Ecological and Physiological Effects of Proximity to Roads in Eastern Box Turtles (*Terrapene carolina carolina*)

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ABSTRACT

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Roads are ubiquitous in the United States, and their ecological effects are conspicuous. Turtles are among the vertebrate taxa most affected by roads because of their low vagility and use of road and road-side habitats. In 2013, Wayne National Forest in southeastern Ohio was bisected by a new highway, affecting a road-naïve population of eastern box turtles (*Terrapene carolina carolina*), a species of concern in Ohio and vulnerable throughout its range. The goal of this study was to evaluate ecological, physiological, and behavioral effects of proximity to this new road in this road-naïve population of turtles. We used a control-impact study to evaluate potential ecological and physiological effects of proximity to roads, employing radio-telemetry to assess space use, movement behavior, and habitat selection. We used novel bioassay techniques to analyze indicators of chronic stress (across the prior several months) using nail keratin. Overall, we found no significant differences in home range sizes, habitat preferences, or corticosterone concentrations between road-side and control sites. Although the variation in corticosterone concentrations was not explained by site or sex, we did find a significant overall increase in our second year of study. While our work suggests that proximity to roads has limited indirect influence on the ecology and chronic stress responses of eastern box turtles, and that road-naïve turtles demonstrated avoidance of a

high-traffic highway, the road network likely continues to contribute to population declines through direct mortality, and further inquiry is needed to assess road effects, particularly in the areas of stress endocrinology and impacts on demography.

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Eastern box turtles need conservation efforts throughout their range, primarily due to the effects of road mortality. Quantifying potential changes in behavior and space use with respect to a new road structure can provide insight regarding population viability and inform on the types of mitigation, management, and conservation efforts vital for maintaining eastern box turtle populations. Filling the gaps in existing data could inform policy decisions related to wildlife management, direct mitigation strategies for terrestrial and semi-aquatic turtles and other herpetofauna, and further scientific knowledge on the population effects of roads on terrestrial wildlife. Therefore, we examined the ecology of eastern box turtle populations at both road-side and road-free areas of the Athens Unit of Wayne National Forest to provide insight into animal ecology and inform the overarching conservation strategy for the region. The primary goals of the study were to identify and quantify the type and magnitude of potential effects the bypass might have on the affected turtle population, including whether proximity to the road altered turtle space-use and behavior, habitat preference, and long-term stress response. We examined turtle behavior over a two-year period to account for animal life cycle events (mating and oviposition), daily events (foraging, resource acquisition, and thermoregulation), and seasonal events (overwintering and temporal differences). We combined traditional movement and habitat selection research with an evaluation of chronic stress using newly-developed hormone bioassays to assess turtle population health relative to roads. These methods comprised a novel approach to road ecology research and augmented work already undertaken by the Ohio Department of Transportation (ODOT) and Ohio University researchers on wildlife ecology relative to the bypass.

Natural History of Eastern Box Turtles

Eastern box turtles are found east of the Mississippi River from southern Maine and Ontario in the north to Georgia in the south (Dodd Jr. 2001, IUCN 2017). They occupy a wide range of habitats based on thermal requirements, physical needs, and resource availability. Turtles often choose to inhabit forested areas, with optimal habitat conditions including intermittent sunlight, moist soils, and multiple options for cover (Dodd Jr. et al. 1994, Dodd Jr. 2001, Rossell et al. 2006). Forests provide a variety of microhabitats and turtles may take advantage of clearings and wetlands for foraging, mating, thermoregulating, and laying eggs. Eastern box turtles exhibit site fidelity and often return to foraging, reproduction, and overwintering sites where they had previous success (Dodd Jr. et al. 1994, Dodd Jr. 2001). They have a preferred body temperature of 28-30°C, but are able to withstand temperatures extremes approaching both 40°C and 1°C (Dodd Jr. et al. 1994, Plummer 2003). Ideal weather conditions include warm temperatures, high humidity, and dependable precipitation (Dodd Jr. et al. 1994, Dodd Jr. 2001).

Although eastern box turtles are not territorial, their home ranges tend to remain consistent from year to year (Stickel 1950, Dodd Jr. 2001). Individuals do not display sex-specific differences in range size; most ranges extend from 0.1-5 hectares, depending on habitat quality. Life cycle events may occur outside the usual range (Dodd Jr. et al.

1994, Belzer 2000, Dodd Jr. 2001, Donaldson and Echternacht 2005, Budischak et al. 2006).

Turtles demonstrate a variety of movement patterns, including meandering throughout the home range, forging uninterrupted pathways to resources, and remaining motionless for indeterminate periods of time (Iglay et al. 2007, Nazdrowicz et al. 2008). Although these turtles are primarily terrestrial creatures, they may extend their ranges to include small bodies of water and wetlands, especially during drought conditions (Donaldson and Echternacht 2005). Resource availability, sexual dimorphism, imposed constraints, habitat quality, and environmental conditions determine turtle needs and movements (Iglay et al. 2007). A 27-year study of box turtles in Indiana suggested a preference for forested habitats and grasslands depending on temperature and cover availability, with increased usage of diverse habitats with multiple microhabitats available (Williams and Parker 1987). A 50-year study of box turtles published in 1999 also suggested that turtles prefer habitats along the forest edge, providing multiple microhabitat options throughout the active season (Hall et al. 1999). A 2.5-year study of habitat selection for breeding structures by *Terrapene carolina triunguis* determined that temperature, humidity and ground cover are the most important microclimate variables for nesting turtles, with humid sites selected at a higher rate (Reagan 1974). Eastern box turtle nesting sites in a 2006 Illinois study were also uniformly chosen in open habitats with significantly less vegetation, ground cover, and canopy cover than was locally available, suggesting that warmer microhabitats are essential to nesting (Flitz and Mullin 2006). This study also found significant nesting use of artificial clearings, often created by anthropogenic development of the land for transportation infrastructure. This suggested both a positive in increased nesting habitat availability and a negative in increased exposure of gravid females, nests, and offspring to danger and mortality (Flitz and Mullin 2006). Non-gravid turtles also preferred moist substrates and shifted habitat preference from season to season depending on soil and surface humidity; this included burrowing during dry seasons and climate extremes (Reagan 1974).

Ambient temperature has a considerable effect on activity levels of all ectotherms, eastern box turtles included. Many turtles exhibit bimodal patterns of activity during temperature extremes, ceasing activity in the warmest or coolest parts of the day and foraging/performing life cycle events during times of more moderate temperature variation (Dodd Jr. 2001, Converse et al. 2003). As a result, microclimatic conditions, including ambient temperature, moisture, and humidity, drive habitat selection for both thermoregulation and life cycle events, such as nesting. As vegetation and canopy cover are integral components driving microclimates, box turtles can often be relied upon to seek out certain and similar types of microhabitats depending on ambient conditions (Converse et al. 2003, Rossell et al. 2006). In a 2006 study of eastern box turtles in North Carolina, turtles consistently selected habitats with lower ambient temperatures and higher humidity levels than locally available, with both thermoregulatory and moisture-content benefits to the animals; turtles selected moderate canopy cover and minimal vegetation, possibly allowing air to circulate and maintain more consistent ambient temperatures (Rossell et al. 2006). A 2002 study on thermoregulation confirmed eastern box turtle preference for low ambient temperatures and high levels of moisture, suggesting that turtles prefer forest edge habitat with access to both wooded and open areas to allow for maximum thermoregulatory options (Amaral et al. 2002). Eastern box turtles in this study demonstrated an optimal mean active body temperature of 26-28°C and maintained this thermogradient by cycling between available microhabitats, including wooded areas and open grasslands (Amaral et al. 2002). In addition to careful selection of microhabitats based on resource availability and thermoregulatory needs, box turtles display high site fidelity and philopatry, suggesting that anthropogenic encroachments on existing turtle habitats can only have a deleterious effect on populations (Bernstein et al. 2007). Turtles are also at heightened risk from road mortality because of risks to migrating and nesting females and the sensitivity of populations to increases in adult mortality (Steen and Gibbs 2004).

Habitat Selection and Home Ranges

Understanding the driving forces behind habitat selection is vital to understanding animal ecology in general. In addition to species-specific selection parameters, as described for eastern box turtles, animals select habitat based on resource availability, landscape structure, protection (cover, crypsis, absence of predators, heterospecifics), proximity to mates and conspecifics, optimization for behavior and life cycle events, and access (including anthropogenic habitat fragmentation). Quantifying habitat selection using resource selection functions can enable conservation biologists to identify and protect habitats vital to the ecology of targeted species (Manly 1985, Manly et al. 1993, Boyce et al. 2002).

Assessing the relative quality of a habitat for a particular species can be challenging because of the interplay between the factors that engender selection decisions and the presence of unknown but potentially contributing factors. Additionally, the density and distribution of individuals across habitats may influence the quality and desirability of those habitats (Van Horne 1983, Manly et al. 1993). Preference in animals for a given habitat under a given set of circumstances can be approximated by comparing selected and available habitats. Habitat selection is a hierarchical process of decision making that is influenced by factors both innate (site fidelity and natal homing) and learned (previous successes). Additionally, there are different scales at which habitats might be selected, accounting for overall landscape, macrohabitat, and microhabitat (Johnson 1980). For example, at the landscape level, the eastern box turtle inhabits much of the eastern United States, while at a higher resolution of landscape-level habitat, or macrohabitat, individual box turtles have 0.1 - 5-hectare home ranges, which may include forests, streams, fields, and edge habitat. A higher resolution of macrohabitat might include an individual's use of habitat components within its home range. For example, although eastern box turtles might have broad home ranges, much of these home ranges are primarily used for travel. Box turtles typically select specific forested and edge habitats within their home ranges for thermoregulation and daily activities, like foraging. The highest resolution, or microhabitat, examines individual selection of a habitat component from all those available in the home range. Many eastern box turtles have site fidelity to a particular multiflora rose, briar thicket, or grassy edge within their home ranges. In summation, eastern box turtles select the eastern United States at a landscape level, forested regions at a macrohabitat level, edge habitat at a microhabitat level, and we are interested in further defining preferred microhabitats by examining the particular edge habitats selected.

To do so, we must consider not only spatial scales of habitat selection, but also examine these choices on a temporal scale, including daily versus seasonal movements. We must also choose a study design appropriate to our species and parameters. Based on our ability to quantify habitat use by individuals and define overall habitat availability, we might select one of three types of sampling designs: a population-level habitat selection design where we examine habitat use and overall availability for a population as a whole; a population-level habitat selection design where we examine habitat use by individuals and overall availability for a population as a whole; or an individual-level habitat selection design where we examine individual habitat selection versus availability within individual home ranges. Although an individual-level sampling design is more time and labor-intensive, it also provides the most detailed information about both individuals and populations as a whole (Manly et al. 1993).

An individual-level habitat selection design pairs well with the use of radio-telemetry and direct observation of individual animals. While available macrohabitat can be approximated based on aerial photographs and vegetation maps, microhabitat specific to each individual home range requires direct contact with the landscape. Resource selection functions enable us to estimate the probability of occurrence based on use and availability, often by fitting generalized linear models to landscape data. Using (conditional) logistic regression models to examine habitat selection has particular advantages when examining individual-level habitat selection of microhabitat because they are uniquely suited to data collected in pairs, as in our turtle occurrence (used habitat) and matched random point (available habitat) data. Additionally, while eastern box turtles move quite a bit within their home ranges, they are relatively sessile compared to many other animal species (ungulates, large carnivores, etc.), which means conditional logistic regression may provide a more accurate depiction of their habitat selection than other methods (Compton et al. 2002, Rossell et al. 2006, Popescu et al. 2013).

Radio-telemetry has been a primary method of tracking movement and acquiring spatial data about these cryptic creatures. Telemetry has been used for all manner of tracking projects; a 2004 study even used transmitters to track nest dispersal and habitat use by neonates (Forsythe et al. 2004). In 2008, researchers used telemetry to identify a strong road avoidance preference in terrestrial turtles, including eastern box turtles, and expressed concerns about potential reductions in interpopulation gene flow and increasing extinction risks (Shepard et al. 2008).

A 2007 study of eastern box turtles in the fragmented landscape of the mid-Atlantic (Delaware) found that habitat fragmentation significantly compressed the home ranges of turtles as compared to those in less-affected areas; it also suggested seasonal variation in movement distances and patterns and sexual dimorphism in movement patterns, possibly related to life cycle events (Iglay et al. 2007). A 2016 study found that an increase in road usage intensity and type significantly and negatively correlated with road-crossing inclinations in a sample of almost 700 radio-tracked snakes (Siers et al. 2016). Additionally, this snake population exhibited phenotypic plasticity in behavior, as it demonstrated a continual decline in road crossing rates spanning several decades. Implementing this radio-telemetry tracking study allowed researchers to develop predictive road-crossing models (Siers et al. 2016). This research suggests proximity to roads engenders significant negative consequences for reptiles.

Road Ecology and Turtle Mortality

Wildlife populations interact consistently with roadways built through their native habitat, leading to disruption of natural processes, increased mortality, and higher extinction risk (Langen et al. 2009). For example, roadways pose high risks to the population persistence of many turtles in the northeastern United States because road mortality tends to be biased towards females (Steen et al. 2006). Such bias is worrisome because in these long-lived species, even small changes in adult female mortality can lead to sudden declines in local population (Congdon et al. 1993, Gibbs and Steen 2005). For eastern box turtles such data is missing, and this project informs the current conservation status of this species in Ohio. Evaluating road effects on turtle populations often relies on tracking animals using VHF transmitters and evaluating both road crossing frequency and the timing of such crossings (annual, circadian; Siers et al. 2016). While the mortality risk from road interactions can vary widely from one species to another, identifying mortality hotspots has been at the core of existing research aimed at informing best mitigation practices. In a comprehensive study, Beaudry et al. (2008) determined that road mortality can be high in semi-aquatic turtles, but that mitigation efforts can be successful if implemented at the scale of particular road segments (e.g., abutting high quality turtle habitat). This research takes a similar approach for eastern box turtles and will identify the use of areas adjacent to the bypass. Other studies have shown eastern box turtles avoid roadways (Shepard et al. 2008), but it is unclear whether this avoidance is a behavioral response that developed over generations, or is a rapid adaptation to new conditions. Research on the road ecology of herpetofauna is often performed in landscapes where the road network has been around for many generation times (even for the long lived turtles; Marsack and Swanson 2009). As the majority of road ecology studies on herpetofauna are implemented in areas where roads have long been part of the landscape, little is known about roadway effects in naïve animal populations, which have been exposed for short time periods (e.g., < 1 generation).

In addition to focused studies, which evaluate road crossings and mortalities at the road segment scale and can provide reliable information on prioritizing hotspots for mitigation (Langen et al. 2007), broad scale modeling can be used to determine hotspots of herpetofaunal crossing and potential mortality. Geographic Information Systems (GIS) analyses combined with habitat selection information provided an efficient way to predict mortality hotspots for turtles at the scale of Upstate New York (Patrick et al. 2012) for a range of types of roadways and traffic intensities. In addition to prioritizing mortality hotspots for implementing mitigation measures, studies evaluated the effects of the road network on the long-term persistence of herpetofauna populations (Hels and Buchwald 2001). Understanding different types of mortality (e.g., predators, roadways, poaching) is critical to designing conservation plans, as managers can prioritize needs and actions (e.g., managing predators, designing mitigation measures).

Transportation infrastructure has a detrimental effect on the dynamics and structures of ecosystems, and its wide tract of influence renders much of the landscape subject to unintended ecological consequences (Coffin 2007). Roads not only serve as significant barriers to wildlife, fragmenting habitats and creating often impassible obstacles for animals needing to utilize microhabitats on either side of the road, but they also alter the soil chemistry in nearby habitats due to run off from their impervious surfaces. Turtles are globally threatened and road mortality is among the top anthropogenic threats to the population viability (Iosif et al. 2013). This is a result of human expansion and development and is due in large part to some characteristics of turtle populations which make them vulnerable to human-induced change (Beizer and Steisslinger 1999). These characteristics include slow individual growth, late maturity, a low reproductive potential, and an already high natural mortality of juveniles and eggs (Dodd Jr. et al. 1994). Beaudry et al. (2008) identified hot spots of turtle mortality along roads, deriving a 100-year estimate of extinction between 5-59% based on species, directly related to interactions with roads. This takes into account the spatial distribution of turtle mortality using radio telemetry, traffic counts, and road layers at the individual, road segment, and population levels. Additionally, turtles do not move with linear purpose directly perpendicular to roads; these meanders and pauses contribute to their high rates of mortality (Beaudry et al. 2008). Artificial open spaces and edge habitat created by road construction may also contribute to turtle mortality by establishing dangerous areas that are nonetheless appealing for life cycle events, including mating and nesting (Aresco 2005).

Walking and driving surveys have been widely conducted to develop mortality counts and determine prime areas of road interference with reptile and amphibian populations. A 2012 study also documented high levels of road mortality and used spatial models to pinpoint hotspots, which differed depending on the species' life cycles (Patrick et al. 2012). Turtles are at a heightened risk of road mortality, particularly when that road divides two microhabitats utilized for key life cycle events.

Sex bias can also play a substantial role in turtle road ecology. Aresco (2005) found that although male freshwater turtles outnumbered females in ponds near roads, 57-72% of turtles killed on nearby roads were female. The same study further described significantly higher ratios of males to females in ponds affected by roads than in ponds not influenced by road mortality, suggesting that a cumulative reduction in female populations, and thus reproductive potential, may be attributed to adjacent roads (Aresco 2005). Another study linked the significant linear increase of the proportion of males in both terrestrial and freshwater turtles with the expansion of the United States road network, noting that sex ratios are particularly skewed in areas with high road density (Gibbs and Steen 2005). This suggests a relationship between declining turtle populations and the higher likelihood of breeding females compromised by road mortality (Gibbs and Steen 2005). This is especially relevant given the low reproductive potential of eastern box turtle populations, which require a high density of breeding adults to ensure species endurance (Belzer 2002).

Strategies to Mitigate Road Effects on Turtles

Between loss of habitat, overharvesting, and road mortality, eastern box turtles are already facing significant population declines (Beizer and Steisslinger 1999, Marsack and Swanson 2009). They are listed as vulnerable or a species of concern throughout their range (Beizer and Steisslinger 1999, Budischak et al. 2006). Despite this decline, studies have indicated that some populations are maintaining broad genetic variation. This suggests the potential success of future populations, given the mitigation of some of the deleterious effects of roadways on populations (Marsack and Swanson 2009). Although other studies have confirmed the maintenance of genetic diversity in some populations, the potential demographic and genetic corollaries of roads on turtle populations are too significant not to explore further (Marsack and Swanson 2009).

Mitigation measures can be successful if targeted at known herpetofauna hotspots. Some common mitigation measures include barriers to block access to roads, which direct animals toward eco-passages (Benayas et al. 2006, Crawford et al. 2014). These mitigation methods were put into place for the Nelsonville Bypass. During the development of the bypass, ODOT concluded that home ranges would be fragmented, and movements would be impaired for indigenous species. ODOT acknowledged a potential local reduction in species numbers, although it cited the remainder of the forest as possible post-construction habitat, assuming the flexibility of many of these animals to shift their home ranges. However, in order to minimize effects on local wildlife, ODOT identified 12 potential sites along the bypass where it was possible to install wildlife mitigation measures. These included a bridge under the roadway embankment, two large culverts for wildlife crossing, and two small culverts for use by small mammals and reptiles. Additionally, ODOT implemented mesh fencing for reptiles and low berms to direct animals to these eco-passages. While there are four total eco-passages and related infrastructure for wildlife along bypass corridors, only two were present in our study sites: a 38 m bridge beneath the roadway embankment (intended for deer and large mammals), and a 61 - 71 cm diameter culvert with associated fencing (The Ohio Department of Transportation 2005). Eastern box turtles did not make use of these eco-passages during the length of our study. Current research is underway to determine the efficacy of these mitigation measures, and although our turtles did not make use of them, movement data compiled through this study will nonetheless inform their space use in relation to the road and its eco-passages.

The reduction in habitat permeability and increased threat to animals from transportation infrastructure can be reduced through advanced planning and movement metrics for local wildlife. Mitigation structures will be more effective at facilitating healthy wildlife-roadway interactions if they are thoughtfully and ecologically planned (Bissonette and Adair 2008). Some researchers have suggested increasing cover near eco-passages and the number of overall drainage culverts, at low cost and high ecological benefit, and widening verges and barriers near curves with low visibility (Clevenger et al. 2003).

One challenge of mitigation planning is accounting for multiple species. Long-term and diverse research will contribute to the overall efficacy of mitigation systems. It is notable that mitigation efforts and habitat protection regimes ultimately provide no guarantee for the success of a population facing many natural and anthropogenic threats, and that continual wildlife monitoring will be necessary to ensure the success of vulnerable species (Browne and Hecnar 2007).

Stress Responses in Wild Turtles

Signs of stress in turtle populations are not always apparent. Primarily adult populations (indicating a lack of procreation) can be under stress, as can populations facing resource scarcity and predation threats (Browne and Hecnar 2007). Anthropogenic interference in particular can cause persistent and chronic stress that is both unnatural to eastern box turtles and unrelenting. Prolonged exposure to high levels of stress can instigate autoimmune consequences and detrimental effects on reproduction and overall life expectancy, thus seriously affecting generations and populations. Recently, Baxter-Gilbert et al. (2014) developed a novel method for evaluating stress caused to turtles by roads and resulting habitat fragmentation. They measured corticosterone, a glucocorticoid used as a measurement of physiological stress, using keratin samples trimmed from turtle claws. Corticosterone is continually deposited in the structural proteins of keratin as the claws grow, thereby offering insight into long-term stress levels (Alibardi 2006, Baxter-Gilbert et al. 2014). Although they successfully distinguished chronic stress levels and validated the method for turtles, they found no significant differences between turtles at road-side versus control sites; they called for additional reptile endocrinology research (Baxter-Gilbert et al. 2014).

