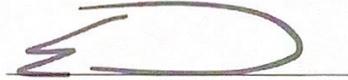


Snack-Sized Turtles? Investigating Size Class Distributions and Predation of the Endangered

Spotted Turtle (*Clemmys guttata*)

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Endangered Spotted Turtle (*Clemmys guttata*)

by

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A thesis submitted to the Department of Biological Sciences
of Salisbury University in partial fulfillment of the requirements for the degree of
Master of Science Applied Biology

November 30, 2021

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By

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2021

ABSTRACT

Reptiles are experiencing decreased population sizes and declines in number of populations. Despite this overall pattern, for many species, there remains insufficient data to understand population trends, let alone the specific causes of these declines. One species experiencing severe declines, but sparse assessment is the spotted turtle (*Clemmys guttata*), a freshwater species found in wetlands in Eastern North America and classified as endangered under the IUCN Red List. This decline likely has occurred because of potential threats such as predation, collection for the pet trade, and habitat loss. I studied the size class distributions of turtles in Maryland and Delaware populations based on frequency of juvenile and adult turtles. I found that *C. guttata* populations had a negatively skewed size distributions at all study sites. However, they were primarily individuals in the 90–100 mm plastron length size range, while larger, older, individuals were not typically present. Additionally, I analyzed the potential predation of *C. guttata* based on predator-caused injuries and assessing predator abundance via camera traps. *Clemmys guttata* had a greater rate of predator-caused injuries compared to other common turtle species; however, there was no correlation between injury rate and predator activity, specifically regarding raccoons (*Procyon lotor*). My results suggest while there was a large proportion of adults in populations of *C. guttata* at my sites, very old adults were absent. This was potentially caused, in part, due to predation, but the explanation for high injury rates of *C. guttata* is complicated and likely involves other factors such as human removal for the pet trade. **Keywords:** Predation rates; Population status; Injury rates; Size class; Endangered species; Adult limitation hypothesis.

Formatted for the journal: Herpetologica.

ACKNOWLEDGEMENTS

I thank the many people who assisted with fieldwork, especially K. Bachran, A. Beam, E. Chaudhry, M. Kinsella, E. Liebgold, G. Leasure, T. Ransom, and C. Woppman. I thank E. Liebgold for mentoring and help with analyses. I also thank Salisbury University for funding as well as S. Smith from the Maryland Department of Natural Resources and N. Nazdrowicz from the Delaware Department of Natural Resources and Environmental Control for their help in sharing information regarding site planning. This study was approved by the Salisbury University Institutional Animal Care and Use Committee Protocol permits 2016-WSC-001, 2017-WSC-003, 2018-WSC-003, 2019-WSC-008, 2020-WSC-004, and 2021-WSC-009.

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THESIS INTRODUCTION

Globally, 18.9% of reptiles are listed as threatened or endangered. However, this is likely an underestimate, as 21% of species lack the data needed to assess their status (reviewed in Böhm et al. 2013). Because of this lack of information, it is imperative that more research is done on wild reptile populations to better evaluate their status. Reptiles are particularly important to ecosystems as predators, prey, grazers, and seed dispersers (reviewed in Böhm et al. 2013). Many reptiles are also indicator species for the health of the environment and provide scientists with an understanding of evolutionary patterns, such as speciation (reviewed in Böhm et al. 2013). With the diverse assortment of niches and roles they fill, it is imperative to improve the survival and conservation of reptile species.

The spotted turtle (*Clemmys guttata*) is a classic example of a declining reptile species. Numbers have decreased by 50% since record keeping began and the species is categorized as “Endangered” by the International Union for Conservation of Nature (IUCN) (van Dijk, 2011). Due to the continued decline of *C. guttata* populations, they are currently under review for Federal listing in the United States (USFWS 2021) and, as of September 2020, the Government of Ontario, Canada has adopted a recovery strategy under the Endangered Species Act for *C. guttata* recovery (Ministry of the Environment 2020). Potential threats to *C. guttata* include destruction, fragmentation, and degradation of habitat, predation, and collection for the pet trade (Lewis et al. 2004). Life history traits of *C. guttata*: small clutch sizes, low recruitment rates, and delayed sexual maturity, can exacerbate these threats, and make population recovery less likely (Lewis et al. 2004; Litzgus 2006). This thesis provides information on predation pressures present on the

Delmarva Peninsula of the Atlantic Coastal Plain. My results could potentially identify populations under threat in Maryland and Delaware, and may be incorporated in conservation strategies, if needed, via amelioration of predator effects, such as meso-predator removal or modification of specific types of habitats.

I hypothesized that 1.) *C. guttata* have fewer individuals in the adult life stage in some sites or compared to other species. Furthermore, I hypothesized that 2.) *C. guttata* experiences higher predation rates compared to other common turtle species which may relate to mesopredator activity.

To research these hypotheses, my study consisted of three goals: 1) to determine if *C. guttata* populations are declining relative to other species in the same habitat, 2) to determine whether a population decline is due to reduced proportion of adults (long-lived adult limitation hypothesis) and 3) to determine if injury rates are concomitant with predator activity.

I found that *C. guttata* populations in Delaware and Maryland had a negatively skewed size distribution; however, there were fewer older adults compared to other species suggesting that something is removing older individuals from the population. Specifically, there appear to be more turtles in the 90-100 mm plastron length range. Furthermore, *C. guttata* were more frequently found with predator caused injuries compared to other common species; painted turtles (*Chrysemys picta*), eastern mud turtles (*Kinosternon subrubrum*), and snapping turtles (*Chelydra serpentina*).

Analysis of predator activity shows raccoons having the highest activity rate compared to red foxes and opossums. Testing for effects of raccoon activity on injury rates however, does not show a significant correlation, likely implying a more

complicated explanation for *C. guttata* having a greater injury rate than other turtle species.

INTRODUCTION

Predation pressure often acts as a top-down control for prey populations (Stevens 2010). As predator populations increase, there are higher predation rates on prey, potentially resulting in population declines (Stevens 2010). In communities, predation of common prey species often has a positive effect on species diversity of prey, however. The mechanism underlying this is the removal of superior or more common competitor species (of prey) prevents that taxon from dominating a community and allows less common or weaker competitors to increase (Hiltunen and Laakso 2013).

However, predation can sometimes have a negative impact on prey populations of conservation concern (Fletcher et al. 2010). The loss of each individual to predation in a small, endangered population can have a larger influence on population persistence, compared to more abundant, and less vulnerable, species (Neel et al. 2012).

One example of predation which results in threats to prey species' survival is mesopredator release (Crooks and Soulé 1999). Mesopredator release occurs when there is a loss of larger, apex predators from an area resulting in increases in mesopredators as there is a reduction in or removal of top-down control of mesopredator populations (Crooks and Soulé 1999; Hiltunen and Laakso 2013). The loss of typical top predators like large carnivores may occur due to fragmentation of landscapes or over-hunting by humans, both of which were common during European colonization of North America (Crooks and Soulé 1999; Hogue and Hayes 2015).

