Overwintering behavior reduces mortality for a terrestrial turtle in forests managed with prescribed fire

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Abstract

Prescribed fire is an essential management practice in pyrogenic ecosystems, but fire can also be a significant disturbance and source of mortality for both target and non-target species. Seasonal periods of animal inactivity may provide opportunities to design burn plans that minimize negative impacts to species of conservation concern, but few studies have rigorously examined this possibility. Using radiotelemetry, we studied overwintering behavior and interactions with fire in a forest-dwelling terrestrial turtle, the Eastern Box Turtle (*Terrapene carolina carolina*), over an eight-year period at two sites that use prescribed fire in forest management. Turtles at both sites selected predominantly hardwood forests and mesic habitats and avoided upland pine forests. Turtles buried deepest (2.9 – 3.2 cm) below the soil-litter interface in late February and then moved gradually shallower until emergence in early April. Emergence timing varied over a 58-day period, but was consistent within individuals from year to year. Turtles also maintained fidelity to refuge locations, but those overwintering in burned areas selected sites over twice as far from refuges used in previous years compared to those in unburned areas. The areas and habitats selected by turtles during winter served as refugia from fire, and those whose refuges did burn remained buffered from lethal temperatures even at shallow burial depths. The only fire-related injury or mortality occurred during seasons of surface activity. Timing burning and other forest management practices during periods of winter dormancy may thus minimize threats to turtle populations, but modifications to prescribed fire regimes must also be balanced with other management objectives.

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Keywords

1. Introduction

Prescribed fire has become a common practice used to achieve a variety of land management goals such as wildfire hazard reduction, control of invasive vegetation, biodiversity conservation, and other objectives (Haines et al. 2001, Pastro et al. 2011). In the case of biodiversity conservation, fire may be used to maintain specific environmental conditions required for target fire-dependent biota (Keeley et al. 2011, Steen et al. 2013, Pausas and Parr 2018), and to provide disturbances that maintain spatio-temporal habitat heterogeneity that supports higher species diversity (Brockett et al. 2001, Darracq et al. 2016). However, fire can negatively modify environments and be a significant source of mortality for target and non-target biota (Webb and Shine 2008, Lyet et al. 2009, Valentine and Schwarzkopf 2009, Humphries and Sisson 2012, O'Donnell et al. 2015). In such cases, fire can present a conflict for land managers that may require conservation trade-offs or careful evaluation of prescribed fire regimes that minimize collateral damage to native biota.

Longleaf Pine (*Pinus palustris*) forests of the southeastern United States are an area of high species diversity, with many plants and animals dependent on periodic fire disturbance (Means 2004, Van Lear 2005). The Longleaf Pine ecosystem has declined by 97 % from its original extent due to overharvest, land clearing, fire suppression, and other factors (Noss 1989, Frost 1993), resulting in the imperilment of many native species (Van Lear 2005). Natural wildfires historically burned at a frequency of one to six years (Frost 1998), but the details of past fire regime (e.g., frequency, seasonality, and severity) are complex and vary depending on interactions between several environmental and anthropogenic factors (Stambaugh et al. 2011, Rother et al. 2020). Currently, forest managers set prescribed fires that mimic the historic disturbance regimes to restore and maintain this system, but fire management is often driven by the habitat requirements of select target species of highest conservation priority, such as the endangered Red-cockaded Woodpecker (*Picoides borealis*) in Longleaf Pine ecosystems (James et al. 1997, Hiers et al. 2014). However, depending on management objectives, there are numerous inter-related aspects of prescribed fire regimes that can be varied to modify fire behavior and its effects on biota, including fire frequency, seasonal timing, spatial configuration and size of burn units, ignition methods, target weather conditions, and others (Lashley et al. 2014). Natural resource managers may thus implement burn plans that reduce the impact of fires on non-target species that are also of conservation concern, while still accomplishing other management goals.

The southeastern United States is an area of high biodiversity and a conservation priority for turtles (Buhlmann et al. 2009). Most turtle species in the region associate with aquatic habitats and would only be vulnerable to fire during typically brief periods of terrestrial activity or wetland drying. However, species that spend the majority of their life cycle in terrestrial environments would be more frequently exposed to and potentially affected by

forest fires. Some terrestrial turtles are capable of coexisting in natural fire regimes (Ashton et al. 2008, Pawelek and Kimbal 2014), while others may experience high mortality or injury (Hailey 2000, Esque et al. 2003, Sanz-Aguilar et al. 2011, Platt et al. 2010, Howey and Roosenberg 2013, Roe et al. 2019). Turtle populations are particularly sensitive to adult mortality as a result of slow somatic growth, delayed sexual maturity, and high natural mortality in the early life stages (Brooks et al. 1991, Congdon et al. 1993, 1994, Heppell 1998). Natural resource managers may thus need to consider the effects of prescribed fire regimes on terrestrial turtle species and tailor burn plans to reduce negative impacts, including mortality resulting from management practices, to their populations.

The activities of reptiles are strongly constrained by seasonal and episodic changes to thermal and hydric environmental conditions. For instance, as ectotherms, turtles do not generate heat sufficient to maintain body temperatures to support activity during the winter, so they enter a period of dormancy (Gregory 1982). Terrestrial reptiles in temperate zones typically overwinter for several months in select habitats and enter underground refuges to escape predators, extreme temperatures, and other physiological stresses during dormancy (Gregory 1982, Claussen et al. 1991, Harvey and Weatherhead 2006, Gienger and Beck 2011). Natural resource practitioners may thus plan management practices, such as prescribed fire, to coincide with reptile inactive periods to minimize disturbance and mortality, as has been suggested by several researchers (Hailey 2000, Lyet et al. 2009, Harris et al. 2015, DeGregario et al. 2017, Hileman et al. 2018). Such limited operating periods, where prescribed fire is timed to avoid critical behaviors or activity periods, are often implemented or suggested to protect various species of native wildlife including amphibians (Humphries and Sisson 2012, O'Donnell et al. 2015), birds (Tucker and Robinson 2003, Tucker et al. 2004, Cox and Jones 2007), and mammals (Thompson and Purcell 2016). However, to design an effective management strategy that minimizes negative consequences for vulnerable non-target biota, managers require detailed information on the locations and environments of refuges and the seasonal timing of their use relative to existing and proposed prescribed fire regimes.

The Eastern Box Turtle, *Terrapene carolina*, is a terrestrial turtle found throughout forests of the eastern United States (Dodd 2001, Kiester and Willey 2015), but comparatively little information is available on their ecology in fire-managed Longleaf Pine systems (but see Greenspan et al. 2015, Roe et al. 2017, 2018, 2019, 2020). *Terrapene c. carolina* populations are in decline throughout much of their range (Stickel 1978, Williams and Parker 1987, Hall et al. 1999, Nazdrowicz et al. 2008), resulting in their listing as a species of conservation priority in more than half of the states in which it occurs (Erb et al. 2015, Kiester and Willey 2015). While not a widespread threat to box turtle populations, fire can have a number of deleterious effects on localized populations, including high mortality, injury, and reduced body condition (Platt et al. 2010, Howey and Roosenberg 2013, Roe et al. 2019). For example, annual survival for *T. c. carolina* was only 45.9 % in areas with the most intensive prescribed fire management at one site in North Carolina (Roe et al. 2019), and prescribed fire resulted in up to 21.6 % mortality in a Florida population of *T. c. baurii* (Platt et al. 2010). Because *T. c. carolina* typically burrows only shallowly in loose soil and ground litter during winter dormancy (Congdon et al. 1989, Costanzo and Claussen 1990, Claussen et al. 1991, Currylow et al. 2013), they may be exposed to extreme environmental conditions

such as temperature fluctuations and other disturbances on the forest floor. Thus, selection of suitable retreat sites and the timing of their use is critical for overwinter survival and other fitness outcomes (Grobman 1990, McCallum et al. 2009, Savva et al. 2010).