Although corticosterone levels in reptiles have historically been measured using blood or fecal samples, quantifying chemical levels using keratin can be less invasive and more accurate (Cash et al. 1997). The deposition of corticosterone stored in turtle claws is not subject to short-term fluctuations seen in blood and fecal samples. As the claw grows away from corticosterone-carrying and depositing capillaries, it provides a record of long-term stress. Claw trimmings eliminate immediate handling and processing stress that shows up in blood samples, and keratin testing provides analogous results to blood and fecal testing (Berkvens et al. 2013). Biochemical, biological, and physiological validation assays demonstrated that keratinized structures were accurate indicators of long-term stress levels and provided insight into individual stress responses to environmental disturbances (Bortolotti et al. 2008). Additionally, corticosterone is maintained in the keratin indefinitely, and the reliability of chemical recovery from claw trimmings is between 89% and 97%, which provides a more accurate view of stress responses (Baxter-Gilbert et al. 2014).

By virtue of their daily and life cycle requirements – including resource acquisition, interaction with conspecifics, reproduction, and thermoregulation – eastern box turtles are exposed to a variety of stimuli across a broad spectrum of forest, edge, and open microhabitats. While direct effects of proximity to roads, i.e., vehicle strikes and resulting mortality, are documented for turtles, there is some precedence suggesting they demonstrate road avoidance, which may or may not be the case when turtles have been exposed to roads for short time periods (< 1 generation; Shepard et al. 2008). As such, this population presents an opportunity to increase our understanding of herpetofaunal endocrinology and assess potential indirect effects that roads may have on individuals and populations. In addition to novel bioassays of stress-related hormones, we examine habitat selection and home range size in eastern box turtles using traditional selection methods and analyses, e.g., Minimum Convex Polygons. We employ radio-telemetry tracking and collect turtle and random habitat data to examine differences in home range sizes, habitat preferences, and corticosterone concentrations relative to proximity to the road. The juxtaposition of these traditional research methods with the novel hormone testing of road-naïve turtles in both road-side and control sites should provide interesting insight into how these animals behave in proximity to the road and inform possible management strategies that might be considered to ameliorate anthropogenic effects on these vulnerable animals.

CHAPTER 2: ECOLOGICAL AND PHYSIOLOGICAL EFFECTS OF PROXIMITY TO ROADS IN EASTERN BOX TURTLES (*TERRAPENE CAROLINA CAROLINA*)

Introduction

Human development has historically created significant encroachment on wildlife populations. Roads are one of the most ubiquitous forms of human impact on wildlife and the effects of roads influence much of the conterminous United States (Forman 2000). The effects of roads on wildlife include habitat loss, when former habitat is converted to asphalt and artificial edge, and habitat fragmentation, which poses challenges for organisms needing to access habitats or conspecifics in previously intact areas (Marchand and Litvaitis 2004, Budischak et al. 2006, Iglay et al. 2007, Shepard et al. 2008, Collins and Russell 2009, Baxter-Gilbert et al. 2015). Additionally, chemicals, runoff, and the effects of construction alter and degrade wildlife habitat (Budischak et al. 2006, Shepard et al. 2008, Collins and Russell 2009, Baxter-Gilbert et al. 2015). Further, animal populations divided by roads face additional long-term vulnerability due to genetic division and decreased population numbers, and are prone to direct mortality from traffic (Steen and Gibbs 2004, Jaeger et al. 2005, Coffin 2007, Benítez-López et al. 2010). As road building and improvement continues largely unabated, it is vital to understand animal behavior, ecology, and physiology in relation to roadways to provide mitigation strategies to reduce the effects of roads on wildlife (Coffin 2007).

Deleterious effects of roads, including habitat alteration and related mortality, have been documented for both terrestrial and freshwater turtle populations (Dodd Jr. et al. 1994, Wood and Herlands 1997, Aresco 2005, Iglay et al. 2007, Nazdrowicz et al.

2008, Crawford et al. 2014). Turtles are among the vertebrate taxa most affected by road mortality in the United States because of their low vagility and attraction to roads for nesting and basking (Steen and Gibbs 2004, Dodd and Dreslik 2008). These behaviors have been found to bias mortality towards females, potentially further skewing populations and increasing population-level effects of exposure to roads (Gibbs and Steen 2005). Additionally, when turtles encounter roads, they seldom take the most parsimonious route, further exposing themselves to injury and direct mortality (Beaudry et al. 2008, 2010). Some studies have suggested that eastern box turtles may demonstrate road-avoidance, but it is unclear whether this avoidance is a behavioral response that developed over generations, or a rapid adaptation to new conditions (Shepard et al. 2008). Even in the case of turtles demonstrating road avoidance, road mortality is still a primary threat to the persistence of eastern box turtles in Ohio and throughout their North American range (Forman and Alexander 1998, Coffin 2007, ODNR 2016, IUCN 2017).

Research on the road ecology of herpetofauna is often performed in landscapes where the road network has been around for many generation times, even for long-lived turtles (Marsack and Swanson 2009). As such, little is known about the direct and indirect effects of roads on naïve animal populations, exposed to new roads for short time periods (e.g., < 1 generation). Working with a road-naïve population provides a unique opportunity to assess the immediate effects of proximity to roads on turtles (Marsack and Swanson 2009, Baxter-Gilbert et al. 2014). In addition to effects on space use and direct mortality from traffic, one of the potential effects of proximity to roads is increased and chronically-high levels of stress, which could alter behavior and decrease individual fitness (Browne and Hecnar 2007, Baxter-Gilbert et al. 2014). Although quantifying chronic stress is challenging due to the multiple factors that comprise stress responses, corticosterone concentrations are often employed as a standard biomarker of physiological stress in ecological studies (Angelier et al. 2010, Tonra et al. 2013, Baxter-Gilbert et al. 2014). Corticosterone is a hormone and glucocorticoid released as part of the response to stress. While it is far from the only physiological response to stress, it is one of the places ecologists start when examining the stress response (Angelier et al. 2010, Tonra et al. 2013, Baxter-Gilbert et al. 2014).

Our goal was to address gaps in understanding the effects new roads have on the ecology and behavior of turtle populations, thus our study focused on a recently-built highway in southeastern Ohio. In 2013, a previously intact, forested, portion of Wayne National Forest was bisected by a 9-mile section of U.S. Highway 33, a high-speed, high-traffic, four-lane highway built to bypass the city of Nelsonville, Ohio (hereafter bypass). At the time the road was built, a wide range of structures were included to mitigate its effects on wildlife, including deer fencing with jump-outs, snake fencing, large mammal underpasses, and small animal underpasses. This section of the forest provides habitat for a broad variety of animal and plant life, some of which, including the endangered Timber Rattlesnake (*Crotalus horridus*), have already experienced effects of proximity to the bypass, including altered movement patterns, habitat selection, and mortality rates (The Ohio Department of Transportation Office of Statewide Planning & Research 2017). For other species, these effects remain unknown. The eastern box turtle (*Terrapene carolina carolina*) is a terrestrial turtle species inhabiting much of the eastern United States, including southeastern Ohio. The eastern box turtle is listed as a species of concern in Ohio, and is vulnerable throughout its range, mainly due to effects of roads (ODNR 2016, IUCN 2017). As such, the context of the bypass presents an ideal opportunity to assess the effects of a new road on the space use and behavior of eastern box turtles and evaluate the potential effects of new roads on herpetofauna. We take a two-pronged methodological approach, combining traditional habitat selection studies via VHF telemetry with novel chronic stress hormone bioassays from nail keratin (Baxter-Gilbert et al. 2014). The juxtaposition of these field and lab techniques provides a more comprehensive approach to understanding animal/road ecology and evaluating potential, subtle, non-lethal effects of exposure to roads. Importantly, in this study, we compare a population at a road-side site to a control population in a similar habitat setting in the same national forest, but completely lacking roads. This control-impact design is ideal for evaluating the magnitude of the effect of a stressor on a naïve and previously unexplored population.

The goal of this study was to evaluate the effects of a new roadway on eastern box turtle space use and stress condition using a control-impact design. Our specific objectives were: (1) to quantify differences in home range sizes of turtle populations at road-side and road-free sites, (2) to evaluate differences in habitat selection between the two populations, and (3) to quantify differences in potential chronic stress via corticosterone concentrations between the two populations. We hypothesized that turtles at the road-side site would have larger home ranges, as they would need to seek out resources made unattainable by the highway, and that their home ranges would be bounded by the highway. We further hypothesized that the same animals would select different types of habitat than would control turtles, as edge habitat opened by the development of the highway could present ideal opportunities for thermoregulation and nesting. Lastly, we hypothesized that proximity to roads, increased anthropogenic presence, and resulting habitat degradation would result in higher levels of potentially chronic stress in turtles at the road-side site, and we anticipated increased concentrations of the stress-related hormone corticosterone in those animals. Overall, this work provides insight into turtle behavior, allowing us to predict specific vulnerabilities to roads in long-lived herpetofauna, and inform conservation strategies and policy decisions related to wildlife road mortality mitigation efforts.

Methods

Study Sites

We conducted our study in the Athens Unit of Wayne National Forest in Athens and Hocking Counties in southeastern Ohio at two sites: a road-side (Impact) site that abuts the bypass (Figure 1), located in national right of way north of the highway on the westbound side (39.47654, -82.27231); and a control (Control) site devoid of paved roads and closed to off-highway vehicles (39.44445, -82.23763). We consider the presence of the bypass as the treatment variable (Figure 2).

The road-side site was divided into two sections based on turtle site selection that, while geographically close (~3 km), displayed some possibly relevant distinctions. The presence of the road, with related deer and snake fencing, was the primary disturbance in the eastern section. The western section contained potentially further-confounding, disturbance-related variables, including increased human presence and a network of off-highway vehicle (OHV) trails. We included both sections because of the presence of turtles and because the disparities between sections provided an accurate representation of conditions in the road-side site, and because the differences could provide insight into how the animals respond to gradients of disturbance.

Turtle Capture, Telemetry, and Habitat Sampling

We began capturing eastern box turtles beginning May 1, 2017, with the assistance of Boykin spaniels, detection dogs trained to find turtles in the field (Somers et al. 2003, Anderson et al. 2011, Kapfer et al. 2012). We selected 30 animals for the study, including the first eight adult males and the first seven adult females found in each site, for a total of 15 per site. We transported research animals in individual bags to Ohio University in Athens, Ohio to measure carapace length, plastron length, shell width, dome height, and weight; and to determine sex and age (by counting scute annuli). If turtles had more than 10 annuli, we considered them adults (Iglay et al. 2007).

Telemetry

We equipped each of the 30 study turtles with a small VHF transmitter (Advanced Telemetry Systems, Isanti MN) with a lifespan of 18 months and an iButton temperature data logger (Embedded Data Systems, Lawrenceburg KY; ~10 g combined, Appendix A) (Forsythe et al. 2004). We attached transmitters and iButtons with epoxy (PC Products, Allentown PA) to single healthy costal scutes near the front of turtle carapaces to minimize interference with mating and daily activities. We housed turtles overnight to allow epoxy to completely set; all animals were release at capture site the morning following processing.

We tracked the turtles at least weekly from May – November 2017 and March – 1 June 2018 using a radio receiver and handheld antenna (Advanced Telemetry Systems, Isanti MN). This included tracking animals in 2017 until every turtle had buried itself beneath the substrate to overwinter, checking periodically over the winter on warm days to see if animals had emerged or moved, and tracking every day in the spring of 2018 until all turtles were above the soil. During the active season, we tracked turtles until we were able to visually confirm their presence, or until we were able to pinpoint their location within a few feet; not sighting animals happened primarily when turtles were located in impassable habitat features such as briar thickets and multiflora roses. While turtles were overwintering, we pinpointed their underground locations within a few feet above the surface. Although we shifted the leaf litter in an attempt to visually confirm turtle presence, we did not dig up animals that remained beneath the soil.

Habitat sampling

We measured habitat characteristics and environmental variables that are ecologically relevant to eastern box turtles at turtle locations and paired random locations (Appendix B). We used a 1 m^2 Daubenmire frame positioned with the turtle at the center to approximate percent ground cover and understory, a densiometer to estimate percent canopy cover, a handheld weather meter to measure temperature and relative humidity (KestrelMeters.com, Minneapolis MN), and a SM150T soil moisture meter to measure the water content of the soil with a 3% accuracy (Delta-T Devices Ltd, Cambridge, UK; Converse et al. 2003). Specifically, we measured volumetric water content for mineral soil, as soils in our study area are primarily clay-rich; under similar precipitation conditions, lower readings suggest lower clay concentrations, and thus a thicker organic layer. Following data acquisition for each turtle location, we selected a randomly-generated location 50 m away and repeated the data sampling process. Random points between 0 - 360° were generated using the Random application for iOS (Version 1.3.6; Rossell et al. 2006). We used the habitat data collected at turtle locations (use) and at paired random locations (availability) to evaluate habitat selection (Compton et al. 2002).

Thermal Selection

We collected ambient air temperatures at ground level every 30 min from May 2017 to May 2018 using iButton temperature data loggers. We downloaded stored data from the loggers approximately once per month using a handheld thermochron downloader (Embedded Data Systems, Lawrenceburg KY). While these data are somewhat fragmented due to equipment malfunctions and turtle accessibility, we have included them in the event they might be of interest for potential thermal selection studies (Appendix A).

Chronic Stress Bioassays

We evaluated free corticosterone concentrations as a proxy for the presence of chronic stress in turtles at each of the two sites using corticosterone bioassays of nail keratin. Corticosterone is one of the hormones released in response to stress and levels of free corticosterone (not bound to receptors) may remain elevated during prolonged exposure to stressors. Therefore, concentrations of corticosterone in keratin may be interpreted as a proxy for stress levels over time (Baxter-Gilbert et al. 2014). In addition to collecting keratin samples from the turtles tracked via VHF telemetry (N = 30), we opportunistically collected nails from 56 other turtles encountered during the 2017 telemetry surveys (33 at road-side site and 23 at unimpacted site). We clipped 4-11 toenails per turtle (depending on size and availability) from the hind and front feet of all turtles. Nails were clipped below the quick and ranged in length from 0.5 - 4.9 mm ($\bar{x}$ = 2.48 mm). The total weight of individual nail samples from each turtle ranged from 1.2 - 35.5 mg ($\bar{x}$ = 15.23 mg). Toenails were clipped using scissor-style, stainless steel nail clippers. Clippers were cleaned with alcohol wipes between uses.

We collected another set of nail samples in May 2018 from each of our telemetered turtles and one incidental recapture sampled the previous year (N = 31). All

2018 samples were collected in the field. We clipped 4-14 toenails per turtle (depending on size and availability) from the hind and front feet of all turtles. Nails ranged in length from 0.9 - 4.9 mm ($\bar{x}$ = 2.57 mm). The total weight of individual nail samples from each turtle ranged from 5.3 - 25 mg ($\bar{x}$ = 14.32 mg). The purpose of resampling was to provide a temporal perspective for comparison. Additionally, we wanted to evaluate the potential effects of handling and tracking on the concentrations of stress response hormones in turtles and determine whether our continued presence had increased corticosterone concentrations.

Following nail clipping, we stored individual keratin samples in 16 x 100 mm vials labelled with individual identification numbers. Samples were stored at -18°C until transported to the Tonra Lab of Avian Ecology at the Ohio State University for bio-assaying. We measured the concentration of free corticosterone in toenail samples using a methanol-based extraction and Enzyme Immunoassay (EIA), as validated by Baxter-Gilbert et al. (2014), using a commercial CORT ELISA kit (Product #402810; Neogen Corporation, Ayr, UK). Briefly, crushed samples were incubated in methanol overnight in an oscillating water bath, following which extracts were separated via pipette (including multiple rinses), evaporated under nitrogen gas, and then reconstituted in Neogen extraction buffer before running through kit assay procedures (Tonra et al. 2013, Baxter-Gilbert et al. 2014). Values are reported in pg/mg of toenail. Samples were run in four separate bioassays over eight days in the Tonra Lab (August 22-23, 2017; August 31-September 1, 2017; September 21-22, 2017; June 14-15, 2018). Assay recovery was assessed by adding 20µL of tritium-labeled CORT in each sample, and mean recovery rate was 0.94. Intra-assay variation based on duplicate samples was 4.1%, and inter-assay variation (N = 4) based on kit standards was 6%. The detailed protocol is presented in Appendix C.

Data Analysis

Home Ranges

We calculated 100% Minimum Convex Polygons for individual home ranges for data pooled across the two years of study. We evaluated differences between male and female home ranges, and between control and road-side sites using non-parametric Kruskal-Wallis tests (Iglay et al. 2007). We also quantified proximity to roads for turtles at the road-side site.

Habitat Selection

We evaluated habitat selection by eastern box turtles using mixed-effects conditional logistic regression analyses in program R (package *mclogit*; R Core Team 2013, Elff 2018). Conditional logistic regression models allowed us to examine microhabitat selection based on used (turtle occurrence) versus available (random) habitat data. By collecting paired sets of data at the same time, we were able to evaluate the differences between selected and available habitats. We built and tested eight competing models incorporating combinations of environmental variables ecologically-relevant to turtles, including ground cover, habitat structure, and weather conditions (Table 1). Variables that demonstrated correlations > 0.7 or < -0.7 were removed from the analysis.

We built four separate model sets: males and females at each of the two sites. For each model set, we used a model selection procedure and determined the best-supported model using Akaike Information Criteria corrected for small sample size (AICc) in program R (Burnham and Anderson 2002, R Core Team 2013). If a top model was identified (> 2 AICc units from second ranked model), we then calculated the estimated coefficients for the best-supported model for each subset of turtles (Compton et al. 2002, Rossell et al. 2006). The estimated coefficients for conditional logistic regression models may be interpreted similarly to those of traditional logistic regression models; the odds ratios express the likelihood of occurrence given an n-unit increase in each explanatory variable. For example, a 1% increase in woody debris might suggest a certain percentage increase in the likelihood of occupancy by a given subset of turtles. However, unlike in traditional logistic regression, the explanatory variables in conditional logistic regression are the differences between paired sets of data, as opposed to the values of the data themselves. Therefore, these models must be examined in the context of differences in selected versus available habitat rather than the actual values as measured (Compton et al. 2002, Popescu et al. 2013).

Chronic Stress Response

We calculated corticosterone concentrations using CORT Elisa Kits (Neogen, Lexington KY). We built and tested competing generalized linear models (N = 9) using site, sex, and physical covariates (carapace length, weight, age) as explanatory variables (Table 3). We built four separate model sets for males and females at each of the two sites. For each model set, we used a model selection procedure and determined the best-supported model using Akaike Information Criteria corrected for small sample size (AICc) in program R (Burnham and Anderson 2002, R Core Team 2013). We also compared corticosterone concentrations in our tracked turtles across time (Spring 2017 and Spring 2018) using a paired t-test. By measuring the nails before and after clipping and calculating the time taken to regrow, we estimate we are obtaining 5 - 6 months of data for each keratin sample. Based on this timeline, it is possible that higher corticosterone concentrations indicate either larger and more protracted stress responses or more frequent and chronic stress responses.

The presence of the highway is not the whole story of disturbance in our road-side site; other potential or confounding sources of stress could include increased human presence and the broad network of OHV trails in part of our road-side site. In an effort to parse out potential road effects on stress versus those of other impacts, we took a closer look at the road-side site, which is divided into two areas – one with and one without OHV trails. The purpose of this was to determine where higher hormone levels occur, and if stress response is related to the road, the OHV trails, or a combination of the two, in addition to any as yet unknown factors. This variation in intensity of disturbance at the road-side site provided us with an opportunity to examine potential road effects in the context of other prospective confounding variables that could be of interest, in addition to a control site. We examined the relationship between corticosterone concentrations and proximity to the road versus other potentially confounding variables; we built and tested another set of competing generalized linear models (N = 16) for turtles in the road-side site, using disturbance level, sex, physical covariates (carapace length, weight, age), and nearest distance to road as explanatory variables (Table 4).

Results

Home Ranges

Based on a total of 880 turtle locations collected between May - November 2017 and March - 1 June 2018 ($\bar{x}_{Impact}$ = 28 points per turtle, $\bar{x}_{Control}$ = 30.67 points per turtle; Appendix D), females at the road-side site had the largest home ranges (100% MCP = 6.19 ± 2.02 ha), followed closely by females at the control site (4.56 ± 2.79 ha), and distantly by males at the road-side site (1.85 ± 0.64 ha) and males at the control site (1.37 ± 0.24 ha) (Figures 3, 4, 5). There were no differences in turtle home range sizes between road-side and control sites (Kruskal Wallis $\chi^2_1 = 1.1187$, $p = 0.2902$). While females exhibited larger overall home ranges than males (Kruskal-Wallis $\chi^2_1 = 6.4303$, $p = 0.0112$), and females at the road-side site exhibited larger home ranges than males at the road-side site (Kruskal-Wallis $\chi^2_1 = 6.4821$, $p = 0.0109$), females and males at the control site demonstrated no differences in home range size (Kruskal-Wallis $\chi^2_1 = 1.3393$, $p = 0.2472$).

