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Reintroduction Biology of Head-Started Ornate Box Turtles

Eric R. Sievers

REINTRODUCTION BIOLOGY OF HEAD-STARTED ORNATE BOX

TURTLES

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Eric R. Sievers

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REINTRODUCTION BIOLOGY OF HEAD-STARTED ORNATE BOX

TURTLES

Biology

Missouri State University, December 2015

Master of Science

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ABSTRACT

The ornate box turtle (*Terrapene ornata*) is a prairie-dwelling species that has experienced population declines, especially near the northern edge of its range. In order to provide supporting research for a reintroduction program at the Upper Mississippi River National Wildlife and Fish Refuge in northwestern Illinois, I compared the relative success of different approaches to reintroduction. Specifically, I tested the null hypothesis that reintroduced turtles exhibited equal reintroduction success when, 1) released at a site that is known to support a viable ornate box turtle population, 2) 'soft-released' in a fenced enclosure at a site where very few ornate box turtles persist, or 3) 'hard-released' at the same site without the protection of a fence. I also characterized important habitat components that are likely to maximize the quality of a reintroduction site. By many measures, the three treatments returned comparable results. Home ranges were not smaller when turtles were confined to a soft release enclosure, growth rates were not significantly influenced by either the enclosure or by whether or not the release location already supported an ornate box turtle population, diet was similarly varied among all three treatments, and mortality rates during the activity season were similarly low across the three treatments.

KEYWORDS: ornate box turtle, *Terrapene ornata*, reintroduction biology, head-starting, soft-release, sex determination, home range, microhabitat, activity, diet

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Dr. Day Ligon
Chairperson, Advisory Committee
Missouri State University

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INTRODUCTION

Reptiles are declining on a global scale due to anthropogenic effects such as habitat alteration and fragmentation, as well as introduction of invasive species, unsustainable harvest for the pet trade, and infectious diseases (Gibbons et al. 2000; Mitchell and Klemens 2000). Of these threats, habitat loss and fragmentation is the most substantial threat (Gardner et al. 2007). This is especially true in the Midwestern United States, where agricultural expansion and land development have left less than 0.01% of the native prairie habitat (White 1978; Samson and Knopf 1994; Corbett and Anderson 2006). Species extinction due to the loss of prairie habitat is a major concern, as 55 grassland species in the United States are threatened or endangered (Samson and Knopf 1994).

Head-starting is one tool that is used to bolster declining wildlife populations, and typically involves propagating individuals of a species in captivity from wild-harvested eggs or young, and then releasing them back into the wild when they are larger and older and have a greater chance of survival (Jones 2002). Despite the prevalence of head-starting across many taxa, its conservation value has been questioned and criticized (Dodd and Siegel 1991; Reinert 1991; Wilson et al. 2004; Enneson and Litzgus 2008). Major concerns include the loss of fear of humans and other predators by head-started animals, difficulty in adjusting to natural food sources after extended periods in captivity, and the potential to spread diseases to wild populations (Reinert 1991; Alberts et al. 2004). Additionally, head-start programs often also lack adequate post-release

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monitoring, which is essential to properly assess the success of such projects (Dodd and Siegel 1991; Reinert 1991; Seddon 2007; Nichols and Armstrong 2012).

In spite of these criticisms, head-starting has become a popular and successful conservation tool used by wildlife managers in reintroduction plans for threatened and endangered reptiles, including snakes (King and Stanford 2006), iguanas (Escobar et al. 2010), and turtles (Spinks et al. 2003; Fontaine and Shaver 2005; Anthony et al. 2015). To be an effective management tool, head-starting must result in greater recruitment of reproductive adults into the population than would occur without intervention (Heppell et al. 1996; King and Stanford 2006).

The ornate box turtle (*Terrapene ornata*) is a prairie-dwelling species that has experienced population declines, especially near the northern edge of its range in Wisconsin and Illinois (Levell 1997; Conant and Collins 1998; Dodd 2001; Redder et al. 2006; United States Fish and Wildlife Service [USFWS] 2013). Due to these declines, ornate box turtles were listed under Appendix II of the Convention on International Trade of Endangered Species of Wild Flora and Fauna in 1994 (CITES, USFWS 1995), and are protected in Colorado, Illinois, Iowa, Indiana, Nebraska, Kansas, and Wisconsin (Redder et al. 2006). Because of its conservation status several reintroduction programs have been initiated on its behalf.

My research took place at the Upper Mississippi River National Wildlife and Fish Refuge in northwestern Illinois in conjunction with an on-going reintroduction effort conducted by the Refuge. In 2008, the United States Fish and Wildlife Service initiated efforts to reestablish a viable population of ornate box turtles on a patch of remnant prairie located at a former army depot that was decommissioned in 2000. The project

uses head-started juveniles that were hatched from eggs collected from Thomson Sand

Prairie, a nearby prairie that is also managed by United States Fish and Wildlife Service.

In 2010, a population viability study concluded that the ornate box turtle population at

Thomson Sand Prairie could sustain the harvest of eggs for a head-start program to

repopulate Lost Mound Sand Prairie (USFWS 2013).

My overall objective was to compare the relative success of different approaches

to reintroduction. Specifically, I tested the null hypothesis that reintroduced turtles

exhibited equal reintroduction success when, 1) released at a site that is known to support

a viable ornate box turtle population, 2) 'soft-released' in a fenced enclosure at a site

where very few ornate box turtles persist, or 3) 'hard-released' at the same site without

the protection of a fence. I also conducted diet analyses from fecal samples collected

from each turtle and did plant and invertebrate surveys at each site to characterize

important habitat components that are likely to maximize the quality of a reintroduction

site.

MATERIALS AND METHODS

Prior approval for this project was obtained from the Missouri State University IACUC (27 September, 2011; protocol #120011) and the Illinois Endangered Species Protection Board (permit number 10-06A).

Study Sites

Research was conducted at two units of the Upper Mississippi River National Wildlife and Fish Refuge, both of which lie on the eastern bank of the Mississippi River. Thomson Sand Prairie (TSP) is a 146-ha unit in Carroll County, Illinois, that includes both remnant and reestablished sand prairie. The site contains sand prairie, sand dune, and blowout communities dominated by needlegrass (*Stipa* spp.) and little bluestem (*Schizachyrium scoparium*), with interspersed patches of prickly pear cactus (*Opuntia humifusa*), aromatic sumac (*Rhus aromatica*), and spiderwort (*Tradescantia ohiensis*). A strip approximately 10 m wide immediately bordering the river is dominated by a variety of deciduous trees, black raspberry (*Rubus occidentalis*), and poison ivy (*Toxicodendron radicans*). Isolated raspberry patches and eastern red cedar (*Juniperus virginiana*) are scattered throughout the study site (Bowen et al. 2004; Refsnider et al. 2012; USFWS 2013). The site is bordered by the Mississippi River to the west, a railroad right-of-way containing remnant prairie to the east, a residential development to the north, and a pine plantation to the south that separates Thomson Sand Prairie from another remnant sand prairie, Thomson Fulton Sand Prairie. A narrow corridor of prairie associated with the

railroad right-of-way and a public bike path connects Thomson Sand Prairie and Thomson Fulton Sand Prairie.

Lost Mound Sand Prairie (LMSP) is a 1,619-ha unit in northwestern Carroll and southwestern Jo Daviess counties on the former Savanna Army Depot, and is the largest remnant sand prairie in Illinois (Ebinger et al. 2006; USFWS 2013). The area is bordered on the west by the Mississippi River, on the east by railroad tracks, on the north by a campground and day use area managed by the U.S. Army Corps of Engineers, and on the south by privately owned semi-developed sand prairie. Ornate box turtles were once common at LMSP, but decades of military activity nearly extirpated them from the area (McCallum and Moll 1994). LMSP is jointly managed by United States Fish and Wildlife Service and Illinois Department of Natural Resources and contains sand prairie, sand dune, sand savanna, and blowout communities dominated by prairie junegrass (*Koeleria macrantha*) and little bluestem (*Schizachyrium scoparium*) with interspersed patches of prickly pear cactus (*Opuntia humifusa*), aromatic sumac (*Rhus aromatica*), redroot (*Ceanothus herbaceus*), and spiderwort (*Tradescantia ohiensis*).

Head-starting

In 2012, 19 eggs were harvested from eight ornate box turtle nests at Thomson Sand Prairie. All of the eggs were incubated at the Lincoln Park Zoo in Chicago, IL, and 18 hatched. In 2013, 23 eggs from eight nests were incubated at the Lincoln Park Zoo, of which 20 hatched. Hatchlings were maintained in a clear plastic container with a thin layer of damp sphagnum moss for the duration of the head-starting period. Additional moss was added as the turtles grew larger to help keep moisture at a proper level for

healthy shell growth (Wiesner and Iben 2003) and to provide adequate cover. Another plastic container was nested beneath the upper container and filled with 4.5 cm of water so that the bottom of the upper container made full contact with the water in the lower container. A 100-watt water heater was used to maintain the water temperature at 28°C. Turtles were fed a commercially available turtle food (Reptomin Sticks, Tetrafauna, United Pet Group; Blacksburg, VA, USA). Dampened food sticks were offered daily in the early morning. Every evening unconsumed food was removed to help maintain container cleanliness. The photoperiod was set to 8.5 hours of light per 24-hour cycle, and turtles were maintained under these conditions from the time that they hatched in August until their release the following June. A six percent of body weight threshold was set for a turtle to carry a transmitter and at the time of release, one of the 18 turtles from 2012 cohort was not large enough to equip with a radio transmitter (Schubauer 1981).

As a result 17 10-month-old head-started turtles were released in June 2013: five were released at the TSP donor site, six were released inside of the soft-release enclosure at LMSP (LM IN), and six were released outside the enclosure (LM OUT). Nine head-started turtles from the 2013 cohort were released in June 2014, with three added to each treatment. Radio transmitters (model SOPR-2190, Wildlife Materials International, Inc., Murphysboro, IL) were affixed to costal scutes three and four with epoxy. Before release, each turtle was marked with a unique combination of notches filed into the marginal scutes (adapted from Cagle 1939). Although location and activity data were collected until the end of the 2014 activity season, for the purposes of my study data collected after 31 August, 2014 were not included in analyses.