Here, we use a long-term dataset (8 yrs.) to investigate individual variation and repeatability in several aspects of *T. c. carolina* overwintering ecology that could influence vulnerability to prescribed fire. We examine habitat selection, burial depth, emergence timing, and survival relative to prescribed fire regimes for two nearby *T. c. carolina* populations in the sandhills and coastal plain regions of North Carolina. The study sites differ in natural environments and their historic and current use of fire, with the sandhills site comprised of predominantly xeric pine forests that have been managed with prescribed fire for several decades, and the coastal plain site comprised of mostly unburned bottomland hardwood forests and a recently initiated and less extensive prescribed fire program. We hypothesize that turtles would be least vulnerable to fire during overwinter dormancy, and that they would select habitats and bury to depths that would confer protection from environmental extremes (including fire) during winter. We expect selection of mesic environments (hardwood forests and aquatic habitats) would be strongest at the more xeric fire-managed site, in part due to the limited availability of these habitats in the sandhills region and the refuge such environments may offer from fire (Roe et al. 2018, 2019). We also hypothesize that turtles would be consistent in their selection of refuge sites and timing of emergence over time given the individual repeatability of overwintering (Refsnider et al. 2012, DeGregorio et al. 2017) and other behaviors (Rittenhouse et al. 2008, Kashon and Carlson 2018, Roe et al. 2020) in *Terrapene* populations. Such information would not only improve our understanding of overwintering ecology in *T. c. carolina*, but it could assist land managers in tailoring fire management plans that minimize negative effects to *T. c. carolina* in Longleaf Pine and other fire-managed systems.

2. Materials and Methods

2.1. Study sites

We conducted the study at two sites, including Weymouth Woods Sandhills Nature Preserve (hereafter Weymouth Woods) and the Lumber River State Park (hereafter Lumber River). Weymouth Woods is an approximately 200-ha site in the Sandhills Level IV Ecoregion (Griffith et al. 2002), and is comprised of a forest mosaic of mixed pine and hardwood forests (Fig. 1), including Longleaf Pine (*Pinus palustris*) and Loblolly Pine (*P. taeda*), along with several species of hardwood such as oak (*Quercus* spp.), hickory (*Carya* spp.), Red Maple (*Acer rubrum*), Sweetgum (*Liquidambar styraciflua*), American Holly (*Ilex opacum*), Sassafras (*Sassafras albidum*), and Tuliptree (*Liriodendron tulipifera*). The majority of the site is xeric uplands with a small stream network and associated bottomlands (Fig. 1). Prescribed fire has been used regularly in forest management since 1974, with 76% of the area being managed using low-intensity controlled burns ranging in size from 0.9–23.9 ha (5.2 ± 3.2 ha; mean ± standard deviation), with a historic mean burn frequency of every 5.8 y (range 1.8–20 y) from 2000–2019 (Weymouth Woods Sandhills Nature Preserve, unpubl. data).

The Lumber River site is an approximately 225-ha reserve in the Atlantic Southern Loamy Plains and Southeastern Floodplains and Low Terraces Level IV Ecoregions (Griffith *et al.* 2002). The habitat of Lumber River includes extensive riverine bottomland swamp forests with Bald Cypress (*Taxodium distichum*), tupelo (*Nyssa* spp.), Tuliptree, Sweetgum, Red Maple, and Atlantic White Cedar (*Chamaecyparis thyoides*) along with upland mixed pine and hardwood forests comprised of Loblolly and Longleaf Pine, oaks, and hickory (Fig. 1). A single controlled burn conducted in March 2017 covered 17 % of the park area (37.2 ha), but fire had not been previously used in management since designation as a state park in 2001 (Lumber River State Park, unpubl. data).

2.2. Habitat mapping

We determined the spatial distribution of forest types and aquatic habitats within park borders by walking transects along an established grid dividing each site into 50×50 m cells using ArcMap 10.2.2 (Esri, Redlands, California, USA). At the center of each grid cell, we counted trees in the surrounding area using a CRUZ-ALL angle gauge (Forestry Suppliers, Inc., Jackson, Mississippi, USA) by rotating 360° while holding the gauge at head height and counting the number of tree trunks that completely filled (or more than filled) the 10-factor gauge opening. We divided trees into either pine or hardwood classes and calculated the relative proportion of each category in each cell. We also determined whether each grid cell was in a bottomland habitat by assessing several field indicators, including surface water presence, signs of recent flooding (watermarks, debris and substrate scouring), plant communities, and animal sign (e.g., crayfish burrows). We mapped river and stream networks by paddling a canoe or walking each watercourse following heavy rainfall. We collected coordinate positions of each river or stream channel using hand-held GPS units (GPS 72H, Garmin, Olathe, Kansas, USA) and digitized the network as polylines using ArcMap 10.2.2. By mapping during high water, we were able to include areas where surface water flows were present on a temporary basis. For more details about habitat mapping and classifications, see Roe et al. (2018). Finally, we delineated fire areas at Lumber River by walking the perimeter of controlled burns and recording coordinate positions using GPS. We then digitized fire borders as polygons using ArcMap 10.2.2. At Weymouth Woods, the state park provided data on the spatial coverage and timing of controlled burns.

2.3. Turtle capture and radiotracking

We initially captured turtles during visual searches from a variety of forest types at each site, including from historically burned and unburned areas and bottomland and upland areas. We measured midline carapace length (CL) to the nearest 0.1 mm using vernier calipers and mass to the nearest gram using a spring scale. We determined sex by observing several sexually dimorphic features, with males typically having a concave posterior plastron, stouter and longer curved claws on hind feet, a red iris, and thicker and longer tails compared to females (Palmer and Braswell 1995). We attached radiotransmitters (RI-2B, 10 – 15 g, Holohil Systems Ltd., Carp, ON, Canada) to the posterior of the carapace (offset to either the right or left side) using 5 minute epoxy gel (Devcon, Solon, OH). From April 2012 – May 2020, we tracked 57 turtles (15 males and 16 females from Weymouth Woods, 14 males and 12 females from Lumber River) for periods of three months to 8 years. Turtles from Weymouth Woods had mean (± standard deviation) initial CL of 129.5 ± 8.9 mm

(range: 109.3 – 149.6 mm) and body mass of 415.9 ± 73.9 g (range: 260 – 575 g), while turtles from Lumber River had initial CL of 136.0 ± 9.6 mm (range: 124.1 – 151.7 mm) and body mass of 451.5 ± 74.9 g (range: 330 – 615 g).

We located telemetered turtles using a receiver (R-1000, Communication Specialists, Orange, CA) and Yagi antenna once per week during the active season (May – September), every two weeks during overwinter ingress (October – November) and egress (March – April), and once per month for the remainder of the overwintering period (December – February). At each location, we determined the coordinate position using GPS and plotted locations on maps using ArcMap 10.2.2. We classified refuge locations as those where the turtle became inactive underground for an extended period, typically lasting several months, between October and May. Following fires, we located each turtle within 24 h and assessed whether it was alive, dead, or had suffered a fire-related injury.

2.4. Temperature monitoring

In October of each year, we equipped a subset of radiotracked turtles with temperature data loggers (Thermocron iButton, Dallas Semiconductor, Dallas, TX). We sealed all temperature loggers with rubber coating (Plasti Dip International, Blaine, MN) and attached them with epoxy to the posterior of the carapace opposite the radiotransmitter, approximately at the midpoint along the dorsal and ventral axis of the body. When turtles became inactive, we monitored environmental temperatures within 1.5 m of refuge locations using iButtons attached to a wooden stake buried in the ground along a depth gradient. Temperature loggers at each station recorded air temperature 10 cm above the ground surface, at the litter-soil interface (0.0 cm), and at depths of 2.5, 5.0, 10.0, and 20.0 cm below the litter-soil interface. We programmed all turtle and environmental temperature loggers to record temperature at 180-minute intervals.

We compared shell temperature (Ts) to environmental temperatures (Te) at the local monitoring station to estimate daily burial depth and timing of emergence on the surface using methods similar to other studies (Bernstein and Black 2005, Currylow et al. 2013, Frega and Haas 2015, Harris et al. 2015). We calculated mean daily temperatures for each turtle (Ts and Te for air, litter, and the various burial depths) and determined which Te monitoring position was most similar to Ts. We considered turtles to be at the depth where Ts matched Te most closely; when Ts matched Te at two depths simultaneously, we assumed the turtle was midway between the two depth locations. We considered turtles to have surfaced when Ts most closely matched Te of the air. The temperature of the shell and body closely matches temperature of the immediate surrounding environment under most circumstances in *T. carolina* (Parlin et al. 2017, Roe et al. 2017).