Habitat Selection

We collected habitat data at 720 turtle points and 720 paired random points between May 2017 and 1 June 2018. Contrary to our hypotheses of differential use of habitat between the two study sites, the same combination of variables emerged as the best-supported model for three of the four subsets (Table 1). Based on the best model for females at the road-side site, and both males and females at the control site, eastern box turtle occurrence was positively associated with percent woody debris cover, percent herbaceous vegetation cover, and percent understory; occurrence was negatively associated with percent leaf litter, percent bare/rocky/stream microhabitat, and percent canopy cover (Table 2). We calculated the odds ratios for all variables in the best supported model for each subset (Table 2). All turtles selected for higher percentages of woody debris and understory cover. While turtles at the road-side site demonstrated a significant preference for higher percentages of herbaceous vegetation where control turtles did not, there were no overall significant differences between control and impact sites (Table 2). For each 1% increase in woody debris covering the ground, there was a commensurate 3.1% - 5.3% increase in the likelihood of turtle occupancy; for each 1% increase in understory, there was a 2.5% - 4% increase in the likelihood of turtle occupancy, depending on population subset (Figure 6).

Although different variables were included in the top model for males at the road-side site, they also selected for higher percentages of woody debris, herbaceous vegetation, and understory and against leaf litter and bare/rocky/stream microhabitat, although they showed a slightly stronger preference for greater canopy cover than other turtles (Figure 6). All three sets of variables (forest floor, forest structure, and weather conditions) comprised the top model (Table 1). While only forest floor and forest structure variables comprised the top model for female turtles at the road-side site, the model incorporating all three sets of variables (including climate conditions) was also within two AICc of the best-supported model for females at the road-side site – who tended toward cooler and wetter conditions – suggesting that weather plays a stronger role in habitat selection for these turtles than for control turtles (Table 1). The top model(s) explained 77 – 100% of the variation seen in habitat selection by each subset of turtles (Table 1).

Corticosterone as a Proxy for Chronic Stress

The top two models explaining corticosterone concentrations (log-transformed) in nail keratin included the null model and site as an explanatory variable (Table 3). While site as an explanatory variable was within two AICc of the best-supported model and carried more weight than any other variable (cumulative weight = 0.46), the fact that that the top model was the null model suggests that none of the variables we quantified explained variation in corticosterone concentrations. Therefore, contrary to our expectations, site was not a reliable predictor of corticosterone concentrations, and there were no significant differences between population subsets.

Although corticosterone levels did not differ between sites (Kruskal Wallis $\chi^2_1 = 3.147$, $p = 0.076$) or sexes (Kruskal Wallis $\chi^2_1 = 0.2538$, $p = 0.6144$), male turtles in both sites exhibited a broader range of variation than female turtles (Figure 7). We also found no differences when we examined the road-side site based on intensity of perturbation (areas with and without off-road vehicle trails, Figure 7; post hoc Kruskal Wallis $\chi^2_1 = 0.32$, $p = 0.5716$).

Several turtles spent substantial time (> 6 weeks) in close proximity (within 100 m) to the road (Figure 8). Additionally, turtles moved to road-side habitat earlier in 2018 than in 2017. As of early June 2017, we logged fewer than 10 turtle points within 100 m of the road; as of early June 2018, we had logged 47. Despite the near proximity of many turtles to the bypass for much of the summer seasons, the nearest distance turtles were found from the bypass demonstrated only a weak positive correlation with corticosterone concentrations in the road-side site (Figure 9; $r_s = 0.2747$, p = 0.3411). Despite this weak correlation, distance to roads was within two AICc units of the top model explaining 2018 corticosterone concentrations (log-transformed) in turtles at the road-side site (Table 4). While models containing the variables sex and disturbance level were also within two AICc units of the best-supported model, the best-supported model was the null model, which suggests that corticosterone concentrations were subject to random variation. Disturbance level, or section of the road-side site where turtles spent the majority of their time, carried more weight than any other variable (cumulative weight = 0.43) and was within two AICc units of the top (null) model. Interestingly, three of the four independent variables (sex, distance to road, and disturbance level) performed within two AICc units of the best-supported model, while physical covariates alone were not at all explanatory of corticosterone concentrations (Table 4).

We took an additional set of keratin samples from our telemetered population one year after taking the first samples. We compared the temporal differences in corticosterone concentrations (log-transformed) by site and found that overall 2018 levels were significantly higher than 2017 levels ($t_{30} = 3.953$, p = 0.0004). When we examined those differences based on site, there were no significant differences in corticosterone concentrations between 2017 and 2018 for turtles at the control site ($t_{14} = 1.975$, p = 0.0683); however, 2018 corticosterone concentrations were significantly higher for turtles at the road-side site ($t_{15} = 3.578$, p = 0.0027).

Males exhibited significantly higher concentrations of corticosterone in 2018 across both sites ($t_{15} = 3.697$, $p = 0.0022$), while females did not ($t_{14} = 1.921$, $p = 0.0753$). Females in the control site demonstrated a slight decrease in corticosterone concentrations between 2017 - 2018 and the least amount of variation (Figures 10, 11).

We also examined the relationship between 2018 corticosterone concentrations and proximity to the road and found that the change in corticosterone concentration from 2017 to 2018 demonstrated no correlation with the nearest distance turtles were found from the road at the road-side site (Figure 12; $r_s = - 0.2536$, $p = 0.3607$). Further, corticosterone concentrations demonstrated no correlation with home range size, either for 2017 (Figure 13; $r_s = 0.3229$, $p = 0.0818$), 2018 (Figure 13; $r_s = - 0.0779$, $p = 0.6825$), or for the difference between 2017 - 2018 (Figure 13; $r_s = - 0.0939$, $p = 0.6216$). These results suggest that none of the variables we examined are particularly explanatory for concentrations of free corticosterone as a proxy for chronic stress.

Discussion

Overall, while turtles at the road-side site demonstrated no significant differences from turtles at the control site regarding home range size, the trend toward larger female home ranges with broader variation in size informed our understanding of turtle space use and the potential influence of the highway on overall turtle behavior and ecology. Turtles used the same types of microhabitats, irrespective of site and sex, but space use at the road-side site was bounded by the highway, with turtles showing no attempts to cross the road. In fact, although two different types of eco-passages (underpass bridge and culvert) were present within areas used by turtles, and turtles frequently crossed the snake fencing mitigation structures designed to direct them to the culvert passage, no turtles made use of these passages to reach the other side of the road. However, we observed several female turtles spending substantial amounts of time during the oviposition and nesting periods (> 6 weeks) within close proximity to the road, using open habitat in the road right-of-way. Males also used open habitat opened near the road, but they did not get as close to the road as females and tended to congregate on a ridge-side several meters higher in elevation than the road. The chronic stress analyses revealed that while there were no differences in corticosterone concentrations in turtles at between road-side and control sites, we did find broader variation in males at both sites. Additionally, further potentially confounding variables other than the road appeared to have limited impact on the corticosterone concentrations of turtles at the road-side site.

Home Ranges and Seasonal Activity Patterns

Notably, we found that female eastern box turtles had much larger ranges than males, which does not corroborate other studies, which suggest that generally males and females have similar home range sizes (Stickel 1950, Dodd Jr. 2001). Although we did not find home range size differences between sites, the centers of turtle activity suggest some interesting patterns. Clusters of activity appear in both sites and are typically located where canopy openings coincide with proximity to edge habitat (Figure 4). While clearings provide quality basking, mating, and oviposition habitat, the proximity of edge habitat provides for thermoregulatory options, cover, and foraging opportunities. During overwintering (November - March), turtles at both sites retreated to forested habitat near their previous brumation sites. While turtles remained in close proximity to their original overwintering sites throughout the brumation season, they emerged on warmer days (12 - 13°C) – possibly to forage – before retreating into the soil overnight.

There were differences between the types of clearings accessed in each site: two clearings in the control site were naturally-vegetated forest openings (0.25 - 0.5 ha in size) that were compact, largely circular, and surrounded by forest; at the road-side site, turtles used artificial openings that fell largely within the right-of-way, created when the road was cut into the surrounding hills. Although abutted by forest on one side, the other side is bounded by the highway, often including steep hillsides that serve as natural barriers between turtles and the road (Adams et al. 1989, Claussen et al. 2002). While several female turtles spent the majority of the summer months within 25 meters of the highway, we never encountered individuals attempting to cross the road. This behavior was previously recorded in box turtles and raises interesting questions about the implications for road mortality and potential population fragmentation (Shepard et al. 2008). Anecdotally, eastern box turtles are often found on smaller roads, and studies have found that box turtles utilize roads in addition to roadway habitats, leading to higher direct mortality from traffic (Nieuwolt 1996, Converse et al. 2005, Marsack and Swanson 2009). These deleterious effects suggest that any viable management strategies for maintaining populations will require broadly forested areas (~ 100 ha) free of roads and rich in microhabitat diversity (Doroff and Keith 1990, Nieuwolt 1996, Beizer and Steisslinger 1999, Steen and Gibbs 2004). Thus, there may be particular characteristics of roads that engender avoidance, an interesting and important avenue for further study, particularly regarding the loss of genetic variability following habitat bisection by high-traffic roads (Gibbs and Shriver 2002, Steen et al. 2006, Shepard et al. 2008).

While turtles at both road-side and control sites utilized similar habitat, we observed notable differences in movement patterns between the groups. Turtles at the control site often moved between clearings and forest in circuitous and meandering pathways. In contrast, the pathways of turtles at the road-side site between forest and edge habitats tended to be more linear. While turtles at the control site made frequent movements between various forest types – including grassy clearings, early successional habitat, and more mature, closed-canopy sections – turtles at the road-side site tended to move from closed-canopy sections of the forest to open road-side habitat and back. Overall, turtles in the road-side site demonstrated slightly broader variation in their preferences and tended to make more consistent use of edge habitat, demonstrated by their significant preference for vegetation ground cover, corroborating other studies (Figure 6; Dodd Jr. et al. 1994, Compton et al. 2002, Dodd and Dreslik 2008, Stickel 2010, Kapfer et al. 2013).

Habitat Selection

While habitat selection by turtles did not support our hypotheses that usage would differ between sites, turtles did demonstrate a marked preference for habitat that provides ample cover and microhabitats that offer a variety of options based on daily needs and life cycle events (Reagan 1974, Dodd Jr. et al. 1994, Dodd Jr. 2001, Rossell et al. 2006, McKnight 2011, Kapfer et al. 2013). We built our candidate models focusing on ground cover and soil condition selection at the forest floor, forest structure variables providing both immediate and canopy cover (which influences forest type), and ambient weather conditions which, while more variable on a day to day basis than cover and structure, can provide insight into turtle preference when similar microhabitats present with different weather states.

Overall, all turtles demonstrated the strongest selection at the forest floor and undercover levels, which is not surprising considering these microhabitats define their daily and life cycle movements. Eastern box turtle occurrence for each subset was positively associated with percent woody debris, ground cover which includes sticks, logs, and fallen trees on the forest floor, and understory, which includes any cover that obscures at least a foot above the forest floor (e.g., dense vegetation, forbs, and greenbrier and other thickets). Based on the odds ratios for top models, which evaluate occupancy potential based on the difference between selected and available habitat, each one percent increase in woody debris covering the forest floor had a commensurate 3.1 - 5.3% increase in the likelihood of occupancy depending on population subset. By far, the most significant preference displayed by any subset of turtles was for understory, with each one percent increase therein leading to a 2.5 - 4% increase in occupancy potential (Figure 6). Turtles selected understory two to three times more often than it was available, far more than for any other microhabitat variable (Table 2).

Interestingly, while both males and females in the control site exhibited significant preference for only woody debris cover and understory (based on overall availability), both males and females in the road-side site displayed a broader range of selection preferences. In addition to demonstrating significant selection for woody debris and understory, females in the road-side site exhibited significant preference for leaf litter and herbaceous vegetation ground cover, with 2.3 and 2.2 % increase in occupancy likelihood, respectively, per percent increase in difference in cover variable. Males in the road-side site also preferred woody debris, vegetation, and understory while additionally displaying significant selection for increased canopy cover. Although there were slight negative trends in preference, primarily related to weather conditions and soil moisture, males in the road-side site were the only subset of individuals to significantly select against any habitat variable (Table 2).

While ground cover and forest structure variables comprised the top models for all control turtles and females at the road-side site, weather conditions played a role in the top model for males at the road-side site and appeared in a model within two AICc of the top model for females at the road-side site. This suggests that while floor and structure variables are driving selection for all turtles, weather is further informing selection in turtles at the road-side site, possibly because the broader variety of microhabitat availability created by the presence of the road lends itself to more variability in weather conditions. Additionally, turtles at the road-side site are likely taking advantage of a broader variety of microhabitats because there are more available, as compared to the relatively homogenous microhabitat availability at the control site. These results, for the most part, corroborate other box turtle habitat studies in demonstrating preference for forested habitats with plentiful cover (Reagan 1974, Dodd Jr. et al. 1994, Dodd Jr. 2001, McKnight 2011). However, while box turtle occupancy is often positively associated with increased soil moisture, we found the opposite to be true for two population subsets: males at the road-side site (1% increase in difference in soil moisture content engendered 1.5% decrease in likelihood of turtle occupancy) and females at the control site (Table 2, Figure 6; 1% increase in soil moisture suggested 1.5% decrease in occupancy). Because the soils in southeastern Ohio are characterized by their mineral content – primarily clay, silt, sand, and loam – we measured the volumetric water content for mineral soil when taking soil moisture readings. However, while mineral is the most common soil type in this area, box turtles were seldom (but sometimes) found nestled into water-rich clays. Instead, turtles were most often found utilizing loose organic soils, including near forbs and where thick vegetation engendered less-densely packed soils. Therefore, our mineral soil readings may have been recording the moisture content of thickly-packed clay soils, not adequately reflecting that of the looser soils that turtles often selected. These results have interesting habitat management implications, suggesting that maintaining organic soil layers and leaf litter are vital to preserving box turtle habitat. Especially in the case of prescribed burns, these soils and ground cover are often easily overlooked and lost, which could have detrimental effects on the population persistence of resident turtle populations (Russell et al. 1999, Platt et al. 2010, Howey and Roosenburg 2013).

Chronic Stress

Although concentrations of corticosterone in turtles did not differ between sites, male turtles in both sites exhibited a broader range of variation in hormone levels than female turtles (Figure 7). This seems to suggest that males turtles may encounter a broader range of potential stressors, despite having significantly smaller home ranges than female turtles; this signal of broader ranges of corticosterone concentrations in male animals warrants further consideration. This variation could also be a result of the proximity of some turtles to the road. It is also possible that the higher corticosterone levels in males are related to displacement due to the highway construction; they could still be experiencing heightened hormone levels as a result. Males exhibited smaller overall home range sizes than females but also demonstrated strong site fidelity. Although females also exhibit both site fidelity and natal homing, they travel considerably farther than males in this population (Hester et al. 2008, Rittenhouse et al. 2008, Kapfer et al. 2013). While this may expose them to a broader variation of stressors and novel circumstances, including predators, it seems possible that males may be more attuned to their smaller home ranges and possibly more sensitive to small environmental alterations therein, because the additional disturbance layer of OHV trails did not appear to affect overall corticosterone concentrations (Figure 7). Additionally, for turtles at the road-side site, although we examined a variety of variables (sex, size, age, proximity to road, and degree of disturbance), our data suggest that corticosterone concentrations may be more random than linked to any particular factor (Table 4). Assuming that we did not miss the time window for identifying increased stress potentially resulting from habitat fragmentation and the construction and presence of the road, and that the turtles have not returned to metabolic equilibrium, it is important to isolate other indicators of long-term stress and build a stronger understanding of the physiological underpinnings of stress responses (Romero 2004, Owen et al. 2014).

Corticosterone concentrations increased almost universally for our tracked (30) and recaptured (1) animals between 2017 and 2018 (Figure 11). With the exception of females at the control site, our second bioassay of nail keratin suggested significantly higher concentrations of this biomarker for chronic stress. Although year effects may have many ecological and research-related underpinnings, it seems unlikely that the proximity of the road could have resulted in increases across the board. While it is likely that at least some of the differences observed can be attributed to inter-assay variation, some interesting and potentially ecologically-relevant trends emerged (Tonra et al. 2013). Corticosterone concentrations for male turtles across both sites exhibited significant upturns in 2018, possibly lending additional weight to our hypothesis of increased male sensitivity to environmental changes. Changes in their environment may have included the continuous presence of our research team (at least once weekly May – November 2017). Although interaction was kept to a minimum, monthly handling to download temperature data, shifting of cover objects to confirm presence, and our periodic presence may be at least partially responsible for the results we are seeing (Langkilde 2006). It could also be the case that while the road engenders more or less continual stress responses, the personal nature of our interactions with turtles had a more acute effect. Although the total weight of tracking and datalogging equipment carried by turtles comprised < 5% of their total body weight, the constant presence of the equipment may also have triggered stress responses. These are only some possibilities for the changes we are seeing, and there are likely additional, more parsimonious, and potentially undetermined ecological stimuli. However, if we are seeing a research effect on the animals, the discrepancy between population subsets is of particular interest. Every population subset excepting females at the control site exhibited higher concentrations of corticosterone in the second assay. This might suggest that these turtles are, for some reason, already at capacity for free corticosterone, or that they experienced our presence differently than other population subsets. These results are not definitive and suggest that turtle endocrinology research has the capacity to provide insight into turtle ecology and responses to environmental stimuli and further inform our research and conservation planning.

Evaluation of corticosterone-binding globulin (CBG) and other relevant hormones might provide a more complete picture of chronic stress in these animals; for example, an animal normalized to chronic stress might have high concentrations of free corticosterone, but a lower CBG (Angelier et al. 2010, Clinchy et al. 2011). We postulate, however, that the use of keratin samples in extracting concentrations of free corticosterone to examine a commonly-assessed facet of the stress response is a viable alternative to other extraction methods, including blood and fecal sampling. Keratin samples provide more information and insight into the potentially chronic nature of stress-related hormones by extending the temporal scale of sampling beyond the minute spectrum readable through other sampling techniques. Further, keratin sampling is less invasive and indicates little-to-no stress response due to handling and sampling, due to its longer time scale (Baxter-Gilbert et al. 2014). An increased understanding of chronic stress responses will be helpful in assessing the effects that heightened hormone levels might have on long-term fitness and population persistence.

Management and Conservation Implications

Further research into the effects of roads on naïve populations could inform pre-planning and mitigation efforts that could ameliorate potential road effects on turtles. Some of the most important mitigating elements are protecting and preserving existing turtle habitat and promoting and providing appropriate and appealing habitat elements following anthropogenic disturbance. While this might include creating functional corridors (or revamping existing passages) that allow turtles to access habitat on either side of the road, it seems likely that focusing on the protection and improvement of existing available habitat would be safer and more beneficial for animals and potentially less costly from a management perspective. The results of this study have some potentially interesting management implications for promoting essential eastern box turtle habitat and encouraging animal safety throughout the range. Much of the Athens Unit of Wayne National Forest seems to be at a similar stage of forest progression, with oaks and hickories obstructing the canopy and fighting for space, and with little in the way of natural clearings in the forest. It seems probable that a few strategically-placed and seasonally-maintained areas of open canopy and early successional habitat in the forest might provide turtles a viable alternative to performing life cycle tasks within sight of 70 mph traffic. It is important, however, when creating and maintaining clearings and edge habitat to keep in mind turtle usage of organic soils and ground cover that might be negatively impacted by some common management techniques, including prescribed burning and the construction byproduct of packing soils with heavy machinery. Interestingly, some plants and forbs that are considered invasive in Ohio – multiflora rose, for instance – provide particularly good cover, accumulation of leaf litter and other organic material, and soft soils for box turtles. Management decisions will need to weigh the costs of invasive vegetation versus the benefits it provides to local fauna.

Our habitat selection results seemed to provide similar implications. If all turtles strongly prefer the same types of microhabitat, management could be standardized to the tune of allowing fallen trees to be retained (creating ample woody debris), and not eliminating tall grasses and forbs, which provide necessary understory to these animals (Figure 6). Refining the understanding of habitat requirements for herpetofauna in the bypass area and comparing them to the control site provides a foundation for improved conservation efforts and enables us to address wildlife management and conservation mandates. For example, the broader variety and expanse of certain microhabitat types in the road-side site provides appealing options to animals, who will utilize habitats as necessary regardless of their proximity to the road. Therefore, creating these types of habitats further into forested areas could mitigate potential road effects by creating comparably appealing, but less risky and exposed habitat. Determining baselines for turtle interactions with roadways and investigating the risk of road mortality helps us to predict changes within populations and could assist with the development of policy and mitigation strategies, including increased capacity for planning, innovating, cost-benefit analyses, deployment of resources, and project delivery. Based on our assessment of space-use, habitat selection, and chronic stress indicators in the control-impact setting, we are able to inform policy makers on turtle/road ecology (e.g., turtles will access right of way, making them subject to direct mortality, but indirect effects have a potentially more limited influence), predictors for habitat preference (including edge habitat with ample cover and thermoregulatory options), and habitat management strategies (such as maintaining clearings, limiting prescribed burning, and allowing for multiple simultaneous stages of successional forest).

Overall, although no one parameter of eastern box turtle persistence that we examined confirmed the existence of indirect road effects, combining home range measurements, traditional habitat selection studies, and novel assessments of chronic stress suggests the need for further inquiry. We have ascertained that turtles in the road-side site are utilizing road right-of-way habitat, and although none of our research animals crossed the road or were killed by traffic, proximity to highways increases the opportunity for incidences of direct mortality. Further, we have pinpointed habitat management strategies focused on opening forest clearings, maintaining appropriate ground cover, and managing edges that might entice and help protect this imperiled species. Finally, we have identified a signal of broader variation of hormonal responses in male turtles which, when considering in the context of their small home ranges and exposure to road-side habitats, may serve as a launch pad for further inquiry. Although we were unable to draw conclusive connections between the road and deleterious effects on turtle health, this should not be considered an unrestricted license to continue building roads through contiguous habitat. Turtles worldwide face substantial threats from habitat loss, road mortality, invasive species, disease, exploitation, and changing climates. Future research should further explore the effects of chronic stress and evaluate the additive and cumulative effect of this multitude of stressors. A better understanding of stress ecology will inform conservation plans and ensure that the anthropogenic march of progress does not rest on the backs of turtles.