Mass and Growth

Turtles were weighed with a digital scale and straight carapace length was measured using digital calipers at the beginning of each activity season or when released and again in late summer before hibernation. I compared the changes in mass and length among treatments using one-way ANOVAs and between cohorts using two-sample t-tests. I was unable to obtain late summer carapace length measurements for the 2013 cohort due to instrument malfunction.

Sex Determination

Adult male ornate box turtles exhibit secondary sex characteristics that make it possible to visually distinguish them from adult females. However, the head-started turtles in my study were all juveniles and none had developed identifiable secondary sex characteristics. Therefore, to determine the sex ratio that resulted from the incubation conditions used, I collected blood samples from 28 1–5-year-old head-started turtles in June 2014 and measured plasma androgen concentrations for the purpose of differentiating males from females (Rostal et al. 1994). The group of turtles used for this component of my study was expanded by including older head-started turtles that were present at my study site but were not included in any other aspects of this study. I collected 200 μL of whole blood from each animal. These samples were stored on ice and then centrifuged for 1–2 minutes. The plasma fraction (~100 μL) was then transferred to a clean microcentrifuge tube and frozen for later analysis. All blood samples were drawn from the central subcarapacial sinus using a 22-g needle (Hernandez-Divers et al. 2002).

Although population sex ratio is important to consider in the context of wildlife reintroductions, sex was not included as a factor in my analyses because subadult turtles are unlikely to exhibit sex-specific behavior before reaching maturity. Additionally, the small sample sizes in my study precluded the further partitioning of the dataset. I therefore elected to pool sexes for all analyses.

Telemetry

I used radio telemetry to monitor turtles using a receiver and a hand-held yagi antenna (Advanced Telemetry Systems, Isanti, MN). During the study, head-started turtles were tracked at least once per week, beginning when they were released in June 2013 and continuing until they entered hibernation. Turtles were also located once in the winter to confirm overwintering locations. In 2014, turtles were located at least once weekly from 19 May until 31 August. Locations of turtles were visually confirmed, and I tracked turtles at different times of the day to minimize diel biases on the locations of individuals. Geographic coordinates were recorded using a handheld global positioning system unit.

Home ranges for each individual were calculated using 95% minimum convex polygons (MCP) and adaptive kernel estimates (both 50% and 95% kernels) using the ad hoc approach to bandwidth selection (Kie 2013) within the Home Range Tools Extension of ArcMap (Version 9.3.1, ESRI, Redlands, CA). The 95% MCP and 95% kernel estimates were used to characterize a turtle's annual home range, while the 50% kernel estimate defined the core activity area. I included both MCP and kernel estimates to facilitate comparisons with other studies.

The bandwidth selection is considered the most important decision when calculating a kernel estimate, yet no consensus exists on how to choose an appropriate bandwidth value (Silverman 1986; Worton 1989). I chose to use the ad hoc method ($h_{ad\ hoc}$) due to the variable size and shape of turtle home ranges and because it allowed for the delineation of a single, contiguous home range. The value of $h_{ad\ hoc}$ was calculated by decreasing the value of the reference bandwidth (h_{ref}) by increments of 0.05 to 0.95, then 0.9, 0.85, and so on until the isopleth fragmented into multiple polygons. The smallest, continuous proportion of h_{ref} was then used as the bandwidth parameter to define an individual turtle's home range boundary. To minimize the exaggeration of estimated home ranges, individuals with disjunct 50% kernel estimates were accepted if the distance between them was greater than the average distance moved between monitored locations. Although subjective, this method is repeatable and easily standardized (Berger and Gese 2007; Jacques et al. 2009).

Turtle locations recorded in the first week following release were not included in home range calculations to eliminate movements related to any initial flight response the turtles may have exhibited (Stamps and Swaisgood 2007; Hester et al. 2008; Dickens et al. 2009; Bennett et al. 2013; Bauder et al. 2014). The effects of release site location on home range estimates were tested using one-way ANOVAs. I used linear regression to examine the relationship between the mass of the turtle and the size of the MCP home range. Year-to-year home range fidelity was examined by calculating the percent overlap of MCPs from subsequent years for eight turtles (five at LM IN and three at TSP) for which data were generated using the following formula: [Area$_{overlap}$/ (Area$_{year1MCP}$ + Area$_{year2MCP}$ - Area$_{overlap}$)] × 100 (Refsnider et al. 2012). A two-sample t-test was used to

compare overlap between LM IN and TSP treatments. A paired t-test was also performed to compare home range size in 2013 versus 2014 for these eight individuals.

Activity Patterns

That box turtle activity is constrained by weather conditions is well documented (Legler 1960; Reagan 1974; Tucker et al. 2015). During the summer, turtles are often active in the morning and late afternoon but avoid the warmest temperatures in the middle of the day (Legler 1960; Neieuwolt 1996; Converse and Savidge 2003; Tucker et al. 2015). I compared activity levels among the three treatment groups by recording activity status (active or inactive) at one-minute intervals using automated radio telemetry and the signal change method (Tucker et al. 2014). A vertically positioned omnidirectional antenna was placed on top of each of six 9.2-m towers and connected to an automated receiving unit (Sparrow Systems, Fisher, IL). Four automated receiving units and their associated towers were located at TSP and two were positioned at LMSP, with one positioned inside and one outside the soft-release enclosure. Activity data were then paired with the meteorological data from weather stations located at LMSP and TSP. However, weather data for TSP in 2014 were obtained from nearby Tri-Township Airport due to a wildfire that damaged the on-site weather station.

Turtles were grouped by release location (LM IN, LM OUT, or TSP) and repeated-measures ANOVAs were conducted to test the effects of location and time (hour of day). Activity data were pooled by hour for each individual to test for differences in activity levels among treatments, as well as between days when rain did or did not occur. Average activity levels were pooled for each turtle for each week to examine seasonal

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changes in activity levels. T-tests were used to compare meteorological data from Tri-Township Airport during the true activity season (April 15–October 15) between 2013 and 2014.

Microhabitat

To characterize habitat selection by head-started ornate box turtles, I identified available microhabitat structure of each turtle's home range by pairing randomly selected sites with turtle-selected locations. Paired random points were selected by designating a random direction (1–360°) and paced distance (1–20 paces) from the turtle's location using computer-generated random numbers tables. Microhabitat data were gathered using a 1×1-m PVC frame centered over the turtle's location. Within the frame, ground cover was analyzed by estimating percent bare ground, grass, forbs, shrub, and litter. Vegetation height was also recorded by averaging the measurements from four points along the center of the frame at 20, 40, 60, and 80 cm.

Microhabitat structure at turtle-selected sites and random locations were compared using MANOVAs. Discriminant function analysis was then used to identify which variables contributed most to the separation of group centroids. The percent cover of forbs was not included in the discriminant function analysis as it was not significant in any of the preceding MANOVAs. All multivariate analyses were conducted in SPSS (Version 22, IBM, Armonk, NY).

Diet

I collected two fecal samples annually from each of the head-started turtles to identify key dietary components. Sampling times occurred during the active season and were spaced approximately one month apart to account for seasonal variation in available resources. Upon capture, each turtle was thoroughly rinsed to remove externally adhered particles that could contaminate the fecal sample and then retained overnight in a 19-L bucket containing 1–2 cm water. All turtles defecated in the allotted time. The following morning, the contents of the bucket were filtered through a 250-μm wire sieve and stored in alcohol for later identification.

Concurrent with collecting fecal samples I also collected arthropod reference samples using pitfall traps. These traps consisted of 85-mL plastic cups containing a small amount of propylene glycol. Each cup was buried with the rim flush with the ground surface. Ten traps were placed in a straight-line transect and spaced 50 m apart at each release site. Representative plant specimens were also collected and preserved in a plant press for later reference to aid identification of fecal material.

RESULTS

All 17 transmittered turtles from the 2012 cohort survived the 2013 active season and entered hibernation. However, seven of these turtles (5 LM OUT, 1 LM IN, 1 TSP) did not survive the winter. In addition to the winter mortalities, one turtle was depredated at TSP and another died after contracting an unknown disease at LM IN in 2014. Furthermore, the surviving individual from the 2012 LM OUT cohort lost its transmitter early in the 2014 active season, preventing comparisons involving this cohort.

Mass and Growth

Mean increase in mass was not significantly different among treatments for the 2013 cohort ($F_{2,4}$ = 2.03, P = 0.246). However, the mean mass increase was almost five times greater for LM OUT turtles (15.28 ± 2.39g) than those at TSP (3.13 ± 1.29g), with LM IN turtles (8.37 ± 8.44g) exhibiting intermediate growth. In the 2012 cohort, the difference in average mass gain of LM IN (19.80 ± 5.21g) and TSP (3.00 ± 2.89g) turtles was large (19.80 ± 5.21g versus 3.00 ± 2.89 g, respectively) but non-significant (t_6 = 2.31, P = 0.060).

Turtles from the 2012 cohort gained significantly more mass in 2014 (6.43 ± 4.87g) than in 2013 (-1.06 ± 1.08g) (t_{21} = 2.12, P = 0.046), and gained an average of 13.50 ± 4.48g over the duration of the study. In comparison, the 2013 cohort gained an average of 9.84 ± 2.94g in 2014, significantly more than the 2012 cohort increased in their first year after release (t_{21} = 4.34, P = 0.0003). Turtles from the 2012 cohort gained an average of 0.30 ± 0.05cm in straight carapace length during the study. There was no

significant difference in mean carapace growth between LM IN and TSP ($t_6 = 0.53$, P = 0.615).

Sex Determination

Radioimmunoassays were conducted with large plasma volumes (typically 30 μL) and recovery efficiency was 90%. There was substantial variation in circulating androgen levels among individuals and among year classes (Figure 1). Although androgen concentrations appeared to increase with age in both sexes, across all juvenile year classes included in my study it appeared that circulating androgen levels were ≤ 0.12 pg/mL among females and above this threshold among males.