In February 2019, we recorded environmental temperatures at 1-minute intervals using iButtons at five locations during two controlled burns. At each location, we placed temperature loggers at the litter-soil interface (0.0 cm), and at depths of 1.5, 2.5, and 4.0 cm below the ground. We placed three of these stations within 1.5 m of turtle refuge locations, and the other two in similar habitats nearby.

2.5. Data analyses

We performed statistical analyses with SPSS v. 25.0 (SPSS Inc., Chicago, Illinois, USA). Where appropriate, we examined assumptions of homogeneity of variances and normality and made appropriate transformations when data failed to meet assumptions. We accepted statistical significance at α 0.05 unless otherwise noted. We report values as mean $\pm$ 1 SE unless otherwise stated. Even though we did not develop any explicit hypotheses regarding sex or body size, we include these independent variables in analyses given their important influence on turtle behavior and vital rates in our study system (Roe et al. 2018, 2019, 2020).

For turtles studied over multiple years, we measured the straight-line distance between sequential refuge locations as an estimate of geographic fidelity. When turtles moved during the overwintering period, we used the location where the turtle remained for the longest period in analyses. To examine sources of variation in geographic fidelity to refuge locations, we used linear mixed effects models with distance between locations as the dependent variable, site, sex, site × sex, and year as independent variables, CL as a covariate, and individual as a repeated variable. At Weymouth Woods, where prescribed fire was used more regularly in forest management, we examined the effect that fire had on site fidelity using a linear mixed effects model with fire exposure as an additional independent variable. We $\log_{10}$-transformed all distance and CL values prior to analyses.

To examine sources of variation in habitat use, we calculated the relative proportion of pine and hardwood trees (forest classes) in the overlapping grid cell, and measured the straight-line distance to the nearest stream and bottomland habitat for each refuge location. We used a series of linear mixed effects models with forest class, stream, and bottomland measures as dependent variables, site, sex, site × sex, and year as independent variables, CL as a covariate, and individual as a repeated variable. To assess whether individuals selected habitats different from that habitat's availability, we then compared forest class, stream, and bottomland measures at turtle locations to paired random points using linear mixed effects models with proportion (forest class) or distance (stream and bottomland) as the dependent variable, location (turtle or random) as the independent variable, and individual as the repeated variable. We generated paired locations using the create random points tool in ArcMap and constrained points within each individual's home range, defined as the minimum convex polygon (MCP) encompassing all active and overwintering season locations. We used Geospatial Modeling Environment (Beyer 2012) and R (R Core Team 2017) as extensions of ArcMap to generate MCPs. For comparisons of use to availability (i.e., selection), we used mean values of habitat measures for each individual if there was no variation among years, and only retained independent variables in statistical models that were identified as significant sources of variation in the analyses of habitat use (above). Only locations within the state park borders where habitats were delineated were used in analyses of habitat selection. We arcsin-transformed all forest class proportions and log10-transformed all distances and body sizes prior to analyses.

To examine sources of variation in burial depth among and within individuals over time, we used linear mixed effects models with depth as the dependent variable, site, sex, time, and interactions as independent variables, CL as a covariate, and individual as a repeated variable. We used the mean burial depth over nine two-week intervals from 1 Jan to 1

May for each individual in this analysis, including only one year for each individual. We log$_{10}$-transformed depth prior to analyses.

We examined sources of variation in emergence timing using linear mixed effects models with date (number of days past 1 Jan) as the dependent variable, site, sex, year, and interactions as independent variables, CL as a covariate, and individual as a repeated variable. We log$_{10}$-transformed number of days prior to analyses. For 16 individuals (9 from Weymouth Woods, 7 from Lumber River), we assessed emergence timing for two years, and examined whether emergence date was repeatable using a Spearman's rank-order correlation test, running one overall analysis combining sites and sexes.

3. Results

3.1. Site fidelity

Male and female turtles used locations 114.0 ± 35.5 m and 82.1 ± 16.7 m (range: $0 - 997$ m) from their previous years' overwintering refuges at Weymouth Woods, and 110.1 ± 22.2 m and 114.5 ± 28.5 m (range: $4 - 676$ m) from their previous year's refuges at Lumber River. Fidelity to refuge sites was consistent over years and did not vary by site, sex, body size, or any interactions among variables (site: $F_{1,191.262} = 1.932$, $P = 0.166$; sex: $F_{1,192.612} = 0.006$, $P = 0.940$; site $\times$ sex: $F_{1,193.077} = 3.346$, $P = 0.073$; year: $F_{6,20.990} = 0.326$, $P = 0.916$; CL: $F_{1,183.822} = 0.055$, $P = 0.815$). Fire had a strong effect on fidelity to overwintering refuges at Weymouth Woods ($F_{1,53.441} = 27.287$, $P < 0.001$), with distances between refuges of 161.7 ± 43.7 m for individuals in fire-maintained areas compared to 72.5 ± 6.8 m in unburned areas.

3.2. Habitat selection

Turtles at Weymouth Woods used forests comprised of 59.2 ± 5.8 % hardwood and 40.2 ± 5.8 % pine (Fig. 1), while turtles at Lumber River used forests comprised of 66.4 ± 4.4 % hardwood and 33.4 ± 4.3 % pine (Fig. 1). Use of hardwood forests did not differ between years, body sizes, sexes, or sites, but did vary according to the site $\times$ sex interaction (Table 1). Females at Lumber River used forests with more hardwood (64.0 %) compared to Weymouth Woods (55.2 %), whereas differences were less pronounced in males (68.9 % at Lumber River, 63.1 % at Weymouth Woods). Turtles at both sites used hardwood forests more frequently than this habitat's availability (location: $F_{1,91.954} = 7.631$, $P = 0.007$), but the degree of difference between use and availability differed between sites, with turtles from Weymouth Woods using hardwood forests 19.2 % more than expected from random, and turtles from Lumber River using hardwood forests only 10.2 % more than expected from random (site $\times$ location: $F_{2,46} = 3.243$, $P = 0.048$; sex $\times$ location: $F_{2,46} = 0.271$, $P = 0.764$; site $\times$ sex $\times$ location: $F_{2,46} = 0.049$, $P = 0.952$).

Use of pine forests did not differ between years, body sizes, sexes, or sites, but did vary according to the site $\times$ sex interaction (Table 1). Females at Weymouth Woods used forests with more pine (44.9 %) compared to Lumber River (35.6 %), whereas differences were less pronounced in males (36.9 % at Weymouth Woods, 33.1 % at Lumber River). Turtles at both sites used pine forests less frequently than this habitat's availability (location: $F_{1,91.999} = 8.026$, $P = 0.006$), but the degree of difference between use and availability differed between

sites, with turtles from Weymouth Woods using pine forests 19.7 % less than expected from random, and turtles from Lumber River using pine forests only 10.7 % less than expected from random (site × location: $F_{2,46}$ = 3.427, P = 0.041; sex × location: $F_{2,46}$ = 0.244, P = 0.785; site × sex × location: $F_{2,46}$ = 0.023, P = 0.978).

Refuge locations were 36.6 ± 5.3 m from streams and 146.9 ± 41.6 m from bottomland habitats at Weymouth Woods, and 92.2 ± 14.1 m from streams and 43.0 ± 12.6 m from bottomland habitats at Lumber River. Proximity to streams differed between sites and body sizes, but not according to sex, year, or any interactions among variables (Table 2). Turtles at Weymouth Woods were found 55.6 m closer to streams than those at Lumber River, and smaller turtles were found closer to streams than larger turtles (t = 2.311, P = 0.022). Turtles were found closer to streams than expected from random (location: $F_{1,93.834}$ = 19.534, P < 0.001), but the degree of difference between use and availability differed between sites (location × site: $F_{2,48}$ = 7.858, P < 0.001). Turtles at Weymouth Woods were 53.8 m closer to streams than expected from random, compared to 34.5 m closer than expected from random at Lumber River.