On the Delmarva Peninsula of Maryland, Delaware and Virginia, all large and apex carnivores have been extirpated since European colonization: gray wolf (*Canis lupus*), cougar (*Puma concolor*), American black bear (*Ursus americanus*), and, likely, bobcat (*Lynx rufus*) (Hogue and Hayes 2015). Because large carnivores were commonly targeted for removal by humans, mesopredator release occurred with populations of smaller carnivores (mesopredators) increasing over time (Hogue and Hayes 2015). Hogue and Hayes (2015) observed raccoons (*Procyon lotor*) increased in more than half of the study sites (54%), more than any other observed mesopredator species including red fox (*Vulpes vulpes*), gray fox (*Urocyon cinereoargenteus*), striped skunk (*Mephitis mephitis*), and river otter (*Lontra canadensis*) (Hogue and Hayes 2015).

With the change in predator populations and species' presence on the Delmarva Peninsula, it is possible this increase in mesopredator populations, at least of raccoons, was followed by a decrease in populations of mesopredators' prey species, as has occurred in other systems (Matter and Mannan 2005). This increase in predation risks for prey may differentially affect endangered species vulnerable to mesopredators compared to more common prey species (Macdonald et al. 1999; Smith et al. 2010). Already diminished prey populations (due to habitat loss or other factors) are more likely to be further reduced by larger numbers of mesopredators (Matter and Mannan 2005), making plans for conservation and recovery more difficult (Macdonald et al. 1999).

In addition to mesopredator release, increased habitat edge due to fragmentation of forested habitats can benefit mesopredators by increasing predation success (Paton 1994; Hartley and Hunter 1998). Fragmentation occurs when a formerly continuous habitat is converted into smaller, isolated patches which leads to increased edges within

that area (Batáry and Báldi 2004). Predators preferentially use these habitat edges to search for prey and have increased odds of finding prey as prey are more exposed on edges (Paton 1994; Robinson and Wilcove 1994; Hartley and Hunter 1998). Many mesopredators are suspected of using this behavior including skunks, raccoons, and opossums as well as fox, coyote, and dog (Temple, 1987).

Humans may also increase predator populations by providing supplemental food (Smith et al. 2010), typically through food waste in urban areas and crops in agricultural lands (Demeny et al. 2019). In Europe, raccoons have increased population densities in suburban areas as they gain extra food from human environments (Bartoszewicz et al. 2008). Their diet in these areas consists of rodents, water insects, garden fruits, and garbage (Bartoszewicz et al. 2008). Access to anthropogenic food sources allows raccoons to have high population densities and increased body condition (Demeny et al. 2019). With the urbanization of raccoons and the success it allows populations, predation pressure may increase for native prey in the area that the raccoons are foraging (Bartoszewicz et al. 2008).

Predation can impact prey populations by reducing numbers or affecting their ability to recover from already declining population rates due to other factors such as habitat loss or degradation (Roby et al. 2003). Increases in land use due to human development and deforestation lead to habitat loss and fragmentation (Spellerberg 1998). Furthermore, any predation population increases that occur with habitat fragmentation, due to increases in ecological edges in reduced and fragmented habitats (Lewis et al. 2004; Bartlett et al. 2016), can act synergistically with prey habitat loss, decreasing populations further than either threat alone (Roby et al. 2003).

While turtle nest predation is common and well-studied (Temple 1987), little is known about predation after hatching and as adults (reviewed in Ernst and Lovich 2009). Adult turtles can be maimed and killed by predators including by raccoons (e.g., *Chrysemys picta*: Wilbur 1975; *Emydura macquarii*: Spencer and Thompson 2006). Furthermore, adult turtle predation may occur more frequently after human disturbances of habitat (Miyata 1980). This may lead to large reductions or even eradication of local turtle populations impacted by habitat alteration (Miyata 1980).

Understanding age distributions of populations in areas where species of conservation concern persist can be helpful in predicting their future population dynamics (McClure et al. 2016). Age distribution patterns can also be compared to presence of species' threats to understand causes of population declines and potential amelioration of future declines through protecting habitat (Sánchez-Azofeifa et al. 2001; Friedlander et al. 2003; Thomas and Gillingham 2015) or predator removal or control (Fletcher et al. 2010; Smith et al. 2010; O'Connor et al. 2017).

Categorizing individuals into age classes, such as hatchling, juvenile, or adult, is the first step in understanding the level of recruitment of younger individuals and how this may impact the overall population growth compared to persistence of adults (Schueller and Peterson 2011; Gamelon et al. 2016). When there are many juveniles recruited in a population, those juveniles can make up for any loss in the population and perpetuate its existence (Votier et al. 2008; Mourocq et al. 2019). However, if juvenile recruitment is low, there are fewer young turtles surviving or being born to replace the loss of older individuals. On the other hand, population persistence of long-lived species

often hinges on adult survival (Shulman and Ogden 1987; Hughes 1990; Mourocq et al. 2019).

In long-lived species, although decreased juvenile recruitment can have a short-term, immediate effect on population size, the impact of juveniles is often limited because recruitment is typically low; instead, the mortality of adult individuals is more critical to long-lived population densities because they reproduce annually for decades (Shulman and Ogden 1987; Hughes 1990; Gaillard et al. 2008). Therefore, for long-lived species it is the loss of established sexually mature adults and all their potential future reproduction, that drives population declines.

Turtles are typically long-lived reptiles, with some species reaching over 50 years in captivity (Gibbons and Semlitsch 1982). In fact, it is possible that one of the oldest known turtles, a giant tortoise (*Geochelone gigantea*) referred to as Marion's tortoise and one of few reliable documented cases, lived for over 150 years (Gibbons 1987). Longevity of turtles is due to several life traits including a slow metabolism, long period of maturation, rapid growth during a long juvenile stage, and development of protective, hardened shells in adults (Gibbons 1987).

It is necessary to monitor turtle population structures as stresses on populations may not always be apparent, due to their long-lived nature (Browne and Hecnar 2007). For instance, the presence of adults in a population may suggest a healthy population; however, there may be a lack of recruitment of juveniles or persistence of adults which may limit population size (Rubin et al. 2004; Gibbons 1968). Altered age structure or size class distributions in certain conditions can indicate whether a population of conservation concern may be decline in the future.

In general, turtles possess life history traits which may make their age structure more vulnerable to the effects of anthropogenic activity. These traits include low reproductive output, late maturity, and strict wetland and terrestrial habitat requirements (Congdon and Gibbons 1996; Marn, et al. 2017). In Point Pelee National Park, Ontario, Browne and Hecnar (2007) found shifts in size structure to more older individuals and less juveniles over the three decades prior to 2007 in Blanding's (*Emydoidea blandingii*) and snapping turtles (*Chelydra serpentina*). These species experienced limited recruitment due to predation from raccoons on turtle nests and anthropogenic threats including road mortality and habitat loss (Browne and Hecnar 2007; Lefebvre et al. 2012).

The spotted turtle (*Clemmys guttata*) is a freshwater turtle species found primarily in wetlands, where there are higher levels of threats to species compared to terrestrial habitats (Bohm et al. 2013). Most reptiles also have a narrow distribution range, and are limited to certain habitats and niches, more so than other terrestrial animals such as birds and mammals (Bohm et al. 2013). *Clemmys guttata* ranges from Florida to Canada and Illinois to the east coast of North America (Lewis et al. 2004). *Clemmys guttata* is a classic example of a declining reptile species. Numbers have decreased by 50% since record keeping began and the species is categorized as "Endangered" by the International Union for Conservation of Nature (IUCN) (van Dijk 2011).