Based on this interpretation of the data, seven of the 28 turtles tested were scored as males, with four, two, zero, and one males represented in each of the four year classes tested (Figure 1). Among the three release sites there are 13 females and three males at LM IN, three females and one male at LM OUT, and five females and three males at TSP.

Home Range

Mean 95% MCP estimates (1.02 ± 0.21 ha) were smaller than mean 95% kernel home range estimates (1.57 ± 0.28 ha) (Figure 1). Core activity areas represented by 50% kernel density estimates (0.22 ± 0.04 ha) were often concentrated around woody vegetation that likely provided turtles with cover from the midday heat (Figure 2). There were no significant differences among release sites in 2013 or 2014 for any of the three home range estimates, but there was a marginally non-significant difference ($t_7 = -1.87$, P

= 0.052) in 95% MCP size between 1-year-olds and 2-year-olds for the eight individuals for which two years of data were available.

Variation among individuals was large regardless of the method used to estimate home range size (95% MCP = 0.01–4.61 ha, 95% Kernels = 0.1–6.25 ha). Part of this variation was attributable to effects of body size, as larger turtles occupied larger home ranges than did smaller turtles ($R^2 = 0.26$, P = 0.011) (Figure 3). Finally, average 95% MCP overlap between consecutive years was 23.23 ± 7.28% SE, and ranged from 0.06–55% overlap. There was no significant difference in MCP overlap between LM IN and TSP ($t_6 = 0.67$, P = 0.525), averaging 27.2 ± 9.9% and 16.6 ± 11%, respectively.

Weather and Activity Patterns

Weather conditions did not differ significantly between years. However, mean daily maximum temperatures during the activity season were marginally warmer in 2013 (25.52 ± 0.45°C) than in 2014 (24.35 ± 0.43°C) ($t_{366} = -1.89$, P = 0.060) (Figure 4). Mean rainfall during the activity season was not significantly different ($t_{366} = 0.74$, P = 0.447). Total rainfall in 2013 was 39.17 cm and in 2014 was 50.55 cm (Figure 5).

Head-started turtles exhibited bimodal daily activity patterns, with activity peaking in mid-morning and late afternoon (Figure 6). There was a significant site × time of day interaction in 2013 ($F_{46,336} = 1.72$, P = 0.0038) that resulted from lower early morning activity levels among LM OUT turtles (Figure 6). There was no such interaction in 2014 ($F_{46,384} = 1.27$, P = 0.1207) but both time and release site were significant factors (time: $F_{23,384} = 14.11$, P < 0.0001; release site: $F_{2,384} = 18.65$, P < 0.0001. In both years turtles were also more active on days when rain occurred than on days without

precipitation (2013: $F_{1,786} = 102.93$, $P < 0.0001$; 2014: $F_{1,851} = 57.78$, $P < 0.0001$) (Figure 7). The greatest difference in activity levels between rain and non-rain days occurred in the middle of the day and afternoon. Seasonal activity patterns exhibited differences both among weeks and among release sites in 2013 (week: $F_{18,229} = 3.11$, $P < 0.0001$; site: $F_{2,229} = 4.86$, $P = 0.0085$). In 2014 there was a release site × week interaction ($F_{43,214} = 2.67$, $P < 0.0001$) due to low activity levels among turtles at TSP during several weeks early in the activity season (Figure 8). Activity patterns measured in the same Julian weeks in 2013 and 2014 were very similar across the three treatments (Figure 9).

Microhabitat

The microhabitat measured at turtle locations was significantly different than that measured at random locations for all treatments (all $P < 0.002$). Pair-wise comparisons among treatments revealed that turtles at TSP were more often associated with grass than turtles at LMSP, and turtles at LMSP were more affiliated with shrub cover (Table 2). Head-started turtles inside the enclose at LMSP were less associated with canopy cover than those at LM OUT and TSP. LM OUT turtles associated with taller vegetation than those in the other treatments, and LM IN turtles tended to associate with warmer soil temperatures. The number of trees near turtle locations was different at each site, with LM IN having the least and LM OUT the most.

Differences among random points were also apparent, as LM IN was characterized by more bare ground, less grassy cover, shorter vegetation, warmer soil temperature, and fewer trees (Table 3). TSP supported a higher density of forbs and fewer shrubs, while LM OUT had more shrubs and fewer forbs. The percent cover of forbs was

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not different between turtle locations and random points within any treatment and thus was omitted from the discriminant function analysis.

Discriminant function analysis significantly described differences between turtle locations and random points (eigenvalue = 0.304, chi-square = 143.416, p<0.0001). This function was strongly influenced by shrub cover (0.714), vegetation height (0.673), and canopy cover (0.429). There was also a strong negative association with bare ground (-0.476) and warmer temperatures (-0.433).

Diet

Arthropods were found in 31 of 33 fecal samples collected in 2013, and 24 of 25 in 2014 (Figure 10). The most common insect, strawberry root weevil (*Otiorhynchus ovatus*), was found in 48% of samples in 2014 and 73% of samples in 2013 (Figure 11). The weevils were consumed at all three release sites in both 2013 and 2014. Sample contents were consistent among sites with a few exceptions. Click beetles (*Agrypnus rectangularis*) were only present in samples collected at TSP in 2014 and were in 67% of samples from the site. In 2014, field crickets (in 24% of all samples) and dung beetles (in 44% of all samples) were both absent from LM OUT fecal samples, although both were present at the site in 2013. Many turtles also consumed ants, grasshoppers, and one-spotted stink bugs (*Euschistus variolarius*) (Appendix). Snail shells were found in three samples, and eggshell fragments were identified in one sample. Almost all samples contained small rocks and pebbles.

Plant matter was found in 27 of the 33 samples collected in 2013, and 24 of 25 in 2014. Much of the plant matter was unidentifiable, but a number of seeds could be

identified, including those of buckthorn, sedge, hackberry, hoary puccoon, mulberry, prickly pear, and *Rubus* spp. (Figure 12). Monocot and dicot plants both commonly occurred in the samples. Most plant matter was found uniformly among all sites with the exception of *Rubus* spp. seeds, which were never found in samples collected at LM OUT.

DISCUSSION

By many measures, the three treatments to which I assigned ornate box turtles in my study returned comparable results. Home ranges were not smaller when turtles were confined to a soft release enclosure, growth rates were not significantly influenced by either the enclosure or by whether or not the release location already supported an ornate box turtle population, diet was similarly varied among all three treatments, and mortality rates during the activity season were similarly low across the three treatments.

Despite the many similarities, there were several important differences in turtles' responses that have important implications for this reintroduction effort and others like it. For example, although turtles placed within the enclosure at Lost Mound did not inhabit home ranges that differed in size from turtles whose movements were not constrained, they were often located directly against the fence and a well-worn path was conspicuous within the perimeter, suggesting that these turtles spent much of their time walking along the barrier. Such behavior may have several negative results. First, efforts to navigate around the fence barrier were futile and cost turtles time that could have been dedicated to more fruitful exploration away from a barrier. Second, although predator density was very low within the enclosure—a single raccoon was observed in 2014—walking the barrier likely greatly increased turtles' chances of being detected, as potential predators were likely to engage in similar behavior.

In spite of the patrolling behavior exhibited by turtles inside the enclosure, release site differences in activity occurred only for a few hours in the morning and groups were separated by only a small number of minutes. For the majority of the day, turtles across

all three sites displayed the same activity patterns. Activity measured during the middle of the activity season (weeks 27–35) differed very little, suggesting that seasonal activity patterns may be quite consistent from year to year provided that weather patterns do not differ too greatly.

In addition to differences among release sites, there were also differences between age classes. One-year-old head-started turtles had larger MCP annual home ranges than 2-year-old animals. Newly released animals commonly make extensive movements as they explore novel environments and search for suitable habitat (Stamps and Swaisgood 2007; Hester et al. 2008; Dickens et al. 2009; Bennett et al. 2013; Bauder et al. 2014). Other studies have also documented reduced movements of Chelonians in the second year post-release compared to the first year, as they have already mapped their resources and do not need to go on as many exploratory ventures (Tuberville et al. 2005; Nussear et al. 2012).

Comparisons between head-started turtles and their wild counterparts yielded similar patterns. Home ranges of juveniles in my study were smaller than the home ranges of adult ornate box turtles reported in a study conducted at TSP (Refsnider et al. 2012), and are consistent with other findings comparing juvenile and adult box turtle home ranges (Schwartz et al. 1984; Doroff and Keith 1990). This is most likely due to a difference in body size, and is similarly exhibited in the increase in home range size as head-start mass increases. Similar correlations between body size and home range size have also been reported in mammals (Harestad and Bunnel 1979) and reptiles (Perry and Garland 2002).

The head-started turtles in my study exhibited bimodal daily activity patterns that were consistent with the patterns reported for wild adult turtles at TSP, indicating that head-started turtles have similar daily activity patterns as their wild counterparts (Tucker et al. 2015). These patterns suggest that head-started turtles elected to be active when environmental conditions were favorable and were mostly inactive during the hottest part of the day.

Head-started turtles were more active on days when rain occurred in both years of my study, a pattern that has proved consistent across years (Tucker et al. 2015). The increase in activity on rainy days is mostly likely due to the release from lethally hot afternoon temperatures provided by precipitation and the associated weather front, as well as the reduction in evaporative water loss. Turtles may also be more active on days with rain to capitalize on foraging opportunities provided by the increased activity of invertebrate prey. Interestingly, rain had a smaller impact on activity patterns in 2014 than in 2013, possibly due to cooler prevailing temperatures in the 2014 activity season.

Activity levels varied across the active season, but seasonal differences were most pronounced early in 2014. These contrasting patterns may be due to differences in emergence time, as turtles exiting hibernation would be more active as they moved toward the surface than those remaining dormant. Further research exploring the relationship between the overwintering behavior and survival of head-started box turtles is needed to clarify the high winter mortality rates, as all three release sites had high quality habitat that included available hibernation sites. Head-started animals were not the only turtles to experience winter mortality, as an adult female at TSP also succumbed to the cold weather.