Proximity to bottomlands differed between sites, but did not vary according to sex, body size, year, or any interactions among variables (Table 2). Turtles at Lumber River were found 103.9 m closer to bottomlands than those at Weymouth Woods. Turtles were found closer to bottomlands than expected from random (location: $F_{1,86.925}$ = 10.140, P = 0.002), but the degree of difference between use and availability differed between sites (location × site: $F_{2,48}$ = 3.624, P = 0.034). Turtles at Weymouth Woods were 63.1 m closer to bottomlands than expected from random, compared to 26.1 closer than expected from random at Lumber River.

3.3. Burial depth and emergence timing

Burial depth varied over time, but did not differ between sites, sexes, body sizes, or any interactions among variables (Table 3, Fig. 2). Turtles were deepest on 24 February at 3.2 ± 0.7 cm and 2.9 ± 0.9 cm below the soil-litter interface at Lumber River and Weymouth Woods, respectively (Fig. 2). However, burial depths varied among individuals, with some individuals buried only under litter above the soil (0.0 cm depth) throughout the entire overwintering period, and others buried up to 11.8 cm into the soil. Burial depths became progressively shallower from late February through early April when turtles began to emerge on the surface (Fig. 2).

Emergence timing did not vary by site, sex, body size, year, or any interactions among variables (site: $F_{1,57.999}$ = 1.332, P = 0.253; sex: $F_{1,57.956}$ = 0.043, P = 0.837; site × sex: $F_{1,57.965}$ = 0.477, P = 0.493; year: $F_{1,24.594}$ = 0.002, P = 0.963; CL: $F_{1,57.797}$ = 0.248, P = 0.620). Mean surface emergence occurred on 5 April (± 3.1 days) and 9 April (± 3.0 days) at Weymouth Woods and Lumber River, respectively, and on 6 April (± 3.1 days) and 8 April (± 3.1 days) for females and males, respectively. However, surface emergence varied among individuals, with some emerging as early as 9 March and others as late as 5 May, a period spanning 58 days (Fig. 3). For the 16 individuals observed for multiple years, timing of spring emergence was repeatable, with an individual's emergence date in one year being a

strong predictor of its emergence date in subsequent years, accounting for 70.3% of variation ($r_s = 0.703$, $P = 0.002$; Fig. 4).

3.4. Fire temperature

The hottest temperatures recorded in the soil column during controlled burns were at the soil-litter interface at the time the fire passed over the monitoring stations, but temperatures remained cooler with increasing soil depth (Fig. 5). Temperatures generally remained between 14.1 – 17.8 °C in all parts of the soil column where turtles were buried, with the maximum recorded temperature at any monitoring station 23 °C at the soil-litter interface.

3.5. Turtle and fire interactions

From 2012 – 2020, 321.5 ha of forest was burned at Weymouth Woods, with the majority (67.1 %) of prescribed fires occurring in the active season from April through November (Fig. 6). There were 17 instances of turtles occupying a burn unit during a prescribed fire. Seven had fires burn over them in January and February while still underground where they remained until emerging uninjured several months later. Ten encountered fire while active on the surface between April and August. Of these ten turtles, six survived uninjured by sheltering in more mesic unburned patches or by moving across burn boundaries, one suffered burn injuries to its carapace but survived, and three died during the fires (Fig. 6).

At the Lumber River, 37.3 ha of forest was burned in March of 2017. Only two radiotracked turtles were located in the burned area, both of which emerged from refuges either during or soon after the fire and were found uninjured in nearby wet areas.

4. Discussion

Prescribed fire is essential in the management of Longleaf Pine and other pyrogenic systems. While several studies have examined responses of non-target wildlife to fire management regimes (Wilson et al. 1995, Russell et al. 1999, Pilliod et al. 2003, Fontaine and Kennedy 2012, Darracq et al. 2016, Thompson and Purcell 2016), few rigorous and systematic studies have focused on the impacts of prescribed fire and potential mitigation options in turtles. Terrestrial turtles, such as *T. carolina*, may be especially vulnerable to surface fires (Platt et al. 2010, Howey and Roosenberg 2013, Roe et al. 2019), but land managers typically lack detailed information on their responses to fire. Our large sample size of turtles tracked over an eight-year period across a range of environmental and management contexts allowed us to rigorously examine interactions between turtle overwintering behavior and fire management regimes, with the purpose of collecting targeted data to assist managers in modifying burn plans to reduce negative outcomes. The most important findings relevant to prescribed fire management were that 1) turtles selected hardwood forests near streams and other mesic habitats that offered refuge from fire during winter, 2) turtles buried only shallowly in underground refuges during winter but at depths that buffered them from exposure to potentially damaging or lethal temperatures during fire, 3) timing of spring egress from overwintering refuges was variable among individuals, with emergence spanning a two-month period that peaked in early- to mid-April, 4) overwintering behaviors did not differ according to sex or body size and were consistent within individuals over

time, but turtles in fire-maintained forests maintained weaker year-to-year fidelity to overwintering sites, and 5) fire-related injury and mortality occurred only during seasons of surface activity after emergence from overwintering refuges. This study provides the strongest evidence yet that periods of winter dormancy present opportunities to schedule fire and potentially other forest management practices to minimize threats to turtle populations.

Several aspects of turtle behavior could influence risks of fire management practices, including the degree to which the population utilizes environments targeted in burn plans. At both study sites, managers primarily target the more xeric upland forests that are populated with Longleaf and other pines in order to suppress invasion of non-pyrophytic vegetation and maintain an open understory of herbaceous groundcover (Fig. 1). Because turtles selected predominantly hardwood forests in close proximity to streams and bottomlands while avoiding the drier upland pine forests, turtles rarely overlapped with fire-managed areas during winter. Despite burning over 320 ha and 76 % of the park property, only 23.7 % of winter refuge locations at Weymouth Woods were in areas burned at any point during the study, with only seven instances of fire burning directly over dormant turtles. Likewise, 37 ha were burned at Lumber River, representing 17 % of the park area, but only 11 % of refuge locations overlapped with fire-managed areas and two instances of fire burning over dormant turtles.

Selection of hardwood forests and mesic habitats is consistent with other *T. carolina* populations across their range (Donaldson and Echternacht 2005, Rossel et al. 2006, ittenhouse et al. 2008, Kapfer et al. 2013, Greenspan et al. 2015, Kiester and Willey 2015, Parlin et al., 2017, Roe et al. 2018), but to our knowledge, this is the first study to explicitly assess habitat selection during winter inactivity. The dense canopy, complex understory structure, and ground debris of hardwood forests likely provide favorable thermal and moisture conditions in the active season (Reagan 1974, Dodd 2001, Parlin et al. 2017, Roe et al. 2017, 2018) that would also benefit *T. c. carolina* during wither dormancy to buffer from exposure to freezing temperatures and offer concealment from predators. Likewise, selection of mesic habitats such as wetlands and streams would allow turtles to maintain positive water balance (Penick et al. 2002), especially given their limited mobility during winter. Indeed, smaller turtles were more closely associated with streams, likely reflecting their relatively high evaporative water loss rates and lower total body water (Foley and Spotila 1978; Finkler 2001). As expected, the selection of hardwood forests and mesic habitats was strongest at Weymouth Woods, which may reflect the limited availability of these habitat types in the Sandhills physiographic region more than increased usage of these habitats by *T. c. carolina*. Prescribed fire is also used more intensively in forest management at Weymouth Woods, which may indirectly influence the quality and spatial configuration of available habitat by the temporary removal or reduction of understory vegetation, woody debris, leaf litter and associated alteration of microclimate conditions (York 1999; Iverson and Hutchinson 2002; Greenberg and Waldrop 2008; Hossack et al. 2009). Fire intensity is highest in the higher elevation uplands and decreases in the lower elevations near bottomlands and streams (Roe et al. 2018), and fires burn hottest and most frequently in the dry, aerated, fine, and resinous litter of pine forests (especially Longleaf Pine) compared to hardwood forests (Williamson and Black 1981, Mitchell et al. 2009). The stronger selection of mesic forests at Weymouth Woods, together with the negative fitness

consequences of fire in this and other fire-maintained systems (Platt et al. 2010, Howey and Roosenburg 2014, Roe et al. 2019) suggests the possibility of behavioral adaptations to avoid fire in *T. carolina*, as has been demonstrated in other animals (Pausas and Parr 2018). However, habitat selection differences may also reflect phenotypically plastic responses in *T. carolina* (Rittenhouse et al. 2008, Roe et al. 2018), and further replication of studies comparing behavior at sites that differ in natural and prescribed fire regimes are necessary to further explore the causes and consequences of *T. carolina* responses to fire. Regardless of the mechanism responsible, the selection of hardwood forests and mesic habitats affords *T. c. carolina* refuge from fire during winter and other inactive periods. However, we caution that hardwood species differ in their responses to fire and environmental associations (e.g., pyrophytic vs. mesophytic; Hiers et al. 2014), and that finer-scale measures of forest composition that distinguish between species or ecotypes would be instructive in elucidating habitat selection in *T. c. carolina* in fire-managed systems.

