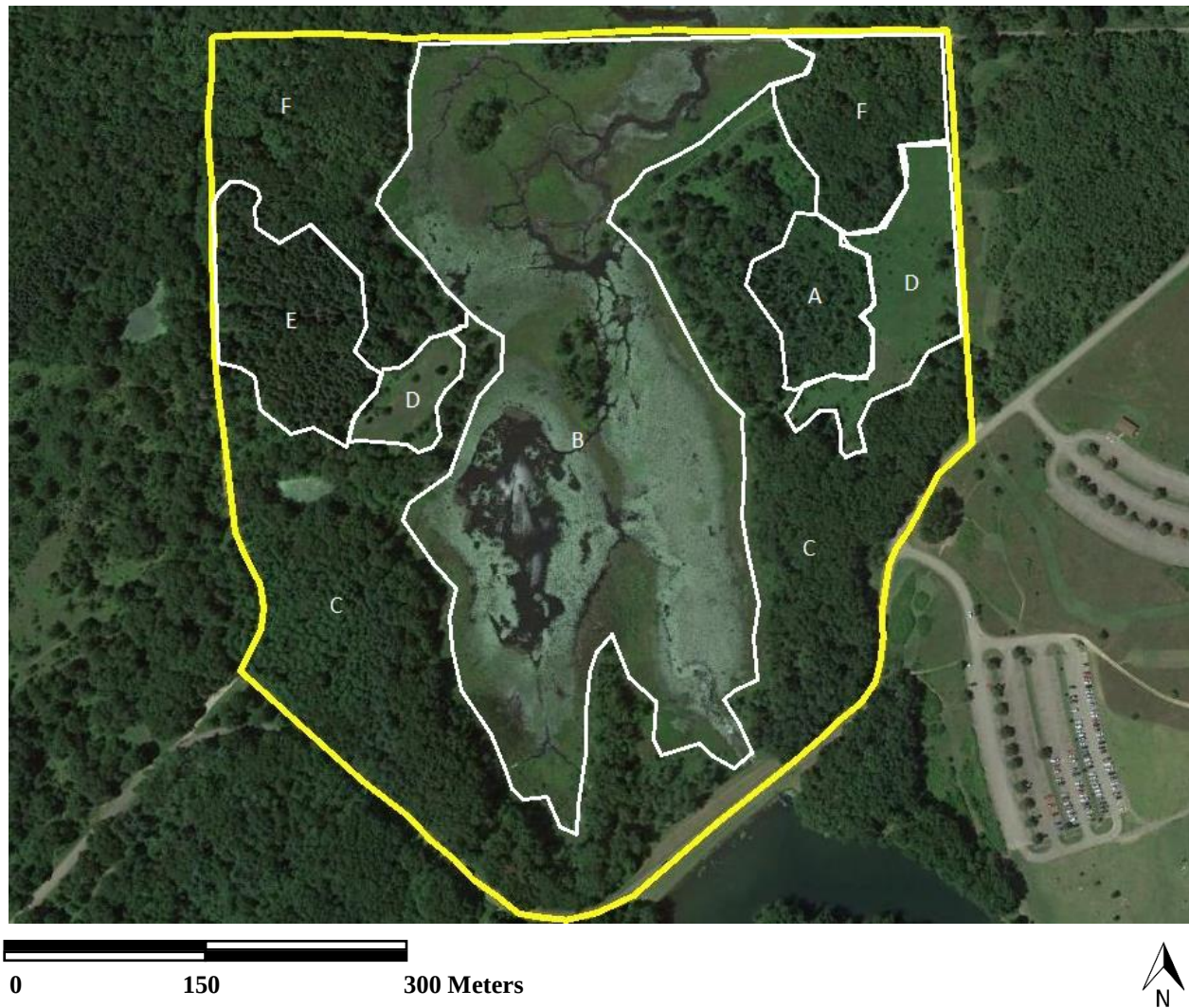


Figure 3.2. An example of a set of 3 Omega TL-10 adhesive, non-reversible labels that indicated temperature (OMEGA Engineering, INC., Stamford, Connecticut) ranging from 87 – 260 ° C. Labels were affixed to a rectangle (11 cm x 20 cm) of aluminum roof flashing. The rectangle was bent to a 90-degree angle and fixed into the soil so that the side with the temperature labels ran parallel to and 4 cm above the soil surface. This set of labels indicated temperatures reached 88° C.