While LM OUT head-started turtles did experience greater mortality rates, most deaths were likely due to the harsh winter conditions and not the protection provided by the enclosure. Only one head-started turtle was depredated in the span of this two-year study, leading me to question the necessity of soft-releasing turtles inside an enclosure, as its principal purpose is to protect the turtles against predators. Without an enclosure, reintroduction projects become more affordable and also allow for the natural dispersal of animals while reducing competition for resources. I detected no evidence of excessive wandering by turtles that were not constrained by an enclosure. As the LMSP population continues to grow, fragmentation of the enclosure boundary should be considered to allow individuals to disperse to previously unoccupied high-quality areas at LMSP.

In order to maximize the success of reintroductions, high-quality release sites must be chosen. While release sites may appear similar to the human eye, they are quite different on a microhabitat scale. Specific criteria for reintroduction sites should carefully consider habitat variables strongly linked with turtle-selected locations. Such locations were strongly associated with cover in the form of shrubs or small trees. Turtles were often found under aromatic sumac (*Rhus aromatica*), a common shrub at both study sites, but more prevalent at LMSP. Results from the discriminant function analysis reveal turtles' preference for tall, shrubby vegetation, while avoiding bare ground and warmer soil temperatures. These landscape features are likely essential components of suitable reintroduction sites, as they provide refugia from midday heat, protection for juveniles from predators, and abundant food (Nieuwolt 1996; Jennings 2007).

In addition to providing a refuge from lethal temperatures, fruit-bearing trees and shrubs such as mulberries and *Rubus* spp. yield a seasonally bountiful food source.

Healthy and diverse invertebrate populations are also likely important, as insects were a key dietary element at all three release sites. The strawberry root weevils seen in over half of the samples are commonly found on *Rubus* spp., and were likely consumed inadvertently while eating the plant. While many plants and invertebrates were identified, only those that have a rigid structure are easily discernable. Food items that lacked rigid structures were most likely not apparent or present in the fecal samples and may have led to results that are biased towards invertebrates with an exoskeleton and highly fibrous plants and seeds. Previous studies and my own observations have recognized the dietary importance of spiderwort (*Tradescantia ohiensis*) due to its water-rich, succulent stems, which was totally absent from my samples (Legler 1960; Doroff and Keith 1990; Gangloff and Nash 2010). While in the field, I also came across an adult box turtle consuming three newly-born mice and another picking at the corpse of an opossum. It appears as though box turtles are highly opportunistic and will eat whatever they can successfully capture.

Another critical component to the success of most reintroduction program— though practically self-evident— is reintroducing both males and females. Achieving mixed sex ratios is of little concern for species that exhibit genetic sex determination; however, assuming production of both males and females of species with temperature-dependent sex may lead to instances where only one sex is used to reestablish a population (Morreale et al. 1982). Most of the juvenile turtles tested that were scored as females expressed androgen concentrations that were below the detection limits of the assay. Additionally, a subset that had very low but detectable levels were also scored as females because of the substantial difference in concentrations between turtles that were

in the same age class but were scored as putatively male. However, it is possible that some or all of the individuals that had detectable circulating androgen levels were in fact males. If this is the case, then the sex ratio of 7:21 (male:female) might in fact be as different as 12:16. In either case, however, the sex ratio is mixed and female biased, and therefore likely appropriate to a reintroduction effort (Nelson et al. 2002; Wedekind 2002; Lenz et al. 2007). Independent verification of the age-specific accuracy of ascertaining sex based on circulating androgens should be conducted for this species via dissection or non-lethal laparoscopic surgery (Ligon et al. 2014).

In conclusion, the continued protection of nests at both LMSP and TSP is vital to the successful hatching and recruitment of new individuals to the population. Both sites experience very high nest predation rates, with mesopredators at TSP and hognose snakes at LMSP consuming most of the nests. Continued nest protection will require that refuge staff continue efforts to track adult females to locate nests. Without this protection, population growth will likely stagnate or decline, undermining the reintroduction efforts of this project.

The primary goal of this project is to reestablish a viable population of ornate box turtles at LMSP, with a target population size of 100 individuals (USFWS 2013). I urge the refuge to continue the head-start program beyond the planned 100 individuals as there are many uncertainties, including high nest predation, adjacent land development, demographic and environmental stochasticity, just to name a few. The likelihood of succeeding in reestablishing a viable population only increases with the number of individuals (Mateju et al. 2012). Continued collaboration with reintroduction biologists at

the Lincoln Park Zoo will help ensure that all factors affecting the population's persistence are being considered.

Most importantly, continued monitoring is necessary to ascertain the success of reintroduction programs. Emphasis should be placed on abundance, survival, dispersal and reproduction of released turtles as a function of their demographic state (age and sex) as well as the location of release (inside vs. outside enclosure), as much is still uncertain as to the effects of head-starting (Nichols and Armstrong 2012). Monitoring data can also aid the decision-making process to determine what level of management is needed to ensure the persistence of the population into the foreseeable future. Furthermore, the successes and failures of this reintroduction program may provide important information for the planning of future ornate box turtle reintroductions.

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Table 1. Summary of home range data for reintroduced turtles at Thomson Sand Prairie (TSP) and Lost Mound Sand Prairie inside (LM IN) and outside (LM OUT) of a soft-release enclosure in 2013 and 2014. All areas are reported in hectares. Values in parentheses represent 1 standard error.

Site	n	95 % MCP	95% Kernel	50% Kernel
2013 TSP	5	1.60 (0.94)	1.45 (0.91)	0.17 (0.10)
2014 TSP	5	0.80 (0.56)	0.88 (0.49)	0.13 (0.06)
2013 LM IN	6	1.42 (0.59)	2.11 (0.72)	0.31 (0.10)
2014 LM IN	8	0.80 (0.31)	1.67 (0.70)	0.25 (0.11)
2013 LM OUT	6	0.53 (0.23)	1.24 (0.44)	0.13 (0.04)
2014 LM OUT	3	1.18 (0.47)	2.17 (0.86)	0.35 (0.15)
1 year olds	25	1.07 (0.27)	1.53 (0.30)	0.21 (0.04)
2 year olds	8	0.85 (0.30)	1.68 (0.70)	0.26 (0.11)
All	33	1.02 (0.21)	1.57 (0.28)	0.22 (0.04)

Table 2. Mean values for microhabitat structural and climatic variables at sites selected by head-started ornate box turtles at Thomson Sand Prairie (TSP) and Lost Mound Sand Prairie, both inside (LM IN) and outside (LM OUT) of the enclosure. Values in parentheses represent 1 standard error. Difference category represents the results from post-hoc pairwise comparisons.

Variable	TSP	LM IN	LM OUT	AVG	Difference
% Bare Ground	4.88 (1.01)	5.37 (1.19)	3.24 (1.08)	4.62 (0.65)	TSP = LM IN = LM OUT
% Grass	40.67 (3.08)	25.41 (2.69)	26.63 (3.14)	30.77 (1.75)	LM IN = LM OUT < TSP
% Forbs	27.58 (2.46)	20.19 (2.40)	16.95 (2.69)	21.74 (1.47)	LM OUT < TSP, LM OUT = LM IN, LM IN = TSP
% Shrub	16.42 (2.50)	40.52 (3.82)	45.97 (4.31)	34.07 (2.22)	TSP < LM IN = LM OUT
% Litter	11.57 (1.43)	8.51 (1.17)	7.21 (0.85)	9.16 (0.71)	TSP = LM IN = LM OUT
% Canopy Cover	12.30 (2.74)	3.17 (1.41)	15.31 (3.18)	9.51 (1.41)	LM IN < TSP = LM OUT
Avg. Veg. Height (mm)	36.02 (1.80)	40.00 (1.68)	46.37 (1.95)	40.44 (1.06)	TSP = LM IN < LM OUT
Soil Temp (°C)	20.68 (0.32)	22.22 (0.22)	20.61 (0.23)	21.27 (0.16)	TSP = LM OUT < LM IN
Trees w/in 10m	2.27 (0.34)	0.68 (0.15)	3.97 (0.59)	2.11 (0.22)	LM IN < TSP < LM OUT

Table 3. Mean values for microhabitat structural and climatic variables at random points at Thomson Sand Prairie (TSP) and Lost Mound Sand Prairie, both inside (LM IN) and outside (LM OUT) of the enclosure. Values in parentheses represent 1 standard error. Difference category represents the results from post-hoc pairwise comparisons.