Tables and Figures

Table 1. Candidate conditional logistic regression models of eastern box turtle microhabitat selection for population subsets at the Athens Unit of Wayne National Forest, southeastern Ohio in 2017-2018. Model statistics include the number of parameters in each model (K), the Akaike Information Criteria corrected for small sample size value/likelihood, the difference in Akaike Information Criteria corrected for small sample size from the best-supported model (ΔAICc), and the AICc weight, denoting the explanatory value of each model. *Variables include: percent woody debris cover (WD), percent leaf little cover (LL), percent vegetation cover (V), percent soil moisture content (MS), percent understory (US), percent canopy cover (CC), air temperature (°C), and percent air relative humidity (RH). Best-supported models (within two AICc units of top model) are italicized.

Model	**Variables***	**K**	**AICc**	**ΔAICc**	**AICc weight**
	Females, road-side site				
Floor + Structure	*WD+LL+V+MS+US+CC*	*6*	*384.36*	*0.00*	*0.53*
Floor + Structure + Climate	*WD+LL+V+MS+US+CC+°C+RH*	*8*	*384.62*	*0.26*	*0.47*
Structure	US+CC	2	395.07	10.72	0.00
Structure + Climate	US+CC+°C+RH	4	395.52	11.16	0.00
Floor + Climate	WD+LL+V+MS+°C+RH	6	398.79	14.44	0.00
Floor	WD+LL+V+MS	4	399.05	14.70	0.00
Null	~	0	418.03	33.67	0.00
Climate	°C+RH	2	419.37	35.01	0.00
	Males, road-side site				
Floor + Structure + Climate	*WD+LL+V+MS+US+CC+°C+RH*	*8*	*393.28*	*0.00*	*0.77*
Floor + Structure	WD+LL+V+MS+US+CC	6	395.98	2.70	0.20
Structure + Climate	US+CC+°C+RH	4	400.56	7.29	0.02
Structure	US+CC	2	403.22	9.94	0.01
Floor	WD+LL+V+MS	4	414.11	20.83	0.00
Floor + Climate	WD+LL+V+MS+°C+RH	6	415.31	22.03	0.00
Null	~	0	453.99	60.71	0.00
Climate	°C+RH	2	457.64	64.36	0.00
	Females, control site				
Floor + Structure	*WD+LL+V+MS+US+CC*	*6*	*467.87*	*0.00*	*0.81*
Floor + Structure + Climate	WD+LL+V+MS+US+CC+°C+RH	8	470.92	3.05	0.18
Structure	US+CC	2	475.89	8.02	0.01
Structure + Climate	US+CC+°C+RH	4	478.56	10.69	0.00
Floor	WD+LL+V+MS	4	493.38	25.51	0.00
Floor + Climate	WD+LL+V+MS+°C+RH	6	497.17	29.30	0.00
Null	~	0	506.56	38.69	0.00
Climate	°C+RH	2	509.90	42.03	0.00
	Males, control site				
Floor + Structure	*WD+LL+V+MS+US+CC*	*6*	*514.20*	*0.00*	*0.87*
Floor + Structure + Climate	WD+LL+V+MS+US+CC+°C+RH	8	518.03	3.83	0.13
Structure	US+CC	2	526.05	11.85	0.00
Structure + Climate	US+CC+°C+RH	4	529.26	15.06	0.00
Floor	WD+LL+V+MS	4	565.42	51.21	0.00
Floor + Climate	WD+LL+V+MS+°C+RH	6	569.39	55.19	0.00
Null	~	0	592.36	78.16	0.00
Climate	°C+RH	2	595.32	81.12	0.00

Table 2. Conditional logistic regression models that best explain microhabitat selection by eastern box turtles for population subsets at the Athens Unit of Wayne National Forest, southeastern Ohio in 2017-2018. Odds ratios refer to a 1% unit increase for each variable. Bolded values denote significant selection (α = 0.05). Starred variables for females at the road-side site were not included in the top model, but were included in the model within two AICc of the top model, and are potentially relevant.

	Measured values $\bar{x}$ ± SE				
Variable	**Turtle Point**	**Random Point**	**Model coefficient ± SE**	**P-value**	**Odds ratio**
		Females, road-side site (n = 153 pairs)			
% Woody Debris	12.22 ± 1.28	8.76 ± 0.93	0.0512 ± 0.0136	**0.0002**	1.0525
% Leaf Litter	43.14 ± 2.73	47.65 ± 2.77	0.0236 ± 0.0095	**0.0127**	1.0239
% Vegetation	41.67 ± 3.12	32.68 ± 2.77	0.0223 ± 0.0096	**0.0202**	1.0225
% Soil Moisture	24.88 ± 2.05	23.07 ± 1.09	0.0034 ± 0.006	0.57	1.0034
% Understory	30.82 ± 2.57	15.16 ± 1.81	0.0249 ± 0.0061	> **0.0001**	1.0252
% Canopy Cover	68.13 ± 2.81	70.09 ± 3.09	-0.0006 ± 0.0056	0.9094	0.9994
Temperature °C*	25.11 ± 0.44	25.27 ± 0.44	-0.0215 ± 0.0262	0.4124	0.9787
% Relative Humidity*	74.01 ± 6.36	64.46 ± 1.42	0.0076 ± 0.0064	0.2389	1.0076
		Males, road-side site (n = 166 pairs)			
% Woody Debris	13.95 ± 1.16	10.21 ± 0.94	0.0448 ± 0.0142	**0.0017**	1.0458
% Leaf Litter	46.96 ± 2.36	54.43 ± 2.59	0.0138 ± 0.0102	0.1771	1.0139
% Vegetation	36.23 ± 2.58	23.16 ± 2.15	0.0269 ± 0.0116	**0.02**	1.0273
% Soil Moisture	22.09 ± 0.87	23.45 ± 1.42	-0.0154 ± 0.0099	0.1192	0.9847
% Understory	33.28 ± 2.37	12.95 ± 1.76	0.031 ± 0.0067	> **0.0001**	1.0314
% Canopy Cover	81.26 ± 2.15	80.14 ± 2.53	0.0133 ± 0.0065	**0.04**	1.0134
Temperature °C	24.45 ± 0.39	24.77 ± 0.37	-0.0728 ± 0.0287	**0.0111**	0.9298
% Relative Humidity	66.05 ± 1.47	66.34 ± 1.4	-0.0056 ± 0.0071	0.4243	0.9944
		Females, control site (n = 185 pairs)			
% Woody Debris	14.16 ± 1.04	11.03 ± 0.98	0.0341 ± 0.0134	**0.0108**	1.0347
% Leaf Litter	58.27 ± 2.15	62.46 ± 2.13	0.0194 ± 0.011	0.0778	1.0196
% Vegetation	25.3 ± 2.33	20.35 ± 1.96	0.0132 ± 0.0123	0.2823	1.0133
% Soil Moisture	22.9 ± 1.13	25.64 ± 1.62	-0.0134 ± 0.0078	0.0863	0.9867
% Understory	25.27 ± 1.91	11.32 ± 1.41	0.0296 ± 0.0065	> **0.0001**	1.03
% Canopy Cover	75.68 ± 2.28	83.34 ± 1.82	-0.0087 ± 0.0049	0.0754	0.9913
		Males, control site (n = 216 pairs)			
% Woody Debris	17.34 ± 1.38	10.49 ± 0.86	0.031 ± 0.0112	**0.0057**	1.0315
% Leaf Litter	53.03 ± 2.13	64.26 ± 2.02	0.0043 ± 0.0087	0.6204	1.0043
% Vegetation	27.11 ± 2.26	20.56 ± 1.96	-0.0029 ± 0.01	0.7746	0.9971
% Soil Moisture	26.98 ± 1.17	27.25 ± 1.26	0.0022 ± 0.0063	0.7328	1.0022
% Understory	30.82 ± 2.16	9.63 ± 1.36	0.0394 ± 0.0062	> **0.0001**	1.0401
% Canopy Cover	77.12 ± 2.09	81.48 ± 1.92	0.0019 ± 0.0046	0.6743	1.0019

Table 3. Candidate linear regression models of eastern box turtle corticosterone concentrations for population subsets at the Athens Unit of Wayne National Forest, southeastern Ohio in 2017-2018. Model statistics include the number of parameters in each model (K), the Akaike Information Criteria corrected for small sample size value/likelihood, the difference in Akaike Information Criteria corrected for small sample size from the best-supported model (ΔAICc), and the AICc weight, denoting the explanatory value of each model. *Variables include: sex (G), site (S), carapace length (CL), and a binary age proxy (A). Mass, though recorded, was not included because of its strong correlation with carapace length. Best-supported models (within two AICc units of top model) are italicized.

Model	**Variables***	**K**	**AICc**	**ΔAICc**	**AICc weight**
Null	*~*	*2*	*116.21*	*0.00*	*0.37*
Site	*S*	*3*	*117.00*	*0.80*	*0.25*
Sex	G	3	118.33	2.12	0.13
Sex, Site	G + S	4	119.21	3.00	0.08
Site, Physical Covariates	S + CL + A	5	119.81	3.61	0.06
Sex, Physical Covariates	G + CL + A	5	120.01	3.80	0.05
Sex-Site Interaction	G * S	5	121.45	5.24	0.03
Sex, Site, Physical Covariates	G + S + CL + A	6	121.46	5.25	0.03
Sex-Site-Physical Covariates Interaction	G * S + CL + A	7	123.90	7.69	0.01

Table 4. Candidate linear regression models of eastern box turtle corticosterone concentrations for turtles at road-side sections Athens Unit of Wayne National Forest, southeastern Ohio in 2017-2018. Model statistics include the number of parameters in each model (K), the Akaike Information Criteria corrected for small sample size value/likelihood, the difference in Akaike Information Criteria corrected for small sample size from the best-supported model (ΔAICc), and the AICc weight, denoting the explanatory value of each model. *Variables include: sex (G), site (S), carapace length (CL), and a binary age proxy (A). Mass, though recorded, was not included because of its strong correlation with carapace length. Best-supported models (within two AICc units of top model) are italicized.

Model	**Variables***	**K**	**AICc**	**ΔAICc**	**AICc weight**
Null	*~*	*2*	*16.94*	*0.00*	*0.26*
Disturbance	*ATV*	*3*	*17.61*	*0.67*	*0.19*
Sex + Disturbance	*G + ATV*	*4*	*17.89*	*0.95*	*0.16*
Distance to Road	*D*	*3*	*18.77*	*1.82*	*0.11*
Sex	G	3	19.06	2.12	0.09
Sex + Distance	G + D	4	19.78	2.84	0.06
Sex + Disturbance + Distance	G + ATV + D	5	20.54	3.60	0.04
Disturbance + Distance	ATV + D	4	20.94	4.00	0.04
Physical Covariates (Carapace Length, Age Proxy)	P	4	21.82	4.88	0.02
Covariates + Distance	P + D	5	24.46	7.52	0.01
Disturbance + Covariates	ATV + P	5	24.98	8.03	0.00
Sex + Covariates	G + P	5	26.21	9.27	0.00
Sex + Disturbance + Covariates	G + ATV + P	6	28.38	11.44	0.00
Sex + Covariates + Distance	G + P + D	6	28.77	11.83	0.00
Disturbance + Covariates + Distance	ATV + P + D	6	29.76	12.82	0.00
Sex + Disturbance + Covariates + Distance	G + ATV + P + D	7	33.72	16.77	0.00

Figure 1. Ohio, major cities, interstates, and study site: Nelsonville Bypass (U.S. Highway 33).

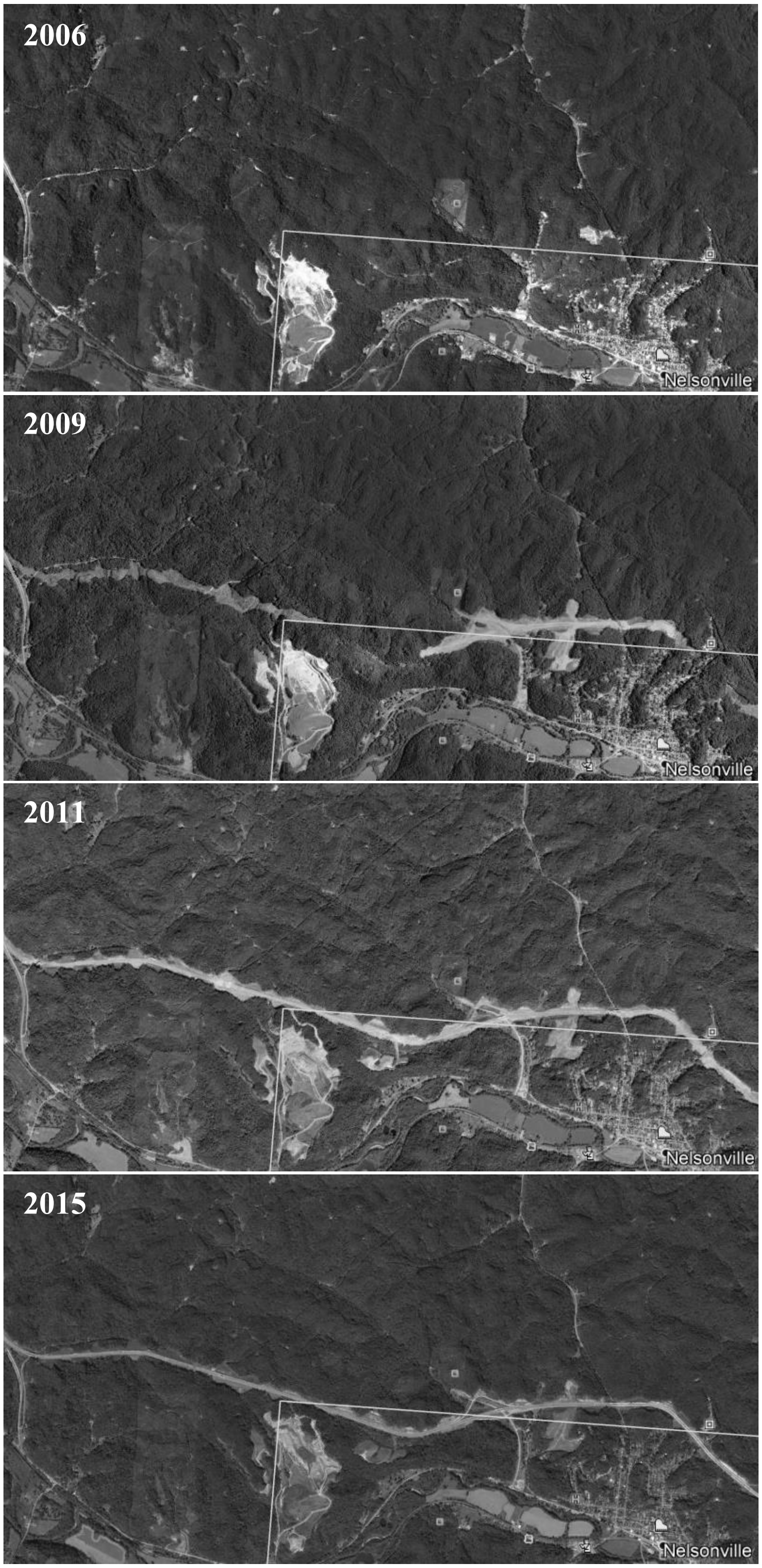

Figure 2. Treatment Site: Wayne National Forest, U.S. Highway 33, Ohio, before (2006), during (2009, 2011), and after (2015) construction (Google Earth 2018).

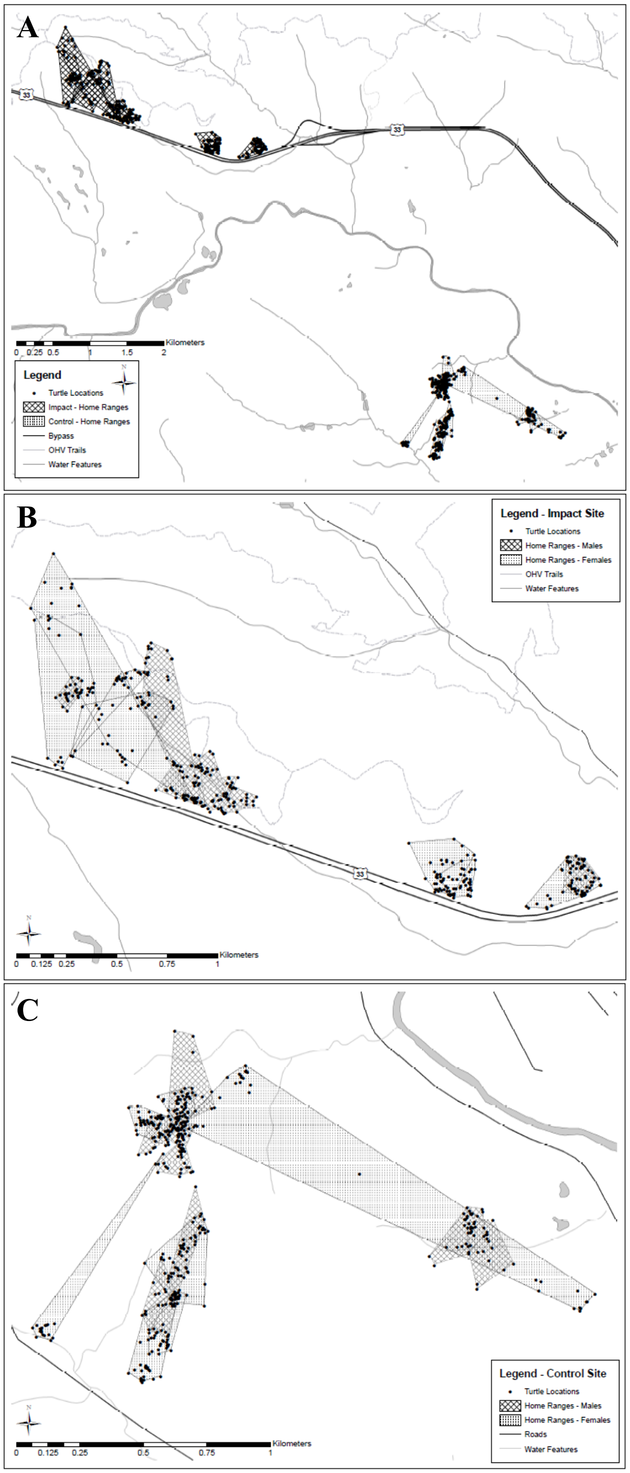

Figure 3. Box turtle locations (black circles) and home ranges as 100% Minimum Convex Polygons. While animals at road-side (lines) and control (dots) sites demonstrated no significant differences in home range size (A), female turtles (dots) exhibited larger overall home ranges than male turtles (lines) in both road-side (B) and control (C) sites.

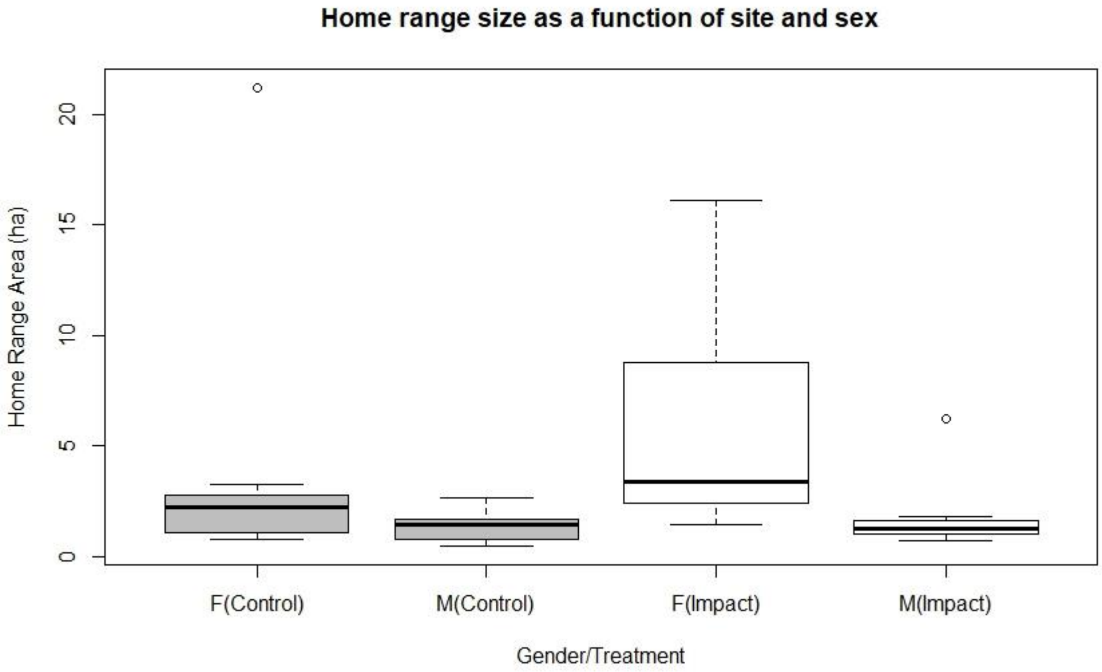

Figure 4. Box turtle home range sizes by population subset based on site and sex.