Another important consideration when assessing the risk that fire and other forest management practices pose to turtles during seasonal inactivity is the burial depth of refuges. For most forest fires, temperature is hottest on the surface and decreases sharply with depth underground, although fuel characteristics, substrate moisture content, weather conditions, and other factors interact to influence fire behavior, maximum temperatures, and below ground heat transfer (Valette et al. 1994, Bradstock and Auld 1995, Neary et al. 1999). Biological disruptions generally occur at 40 – 70 °C (Neary et al. 1999), and extended exposure to temperatures at the lower end of this range (39 – 44 °C) is typically lethal to turtles (Hutchison et al. 1966, Sturbaum 1981, Lagarde et al. 2012). Fire-related mortality during seasonal inactivity was linked in part to burial depth in terrestrial tortoises (*Testudo graeca*), with smaller individuals burying in shallow leaf litter and experiencing higher mortality than larger deeper burrowing individuals (Sanz-Aguilar et al. 2011). Unlike some tortoises (e.g., *Gopherus polyphemus* and *G. agassizii*) that dig extensive burrow systems that can buffer against extreme surface temperatures (Zimmerman et al. 1994, Pike and Mitchell 2013), *T. c. carolina* typically burrows shallowly under leaf litter and loose soil, or in pre-existing burrows as deep as 10 cm underground (Congdon et al. 1989, Costanzo and Claussen 1990, Claussen et al. 1991, Ellington et al. 2007, Currylow et al. 2013). In our study, winter burial depth varied temporally in both populations, with turtles being deepest at 2.9 – 3.2 cm below the soil-litter interface during late February and then moving gradually shallower until emergence in early April, with no differences in burial depth between sexes or among the body sizes examined. Surface temperatures during prescribed fire generally range from 350 – 600 °C in Longleaf Pine and oak hardwood forests (Williamson and Black 1981, Mitchell et al. 2009), but our measures of sub-surface temperature during fires never exceeded 23 °C at the soil-litter interface and decreased with increasing depth as predicted. Turtles would thus not have been exposed to lethal temperatures even at the shallowest burial depths. This mild temporary increase in temperature resulted in minimal disruption, as all turtles that had fire burn over their refuges either remained stationary underground or moved to nearby unburned locations and resumed dormancy. Similarly, overwintering turtles either remained in place or moved short distances after experiencing non-lethal temperature increases in another *T. c. carolina* population exposed to experimental prescribed fires in mixed pine-hardwood forests (Fredericksen et

al. 2015). We caution that our sampling of sub-surface temperatures was limited, and that exposure to lethal temperatures in underground refuges may vary by habitat type, fuel characteristics, weather conditions, fire strategy (e.g., head fire vs. backing fire), and other factors relevant to prescribed fire regimes.

Another factor that could influence fire-related risks is the timing of seasonal activity and behavior. Several studies have suggested timing prescribed fires to coincide with seasonal periods of inactivity, where animals may either be underground or in habitats that offer refuge from fire, may reduce mortality for several species of ectothermic vertebrates, including amphibians (Frese 2003, Humphries and Sisson 2012), snakes (Frese 2003, Lyet et al. 2009, Hileman et al. 2018), and turtles (Dodd 2001, Platt et al. 2010, Sanz-Aguilar et al. 2011, Harris et al. 2015, Reid et al. 2016, DeGregario et al. 2017). Annual mortality rates for *T. c. carolina* in the most frequently burned forests at out study sites are as high as 54 % (Roe et al. 2019), but no fire-related mortalities or injuries were observed during winter dormancy periods despite 33 % of fires occurring at this time. Similarly, *Terrapene carolina bauri* experienced high mortality during wet season prescribed fires when active on the surface, but no fire-related mortality was observed during dry season periods of inactivity (Platt et al. 2010). Timing fires to coincide with turtle inactivity could thus limit or avoid fire-related mortality, but to be most effective, land managers require detailed information on the seasonal timing of surface activity for the population of interest. We did not examine timing of overwintering ingress with the same detail as ingress, but movement rates slow considerably by November (Roe et al. 2020), with most individuals entering sub-surface retreats at this time (J. Roe, unpub. data). Mean emergence date for both populations in our study was in early April, but emergence timing was highly variable within each population, spanning a duration of 58 days. Interestingly, this variability was not related to sex or body size, and those observed over multiple years remained largely consistent in their emergence date. Turtles also emerged asynchronously over a similarly wide time interval (65 days in some years, 108 days overall) in another *T. carolina* population, with individuals demonstrating repeatability in emergence timing across 17 years (DeGregario et al. 2017). Taken together, these long-term studies suggest that individuals may vary in their sensitivity to environmental cues that trigger emergence, or that environmental cues are experienced at different times according to local variation in habitat, topography, burial depth, or other factors (Currylow et al. 2013, DeGregario et al. 2017). In either case, it would be difficult to predict the surface emergence timing of a *Terrapene* population from environmental cues alone (Grobman 1990, Bernstein and Black 2005). Instead, land managers would need to set target dates to avoid burning during periods of turtle surface activity when possible, though the exact timing would vary according to latitude, elevation, climate, and other environmental factors that influence behavior. For example, mean spring emergence date for a more southerly *T. c. carolina* population was 26 March – two weeks prior to our study populations. Further study would be required to determine relationships between emergence timing and regional environmental factors across the broad geographic range of *T. carolina* to inform prescribed fire regimes.

In addition to individual repeatability in emergence timing, male and female turtles at both sites maintained spatial fidelity to refuge sites over multiple years, a behavior consistent with other *Terrapene* populations throughout their range (Cook 2004, Sava et al. 2010,

Refsnider et al. 2012, Currylow et al. 2013). Individual turtles selected locations within approximately 100 m of previous years' refuges despite annual home ranges of 5 – 17 ha for the various site and sex group combinations (Roe et al. 2020). Perhaps as a consequence of spatial fidelity, turtles were also consistent in their selection of habitats from year to year. However, individuals that overwintered in burned areas maintained weaker fidelity to previous years' refuges, selecting sites over twice as far from previous overwintering refuges compared to those in unburned areas. This is the only fire-related disruption to overwintering behavior that we observed, and it is likely due to changes in the location and quality of suitable microhabitat refuge structures (e.g., understory vegetation, woody debris, and leaf litter) following fire (York 1999, Iverson and Hutchinson 2002, Greenberg and Waldrop 2008, Hossack et al. 2009). To minimize fire-related disturbance to *T. carolina* overwintering refuge habitats, land managers could either exclude fire from these areas, burn them infrequently, or burn under conditions that do not promote intense fire (Platt et al. 2010, Roe et al. 2018). Additionally, managers could divide areas into multiple small-scale units and burn them on alternating 2 – 3 year cycles to ensure that at least some areas with suitable refuge structures are available nearby, a practice that could also benefit nest site availability and success in forest-dwelling terrestrial turtles during the active season (Dziadzio *et al.* 2016), including *T. c. carolina* (Roe et al. 2020).