Due to the continued decline of *C. guttata* populations, they are currently under review for Federal listing in the United States and, as of September 2020, the Government of Ontario, Canada has adopted a recovery strategy under the Canadian Endangered Species Act for spotted turtle recovery (Ontario Ministry of the Environment

2020). A study conducted in Central Maryland for over 30 years recently found a 49% decline in estimated total population size (Howell et al. 2019). Furthermore, they found that there was a significant shift from smaller to larger, and likely older, individuals making up the population (Howell et al. 2019). Potential threats to *C. guttata* include habitat destruction, fragmentation, and degradation as well as predation and collection for the pet trade (Lewis et al. 2004). Life history traits of *C. guttata*: small clutch sizes, low recruitment rates, and delayed sexual maturity, can exacerbate these threats, making population recovery less likely (Lewis et al. 2004; Litzgus 2006).

With their current declines in numbers and endangered status, it is important to understand *C. guttata* population patterns across their range; however, most studies on current population status are based in their northern-most range in Canada (Seburn 2003; Enneson and Litzgus 2008). With *C. guttata* habitats located along the entire east coast of North America, it is vital that research is not restricted to the north but reaches across other portions of the species' range.

My goals in this study were to estimate stability of *C. guttata* populations and understand potential predation effects as correlates of declines. **I hypothesized that: 1) *C. guttata* populations are declining relative to other species in the same habitat, 2) the decline is due to reduced frequency of adults (long-lived adult limitation hypothesis) 3) predation rates are higher for endangered *C. guttata* than less vulnerable species and 4) injury rates are concomitant with predator activity.**

To determine the population distribution, and therefore health of the population, I used plastron length and carapace length of *C. guttata* and other sympatric turtles to sort captured turtles into different size classes (Howell et al. 2019). Age class distribution is

useful for understanding stability of species' populations over time, as well as differences among species utilizing the same habitat, and differences in the frequency of juveniles or adults among habitats (Seburn 2003). In this study, I wanted to understand the current population status of *C. guttata* compared to other common, local species. I was also interested in determining if certain sites are more likely to impact turtle populations, specifically that of *C. guttata*, with a decline in numbers.

To better understand why *C. guttata* is experiencing declines, including potentially on the Delmarva Peninsula of the Atlantic Coastal Plain, I tested if there were different rates of predation on *C. guttata* than other, more abundant, local turtle species. For *C. guttata* specifically if the rate of predation on adults varies by general location, it may also provide a hint that ecological factors, such as predator abundance or refuges, may play a role. In a previous study, 13.5% of *C. guttata* showed signs of injury due to predator attacks in Pennsylvania while 31% of *C. guttata* in Cedar Bog, Ohio showed signs of predator-related injuries (reviewed in Ernst and Lovich 2009). Whether this variation in injury rates among sites also occurs on a smaller geographic scale is unknown.

MATERIALS AND METHODS

Species and Study Sites

I studied three species of turtles: *Clemmys guttata*, *Chrysemys picta*, and *Kinosternon subrubrum* (Table 1). Five study sites were located on the Delmarva Peninsula, and all contained freshwater habitats (ponds and/or ditches along roads) that were potential *C. guttata* habitat, which has a disjunct distribution on the Delmarva Peninsula (personal observation). Bodies of water varied from ephemeral to permanent and habitat surround them was mixed deciduous forest, with roads adjacent to some water bodies. Exact locations are not provided due to the endangered status of *C. guttata* at the request of MD DNR and DE DNREC.

Trapping and Processing Turtles

I trapped turtles during the spring breeding season, February–June, from 2016–2021. I set 10–20 handmade D-hoop traps (87 × 50 cm) and minnow traps (61 × 30 cm; Promar, Gardena, CA) at each site, with trap type depending on water depth, and number of traps depending on size and number of water bodies per site. 5–10 traps were set per site depending on the area of each pond with 456.13 +/- 109.90 trap nights per site. Traps were typically located approximately 30 m apart, but in some smaller sites or bodies of water had traps were 15 m apart (sensu Willey et al. 2019). Traps were partially submerged in water, with an 0.3 m air gap and an empty 1 L container included as a float to ensure the traps did not become entirely submerged. I baited traps with punctured cans of sardines in oil (Beach Cliff, San Diego, CA) which I checked and rebaited every 24 h.

I notched the marginal scutes of all captured turtles with a unique notch code (modified from Cagle 1939) using a triangular file. Before release, I took measurements

including carapace length and plastron length, and I determined sex using secondary sexual characteristics. Male *C. guttata* are often shorter than females and have tan chins and brown eyes while females have yellow chins and orange eyes. In *C. picta*, males have longer nails on their forelimbs than females, and *K. subrubrum* males have longer tails than females (Ernst and Lovich 2009). These dimorphic traits aid in determining sex (Rowe et al. 2012). I also determined if there were previous injuries from predation attempts, such as missing or damaged limbs or tails (limb/tail injuries) or bite marks or scratches on the shells (shell injuries; Rowe et al. 2012). Injuries to limbs typically were limb or tail amputations, but also included missing toes or portions of the tail. Damage to the shell included holes (from predatory bites) or scratches on carapace and plastron or if parts of the shell were missing ostensibly from bites.

Statistical Analysis of Population Structure

I used plastron or carapace length to categorize each species of turtle into size classes that represent life stages. Using size to determine approximate life stages has shown to be a more reliable method to approximate population distributions than estimating age by counting annuli (sensu Seburn 2003; Enneson and Litzgus 2008; Howell et al. 2019). Previous studies have found no correlation between the change in the number of annuli rings counted and the years passed between capturing *C. guttata* (Howell and Seigel 2018). Measurements of the carapace length (mm) were used in place of plastron length for *K. subrubrum* when determining size distribution due to their flexible plastron hinge: < 80 mm carapace length = juveniles, > 80 mm = adults (Ernst and Lovich 2009).

For these three species that were consistently captured (excluding incidental captures of *Chelydra serpentina*, *Pseudomys rubiventris*, and *Sternotherus odoratus*), I analyzed differences in plastron length (*C. guttata* and *C. picta*) or carapace length (*K. subrubrum*) size distributions against site using contingency table analysis for each species. Data from only five turtle locations was used for this analysis due to no captures or a limited sample size at the other three sites. Sites F, G, and H with < 25 captures were excluded from the analysis due to a small sample size or no captures of *C. guttata*. Turtles under 50 mm carapace width were excluded as some of those individuals are small enough to escape the larger hoop traps; therefore, we cannot account for that size class of the population from our trap methods.

I ran a contingency table to look for differences in size distributions against species to determine if *C. guttata* has a different distribution from other species. I ran additional contingency tables to analyze the differences in turtle length against five sites for each species, separately (5x5 tables for *C. guttata* and *K. subrubrum*, 5x3 table for *C. picta*) (SPSS v. 28). For recaptured turtles, only the initial measurement was used.

Test for Size Distribution Skewness

I calculated skewness in size for each species at each site and also tested if skewness in size significantly varied from a normal distribution at each site using Shapiro-Wilk (<50 individuals) and Shapiro-Francia (>50 individuals) tests (SPSS v. 28.0).

I ran an ANOVA (SPSS v. 28) to test for differences between species and site for population skewness in *C. guttata* and *K. subrubrum*. Due to *C. picta* not being detected

at site E and having too few individuals to analyze skewness at site B ($n = 8$), I excluded *C. picta* from this comparative analysis.