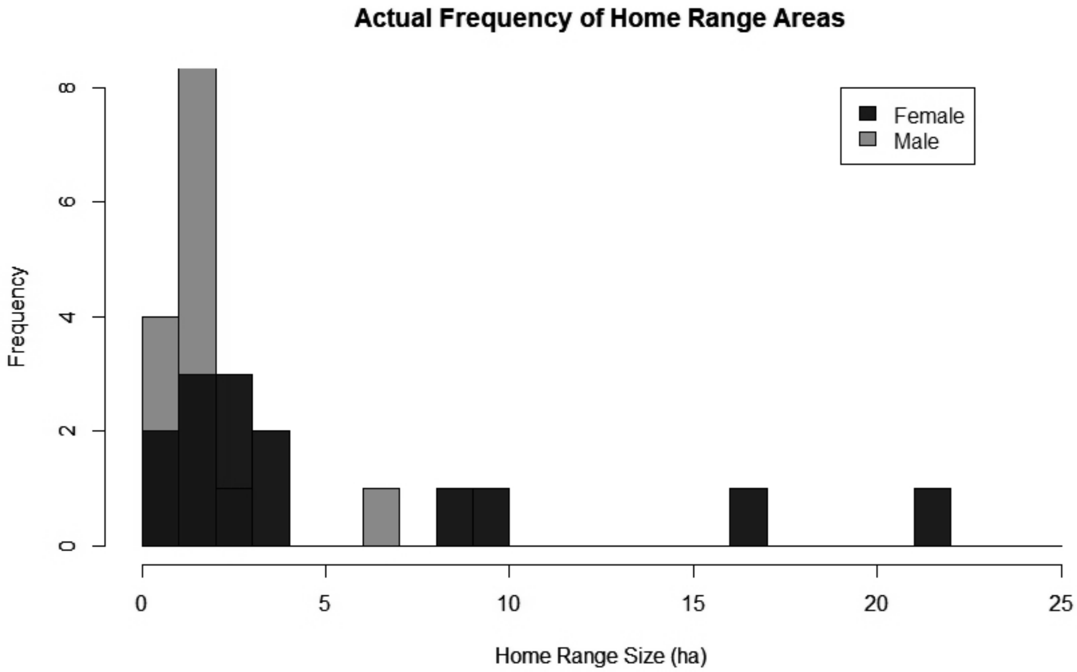

Figure 5. Actual frequency of home range sizes (ha).

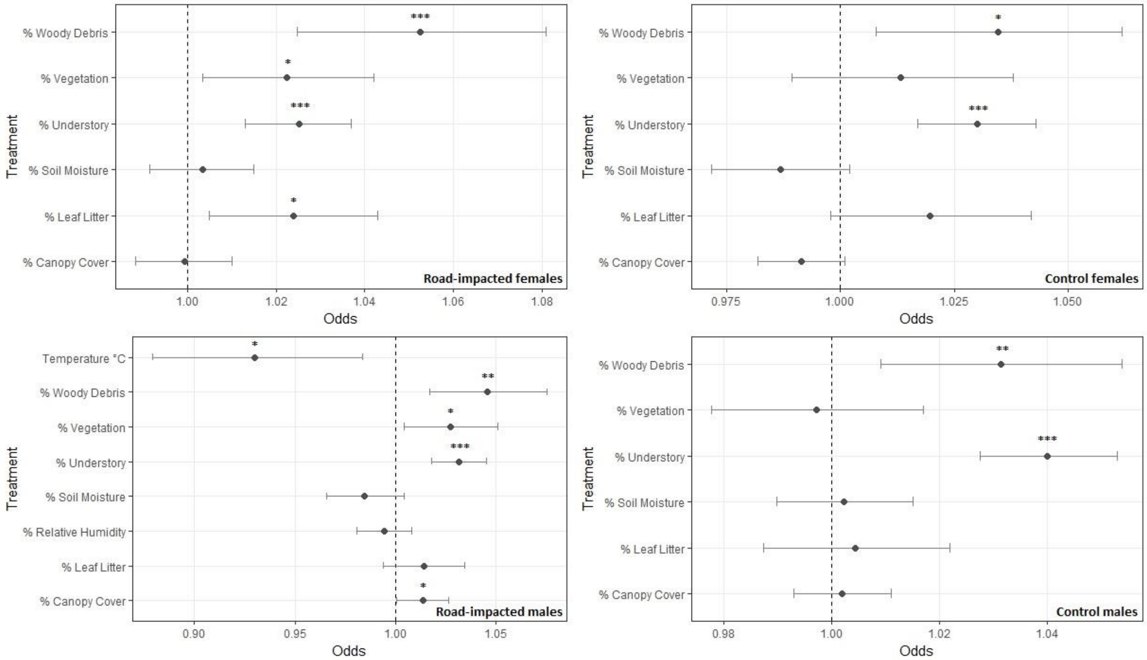

Figure 6. Odds ratios for the best-supported habitat selection model for each subset of turtles, incorporating ground cover and habitat structure variables. All turtles selected for woody debris and understory significantly more than was available; females also selected for vegetation.

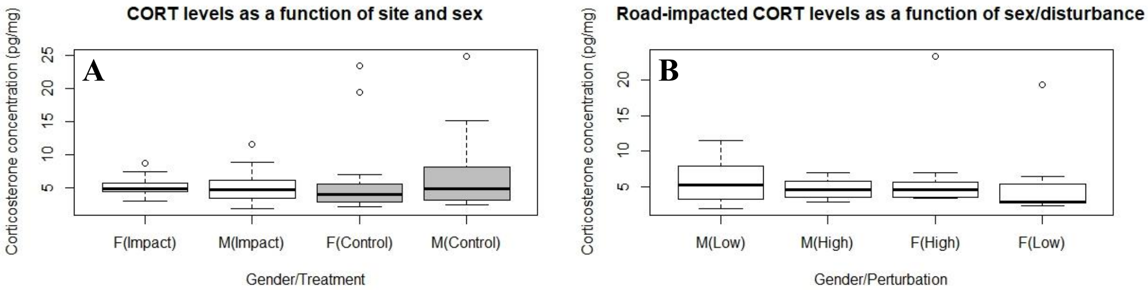

Figure 7. Medians and interquartile ranges of corticosterone concentrations for each subset of the total turtle population (N = 74; N_{IF} = 18, N_{IM} = 26, N_{CF} = 11, N_{CM} = 19), and based on disturbance level for turtles in each section of the road-side site (N = 44; N_{HDF} = 10, N_{LDF} = 8, N_{HDM} = 14, N_{LDM} = 12).

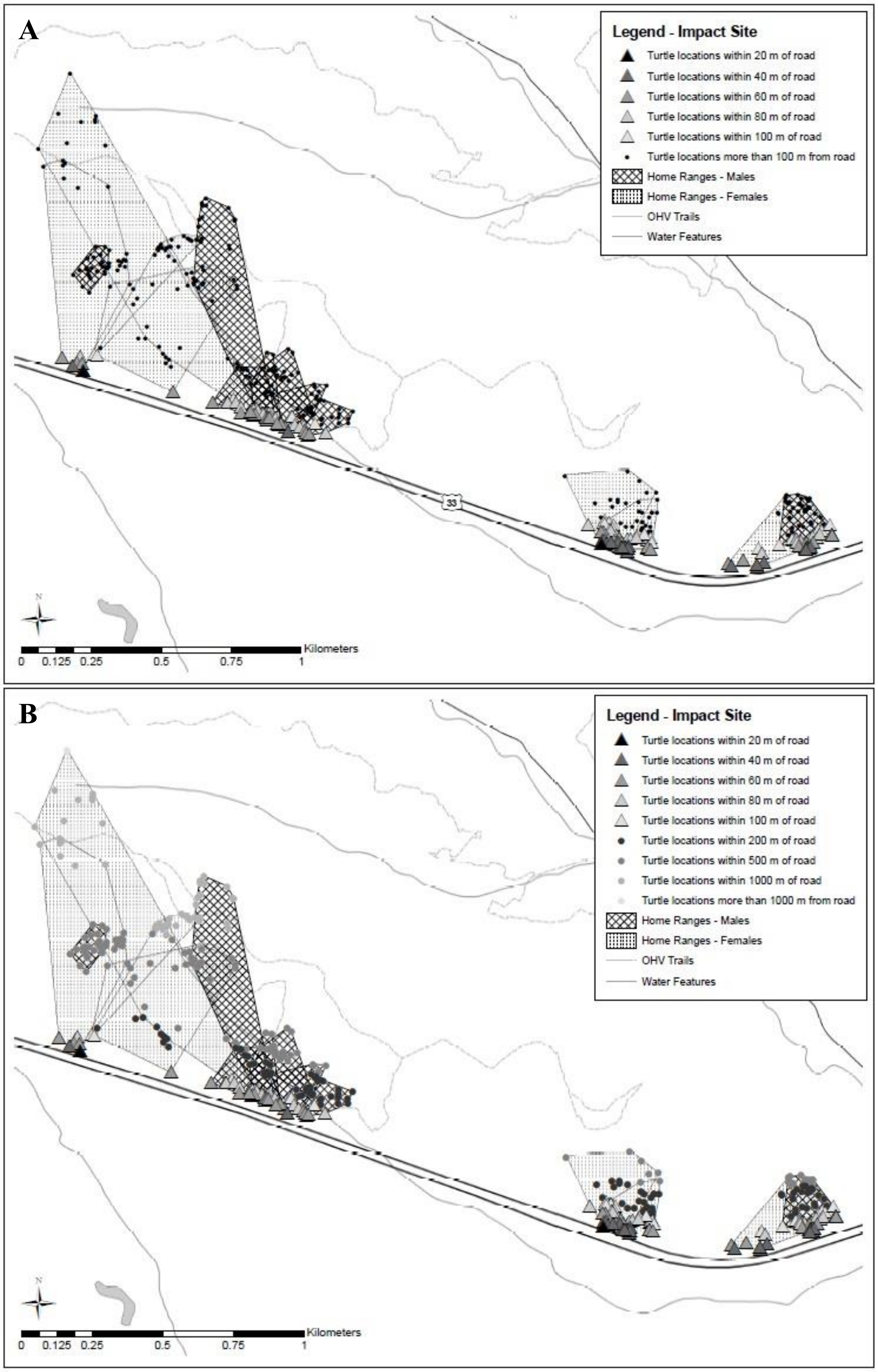

Figure 8. Box turtle locations (black circles) and home ranges showing proximity to the bypass. 147 turtle points were taken within 100 m of the road between May 2017 and early June 2018 (A). 14 of 15 turtles at the road-side site spent time within 200 m of the bypass (B).

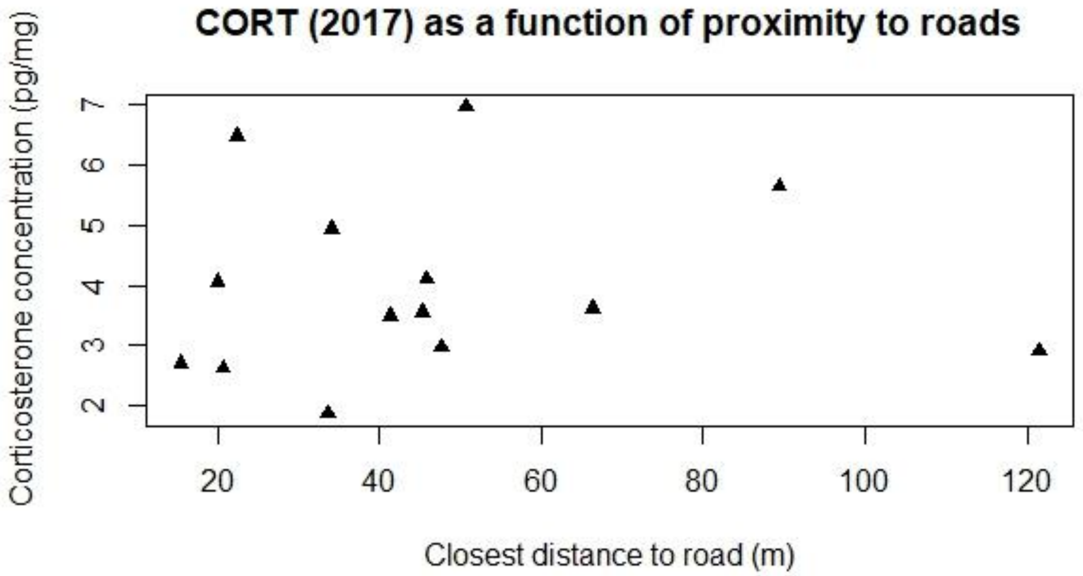

Figure 9. Corticosterone concentrations (as proxies for chronic stress) demonstrated no correlation with nearness to the bypass for turtles at the road-side site.

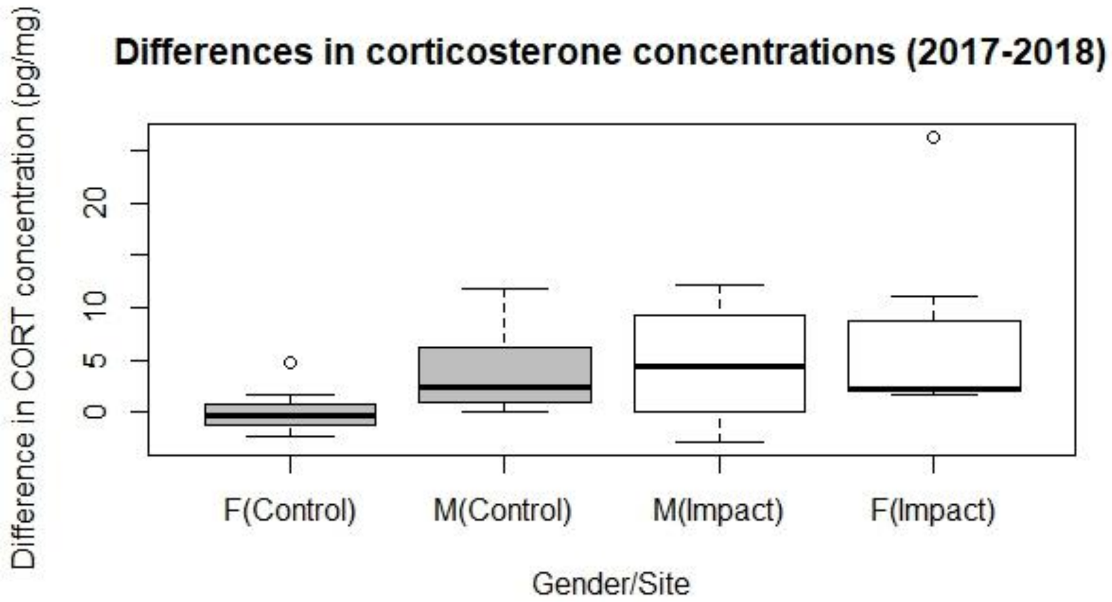

Figure 10. Medians and interquartile ranges for differences in corticosterone concentrations between 2017 and 2018 for each subset of recaptured turtles (N = 31).

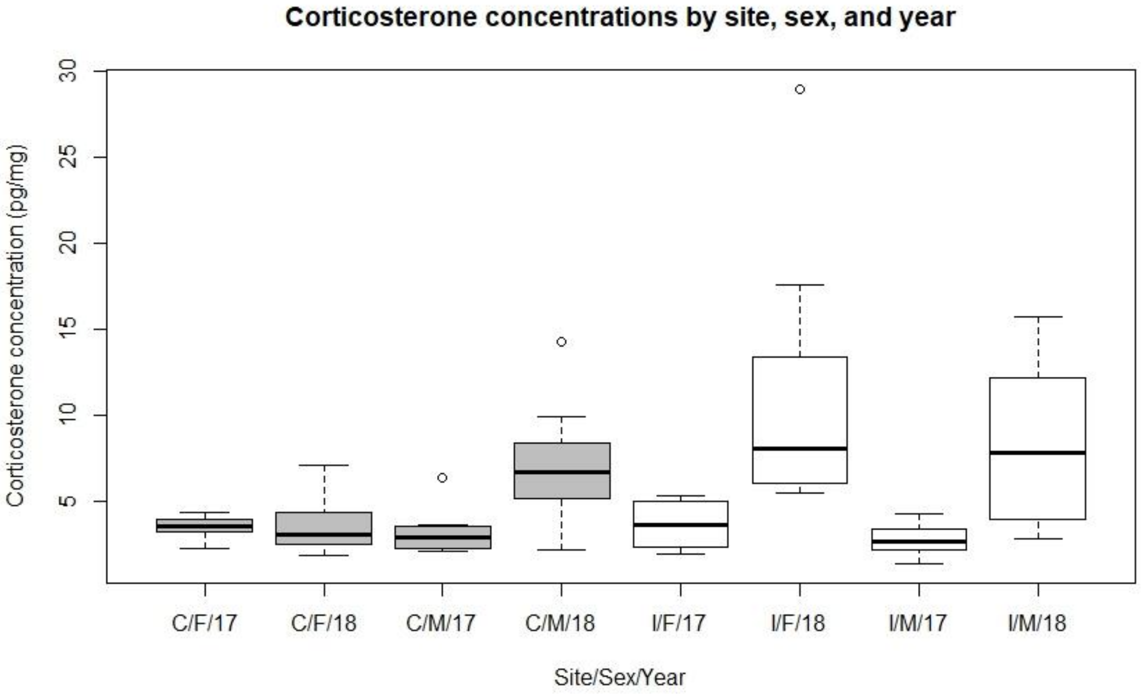

Figure 11. 2017 and 2018 corticosterone concentrations for all subsets of turtles.

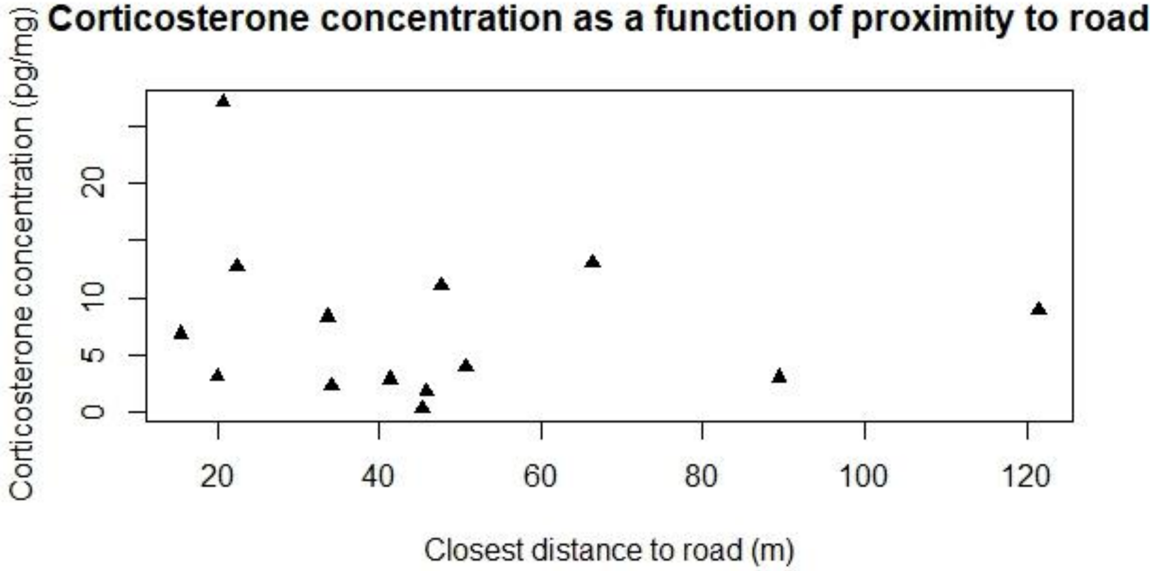

Figure 12. Changes in corticosterone concentrations (as proxies for chronic stress) from 2017 to 2018 demonstrated no correlation with nearness to the bypass for road-side turtles.

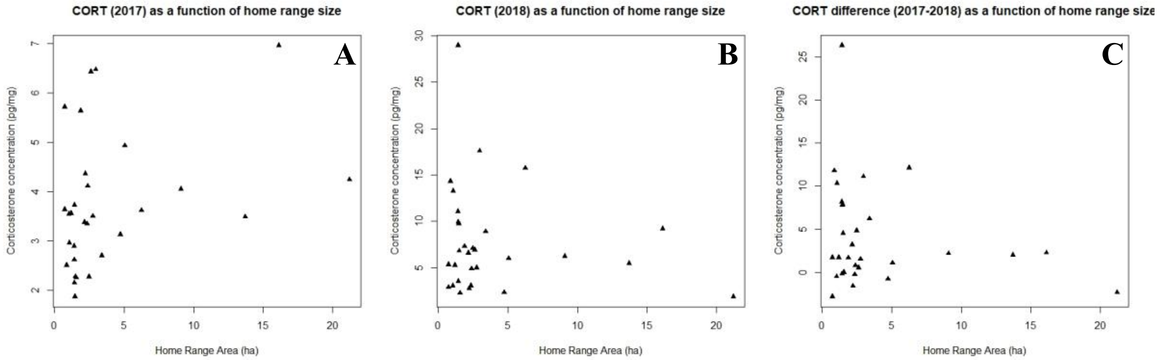

Figure 13. Neither corticosterone concentrations in 2017 (A), 2018 (B), nor the difference between them (C) demonstrated a correlation with home range size for turtles at the road-side site.