5. Conclusions

Terrapene c. carolina may not be as naturally abundant in fire-prone forests compared to other environments, as population densities at the more intensively fire-managed site were approximately half of those at the mostly unburned site and up to six times lower than other regional populations in a concurrent study (Roe et al. in press). It is possible that *T. c. carolina* populations now occur at higher densities in historically fire-prone forests where fire has been recently suppressed or where fire seasonality has changed from natural to anthropogenic schedules (Rother et al 2020), but long-term historical records of *T. c. carolina* populations are inadequate to test this assumption. Nevertheless, *T. carolina* is now a species of conservation concern throughout much of its range (Keister and Willey 2015), and it may serve valuable ecological roles such as seed and spore dispersal and germination and nutrient cycling and transport in forest systems where it still persists (Rust and Roth 1981, Braun and Brooks 1987, Liu et al., 2004, Dodd 2006, Jones et al. 2007). Natural resource managers may thus require information on *T. c. carolina* responses to prescribed fire and other forest management practices along with strategies to mitigate collateral damage if necessary.

When designing management plans to minimize conflict with non-target biota, consistent (i.e., predictable) phenomenon are more easily and effectively incorporated in management practices than episodic events that vary with dynamic environmental conditions. While the variability in emergence timing within and among *T. c. carolina* populations is not ideal for management, the seasonality of winter inactivity is nevertheless a predictable phenomenon of long duration that could provide extended time windows to conduct prescribed fires with minimal impact to turtles. However, modifications to prescribed fire plans that minimize conflict with non-target species must also be balanced with other management objectives. Wildfires naturally occurred during the spring and summer in

Longleaf Pine ecosystems as a result of lightning strikes (Frost 1993, 1998, Stambaugh et al. 2011), and the target vegetative responses, including suppression of hardwood and shrub understory and promotion of herbaceous ground cover, may be best accomplished by more intense growing season prescribed fires that mimic natural disturbance regimes (Platt et al. 1988, Waldrop et al. 1992, Drewa et al. 2002, Fill et al. 2012, Shepherd et al. 2012). Scheduling fires during the growing season may also benefit several species of wildlife that specialize in the open herbaceous understory of Longleaf Pine savannas (Tucker and Robinson 2003, Tucker et al. 2004, Cox and Jones 2007), and minimize mortality of amphibians that become active on the surface during winter breeding migrations (Roznik and Johnson 2007, Humphries and Sisson 2012). However, dormant or early growing season fires under cooler and wetter conditions may be necessary when fuel loads are high after periods of fire suppression, or in areas where managers need to prioritize fire containment, including along the urban-rural interface. Frequent dormant season fires may even result in similar vegetative responses as in the growing season (Brockway and Lewis 1999, Rother et al. 2020), and may be preferred over growing season fires to retain essential nutrients and promote root growth in target plant species (Boring et al. 2004, Sayer and Haywood 2006). In such cases, modifications to prescribed fire regime could be compatible with *T. c. carolina* management while still accomplishing the desired vegetative response. If turtle conservation and management of pyrogenic forests are of equal priority, heterogeneous fire regimes that alternate fire seasonality and frequency, together with small-scale burn units, fire-exclusion zones, or areas with longer fire-return intervals that offer fire refuge in the most critical habitat patches, may be adopted by land managers – a management plan that may benefit other target and nontarget biota of both ecological and economic importance and maximize wildlife biodiversity in Longleaf Pine ecosystems (Hanula et al. 2003, Perkins et al. 2008, Hiers et al. 2014, Lashley et al. 2014, Darracq et al. 2016, Kroeger et al. 2020a, b). Conducting other potentially harmful land management practices such as mowing, timber harvest, invasive vegetation removal, and other habitat modifications during periods of sub-surface inactivity may also reduce negative consequences for *T. carolina* populations in Longleaf Pine and other ecosystems (Dodd 2006, Nadrowicz et al. 2008, Felix et al. 2008, Currylow et al. 2013, Kiester and Willey 2015).

Acknowledgements

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Highlights

- Turtles selected habitats that provided refuge from winter prescribed fires

- Turtles buried deep enough to avoid lethal temperatures during winter fires

- Spring emergence varied over a 58-day period, but was repeatable within individuals