Statistical Analysis of Injury Rates

I determined the proportion of individual turtles in a species with these types of injuries for the four species collected at trap sites (Fig. 2; $n_{\text{spotted}} = 348$, $n_{\text{painted}} = 278$, $n_{\text{mud}} = 234$, and $n_{\text{snapping}} = 103$). I compared injury rates across all captured species for 1. any injury and 2. limb/tail injury only. Limb injuries are more objective to identify as predator injuries compared to some shell injuries because some shell injuries (chipping, scratching, etc.) may be due to other causes. I used Chi-square tests to test for differences among species or site at five sites (A-E) for any injury and only limb/tail injuries.

Predator Activity using Trail Cameras

In 2020 and 2021, to estimate predator activity, I set an average of 4.29 ± 1.02 trail cameras (BCA Strike Force 850 16MP, Browning Trail Cameras) per site $\pm 1\text{SE}$, (approximately one camera per 5 turtle traps) depending on the area of (BCA Strike Force 850 16MP, Browning Trail Cameras) along pond edges at seven sites, with each camera facing a different turtle trap position, approximately 3–5 m from the trap location present in that season or where traps were located in previous years (note that there were no differences in mesopredator activity when traps were present or not: unpublished data). Trail cameras were set throughout the entire turtle breeding season from March through May and removed in June. Photos were visually assessed for the most common mammalian mesopredators including raccoons (*Procyon lotor*), red foxes (*Vulpes vulpes*), and opossums (*Didelphis virginiana*).

As I was unable to identify individual mesopredators (e.g., based on fur patterns), I developed an index of activity based on counts of animals captured in photos. I considered photographed activity to be independent if photos were taken > 30 minutes apart (*sensu* Davis et al. 2010). All photos captured by the camera traps were analyzed and notes were made of each species seen and the time it was captured by the camera. The results from photos were added into totals of each predator; raccoons (*Procyon lotor*), red foxes (*Vulpes vulpes*), and opossums (*Didelphis virginiana*); at each site, and later used for determining total predator activity of each mesopredator species.

I then used linear logistic regressions (SPSS v.28) to test for a relationship between *P. lotor* activity and *C. guttata* injury rates across five sites where *C. guttata* were found. I ran two separate analyses: all injuries and only limb/tail injuries, with *P. lotor* activity per trap night at each location as the independent variable. Finally, I ran a linear regression to compare size distribution skewness of *C. guttata* with *P. lotor* activity for four sites. Sites A, C, D, and E were included for this analysis. Site B was not included as trail cameras' malfunctioned due to wind.

RESULTS

Statistical Analysis of Population Structure

The size distributions of *C. guttata*, *C. picta*, and *K. subrubrum* were analyzed to determine if the distribution for *C. guttata* differed from other species. There was a significant difference in size distribution across species (Fig. 1). Specifically, *C. guttata* have significantly fewer larger, and older, individuals compared to *C. picta* and *K. subrubrum*.

I also analyzed the size distributions of each species to determine if there were differences in size distributions based on site. I observed *C. guttata* populations were primarily made up of individuals in the 90–100 mm plastron length size class in all locations (Fig. 2; $\chi^2 = 83.43$, $P = <0.0001$). Sites A and D also appear to have the fewest old adults in size classes 100–110 and 110+ mm.

I found that the greatest proportion of *C. picta* populations consists of individuals in the 140–160 mm plastron length range with a significant difference in distributions across locations (Fig. 3; $\chi^2 = 83.43$, $P = 0.0052$). There was also a significant difference in size distributions across *K. subrubrum* as populations were made up mostly of adults in size classes 100–110 mm carapace length or 90–100 mm carapace length (Fig. 4; $\chi^2 = 51.14$, $P < 0.0001$).

Test for Population Distribution Skewness

All populations of *K. subrubrum* were negatively skewed with populations at locations B and C being significantly skewed (Table 2). The *C. picta* population at location B was positively skewed while *C. picta* at locations C and D were negatively skewed. All of these were significant differences (Table 2). *Clemmys guttata* populations

at most locations were significantly negatively skewed (sites A, B, C, and E). There was no significant difference in skewness by site ($df = 4$, $P = 0.2308$) or species ($df = 1$, $P = 0.3905$) when comparing *C. guttata* and *K. subrumbrum*.

Statistical Analysis of Injury Rates

There was a significant difference in injury rates among species (Fig. 5; Wald $\chi^2_3 = 10.53$, $P = 0.015$). This difference was a greater proportion of predator injuries in *C. guttata* compared to other species (*K. subrumbrum*, *C. picta*, and *Chelydra serpentina*) (Fig. 5). I found a significant difference across species ($\chi^2 = 8.67$, $P = 0.0131$), with more limb/tail injuries in *C. guttata* than other species (Fig. 6).

Predator Activity using Trail Cameras

Procyon lotor had an average activity of 0.46 ± 0.12 photos/trail camera $\pm 1SE$, *V. vulpes* had an average activity of 0.05 ± 0.01 photos/trail camera $\pm 1SE$, and *D. virginiana* had an average activity of 0.04 ± 0.02 photos/trail camera $\pm 1SE$ across all sites, respectively. There was no significant relationship between mean *P. lotor* activity per camera trap night at each site and injury rates of *C. guttata* for all injuries (Fig. 7; $df = 4$, $t = -0.125$, $P = 0.912$) nor only limb and tail injuries (Fig. 8; $df = 4$, $t = -0.002$, $P = 0.999$). The linear regression of *C. guttata* site distribution skewness against *P. lotor* activity also showed no significant relationship between the two variables ($df = 2$, $t = 1.6$, $P = 0.125$).

DISCUSSION

I found that *C. guttata* populations in the Atlantic Coastal Plain of DE and MD sites were primarily composed of turtles in the 90–100 mm plastron length size range (Fig. 2). Like the turtles I captured of other, more common species, *C. picta* and *K. subrubrum*, the most abundant size class included individuals that were middle to large size adults. Furthermore, these turtle populations, including *C. guttata*, were generally negatively skewed, with populations being made up of primarily these larger, adult turtles. This suggests that populations of turtles in this area are stable due to a larger proportion of adult individuals.

Because turtles are long-lived organisms, it is ultimately the proportion of adult in the population likely determines the stability of the population (Feng et al. 2019). For long-lived species, individual reproductive output and population replacement rates are primarily attributed to longer average turtle lifespans (Shulman and Ogden 1987; Hughes 1990; Feng et al. 2019). This makes it important to protect adult turtles from potential threats such as collection for the pet trade, roadside mortality, and interactions with predators (Feng et al. 2019).

I found that *C. guttata* were significantly less likely to be in the older size classes than the other two species (Fig. 1). As *C. guttata* is likely longer lived than these other species, this is potentially affecting their lifetime fecundity and population recruitment. Some of this lack of oldest possible individuals may be due to differences among sites. At one site, Site D, both *C. guttata* and *K. subrubrum* had fewer individuals than expected in older size classes and were not significantly skewed left. The implication is that some factor(s) are prohibiting aging at that site (and potentially Site A for *C. guttata*). Site D is

a small site among private property and the water bodies used by *K. subrubrum* and *C. guttata* (but not *C. picta*) are small roadside ditches. Potentially, habitat degradation and human collection or predation are having a great effect on adults in that population than at other sites.