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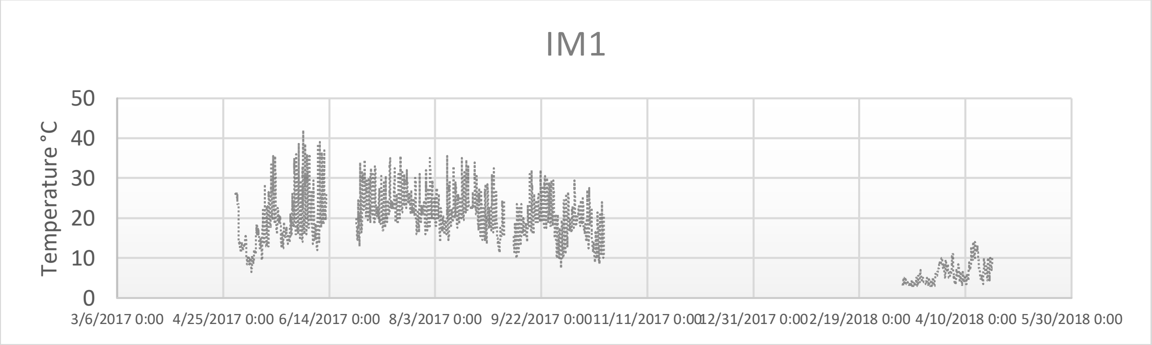
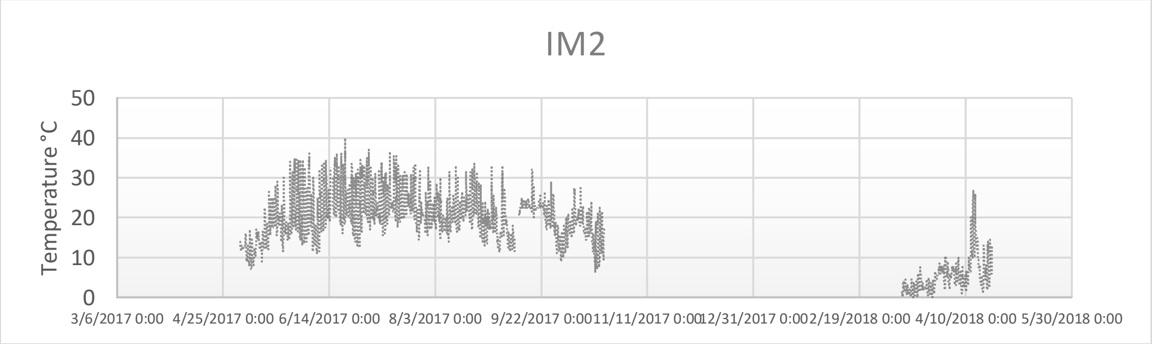
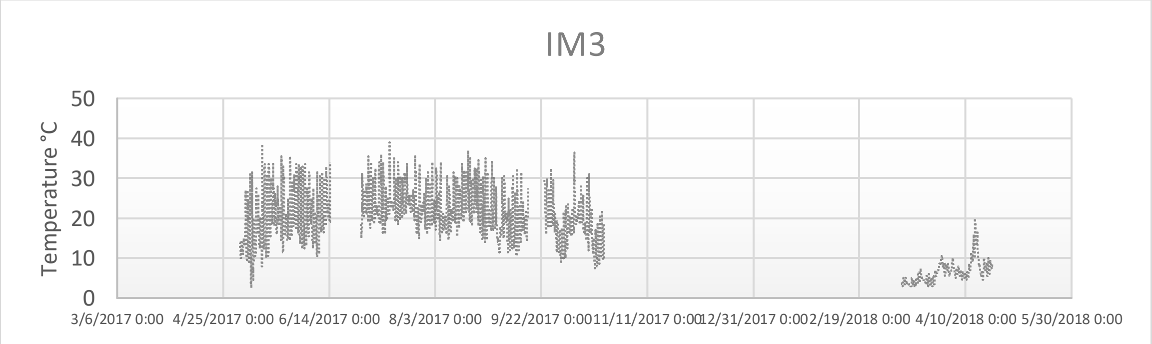
APPENDIX A: AMBIENT TEMPERATURE READINGS FROM IBUTTONS

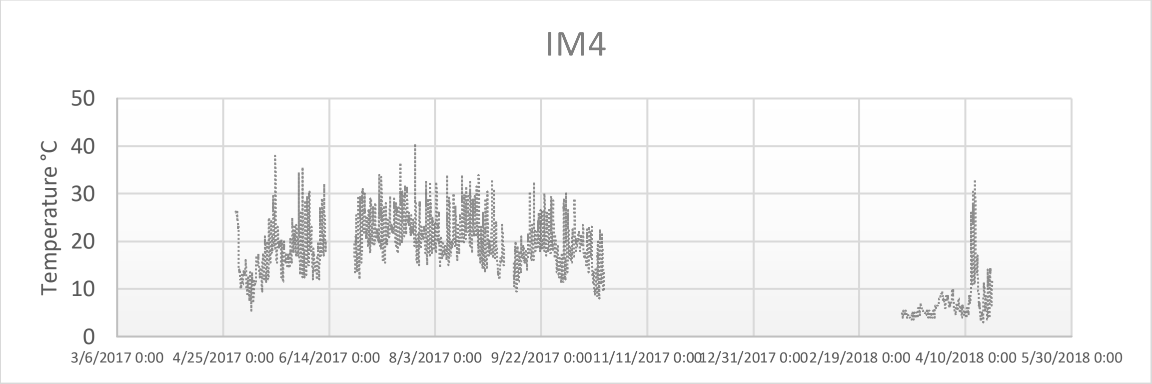
Each tracked turtle carried an iButton datalogger on its left, front costal scute from the time of first capture to May 2018. Temperature data were taken every 30 minutes during this time. Each datalogger stored approximately five weeks of data before recording over previous data. Gaps in data recorded between May and November of 2017 are attributable to the following: temporary loss of turtle location, inaccessibility of turtles (underground, in thickets, or mating), loss of iButton datalogger, malfunction of iButton datalogger, and malfunction of handheld thermochron downloader.

Data were not taken during the brumation period, while turtles were overwintering beneath the substrate, but were taken immediately following the cessation of brumation. These few weeks of data near the end of overwintering provided the most interesting insights into turtle behavior, including the temperatures at which turtles overwintered, temperatures that prompted them to return to the surface, and frequency with which turtles emerged during the final few weeks of the brumation period.