- Turtles maintained less spatial fidelity to refuges in burned areas

- No fire-related injury or mortality occurred during winter dormancy

- Burning during winter dormancy may minimize threats to turtle populations
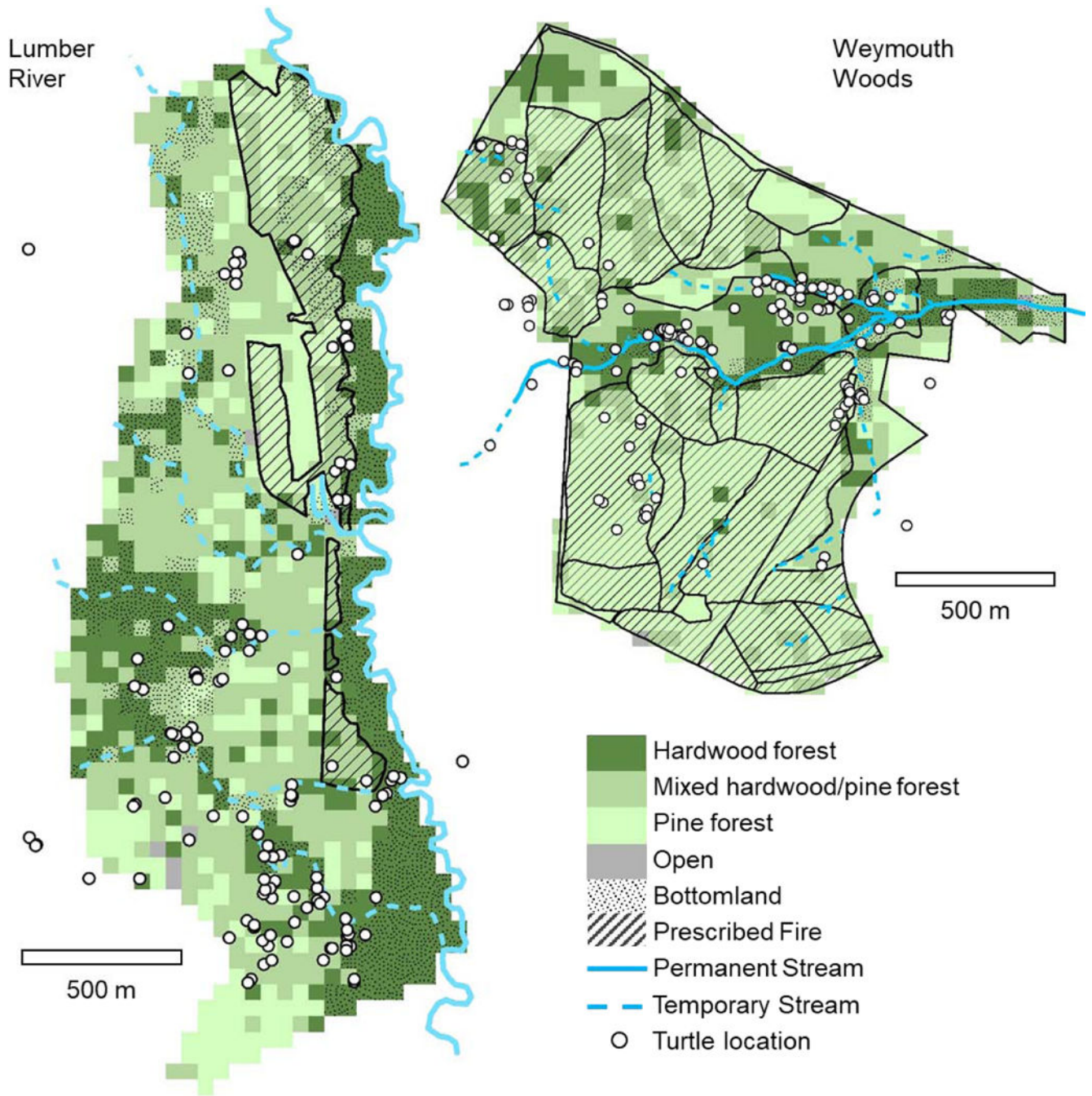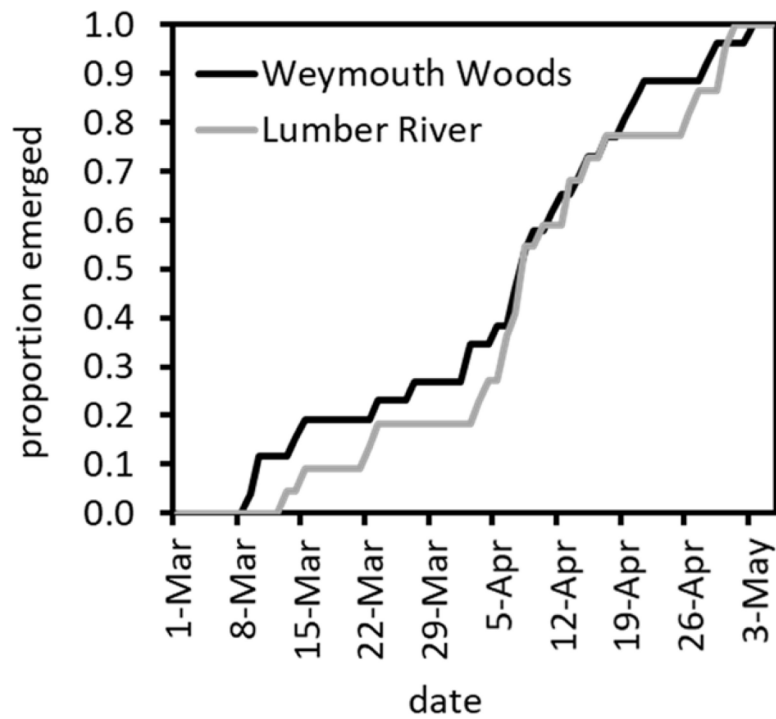
Figure 1.
Eastern Box Turtle (*Terrapene carolina carolina*) winter locations relative to habitat and prescribed fire at Weymouth Woods and Lumber River study sites in North Carolina, USA. Hardwood and pine were included in forest classification if they comprised at least 25 % of basal area in the 50×50 m grid cell.
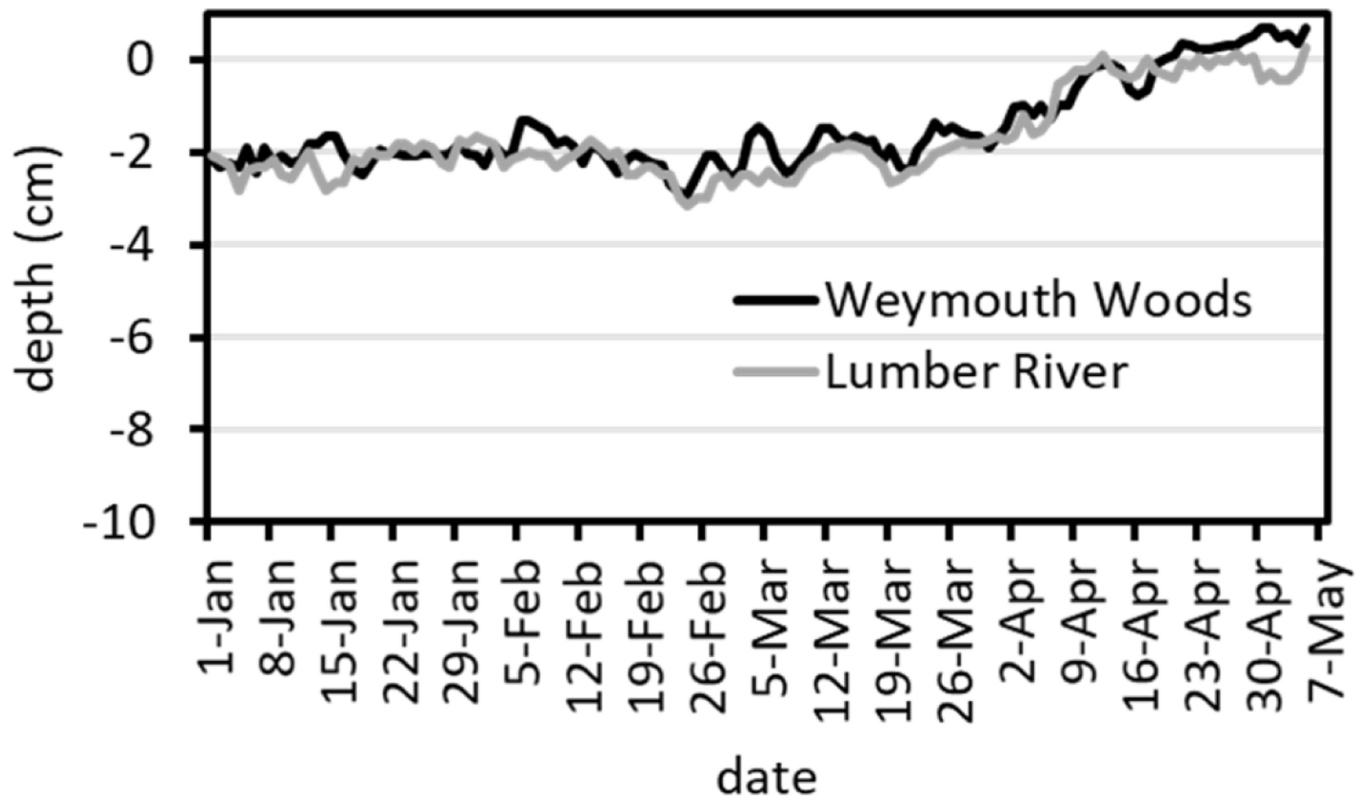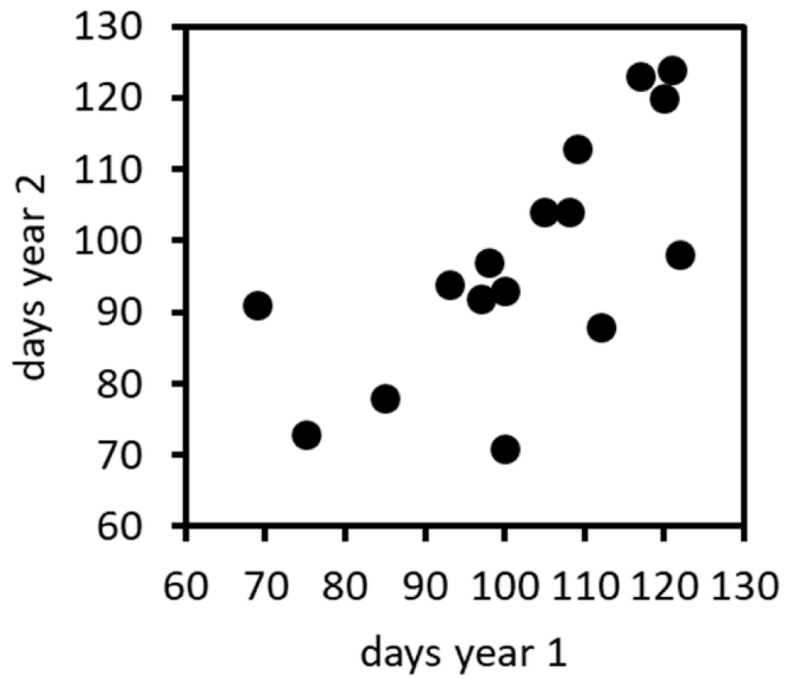
Figure 2.
Temporal variation in Eastern Box Turtle (*Terrapene carolina carolina*) overwintering burial depth estimated by temperature data loggers at Weymouth Woods and Lumber River study sites in North Carolina, USA. Zero depth indicates the soil-litter interface.
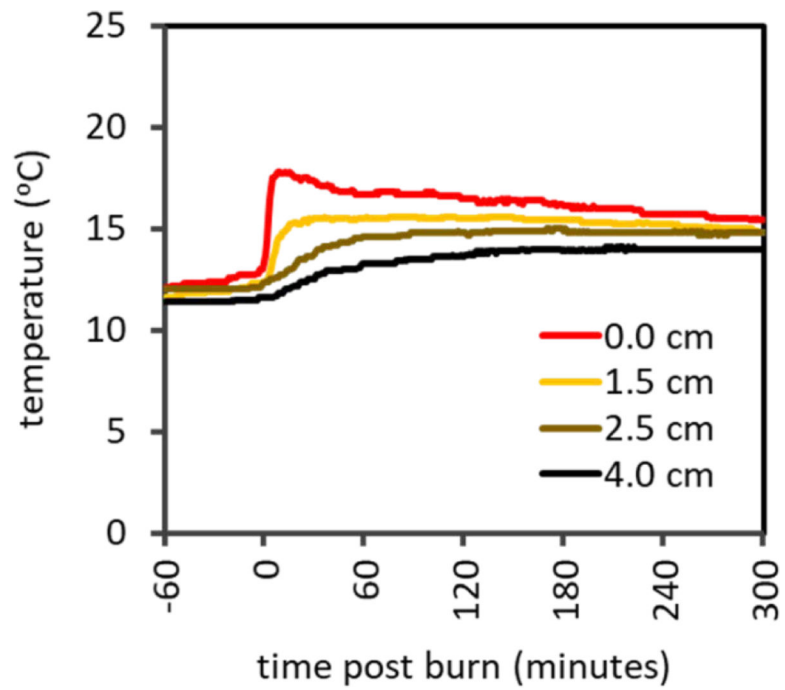
Figure 3.
Timing of surface emergence from overwintering refuges for two populations of Eastern Box Turtles (*Terrapene carolina carolina*) in North Carolina, USA.
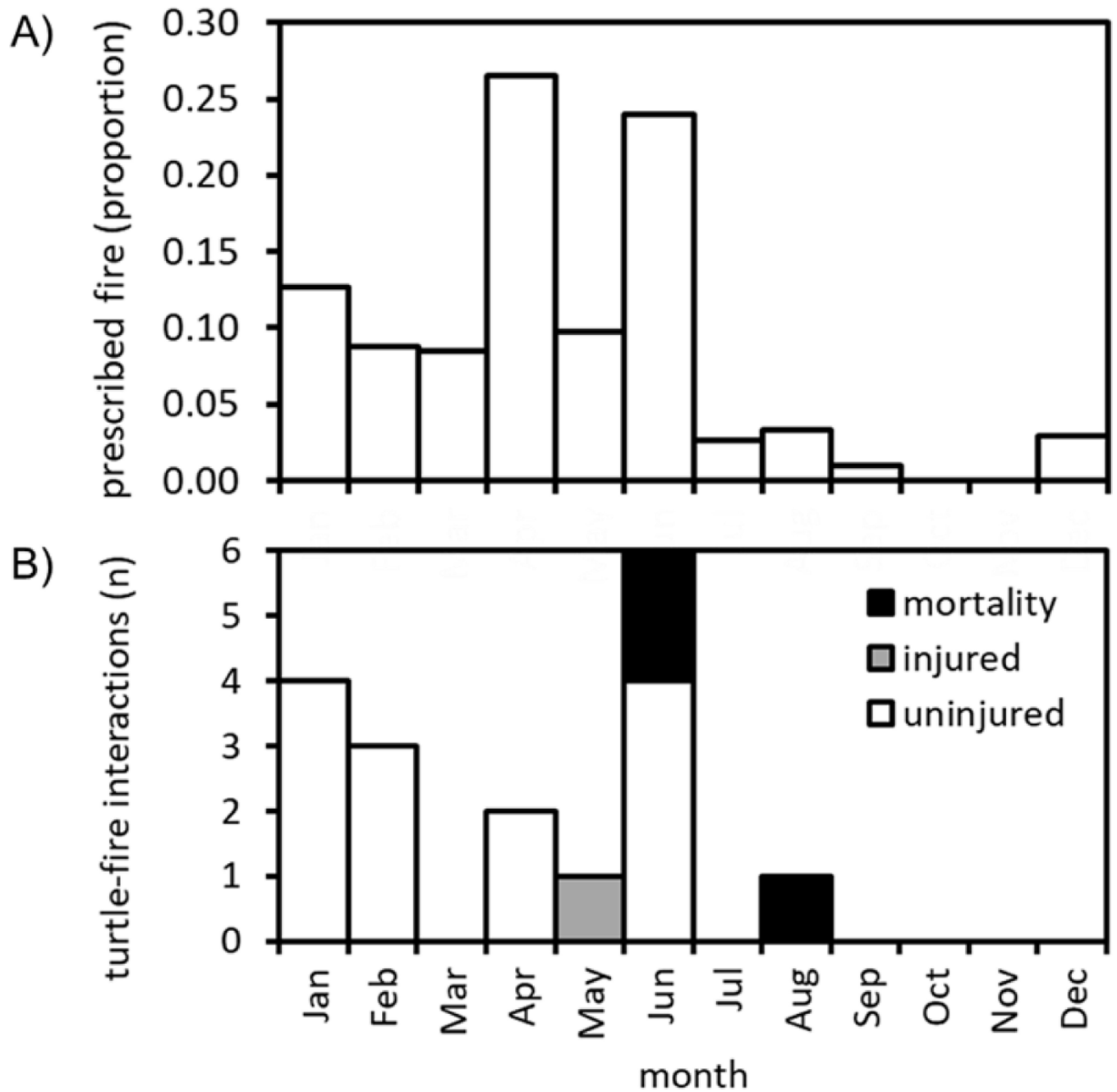
Figure 4.
Repeatability in date of surface emergence (days past 1 January) from overwintering refuge for individual Eastern Box Turtles (*Terrapene carolina carolina*) radiotracked for multiple years in North Carolina, USA.