To potentially determine what factors affected the population size distributions of *C. guttata* and other species, I investigated predation pressures by looking at both injuries and predator activity levels. I found that there is a greater proportion of *C. guttata* with injuries, likely caused by failed predation attempts, compared to other common turtles' species.

A potential reason for the larger proportion of injuries in *C. guttata* compared to *C. picta* and *K. subrubrum* is that they are more terrestrial than the other turtle species (reviewed in Ernst and Lovich 2009). This may contribute to a greater risk from terrestrial predators. Alternatively, there may be other reasons why they are selectively preyed upon, such as their small size as smaller species are more vulnerable to a wider range of predators (sensu Khan and Balakrishnan 2004). However, *C. gutta* has a longer lifespan (60–110yrs, Feng et al. 2019; 65–110yrs, Ernst and Lovich 2009) compared to these other species (reviewed in Ernst and Lovich 2009), so a greater accumulation of injuries simply due to longer lifespans may explain some or all our findings without higher predation rates.

I detected three potential predators of turtles, including *C. guttata*: *P. lotor*, *V. vulpes*, and *D. virginiana* (reviewed in Ernst and Lovich 2009). Although we did not statistically compare activity of potential predator species, *P. lotor* activity was an order of magnitude greater than the other potential predators I observed through camera trapping in

this area. Previous studies noted the involvement of *P. lotor* in predation of *C. guttata* adults as well as their nests (Ernst 1976) as well as other predation of adults of freshwater turtle species (Wilbur 1975; Seigel 1980; Roe 2018; Platt 2019). Because of the frequency of *P. lotor* predation on turtles across other studies, I was particularly interested in determining if *P. lotor* activity in Maryland and Delaware affected rates of *C. guttata* injuries. However, I found no statistically significant relationship between frequency of *C. guttata* injuries and average *P. lotor* activity (Figs. 7 and 8). This suggests a more complicated pattern of variation in injury rates was present.

One possible reason for this result is that detection of injured individuals is not indicative of mortality as injuries represent only failed predation attempts and the true frequency of predation is unknown. Actual predation rates are often higher than non-lethal injury frequencies (e.g., Sprogis et al. 2018). It is therefore possible that mortality of predated turtles is higher and mesopredators, like *P. lotor*, have higher predation success than represented by injuries. It is difficult to recover deceased turtles as they may be in the water or eaten. If this is the case, it would mean that injury rates are underestimated in my analysis (Fig. 4 and 5; sensu Lavery, Korol, and Litzgus 2016).

It is also possible that not all injuries were caused by predation. Other potential causes include anthropomorphic impacts such as road mortality from automobiles or watercraft activities and crab-trapping (Cecala, Gibbons, and Dorcas 2008; Lavery, Korol, and Litzgus 2016; Roe 2018). Non-predator caused injuries may also be conspecific tail injuries caused by adult turtles vying for dominance and mate selection (Walde et al. 2003). Although the former is not likely based on our injury criteria,

injuries, like partial, but not complete, amputations, due conspecific interactions, are poorly understood in these species of turtles (Ernst and Lovich 2009).

Additionally, predator activity may not have a significant effect on *C. guttata* injury rates because of variation in raccoons' preferential diets if diets vary based on location (Bartoszewicz et al. 2008). *Procyon lotor* are generalists and opportunistic foragers that easily adapt their diet to the food sources that are most abundant in the area they inhabit (Bartoszewicz et al. 2008; Demeny et al. 2019). Plants and invertebrates typically make up the majority of *P. lotor*'s diet although this can be different in and near anthropogenically-developed land especially agricultural areas where they utilize anthropogenic food sources (Demeny et al. 2019). At some of my study sites, there are likely other food sources that are more prevalent or preferred by *P. lotor*, which reduces their predation of turtles. Lacking knowledge of the variance in *P. lotor* diet preferences among my sites, it may not be possible to relate predator activity and injury rates.

Another factor complicating the relationship between predator activity and predation rates is that the opportunity for predators and turtles to interact may also be affected by the size of a habitat or degree of fragmentation. This can be due to predators' increased use of habitat edges to search for food, which increases their odds of finding prey (Temple 1987). For turtles specifically, nests, at least, are more vulnerable to predation when they are laid on ecological edges in fragmented habitats (Temple 1987). As habitats become more fragmented, predator detection of turtles and their nests may increase leaving it less likely that turtles can persist when there are abundant ecological edges because of high levels of predator activity (Temple 1987).

In conclusion, it is clear that long-lived species like turtles, especially *C. guttata* have negatively skewed size distributions (Table 2). In populations with these characteristics, adult survival has the highest proportional impact on population growth (Feng et al. 2019) and loss of older individuals can reduce recruitment into a population (sensu Jones et al. 2018). A population of long-lived species with a high frequency of adults may suggest, to some degree, an adult-heavy population which may be less vulnerable to decline (Feng et al. 2019). On the other hand, even adult-heavy populations still potentially can experience declines (Feng et al. 2019; Jones et al. 2018).

Although *C. guttata* populations were negatively skewed, there were still fewer older turtles in the 100–110 and 110⁺ mm plastron size classes, which may indicate that something was removing these older turtles from the population. This led me to look into potential predation pressures after the egg phase.

I tested for a relationship between predator activity and injury rates of *C. guttata*; however, I was unable to detect a relationship between predator activity and injury rates, I still detected high rates of turtle injuries that were highest in the endangered *C. guttata* and found high rates of *P. lotor* activity. Because of the potential impacts of predators on these long-lived adult turtles, it may be beneficial to consider control of mesopredators (Fletcher et al 2010; Smith et al. 2010) or modification of habitat to reduce mesopredators in locations where they have the largest impact on this endangered species (sensu Smith et al. 2010; O'Connor et al. 2017).

Additional protection of particular habitats where endangered species are found that also have low levels of predators may improve nesting and recruitment (Marchland and Litvaitis 2003), which may still be beneficial to adult-skewed populations of *C.*

guttata (Feng et al. 2017). It is important to note that any removal of predators needs to be a long-term strategy (Smith et al. 2010), thus making it important to consider these options as not mutually exclusive, such as balancing predator removal with habitat creation or improvement. This may include reducing habitat fragmentation and habitat edges used by predators (reviewed in Chalfoun, Thompson III, and Ratnaswamy 2002), removing predator breeding sites (reviewed in Gibbons et al. 2007), as well as increasing food resources (Martin 1992) and nest protection (Brickle and Peach 2004) for species of conservation concern. Follow-up studies should investigate population size distributions before and after reducing predator impacts. Geographic information systems studies should also be considered to determine levels of habitat fragmentation and the implications those may have on declining species or predation levels.

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TABLES AND FIGURES

TABLE 1. Turtle Species Overview. Clutch size, number of clutches, ages at sexual maturity, sizes (carapace and plastron length) at sexual maturity, and time on land reviewed in Ernst and Lovich, 2009. Longevity and IUCN status reviewed in IUCN: *C. guttata*: van Dijk 2011A; *K. subrubrum*: van Dijk 2011B; *C. picta*: van Dijk 2016).