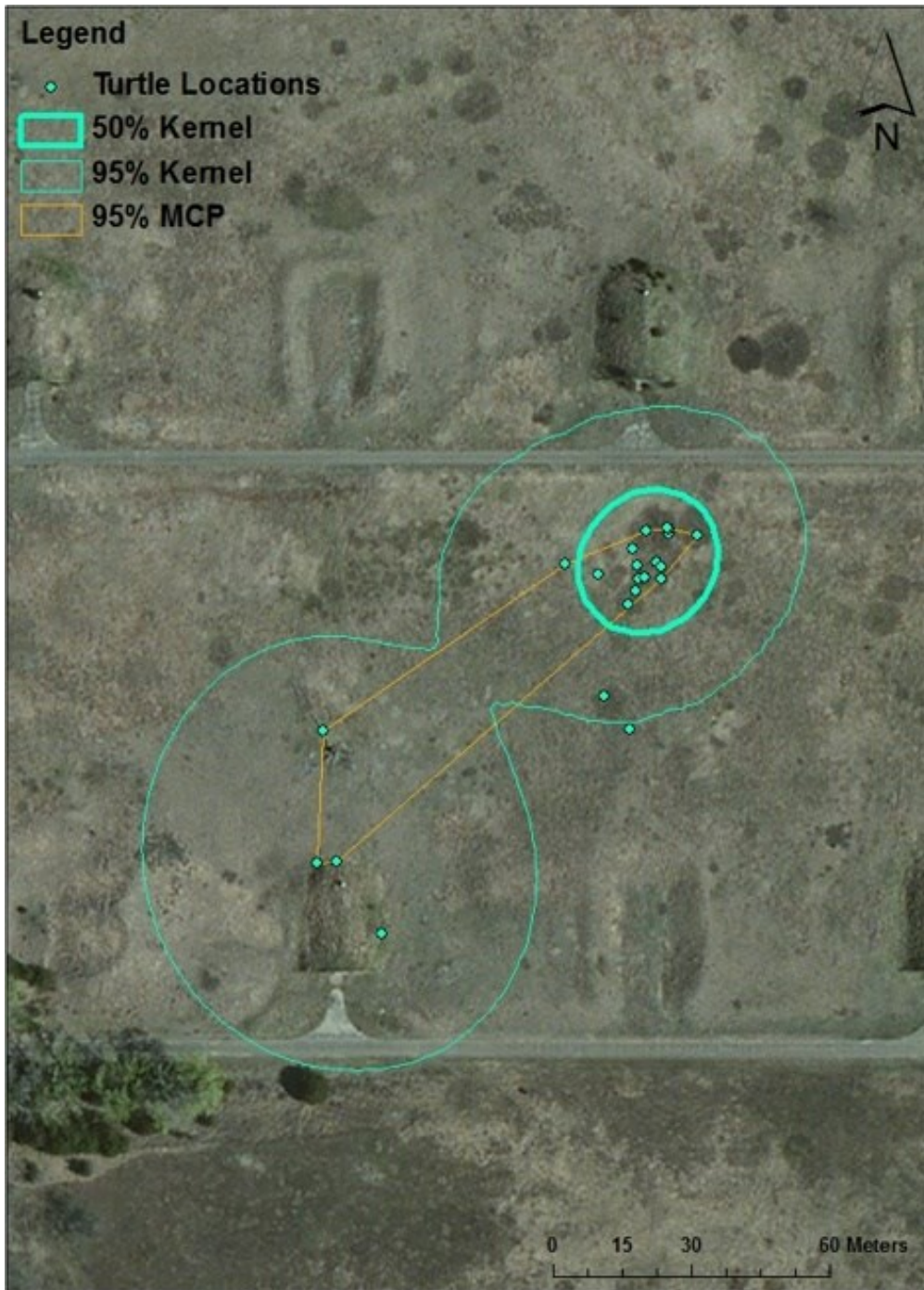| Variable	TSP	LM IN	LM OUT	AVG	Difference
% Bare Ground	9.66 (2.34)	22.67 (2.72)	8.79 (2.04)	14.56 (1.49)	TSP = LM OUT < LM IN
% Grass	42.37 (3.02)	30.06 (2.32)	46.23 (3.40)	38.56 (1.69)	LM IN < TSP = LM OUT
% Forbs	29.39 (2.82)	22.15 (1.85)	18.01 (2.66)	23.40 (1.41)	LM OUT = LM IN < TSP
% Shrub	6.33 (1.67)	11.7 (2.35)	13.16 (2.81)	10.33 (1.33)	TSP = LM IN, TSP < LM OUT, LM IN = LM OUT
% Litter	12.26 (1.50)	13.43 (1.01)	13.81 (1.25)	13.15 (0.72)	TSP = LM IN = LM OUT
% Canopy Cover	1.50 (0.99)	0	2.87 (1.35)	1.28 (0.50)	LM IN = TSP, LM IN < LM OUT, TSP =LM OUT
Avg. Veg. Height (mm)	30.52 (1.81)	24.09 (1.61)	29.42 (1.85)	27.67 (1.02)	LM IN < TSP = LM OUT
Soil Temp (°C)	21.87 (0.42)	24.02 (0.34)	22.09 (0.38)	22.78 (0.22)	TSP = LM OUT < LM IN
Trees w/in 10m	1.67 (0.24)	0.60 (0.20)	1.73 (0.34)	1.26 (0.16)	LM IN < TSP = LM OUT

Figure 1. Plasma testosterone concentrations for 28 head-started ornate box turtles at Thomson Sand Prairie and Lost Mound Sand Prairie, including 14 1-year-olds, 10 2-year-olds, 1 3-year-old, and 3 5-year-olds. Seven of the 28 turtles were scored as males, with 4, 2, 0, and 1 males represented in each of the 4 year classes. Tick marks unaccompanied by bars represent individuals for which testosterone concentrations were below the detection limit.