Figure 5.
Below-ground temperatures before, during, and immediately after prescribed fire near overwintering refuges for Eastern Box Turtles (*Terrapene carolina carolina*) in predominantly pine forests in North Carolina, USA. Zero depth indicates the soil-litter interface.

Figure 6.
Monthly frequency of prescribed fire (A) compared to number and fate of Eastern Box Turtle (*Terrapene carolina carolina*) interactions with fire (B) at Weymouth Woods from 2012 – 2020.

Table 1.

Results for linear mixed effects models examining sources of variation in overwintering forest habitat use in *Terrapene carolina carolina* radiotracked from 2012 – 2020 from two sites in North Carolina, USA.

source	hardwood				pine			
	num *df*	den *df*	F	P	num *df*	den *df*	F	P
site	1	223.830	2.342	0.127	1	223.849	2.434	0.120
sex	1	225.013	0.474	0.492	1	224.926	0.464	0.496
site × sex	1	220.762	6.048	0.015	1	220.687	6.284	0.013
$\log_{10}$ carapace length	1	222.301	1.439	0.232	1	221.895	1.475	0.226
year	7	20.565	1.247	0.324	7	20.548	1.225	0.334

Table 2.

Results for linear mixed effects models examining sources of variation in overwintering aquatic habitat use in *Terrapene carolina carolina* radiotracked from 2012 – 2020 from two sites in North Carolina, USA.

source	distance to stream				distance to topland			
	num *df*	den *df*	F	P	num *df*	den *df*	F	P
site	1	216.335	18.606	< 0.001	1	219.253	10.324	0.002
sex	1	215.502	2.833	0.094	1	222.919	3.161	0.077
site × sex	1	214.676	0.260	0.610	1	224.121	0.012	0.913
$\log_{10}$ carapace length	1	208.558	5.339	0.022	1	213.947	0.859	0.355
year	7	17.736	1.878	0.134	7	17.147	0.352	0.918

Table 3.

Results for linear mixed effects models examining sources of variation in overwintering burial depth in *Terrapene carolina carolina* radiotracked from 2012 – 2020 from two sites in North Carolina, USA.

source	num *df*	den *df*	F	P
site	1	231.614	1.198	0.275
sex	1	234.208	0.010	0.921
time	8	62.613	10.308	< 0.001
site × sex	1	217.250	0.000	0.991
site × time	8	62.613	0.042	1.000
sex × time	8	62.613	0.087	0.999
site × sex × time	8	62.613	0.546	0.817
$\log_{10}$ carapace length	1	175.481	0.919	0.339