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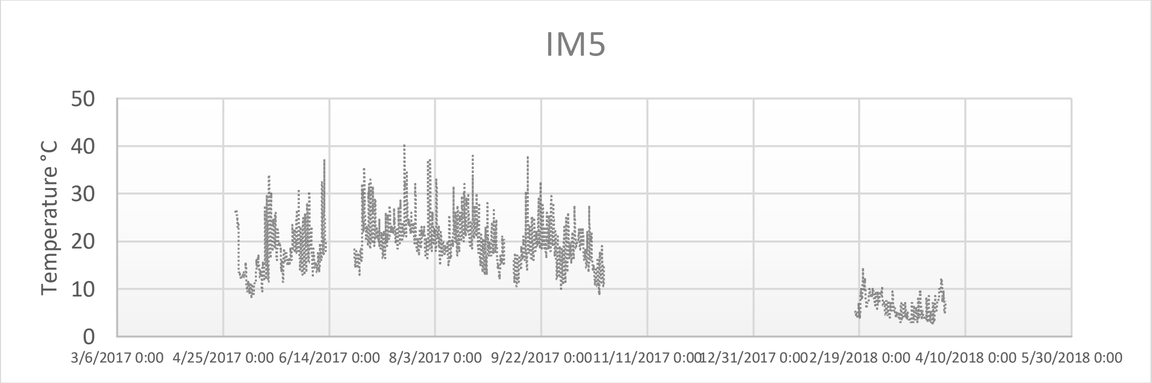
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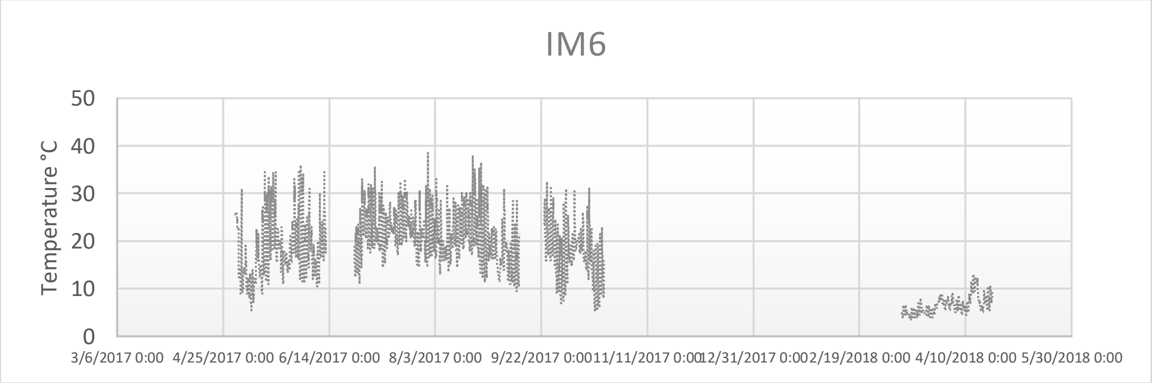
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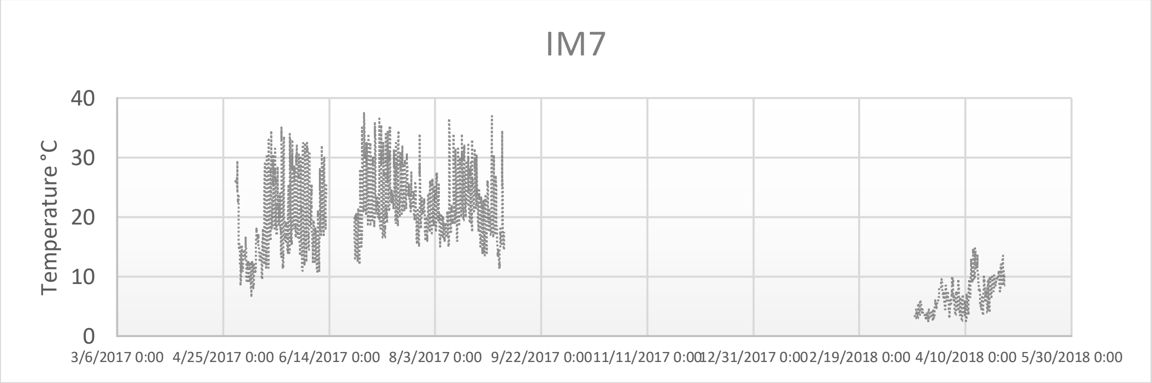
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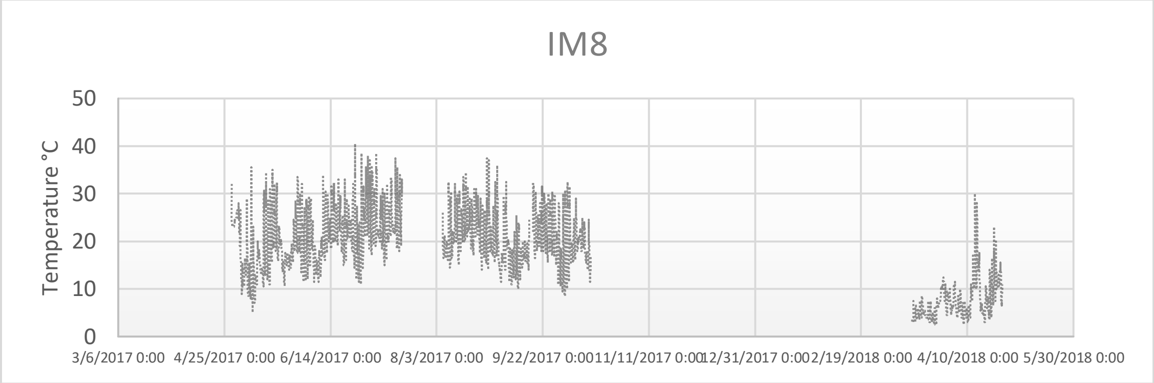
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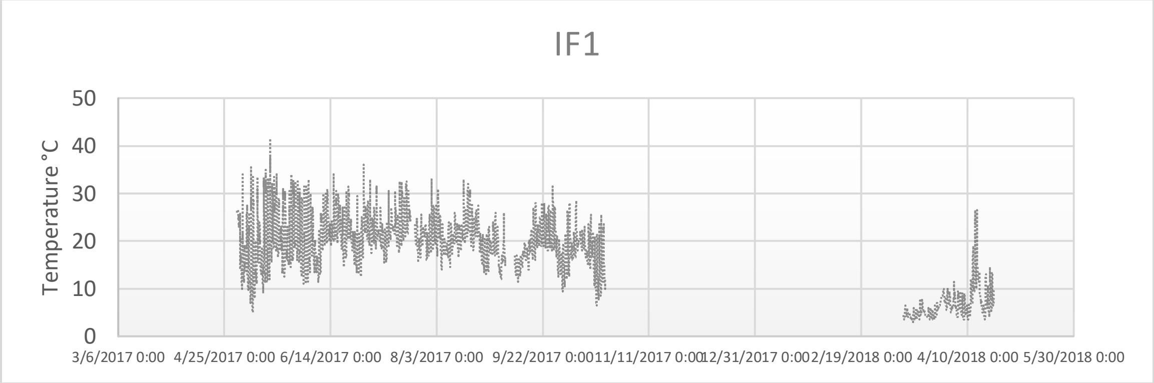
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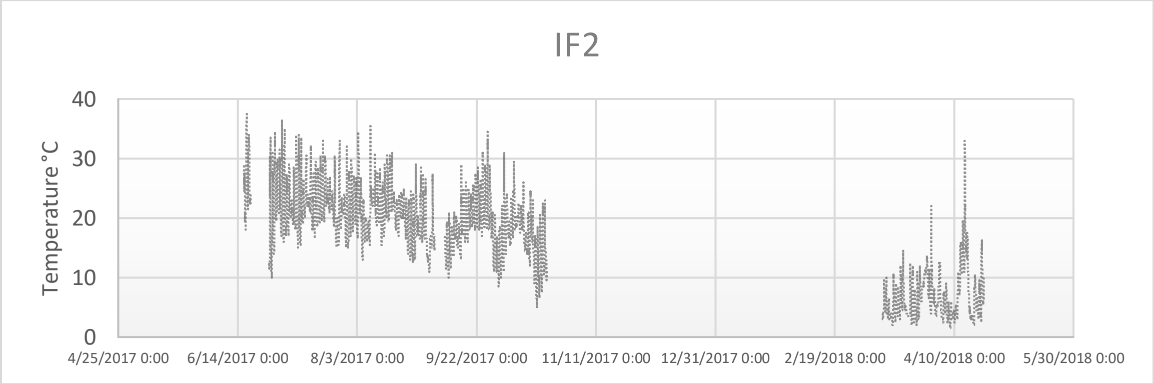
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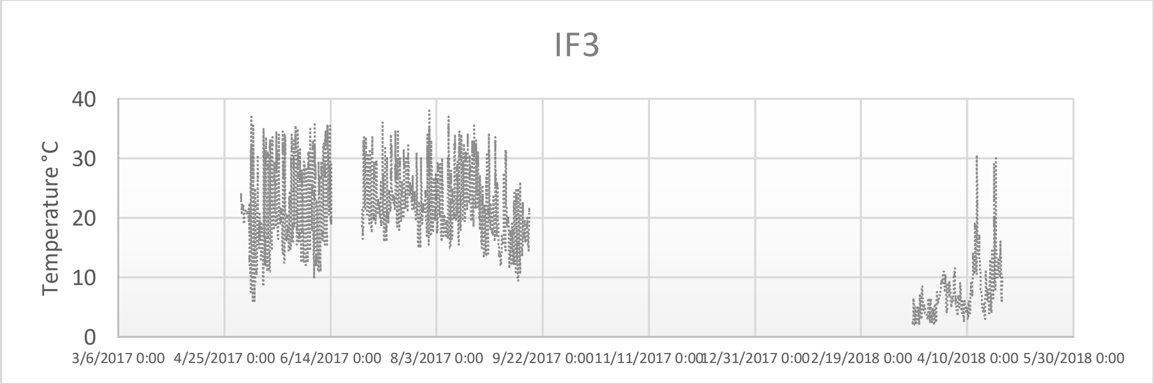
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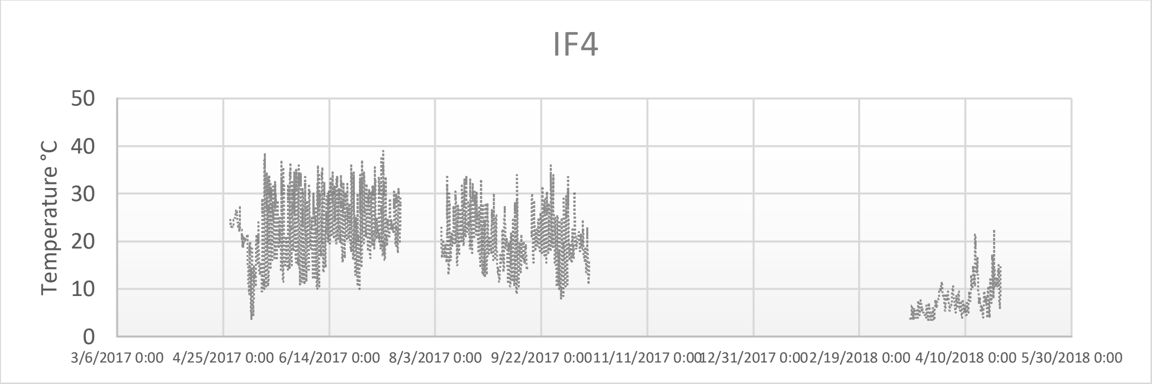
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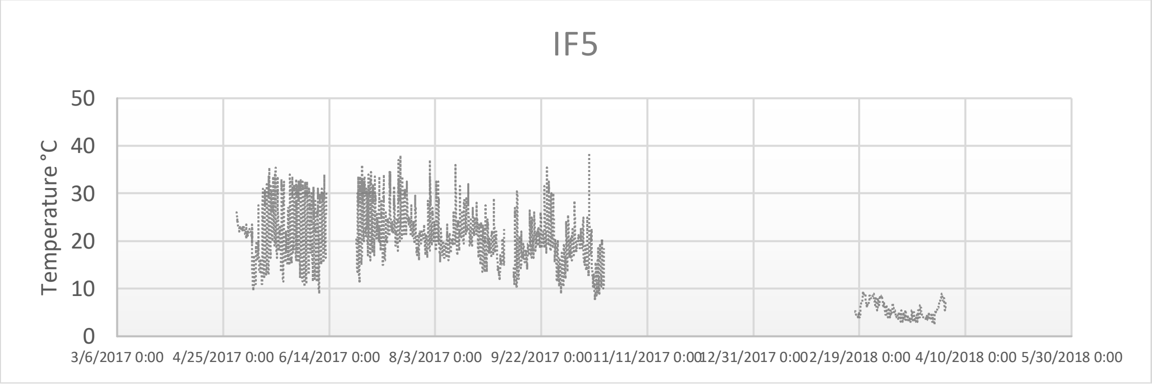
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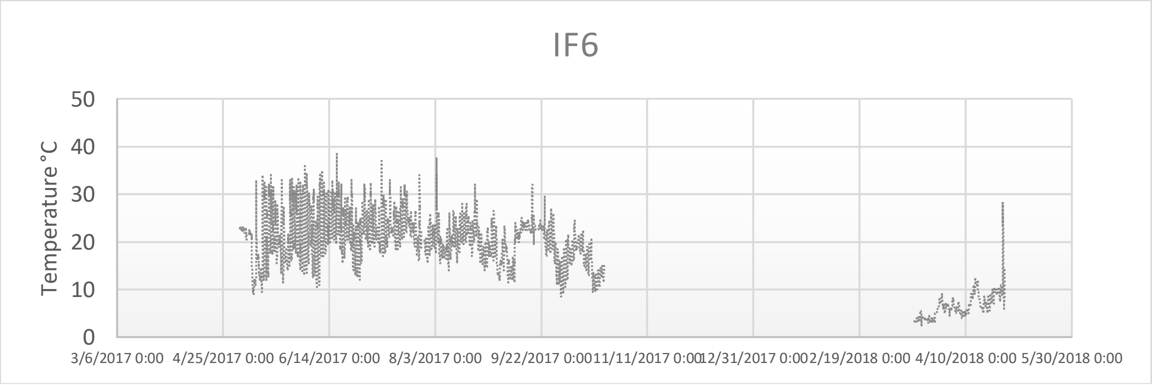
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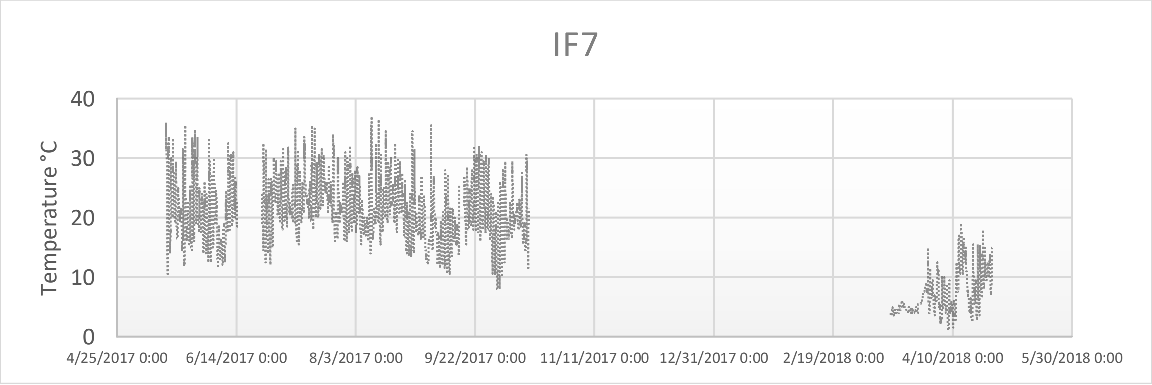
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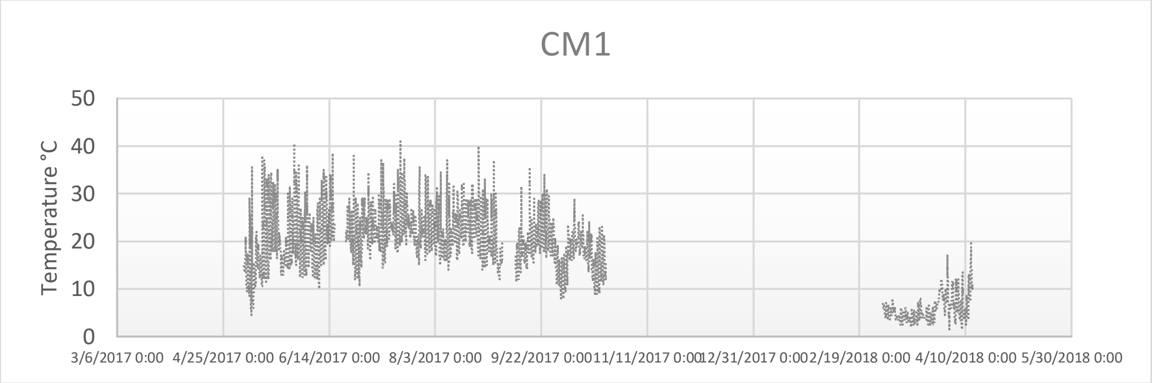
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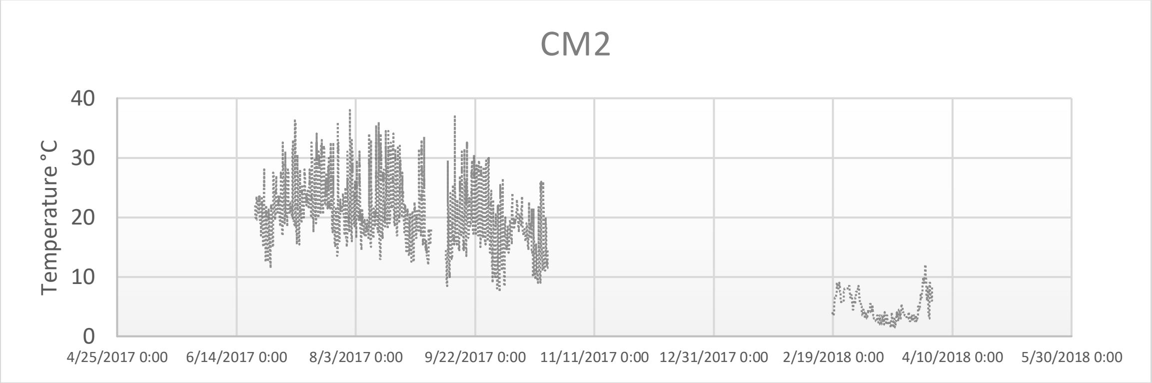
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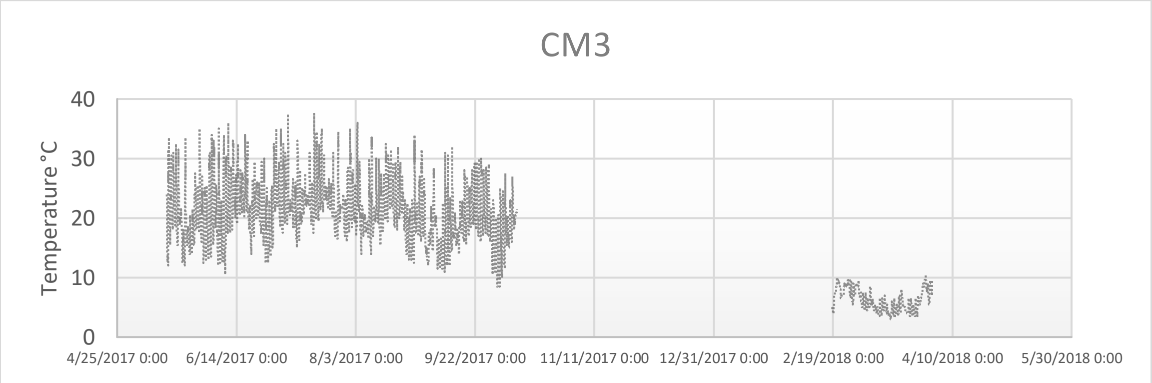
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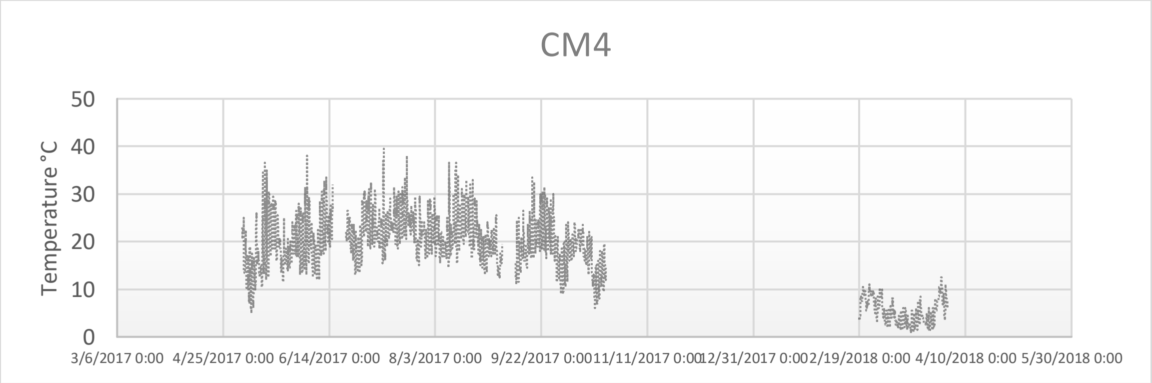
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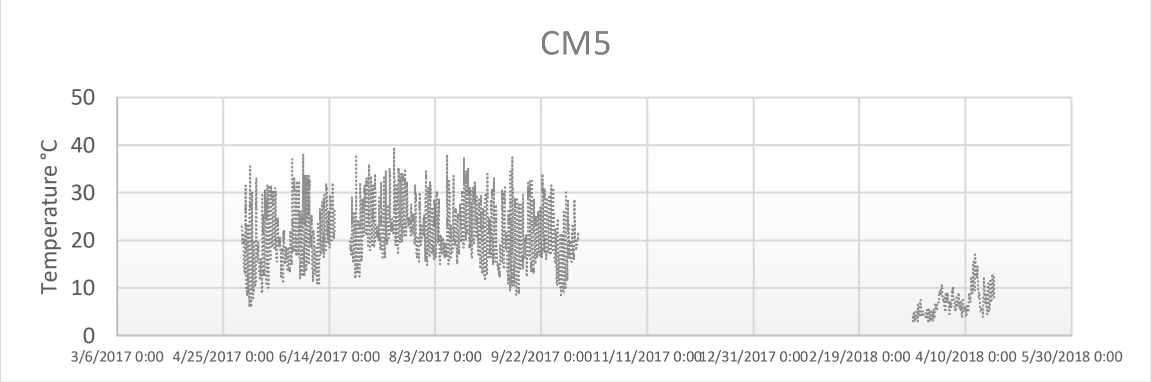
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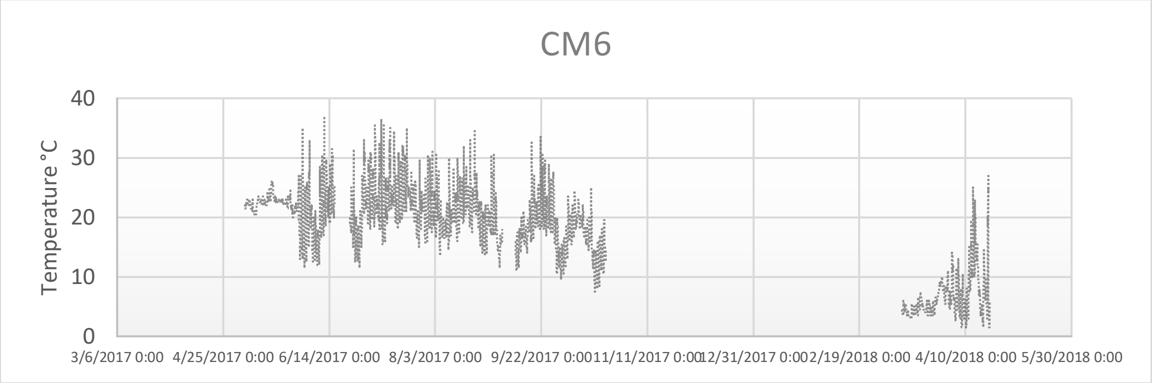
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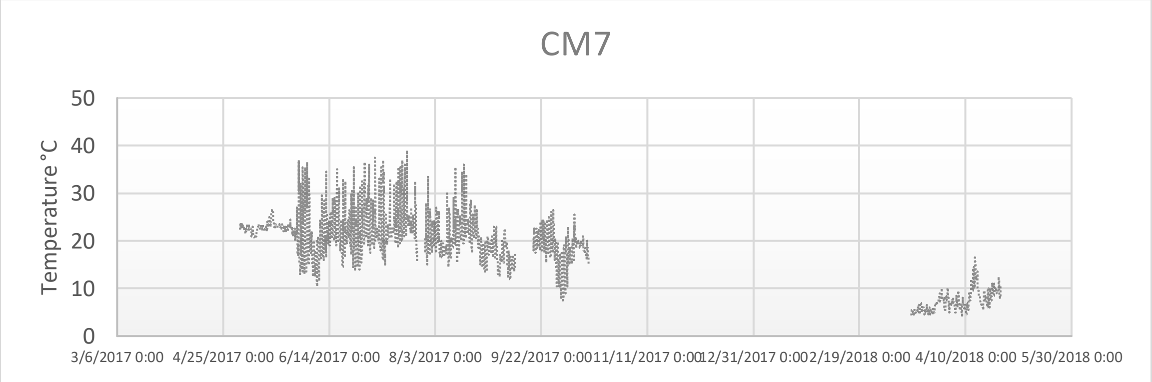
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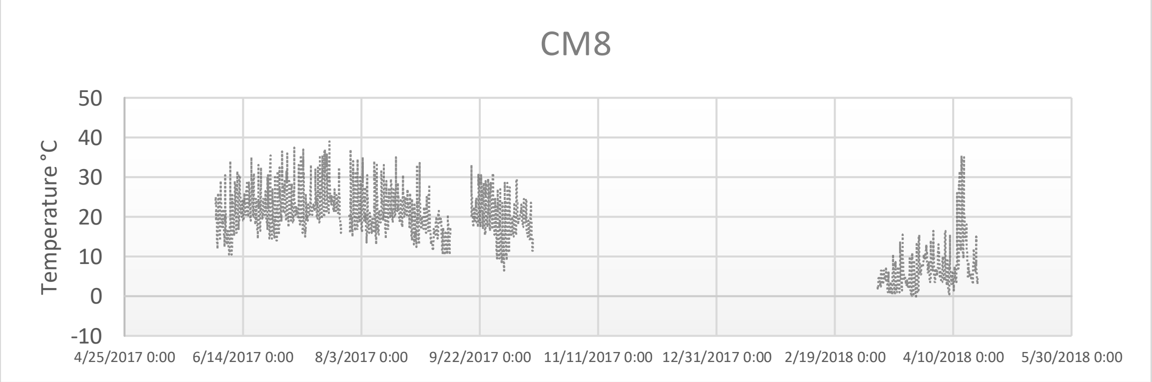
2/19/2018 0:00
4/10/2018 0:00
5/30/2018 0:00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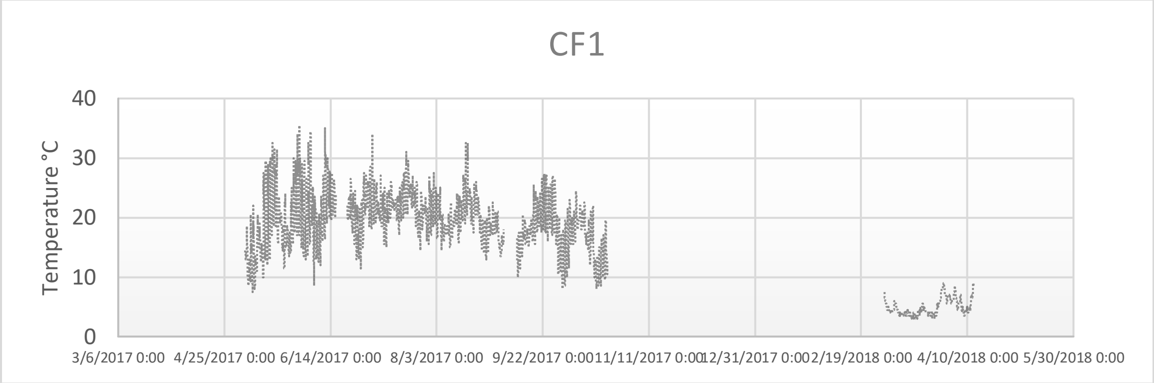
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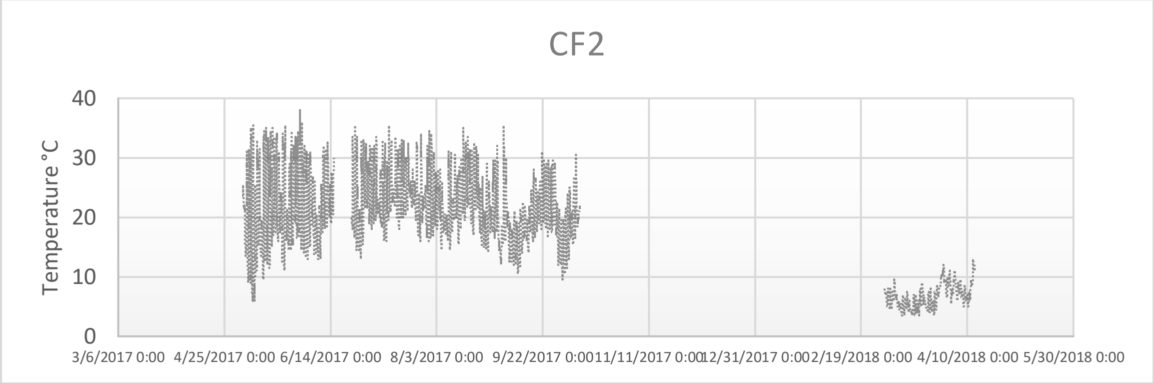
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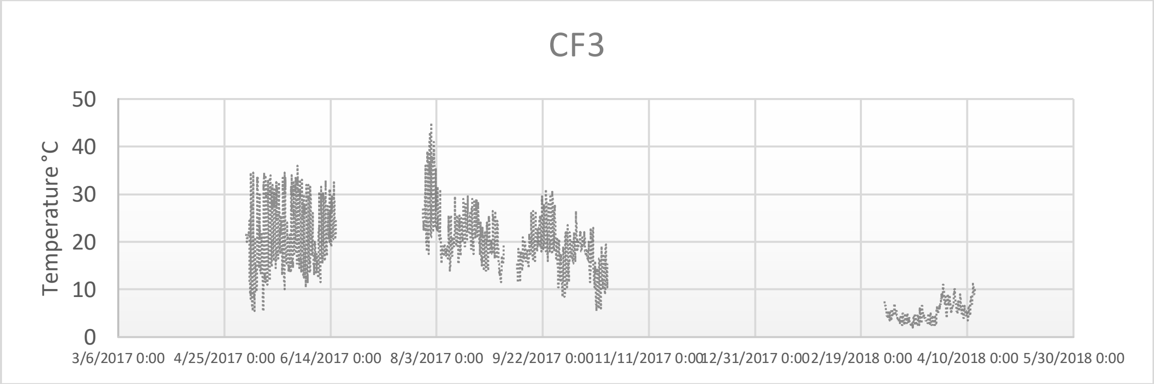
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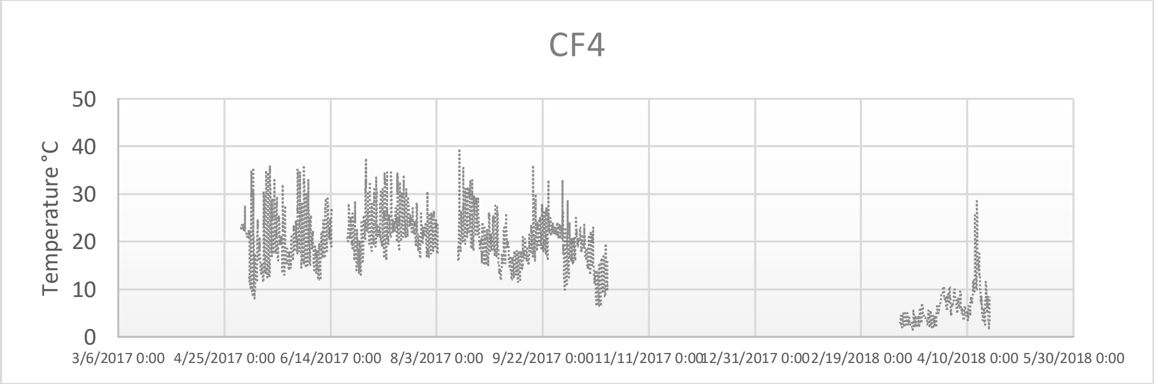
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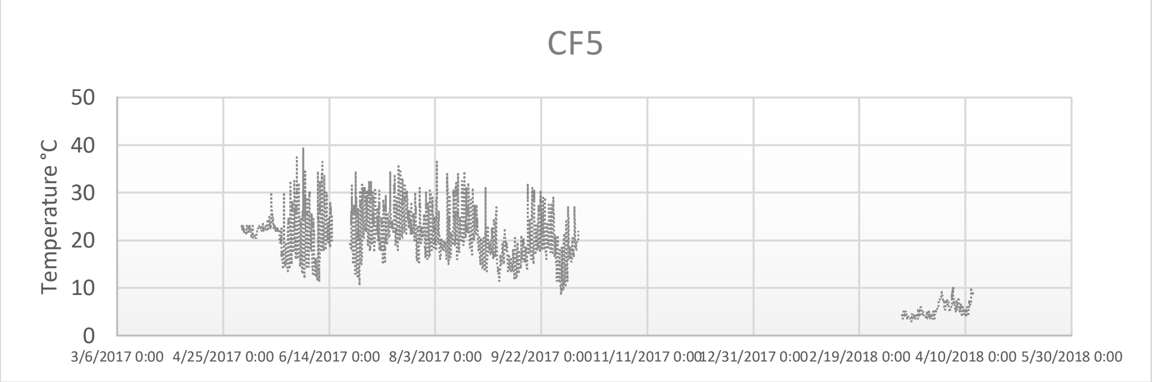
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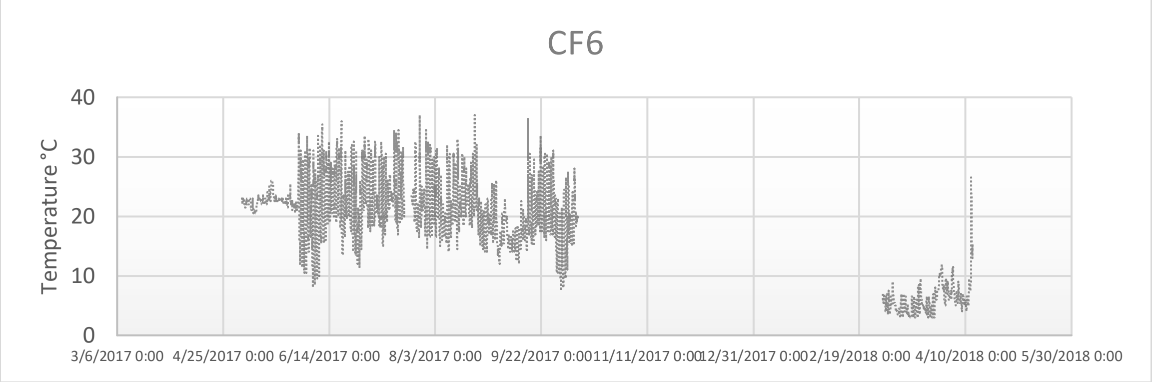
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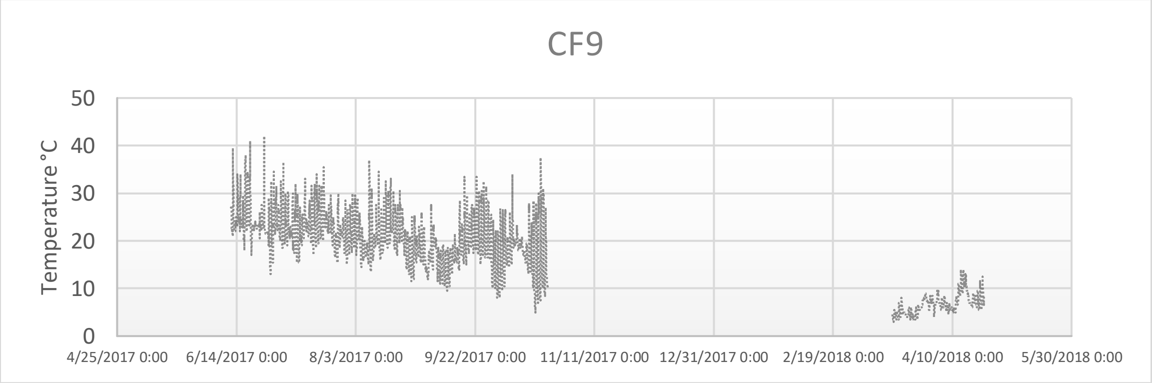
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2/19/2018 0:00
4/10/2018 0:00
5/30/2018 0:00

APPENDIX B: HABITAT SELECTION DATASHEET

Date	ID	VHF	Lat	Long	Order	Random°	Habitat	%W	%L	%V	%O	%US	%CC	°C	%RH	%SM
	IF4	112														
					R											
	IM8	091														
					R											
	IF7	233														
					R											
	IF3	103														
					R											
	IF6	222														
					R											
	IM7	073														
					R											
	1F2	082														
					R											
	IF1	012														
					R											
	IM2	023														
					R											
	IM4	042														
					R											
	IM5	053														
					R											
	IM6	061														
					R											
	IM3	031														
					R											
	IF5	210														
					R											
	IM1	331														
					R											

Date: Month/Day/Year the turtle and random points were collected.
ID: Indicates site, sex, and ordinal number of each individual.
VHF: The final three digits of each turtle's transmitter number, as programmed into the receiver.
Lat: Latitude.
Long: Longitude.
Order: Daily tracking order based on last known location.
Random: Randomly-generated compass point (0-360°) indicating in which direction the random point data should be taken based on the turtle location.
Habitat: Overall habitat type (e.g., forest, field, edge).
%W: Ground cover variable, percentage woody debris within 1m^2.
%L: Ground cover variable, percentage leaf litter within 1m^2.
%V: Ground cover variable, percentage vegetation within 1m^2.
%O: Ground cover variable, percentage other ground cover (e.g., bare, rocks, water) within 1m^2.
%US: Habitat structure variable, percentage understory cover within 1' above the ground within 1m^2.
%CC: Habitat structure variable, percentage canopy cover (collected via densiometer readings).
°C: Ambient air temperature, degrees Celsius (collected via Kestrel wind meter).
%RH: Ambient relative humidity (collected via Kestrel wind meter).
%SM: Percentage moisture content in soil (collected via soil moisture meter).