Figure 2. 2013 home range estimates of a representative head-started turtle released in June 2013 outside of the enclosure at Lost Mound Sand Prairie. The core activity area, represented by the 50% kernel, overlaps a patch of aromatic sumac.
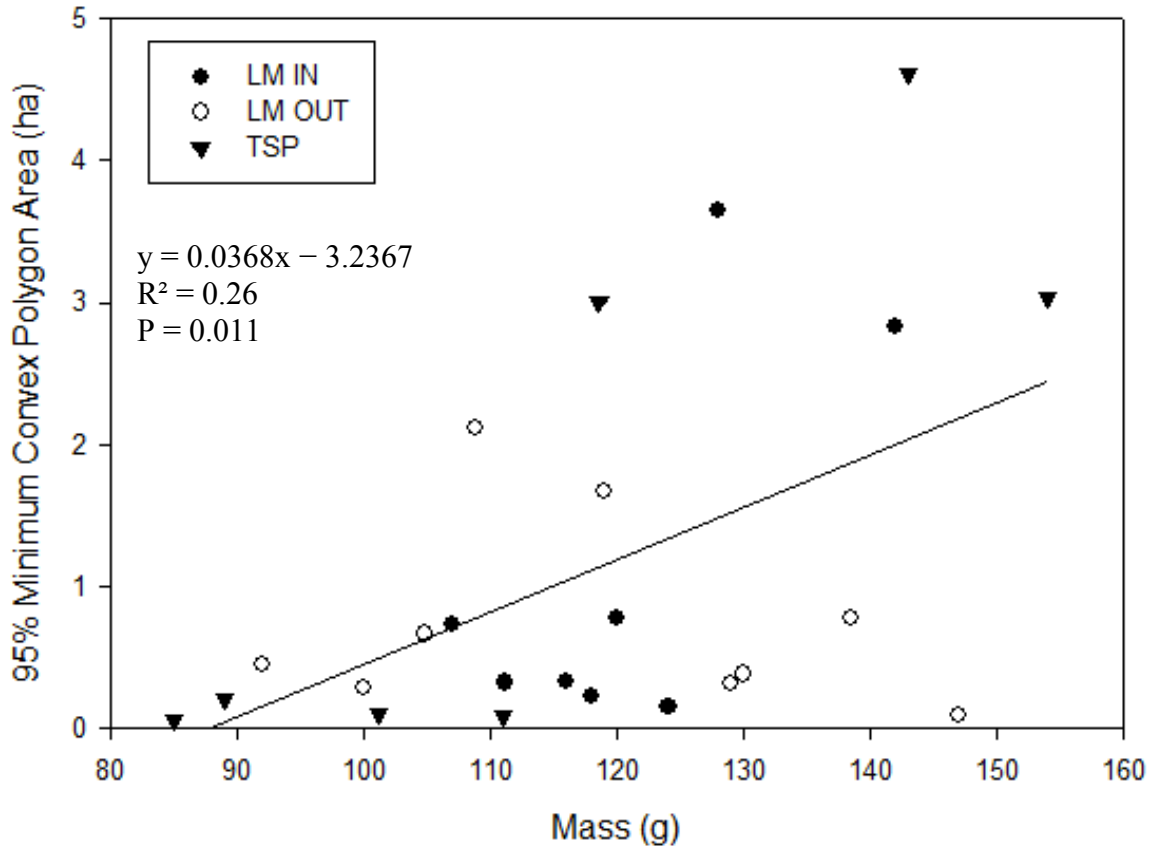
Figure 3. Regression of pre-release ornate box turtle mass against first-year 95% MCP estimates for turtles at Thomson Sand Prairie (TSP) and Lost Mound Sand Prairie, both inside (LM IN) and outside (LM OUT) of the enclosure.
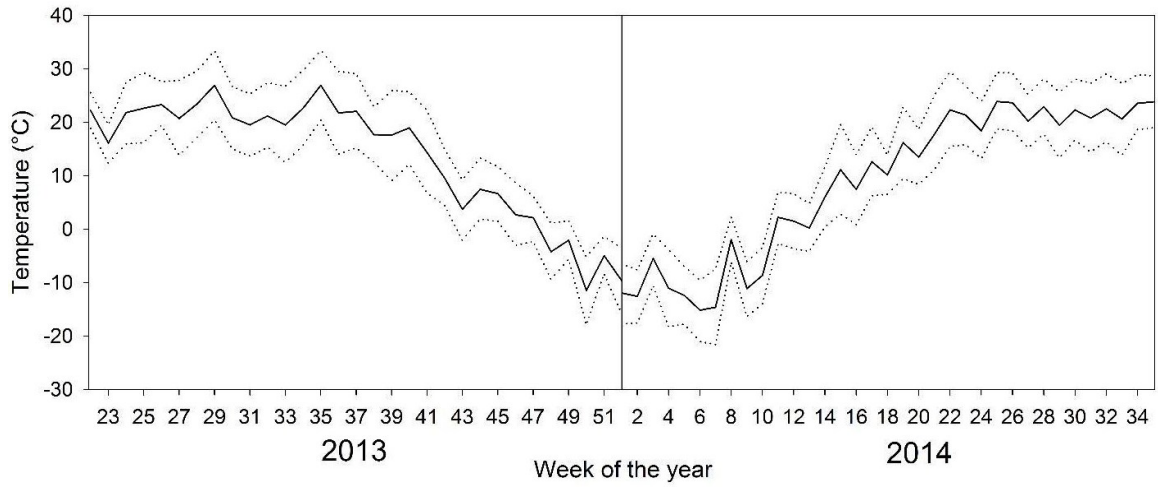
Figure 4. Weekly temperatures for the duration of the study taken at Tri-Township Airport near Thomson Sand Prairie. The solid line is the mean weekly temperature. Dotted lines indicate the mean weekly high and low temperatures.
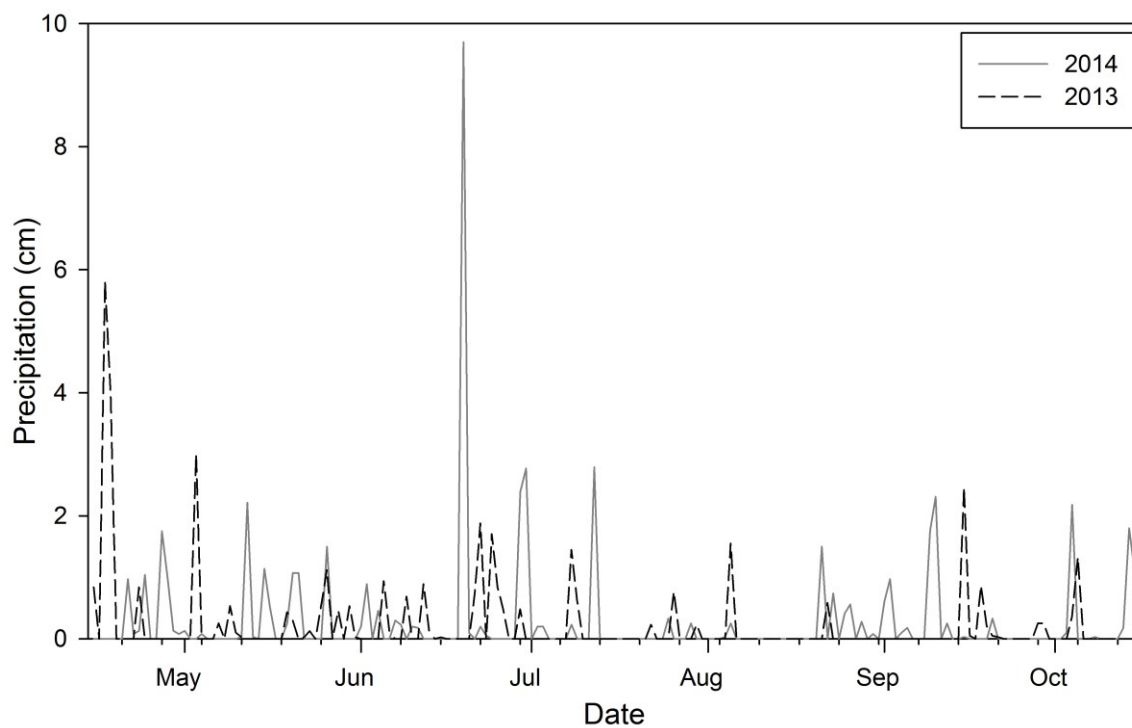
Figure 5. Daily precipitation totals in 2013 and 2014 from April 15–October 15. This period represents the actual activity season of ornate box turtles in northern Illinois. Data obtained from Tri-Township Airport near Thomson Sand Prairie.
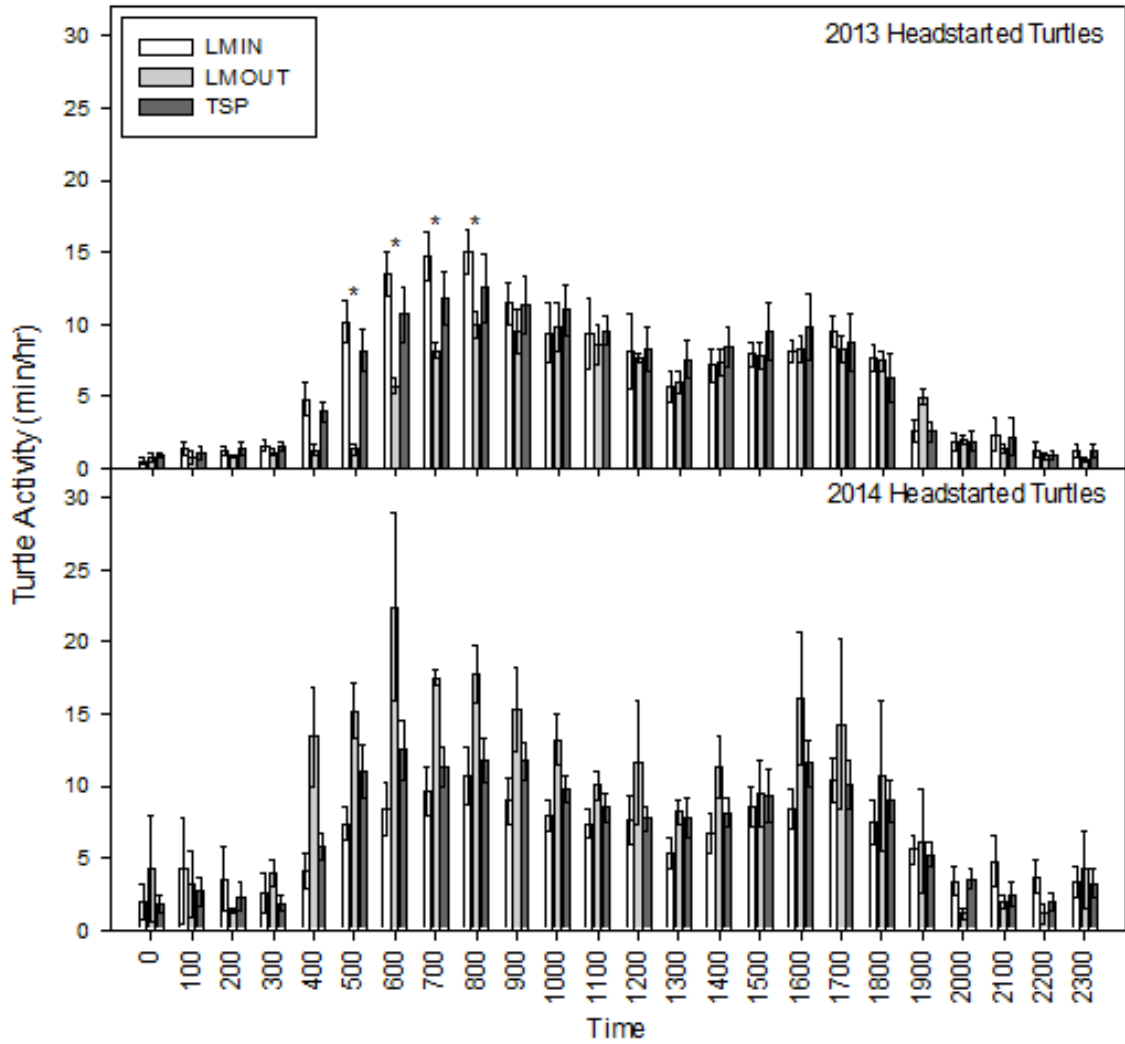
Figure 6. Mean hourly activity of head-started turtles at Thomson Sand Prairie (TSP) and Lost Mound Sand Prairie, both inside (LM IN) and outside (LM OUT) of the enclosure. Hours in which there was a significant effect of treatment are marked with an asterisk. Error bars represent ± 1 S.E.
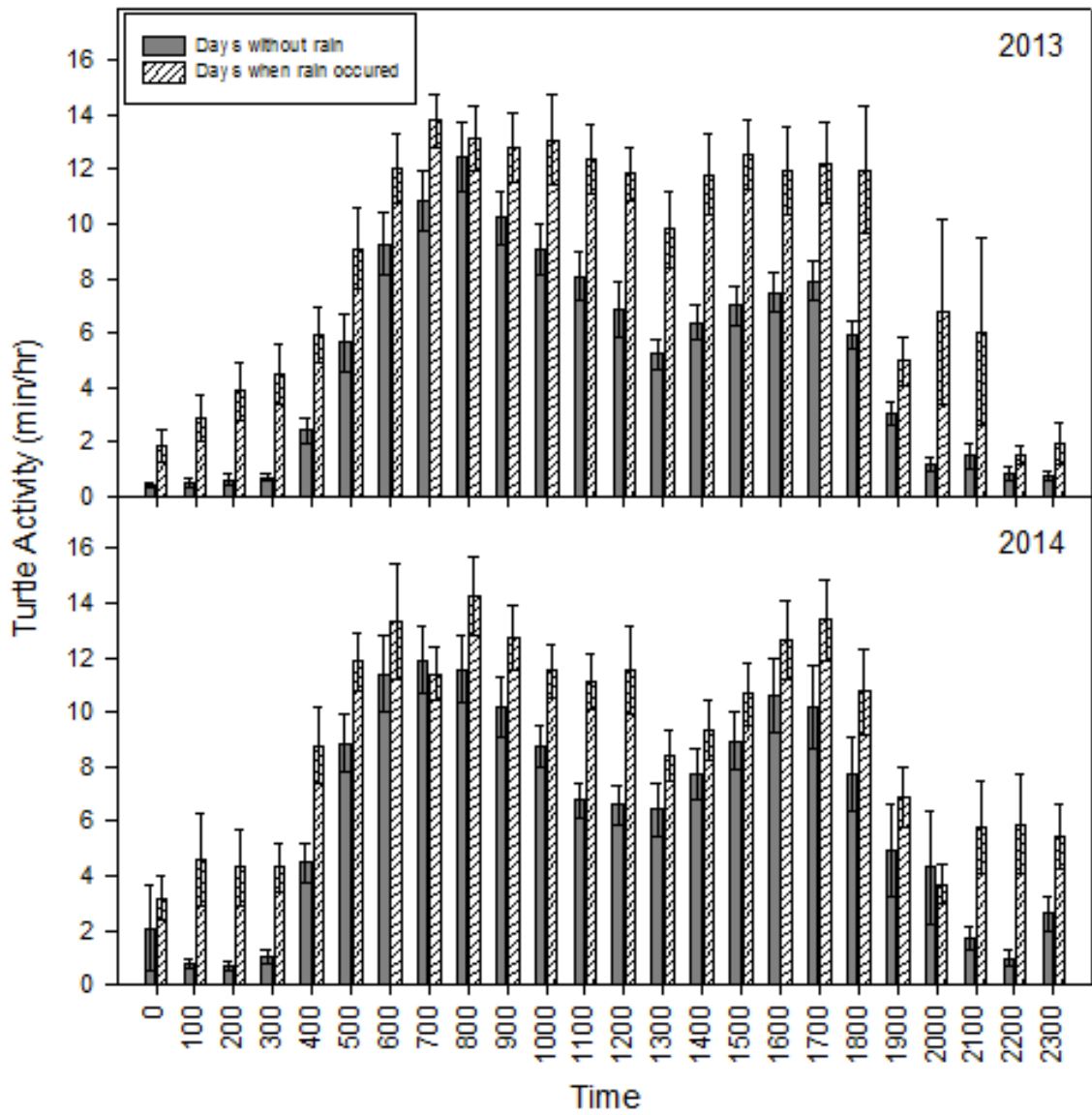
Figure 7. Mean hourly activity of head-started turtles for days with rain and days without rain. Error bars represent ± 1 S.E.
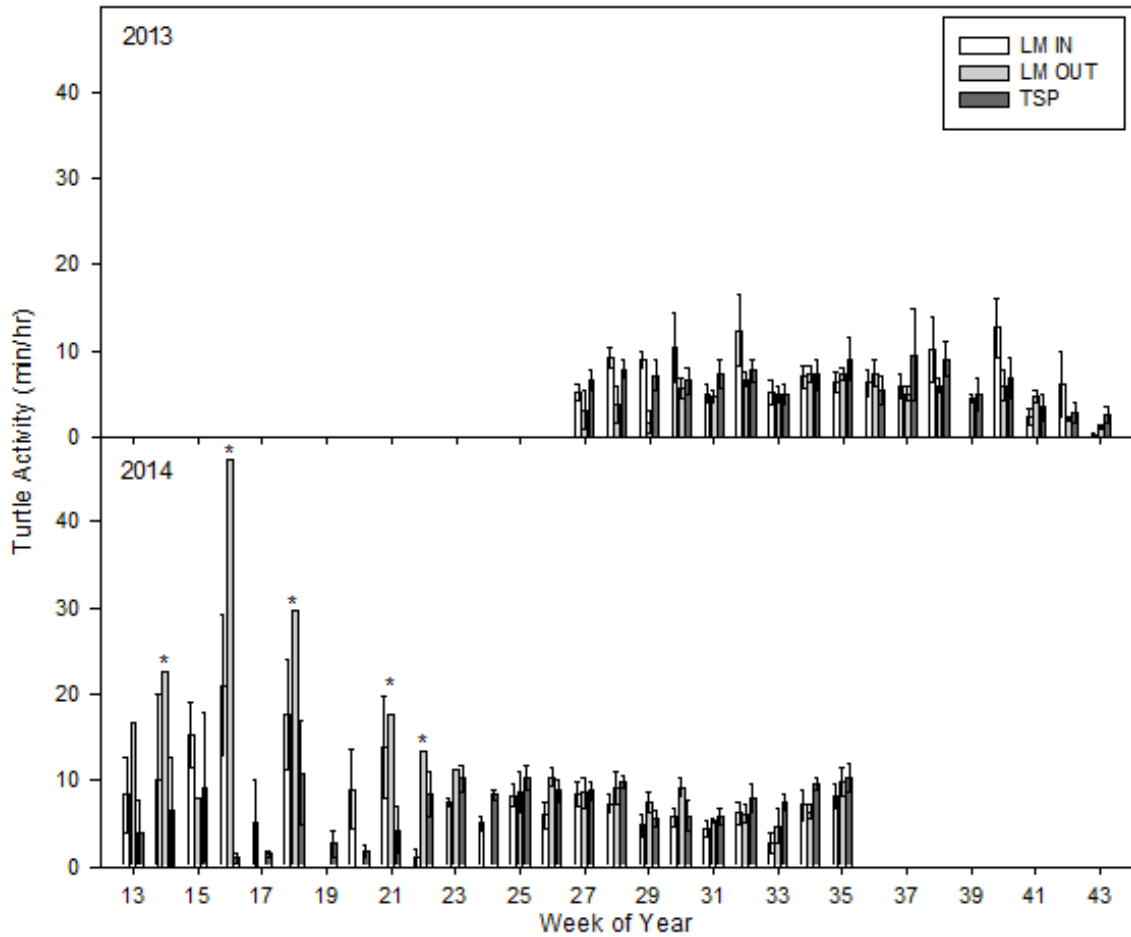
Figure 8. Mean weekly activity of head-started turtles at Thomson Sand Prairie (TSP) and Lost Mound Sand Prairie, both inside (LM IN) and outside (LM OUT) of the soft release enclosure in 2013 and 2014. * denotes weeks in which there was a significant effect of treatment. Because of high winter mortality rates, only one turtle (n=1) represents the LM OUT treatment in 2014 for weeks 13–23. Therefore, statistical comparisons during this period were limited to between LM IN and TSP. Error bars represent ± 1 S.E.
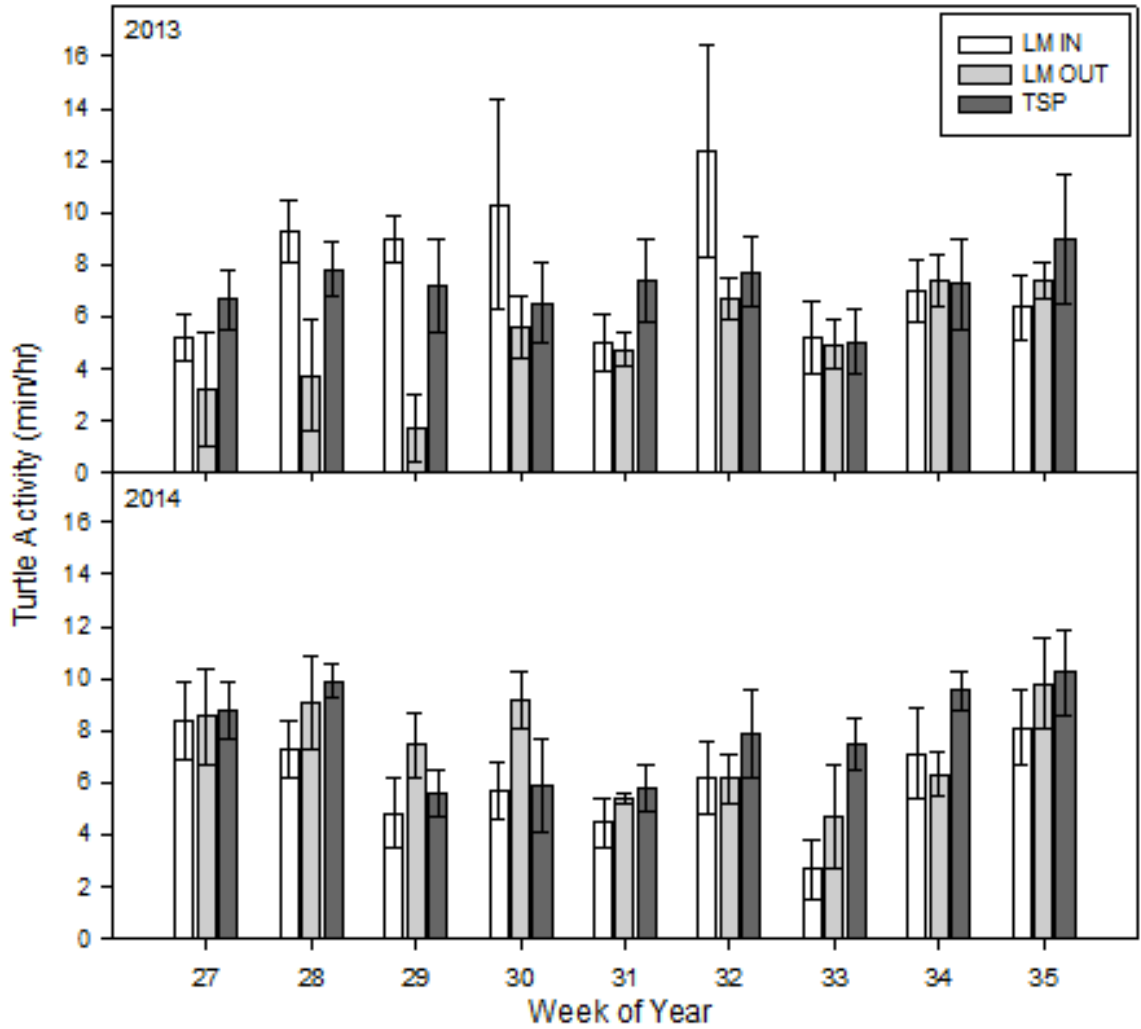
Figure 9. Overlapping mean weekly activity from week 27–35 of head-started turtles at Thomson Sand Prairie (TSP) and Lost Mound Sand Prairie, both inside (LM IN) and outside (LM OUT) of the enclosure in 2013 and 2014. Error bars represent ± 1 S.E.