Species	Common Name	Clutch Size	Number of Clutches per Year	Female Age at Sexual Maturity (yrs)	Male Age at Sexual Maturity (yrs)	Female Size at Sexual Maturity (mm)	Male Size at Sexual Maturity (mm)	Longevity (yrs)	IUCN Conservation Status	Land & Water Habitat
<i>Clemmys guttata</i>	Spotted Turtle	2–5	1–2	7–15	7–13	10.3	10.5	65–110	Endangered (decreasing)	Shallow wetland and terrestrial habitats
<i>Chrysemys picta</i>	Painted Turtle	1–23	1–5	6–10	2–4	9.7–12.8	7.0–9.5	30–40	Least Concern (stable)	Shallow water habitats
<i>Kinosternon subrubrum</i>	Eastern Mud Turtle	2–4	1–2	5–8	4–7	8.0–12.0	8.0–12.0	20–50	Least Concern (unknown)	Shallow water habitats

TABLE 2. Skewness tests of size distribution of each turtle species against a normal distribution at each study site. Shapiro-Wilk test used for <50 individuals, Shapiro-Francia used for >50 individuals in a population. Significant skewness results are bolded. Median size refers to carapace length in *K. subrubrum* and plastron length in *C. picta* and *C. guttata*. More negatively skewed values point towards an adult or older population of turtles.

Species	Site	<i>n</i>	Skewness	Median Size (mm)	<i>W</i> or <i>W'</i>	<i>P</i>
<i>Kinosternon subrubrum</i>	A	140	-0.41	86.30	0.9837	0.3611
	B	104	-1.47	96.18	0.8857	< 0.0001
	C	26	-1.23	90.11	0.9120	0.0294
	D	58	-0.69	89.46	0.9674	0.1203
	E	91	-0.39	89.37	0.9791	0.1523
<i>Chrysemys picta</i>	B	104	0.67	124.74	0.9409	0.0002
	C	106	-0.81	124.20	0.9218	< 0.0001
	D	72	-0.73	123.00	0.9504	0.0066
<i>Clemmys guttata</i>	A	122	-0.99	88.95	0.9410	< 0.0001
	B	203	-1.55	84.06	0.9047	< 0.0001
	C	39	-1.29	85.01	0.9183	0.0077
	D	62	-0.19	82.54	0.9833	0.5707
	E	225	-1.41	86.43	0.9016	< 0.0001

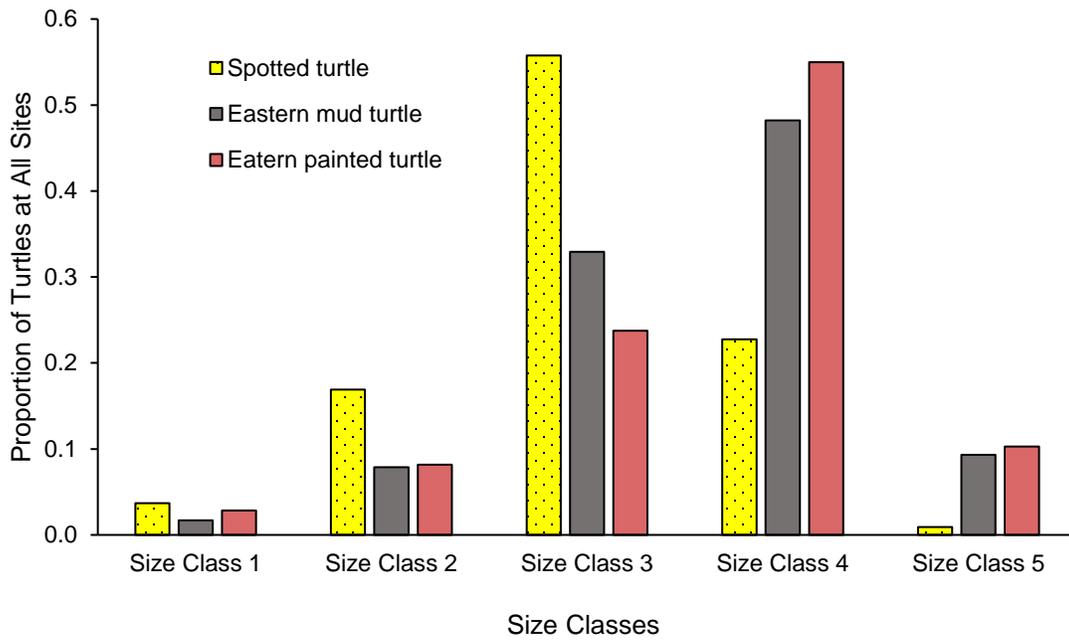


FIGURE 1. Difference in size class distribution across each turtle species ($\chi^2 = 207$, $P = <0.0001$). Carapace (*K. subrubrum*) and plastron length (*C. picta* and *C. guttata*) separated into five size classes using proportion of turtles in that size class.

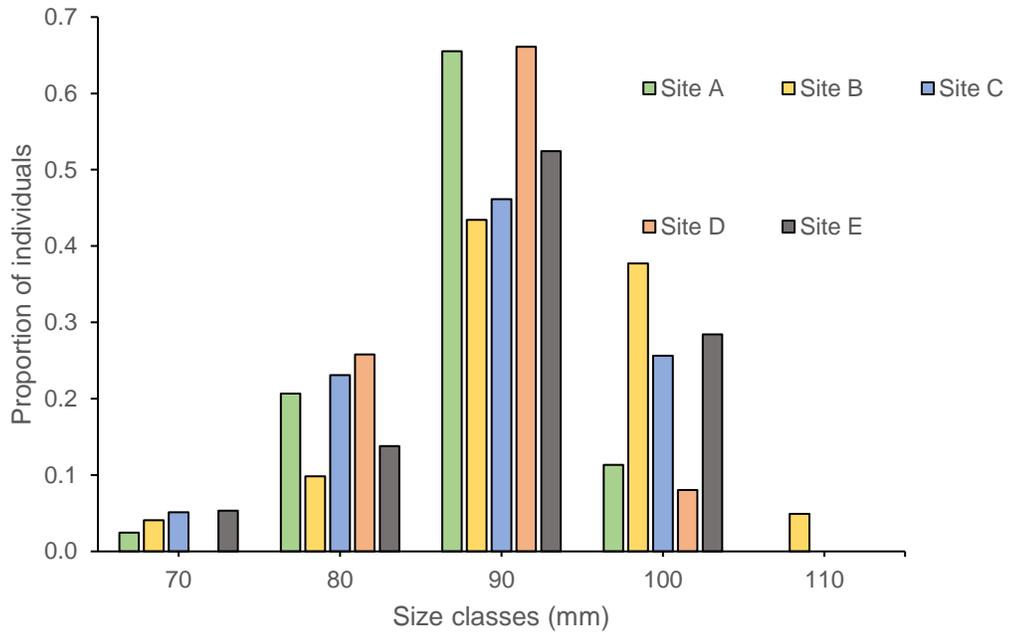


FIGURE 2. Difference in size distributions across five spotted turtle (*Clemmys guttata*) sites using plastron length ($\chi^2 = 83.43$, $P = <0.0001$). Sites A–E present the proportion of individuals categorized under their respective size classes at each site.