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CONCLUSIONS

My thesis focused on the direct and indirect effects of prescribed fire, nesting ecology and hatchling movements, and the efficacy of post-burn surveys on eastern box turtles in a 1226 ha recreation area in Michigan, USA. I summarized the nesting behavior of females, resultant clutch sizes and successes, and hatchling movements using summary statistics and general linear mixed models. I tested the efficacy of post fire surveys and I observed the direct behavioral effects of a prescribed fire on research animals. This study is one of the first to test the efficacy of growing season prescribed fires on both vegetation and animal behavior and survival. The strengths of this research are that: 1) a known cohort of research animals were observed over 3 years, 2) the nesting ecology and hatchling movement research was conducted in relation to current tallgrass prairie restoration practices, 3) the research burn was conducted in relation to a novel prescribed fire season, 4) this is the first research that I am aware of to document post fire detection probability and direct behavioral effects of prescribed fire on eastern box turtles. The limitations of my study include: 1) small sample size ($n = 4$) of adult turtles that were directly subjected to the flame front, 2) inability to directly ascertain whether a female nested or was not detected nesting, 3) inability to determine the known longer-term fates of 51 of 58 hatchlings, and maximum straight line distances from the nest cavity and overwintering sites for 49 hatchlings, and 4) small sample size ($n = 7$ turtles) to determine detection probability.

In Chapter 1, I used radio telemetry on adult females ($n=34$), trailing string and transmitters on hatchlings ($n=58$), and field surveys of nests ($n=25$). I modeled the relationships between: 1) clutch size and physical characteristics of the associated female turtle, 2) number of eggs that developed into hatchlings as a function of year and habitat that contained the nest, and 3) number of hatchlings that emerged from the nest by year and habitat. I failed to find a

significant effect of female physical characteristics on clutch size, and I found no effect of year or habitat on the number of eggs that developed into hatchlings. I found a significant habitat effect on the number of hatchlings that emerged from the nest cavity, with a gravel pit producing significantly more emergent hatchlings than agricultural fields. Although female box turtles used agricultural fields for nesting, those nests failed to produce any emergent hatchlings suggesting that these fields serve as population sinks. My results indicated that female physical characteristics were not reliable predictors of clutch size, and that habitat factors were the primary determinant of hatchling recruitment to the turtle population, emphasizing the importance of preserving and restoring vegetation types and sites that are appropriate for hatchling emergence.

In Chapter 2, I found that average detection probability per survey was low and highly variable among surveyors. I estimated that 26 hour-long surveys per ha would be required to reliably (95% confidence) detect turtles that were present in burned areas. I also found that individual turtles directly exposed to fire remained buried for up to 12 hrs after the fire was extinguished and then rapidly moved to unburned areas. My results suggested that typical visual encounter surveys conducted for eastern box turtles after prescribed burning did not accurately reflect occupancy status or fire caused mortality.

In Chapter 3, I found that the growing season fire reduced leaf litter, and woody vegetation <1 cm diameter, including invasive plant species. Box turtles exhibited varying behaviors in response to the fire including burying and actively evading and negotiating of the flame front. One turtle died from injuries sustained in the fire and females within the management unit where prescribed fire was applied were not observed nesting that year. My

findings emphasized the importance of considering the seasonality, fire frequency, and type of fire and land management techniques in general for the conservation of this species.

I recommend that future research focuses on potential stressors to recruitment of eastern box turtles including predation, habitats that appear to act as population sinks, and hatchling vulnerabilities to prescribed fire. Understanding the nesting ecology and subsequent hatchling box turtle survival, movements and behavior in altered or fragmented landscapes is a necessary precursor to developing management strategies for their protection at the northern boundary of their range. I suggest that future research on recruitment and nest success focus on the role of agricultural fields of any cover crop, better understanding micro-scale differences in soil temperature and moisture in restored tallgrass prairies, and potential manipulation of site fidelity by females with man-made nesting areas. The efficacy of post-fire surveys for eastern box turtles likely varies by fire intensity and the amount and spatial distribution of vegetation and debris, so I encourage additional detection studies in other fire and vegetation conditions. The direct effects of growing season fire on eastern box turtles and vegetation also likely varies by fire intensity and the amount and spatial distribution of vegetation and debris. I encourage additional long-term studies on known cohorts of box turtles, in other fire and vegetation conditions, studies which include effects on body condition, and studies that ascertain the triggers box turtles might have in order to detect oncoming flame fronts, whether this is heat detection, smelling smoke, seeing the flame front, or hearing the flame front. In summary, applied research that recognizes the needs of land managers using prescribed fire to maintain many early successional vegetation types, and recognizes that these management activities may irreparably harm box turtles if not done with care and thoughtfulness, is paramount.