Figure 10. Example of insect specimens to be identified after fecal sample collection. Samples were collected from head-started ornate box turtles at Thomson Sand Prairie and Lost Mound Sand Prairie. Contents that could be identified include: (A) *Zygogramma suturalis* (Fabricius), (B) *Sphragisticus nebulosus* Fallen, (C) *Euschistus variolarius* (Palisot de Beauvois), (D) *Aphaenogaster treatae* Forel, (E) *Melanoplus sanguinipes* (Fabricius), (F) *Canthon* (Melanocanthon) *nigricornis* (Say).
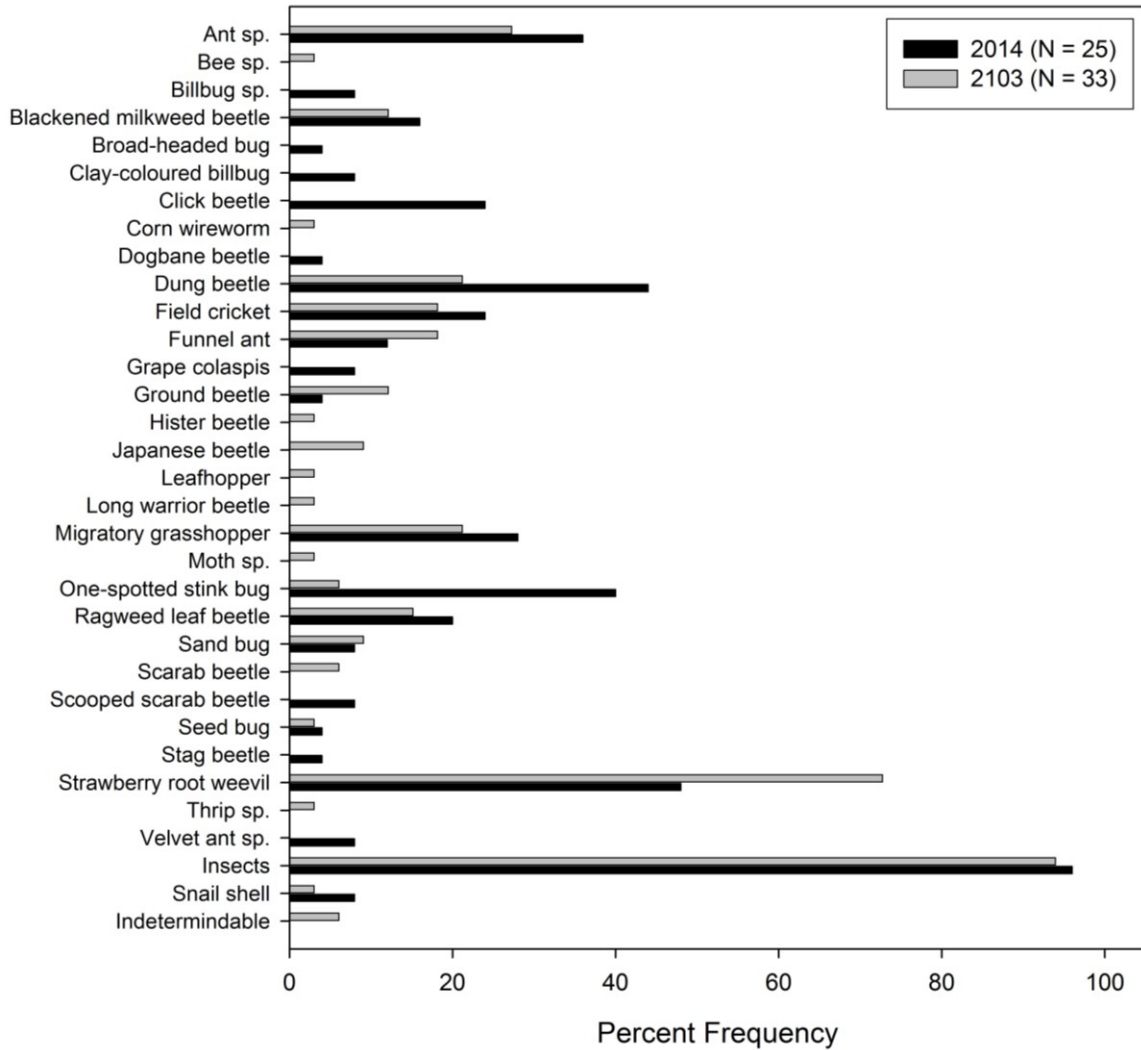
Figure 11. Frequency of invertebrates found in fecal samples of reintroduced ornate box turtles at Thomson Sand Prairie and Lost Mound Sand Prairie. The Insects category represents the total number of samples that contained insects
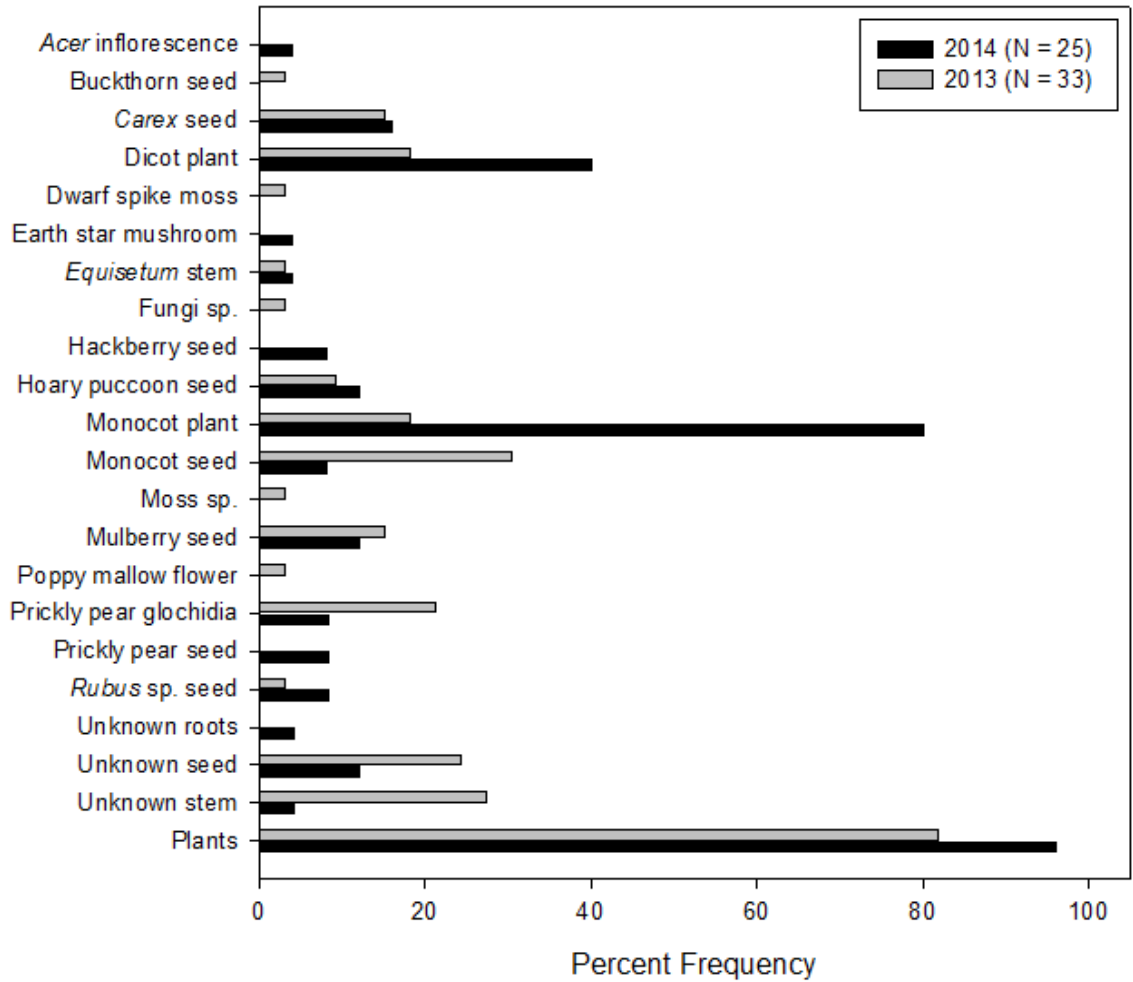
Figure 12. Frequency of plant matter found in fecal samples of head-started ornate box turtles at Thomson Sand Prairie and Lost Mound Sand Prairie. The Plants category represents the total number of samples that contained plant matter.