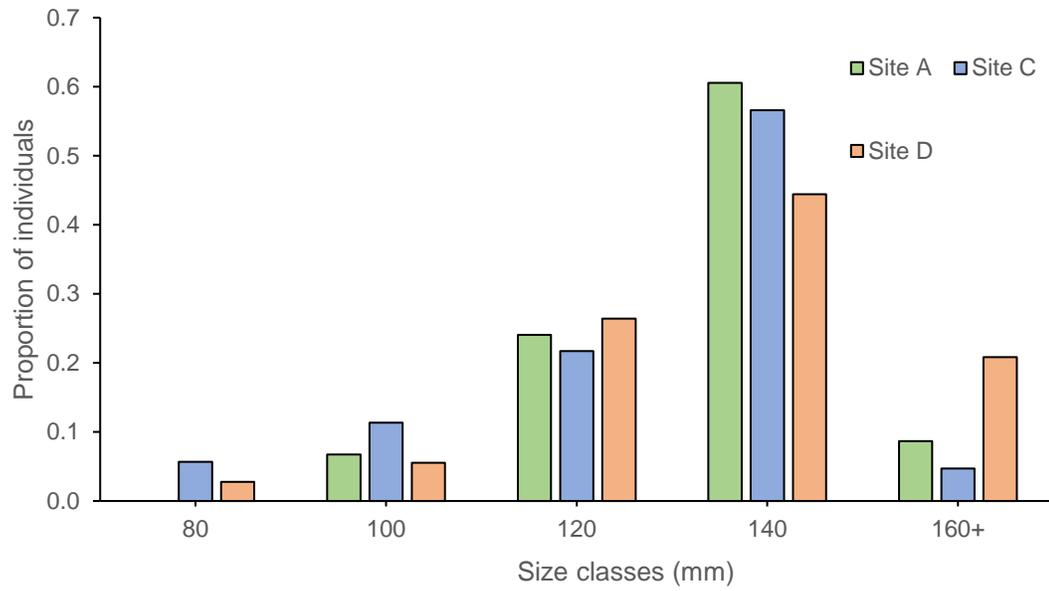


FIGURE 3. Difference in size distributions across three eastern painted turtle (*Chrysemys picta*) sites using plastron length in a contingency table analysis ($\chi^2 = 83.43$, $P = 0.0052$). Sites A, C, and D present the proportion of individuals categorized under their respective size classes at each site.

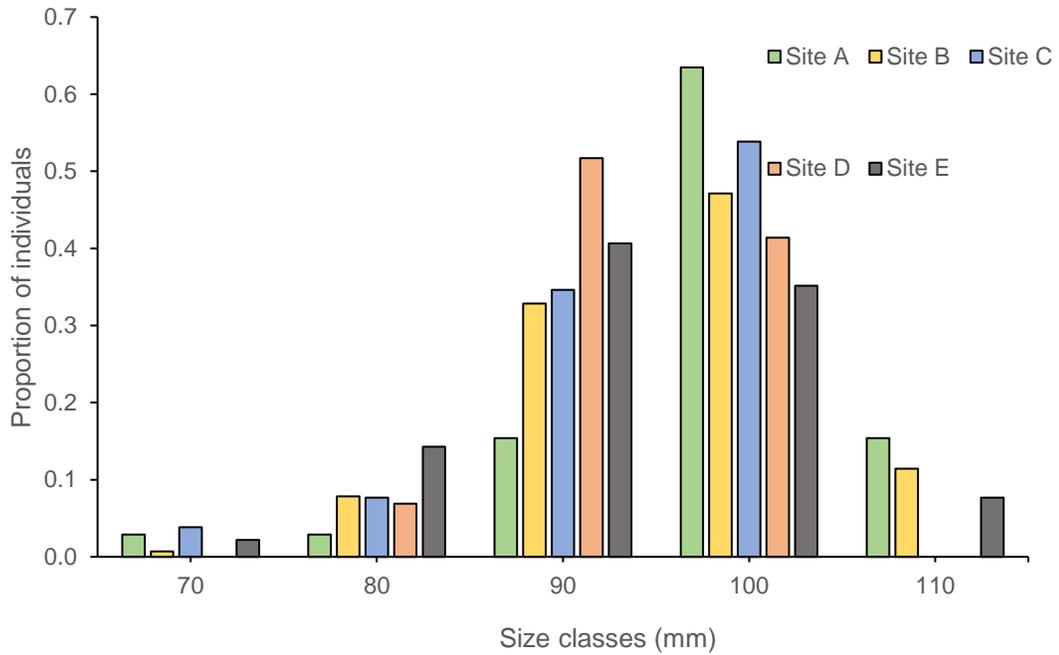


FIGURE 4. Difference in size distributions across five eastern mud turtle (*K. subrubrum*) sites using carapace length in a contingency table analysis ($\chi^2 = 51.14$, $P = <0.0001$). Sites A–E present the proportion of individuals categorized under their respective size classes at each location.

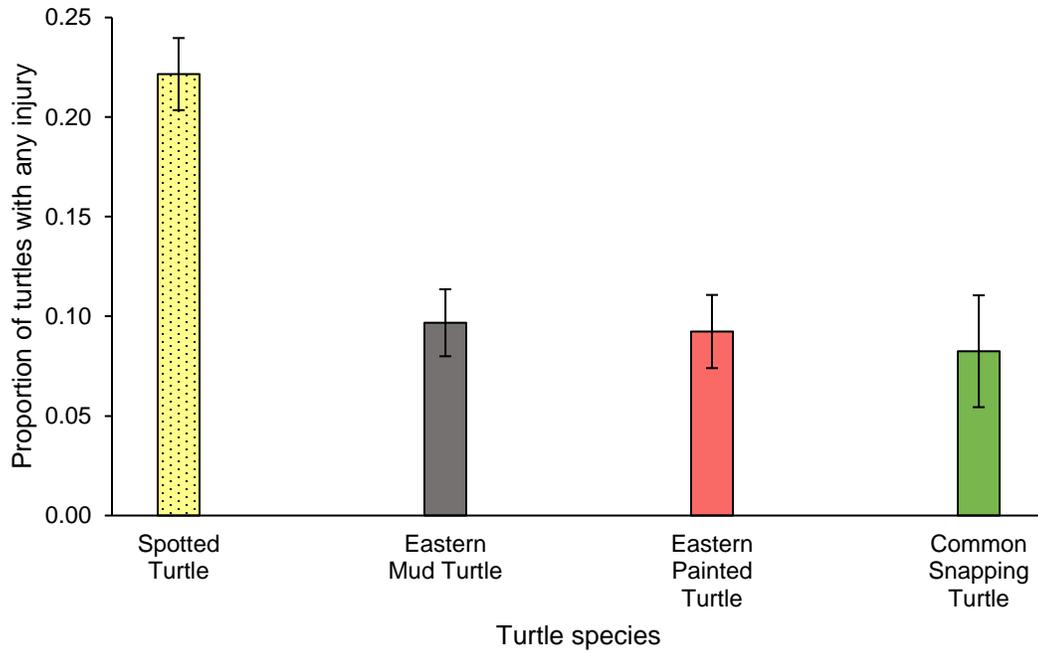


FIGURE 5. Mean (± 1 SE) proportion of individual turtles at seven with any form of injury for each species (Spotted turtle (*Clemmys guttata*), Eastern mud turtle (*Kinosternon subrubrum*), Eastern painted turtle (*Chrysemys picta*), and Snapping turtle (*Chelydra serpentina*)) found at sites A–E (Wald $\chi^2_3 = 10.53$, $P = 0.015$).