APPENDIX C: CORTICOSTERONE EXTRACTION PROCEDURE

1. Measure and record individual nail lengths
2. Transfer measured nails to labelled 7 mL glass scintillation vials
3. Wash nails with deionized H_2O (1 mL); hand agitate glass vial for 10 seconds
4. Wash nails with Methanol (1 mL); hand agitate glass vial for 5 seconds
5. Air dry samples under hood
6. Weigh total individual samples
7. Crush individual samples with mortar and pestle; return samples to 7 mL glass scintillation vials
8. Mix radioisotope (hot steroid) and cold steroid *very* well. Prepare the following:
    a. 2 x BLANK – 5 mL Methanol (HPLC-grade)
    b. 2 x POSITIVE CONTROL – 50 µL CORT Standard (cold steroid)
       20 µL CORT Isotope (hot CORT)
       5 mL Methanol
    c. 2 x REFERENCE VIALS – 20 µL CORT Isotope (hot CORT)
       5 mL Scintillation Fluid
    d. N x SAMPLES – Crushed Nails
       20µL CORT Isotope (hot CORT)
       5ml Methanol
9. Set aside reference vials for Day 2
10. Wrap sample, blank, and positive control vials together in parafilm; seal in Ziploc bag
11. Submerge samples in sonicating H_2O bath for 30 minutes
12. Submerge samples in oscillating H_2O bath at 50°C, 40 RPM overnight

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13. Pipette extract from 7 mL vial into 12 mL test tube
14. Rinse vial and pipette directly into test tube (~2 mL Methanol), hand agitate vial 10 seconds
15. Rinse vial and pipette directly into test tube (~1 mL Methanol), hand agitate vial 10 seconds
16. Dispose of nails using radioactive protocols
17. Evaporate Methanol in oscillating H_2O bath under Nitrogen (N_2)
18. Prepare extraction solution: 150 mL total = 30 mL Neogen extraction buffer + 120 Deionized H_2O (in graduated cylinder); rinse buffer jar/vortex 4x with DI H_2O and pour into solution; add remaining DI H_2O
19. Prepare standard curve (Figure 14) according to protocol established by Neogen; vortex all well
    a. Standard A – provided in green-capped vial 1 µg/mL
    b. Standard B – 20 µL of A + 980 µL of EIA buffer, vortex 20 ng/mL (0.02 µg/mL)
    c. Standard C – 200 µL of B + 1800 µL (1.8mL) of EIA buffer, vortex 2 ng/mL
    d. Standard D – 200 µL of C + 1800 µL (1.8mL) of EIA buffer, vortex 0.2 ng/mL
    e. Standard S_0 – 1000 µL EIA buffer 0 ng/mL
    f. Standard S_1 – 250 µL Standard D + 750 µL EIA buffer 0.05 ng/mL (0.00005 µg/mL)

g.	Standard S_2 – 500 µL Standard D + 500 µL EIA buffer	0.1 ng/mL (0.01 mL)
h.	Standard S_3 – remaining Standard D	0.2 ng/mL (0.0002 µg/mL)
i.	Standard S_4 – 250 µL Standard C + 750 µL EIA buffer	0.5 ng/mL (0.0005 µg/mL)
j.	Standard S_5 – 500 µL Standard C + 500 µL EIA buffer	1 ng/mL (0.001 µg/mL)
k.	Standard S_6 – remaining Standard C	2 ng/mL (0.002 µg/mL)
l.	Standard S_7 – 250 µL Standard B + 750 µL EIA buffer	5 ng/mL (0.005 µg/mL)

20. Reconstitute samples in test tubes; vortex
    a. Add 1000 µL extraction buffer solution to standards/controls, blanks
    b. Add 250 µL extraction buffer solution to evaporated samples
21. Transfer 100 µL reconstituted sample from test tube to clean 7 mL glass scintillation vial
22. Add 5 mL scintillation fluid (Scint Logic U, LabLogic Systems Ltd., Sheffield UK) to 7 mL vial, vortex well
23. Load set of samples in counter (starting with two reference vials previously prepared); load program
24. Begin assay; prep conjugate (full conjugate = 110 µL conjugate + 5.5 mL EIA buffer, vortex)
25. Vortex remaining samples in test tubes; add 50 µL to each of two assigned wells of assay plate
    a. Vortex, Pipette x 2, Change Tip
    b. Corticosterone from samples will begin to bind with plate
26. Batch add 50 µL conjugate to each well
    a. Corticosterone from conjugate will begin to bind with plate
27. Give plate 20 seconds in plate shaker (Epoch Microplate Reader, BioTek, Winooski VT)
28. Cover plate with parafilm; leave set 1 hour
29. Read Counter – Extraction results detail rate and volume of recovery of isotopes
    a. Counter counts to 100, multiply by 2.5 to correct for 250 µL dilution (standards, blanks x10 for 1000 µL dilution), measures CPM radiation recovered
    b. Results = Recovery % of radioactive isotopes in hot CORT (Adjusted Total)
30. Prepare wash buffer solution 200 mL total = 20 mL Neogen wash buffer + 180 Deionized H_2O (in graduated cylinder); rinse buffer jar/vortex 4x with DI H_2O and pour into solution; add remaining DI H_2O
31. Dump plate with hard shake
32. Batch rinse plate with 300 µL wash buffer solution 3x, hard shaking/tapping to dry after each wash
33. Batch add 150 µL substrate to each well, provide gentle shake to mix/10 seconds in plate shaker

34. Cover plate with parafilm; leave set 30 minutes, 45 minutes, 60 minutes
35. Read plate with microplate reader
    a. 10 second plate shake
    b. 10 second rest
    c. Read at 650 and 490 nanometers (nm) wavelengths
36. Correct reader results with dilution factor/recovery figures. Accept values where coefficient of variation (CV) < 20 (ideally < 5)
    a. Raw CORT = mean of two replicates, provides CV between replicates
    b. Total CORT = Raw CORT * # mL in vial (For samples with 250 µL dilution, multiply by 0.25 for a quarter mL)
    c. pg = ng*1000, Recovery line item corrects for differences in recovery (above 90% is excellent)

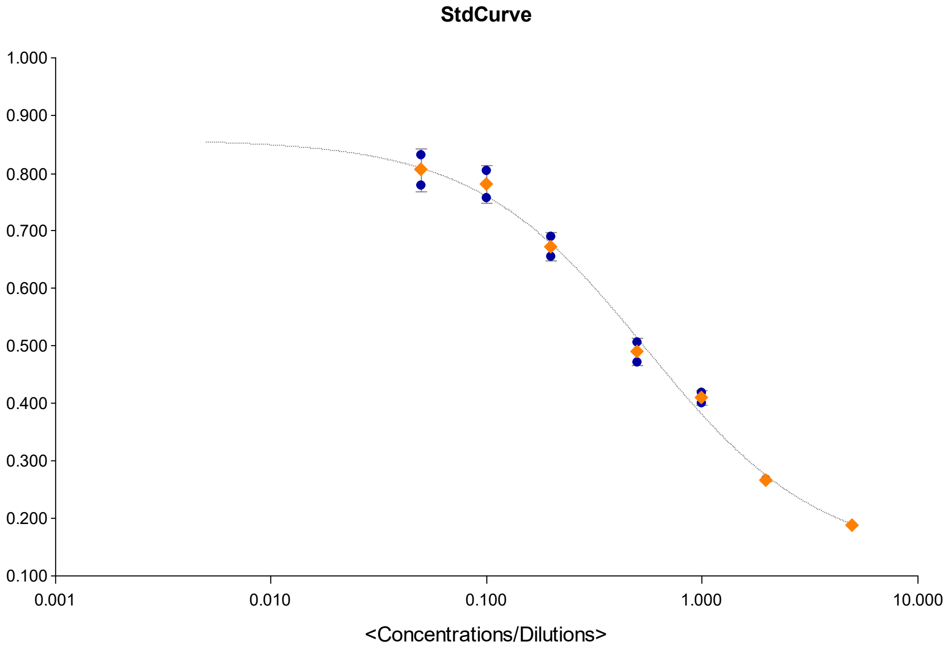

Figure 14. Example of a standard curve from a 2017 bioassay. Points falling closer to the expected curve indicate that corticosterone readings based on known test amounts are accurate. By comparing our sample results to the curve, we were able to calculate the concentration of corticosterone in each well. Nearly all samples values fell within the curve, suggesting our dilutions were appropriate and our accuracy was high (Neogen, Lexington KY; Tonra et al. 2013).

APPENDIX D: STATISTICS FOR PRIMARY RESEARCH ANIMALS

Notch	ID	CL	PL	SW	DH	Annuli	Mass	First Find	Total Finds	Last Find	HR Area	2017 CORT	2018 CORT
2	IM1	138	134	110	68	16	470	5/1	24	5/28	0.72	5.71	2.86
4	IM2	142	131	103	68	16	430	5/1	26	5/29	1.79	4.11	4.87
5	IM3	151	137	116	65	17	490	5/1	19	5/25	1.06	2.97	13.30
6	IM4	134	123	104	58	18	400	5/1	25	5/28	1.39	2.90	11.06
7	IM5	138	124	103	58	22	460	5/1	23	5/28	1.16	4.93	6.01
8	IM6	125	121	103	54	13	370	5/1	23	5/25	1.02	3.55	3.05
9	IM7	124	117	101	58	OLD	335	5/1	22	5/31	6.24	3.62	15.75
R1, L3	IM8	153	143	113	65	19	495	5/1	40	5/29	1.45	1.88	9.76
3	IF1	124	122	93	70	14	390	5/1	25	5/29	8.48	3.49	5.48
10	IF2	132	127	101	71	20	430	5/1	27	5/28	1.87	5.64	7.31
11	IF3	128	123	98	62	20	440	5/2	41	5/29	3.39	2.71	8.91
R1, L1	IF4	136	133	107	75	20	530	5/2	39	5/29	2.95	6.47	17.61
25	IF5	128	112	101	66	OLD	430	5/5	24	5/29	9.07	4.05	6.23
27	IF6	127	117	99	64	OLD	435	5/5	23	5/28	16.11	6.96	9.20
30	IF7	138	121	111	69	17	590	5/10	39	5/29	1.42	2.62	28.98
13	CM1	134	121	94	60	OLD	410	5/3	36	5/30	0.47	3.38	6.57
14	CM2	130	109	92	58	12	360	5/3	33	5/30	2.62	6.43	6.92
18	CM3	131	108	106	61	20	420	5/3	34	6/1	1.48	2.29	6.80
19	CM4	119	106	93	54	19	310	5/3	33	5/30	0.85	2.51	14.32
16	CM5	115	102	84	52	20	160	5/3	35	6/1	1.43	2.16	9.94
39	CM6	130	129	103	69	13	395	5/26	28	5/30	0.72	3.64	5.33
41	CM7	120	114	90	56	11	280	5/26	27	5/29	1.84	3.51	4.99
44	CM8	136	129	101	61	25	330	5/31	25	5/29	1.54	2.26	2.24
12	CF1	131	115	97	60	20	420	5/3	34	5/30	0.95	2.28	7.09
17	CF2	123	110	94	61	18	380	5/3	32	6/1	1.19	3.56	5.26
20	CF3	116	113	96	61	13	370	5/4	34	5/30	0.79	3.73	3.53
22	CF4	123	113	96	63	16	400	5/4	33	5/30	21.21	4.24	1.88
33	CF5	128	116	101	62	18	420	5/17	30	6/1	3.26	3.13	2.33
40	CF6	135	118	101	63	OLD	445	5/26	28	6/1	2.21	4.37	2.73
57	CF9	114	106	94	65	OLD	385	6/23	18	6/1	2.32	3.34	3.06

Notch: Indicates marginal scutes notched for identification.
ID: Indicates site, sex, and ordinal number of each individual.
CL: Carapace length (mm).
PL: Plastron length (mm).
SW: Shell width (mm).
DH: Dome height (mm).
Annuli: Counted to approximately 20, > 20 or smooth scutes indicated by OLD.
Mass: (g).
First Find: All animals were found in 2017.
Last Find: All last find dates are in 2018.
HR Area: (ha).
CORT: (pg/mg).

APPENDIX E: EASTERN BOX TURTLE POPULATION DATABASE

Notch	**ID**	**First Find**	**Sex**	**CL (mm)**	**PL (mm)**	**SW (mm)**	**DH (mm)**	**Annuli**	**Mass (g)**	**Eye**	**A/D**
1	IJ1	5/1/17	F	112	108	86	56	11	240	B	A
2	IM1	5/1/17	M	138	134	110	68	16	470	R	A
3	IF1	5/1/17	F	124	122	93	70	14	390	R	A
4	IM2	5/1/17	M	142	131	103	68	16	430	R	A
5	IM3	5/1/17	M	151	137	116	65	17	490	R	A
6	IM4	5/1/17	M	134	123	104	58	18	400	R	A
7	IM5	5/1/17	M	138	124	103	58	22	460	R	A
8	IM6	5/1/17	M	125	121	103	54	13	370	R	A
9	IM7	5/1/17	M	124	117	101	58	OLD	335	R	A
10	IF2	5/1/17	F	132	127	101	71	20	430	R	A
R1, L3	IM8	5/1/17	M	153	143	113	65	19	495	R	A
11	IF3	5/2/17	F	128	123	98	62	20	440	R	A
R1, L1	IF4	5/2/17	F	136	133	107	75	20	530	R	A
12	CF1	5/3/17	F	131	115	97	60	20	420	R	A
13	CM1	5/3/17	M	134	121	94	60	OLD	410	R	A
14	CM2	5/3/17	M	130	109	92	58	12	360	R	A
15	CJ1	5/3/17	J	94	86	25	42	8	120	R	A
16	CM5	5/3/17	M	115	102	84	52	20	160	R	A
17	CF2	5/3/17	F	123	110	94	61	18	380	R	A
18	CM3	5/3/17	M	131	108	106	61	20	420	R	A
19	CM4	5/3/17	M	119	106	93	54	19	310	R	A
20	CF3	5/4/17	F	116	113	96	61	13	370	R	A
21	CJ2	5/4/17	J	84	75	66	37	7	100	R	A
22	CF4	5/4/17	F	123	113	96	63	16	400	R	A
23	CJ3	5/4/17	J	86	76	67	39	6	110	R	A
24	CJ4	5/4/17	J	63	56	51	31	4	45	B	A
25	IF5	5/5/17	F	128	112	101	66	OLD	430	R	A
26	1M9	5/5/17	M	137	121	103	60	16	420	R	A
27	IF6	5/5/17	F	127	117	99	64	OLD	435	R	A
28	IM10	5/5/17	M	131	127	104	70	22	380	R	A
29	IM11	5/5/17	M	136	118	109	62	OLD	500	R	A
30	IF7	5/10/17	F	138	121	111	69	17	590	B/Y	A
R1, R4	IM12	5/11/17	M	140	136	106	65	14	470	R	A
32	IM13	5/15/17	M	127	113	100	57	OLD	390	R	A
31	IM14	5/16/17	M	130	120	106	64	17	460	R	A
33	CF5	5/17/17	F	128	116	101	62	18	420	R	A
34	IM15	5/18/17	M	131	119	95	60	16	355	R	A
35	IM16	5/19/17	M	160	146	116	73	26	660	R	A
36	IF8	5/24/17	F	118	106	96	60	14	360	R	A
37	IF9	5/24/17	F	117	99	97	68	22	385	R	A
38	IF10	5/24/17	F	104	95	79	51	9	190	B	A
40	CF6	5/26/17	F	135	118	101	63	OLD	445	R	A
39	CM6	5/26/17	M	130	129	103	69	13	395	R	A

41	CM7	5/26/17	M	120	114	90	56	11	280	R	A
100	IF11	5/29/17	F	117	116	98	61	OLD	415	R	A
42	IF12	5/30/17	F	133	127	104	64	14	455	R/B	A
43	IF13	5/30/17	F	121	115	100	60	18	410	R/B	A
44	CM8	5/31/17	M	136	129	101	61	25	330	R	A
45	IM17	6/2/17	M	135	121	105	55	24	450	R	A
46	IF14	6/5/17	F	126	120	95	62	17	230	B	A
47	IM18	6/6/17	M	125	118	93	53	18	355	R	A
48	IF15	6/6/17	F	123	112	100	65	OLD	480	R	A
49	CM9	6/8/17	M	149	127	112	60	24	435	R	A
50	IF16	6/12/17	F	125	110	103	72	19	450	R	A
51	IM19	6/12/17	M	133	117	110	63	20	500	R	A
52	CM10	6/15/17	M	140	116	101	57	25	450	R	A
53	CF7	6/19/17	F	137	125	97	71	12	530	R	A
54	CF8	6/19/17	F	120	117	94	56	20	375	B	A
55	CM11	6/20/17	M	140	122	97	59	OLD	460	R	A
56	CM12	6/23/17	M	124	111	100	58	18	370	R	A
57	CF9	6/23/17	F	114	106	94	65	OLD	385	R	A
58	CM13	6/28/17	M	126	118	86	59	14	330	R	A
59	CF10	6/28/17	F	125	118	92	91	12	380	B	A
60	IJ2	6/30/17	J	71	73	61	40	4	70	B	A
61	IJ3	7/5/17	J	80	80	65	40	6	90	B	A
62	IM20	7/6/17	M	138	121	105	63	20	430	R	A
63	IM21	7/10/17	M	136	120	103	57	OLD	430	R	A
64	CM14	7/11/17	M	136	129	106	63	13	440	R	A
65	IM22	7/12/17	M	140	116	110	66	21	555	R	A
66	CM15	7/15/17	M	134	119	106	63	18	505	R	A
67	IM23	7/21/17	M	135	119	105	63	OLD	480	R	A
68	IJ4	7/24/17	J	95	88	73	46	4	150	R	A
69	CJ5	7/25/17	J	96	84	79	45	8	170	B	A
70	IM24	7/27/17	M	123	108	92	59	18	310	R	A
R1	IM25	8/2/17	M	141	138	104	67	13	440	R	A
71	IM26	8/3/17	M	151	134	59	59	13	435	R	A
72	IM27	8/8/17	M	134	118	105	64	20	455	R	A
73	CM16	8/15/17	M	119	117	102	60	16	380	R	A
74	IM28	8/21/17	M	140	120	102	58	18	460	R	A
75	CM17	9/3/17	M	135	120	104	59	23	430	R	A
76	CM18	9/3/17	M	128	123	106	60	29	420	R	A
77	CM19	9/3/17	M	126	110	106	63	28	395	R	A
78	IF17	9/4/17	F	143	121	107	63	OLD	530	R	A
79	IM29	9/15/17	M	140	116	110	71	22	460	R	A
80	CJ6	10/4/17	F	93	79	76	42	10	140	B	A
81	CF11	10/9/17	F	113	100	92	60	17	355	R	A
82	CM20	10/9/17	M	120	107	96	56	OLD	355	R	A
83	CJ7	10/9/17	F	99	86	74	47	12	115	B	A
84	CM21	10/9/17	M	130	116	106	60	OLD	435	R	A

85	CJ8	10/10/17	F	83	69	65	36	7	95	B	A
101	CM22	10/15/17	M	127	112	107	62	15	385	R	A
86	CM23	10/15/17	M	138	126	106	64	21	470	R	A
87	CF12	10/15/17	F	127	125	98	64	OLD	415	R	A
88	IF18	5/7/18	F	116	104	94	56	OLD	380	---	A
49	CM24	5/10/18	M	151	132	110	64	20+	490	R	A
90	CM25	5/10/18	M	133	119	96	57	15	410	R	A
91	CM26	5/11/18	M	131	114	101	62	OLD	485	R	A
92	CM27	5/12/18	M	127	116	84	57	17	385	R	A
93	CF14	5/12/18	F	136	130	80	68	19	450	B	A
94	CJ9	5/12/18	U	118	107	71	52	11	290	R	A
95	IM32	5/13/18	M	138	123	78	63	17	415	R	A
96	IM33	5/13/18	M	131	122	75	53	12	355	R	A
97	IJ5	5/13/18	U	122	110	70	55	9	240	R	A
98	IM34	5/13/18	M	125	120	80	58	21	370	R	A
99	CM28	5/15/18	M	124	105	78	67	OLD	410	---	A