APPENDIX

Scientific and common names of invertebrates found in fecal samples of head-started ornate box turtles at Thomson Sand Prairie and Lost Mound Sand Prairie.

Scientific name	Common name
Agrypnus rectangularis (Say)	Click beetle
Alydus pilosulus Herrich-Schaffer	Broad-headed bug
Aphaenogaster treatae Forel	Funnel ant
Ataenius miamii Cartwright	Scarab beetle
Atholus falli (Bickhardt)	Hister beetle
Bruchomorpha pallidipes Stal	Leafhopper
Canthon (Melanocanthon) *nigricornis* (Say)	Dung beetle
Chrysochus auratus (Fabricius)	Dogbane beetle
Colaspis brunnea (Fabricius)	Grape colaspis
Coleoptera: Carabidae	Ground beetle
Coleoptera: Elateridae: *Aeolus* sp.	Corn wireworm
Dasymutilla sp.	Velvet ant sp.
Emblethis vicarius Horvath	Sand bug
Euschistus variolarius (Palisot de Beauvois)	One-spotted stink bug
Formica pallidefulva Latreille	Ant sp.
Gryllus pennsylvanicus Burmeister	Field cricket
Hymenoptera: Apidae	Bee sp.
Lepidoptera: Arctiidae (larva)	Moth sp.
Lucanus placidus Say	Stag beetle
Melanoplus sanguinipes (Fabricius)	Migratory grasshopper
Myrmica punctiventris Roger	Ant sp.
Onthophagus hecate (Panzer)	Scooped scarab beetle
Otiorhynchus ovatus (Linneaus)	Strawberry root weevil
Pasimachus elongatus (LeConte)	Long warrior beetle
Pheidole bicarinata Mayr	Ant sp.
Pheidole dentata Mayr	Ant sp.
Pheidole morrisii Forel	Ant sp.
Popilia japonica Newman	Japanese beetle
Selenophorus opalinus (LeConte)	Ground beetle
Sphenophorus aequalis Gyllenhal	Clay-coloured billbug
Sphenophorus sp.	Billbug sp.
Sphragisticus nebulosus Fallen	Seed bug
Tetraopes melanurus Schonherr	Blackened milkweed beetle
Thysanoptera: Tubulifera: Phlaeothripidae	Thrip sp.
Zygogramma suturalis (Fabricius)	Ragweed leaf beetle