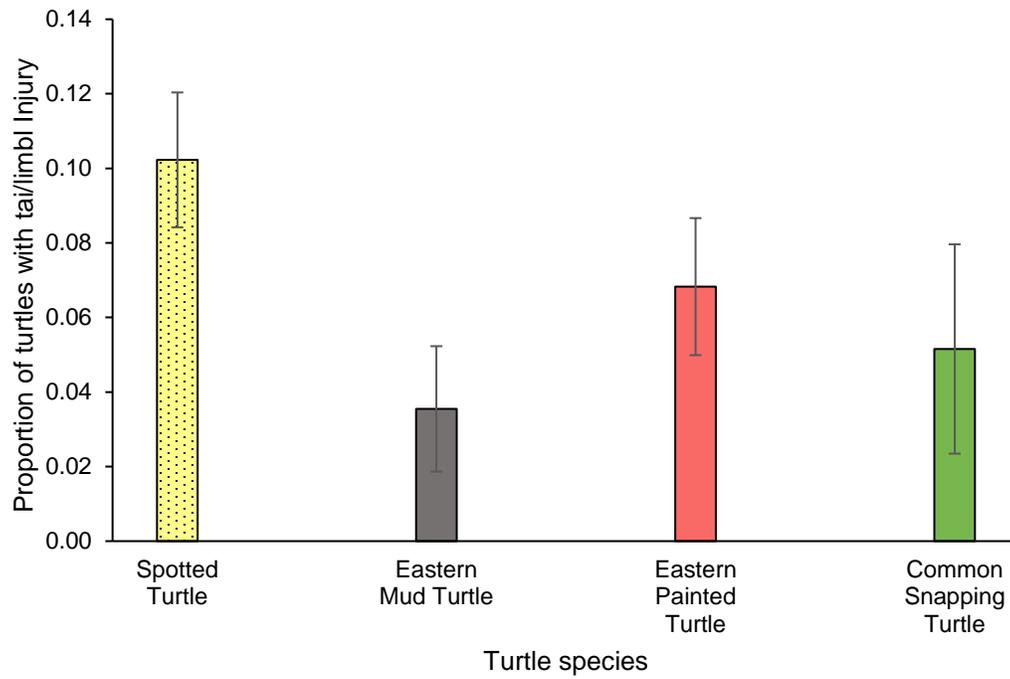


FIGURE 6. Mean (± 1 SE) proportion of individual turtles at seven sites with tail and/or limb injuries for each species (Spotted turtle (*Clemmys guttata*), Eastern mud turtle (*Kinosternon subrubrum*), Eastern painted turtle (*Chrysemys picta*), and Snapping turtle (*Chelydra serpentina*)) found at sites A–E (Wald $\chi^2_3 = 8.67$, $P = 0.0131$).

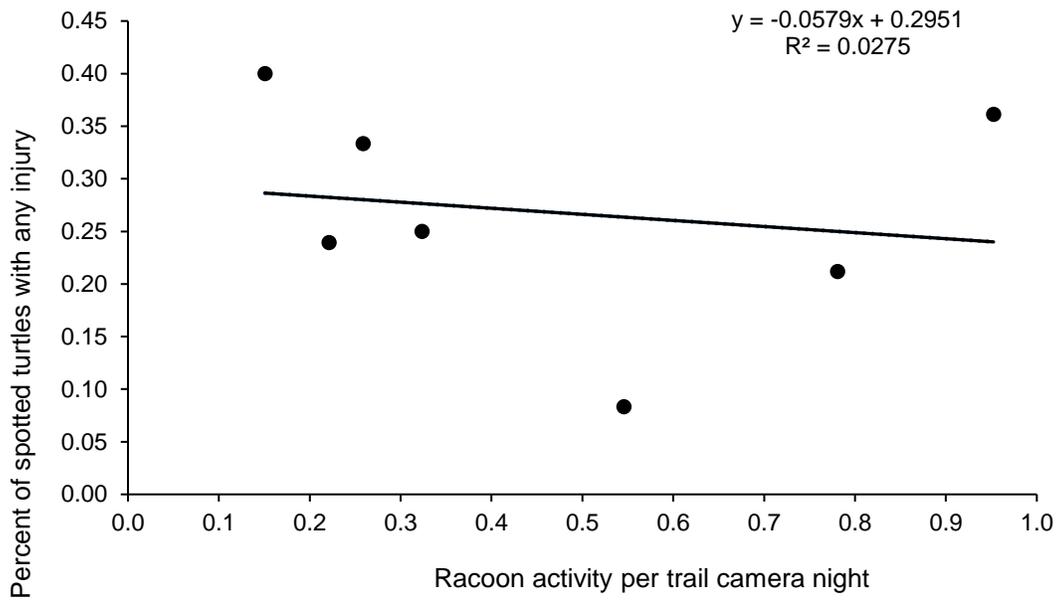


FIGURE 7. Percent of spotted turtles (*Clemmys guttata*) with at least one of any injury types compared to racoon (*Procyon lotor*) activity per trail camera night at sites A–G ($n = 7$).

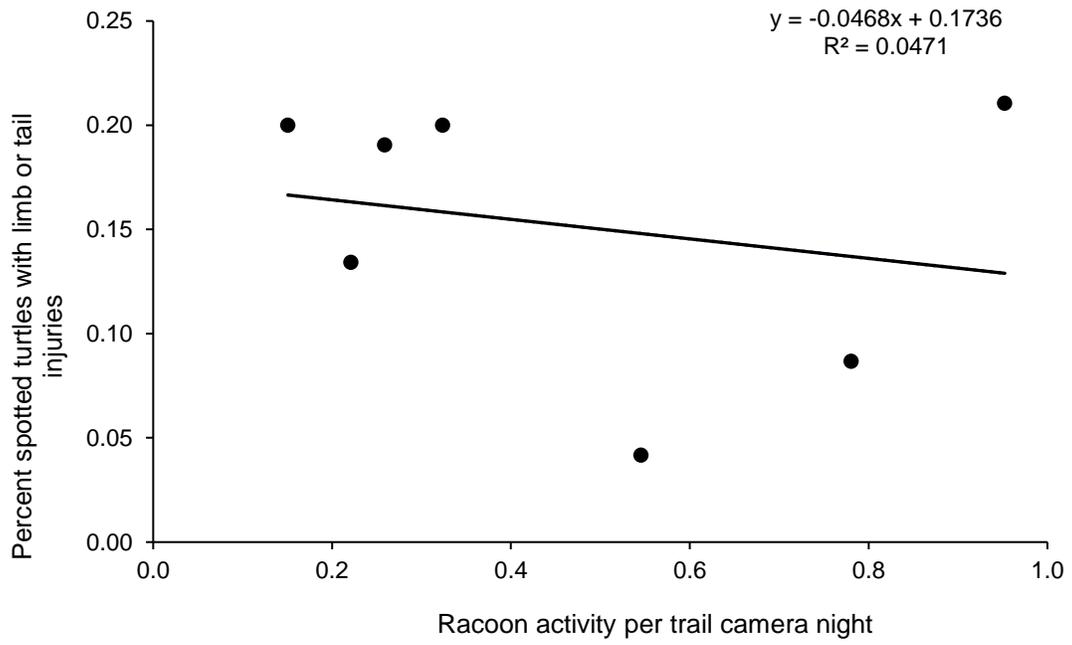


FIGURE 8. Frequency of spotted turtles (*Clemmys guttata*) with a tail and/or limb injury compared to racoon (*Procyon lotor*) activity per trail camera night at sites A–G ($n = 7$).