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Cover Page Footnote

1Department of Biology, University of Mississippi, University, MS 38677. 2Department of Biology, Missouri State University, Springfield, MO 65897. *Corresponding author: Reese Worthington, email: rworthin@go.olemiss.edu. Acknowledgements We would like to thank Joe MacGowan, Bob Anderson, Michael Caterino, JoVonn Hill, Blaine Mathison, and Ed Zuccaro for verification of identified specimens. The Upper Mississippi River National Fish and Wildlife Refuge staff, especially Ed Britton and Jeramie Strickland, provided invaluable support during field work. This research was conducted with the prior approval obtained from the Missouri State University IACUC Board (27 September 2011; protocol number 120011) and the Illinois Endangered Species Protection Board, permit number 10-06A. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service. We would like to thank two anonymous reviewers for their valuable ideas and input on our manuscript.

Insect (Arthropoda: Insecta) Composition in the Diet of Ornate Box Turtles (*Terrapene ornata ornata*) in Two Western Illinois Sand Prairies, with a New State Record for Cyclocephala (Coleoptera: Scarabaeidae)

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Abstract

A study of fecal samples collected over a two-year period from juvenile ornate box turtles (*Terrapene ornata ornata* Agassiz) revealed diets consisting of six orders of insects representing 19 families. Turtles were reared in captivity from eggs harvested from local, wild populations, and released at two remnant prairies. Identifiable insect fragments were found in 94% of samples in 2013 (n = 33) and 96% in 2014 (n = 25). Frequency of occurrence of insects in turtle feces is similar to results reported in previous studies of midwestern *Terrapene* species. A comparison of insect composition presented no significant difference between release sites. There is no significant difference in consumed insect species between turtles released into or outside of a fenced enclosure at the same site. Specimens of *Cyclocephala longula* LeConte collected during this study represent a new state record for Illinois.

Keywords: Ornate box turtle, head-starting, conservation, feces, *Cyclocephala longula*

The ornate box turtle, *Terrapene ornata ornata* Agassiz, 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, Strickland et al. 2013). Habitat loss and fragmentation appear to be leading causes of *T. ornata ornata* decline, especially 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 extirpation due to the loss of prairie habitat continues to be a major concern. Currently, 55 grassland species are threatened or endangered in the United States (Samson and Knopf 1994). Ornate box turtles were listed in Appendix II of the Convention on International Trade of Endangered Species of Wild Flora and Fauna in 1994 still remaining on the list to-date (USFWS 1995, CITES 2017), and are protected in Colorado, Illinois, Iowa, Indiana, Nebraska, Kansas, Wisconsin (Redder et al. 2006), and now South Dakota and Wyoming.

Terrapene spp. are dietary generalists and frequently consume insects and other invertebrates, carrion, vertebrates, and a wide range of plants and fungi (Ernst and Lovich 2009). Insects make up an important portion of *Terrapene carolina carolina* L. diet in Illinois and Pennsylvania, ranging from 60 to 92% frequency of occurrence in digestive tract examinations (Surface 1908, Klimstra and Newsome 1960). Whereas *Terrapene carolina bauri* Taylor fecal remnants from the Florida Keys had an invertebrate occurrence of 10.4% (Platt et al. 2009), a study of *T. ornata ornata* from Kansas found insects in 100% of 23 turtle stomachs examined (Legler 1960), and a study of *Terrapene ornata luteola* H.M. Smith & Ramsey from Texas found insects in 100% of 14 turtles sampled (Platt et al. 2012). However, such ubiquitous consumption of insects may vary seasonally or geographically; the stomach contents of five *T. ornata ornata* collected in Illinois contained partially digested plant material but no insect or other animal material (Cahn 1937). A dietary analysis of insect fragments in fecal samples of ornate box turtles was conducted in this study to ascertain if, in addition to habitat loss, limited diversity in their diet could be exacerbating population declines in Illinois. Diet studies are essential to understanding the ecology of an organism (Rosenburg and Cooper 1990) and help to inform reintroduction efforts as turtle diet directly affects energy allotment, which in turn influences reproductive rates, growth, and survival (Sloan et al. 1996, Ford and Moll 2004, Platt et al. 2009).

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This research occurred in conjunction with an on-going *T. ornata ornata* reintroduction effort conducted by the Upper Mississippi River National Wildlife and Fish Refuge. In 2008, the United States Fish and Wildlife Service (USFWS) 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 used juveniles that were hatched from eggs collected from Thomson Sand Prairie, a nearby prairie also managed by USFWS, and reared in captivity prior to reintroduction, a method termed head-starting. 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 (Strickland et al. 2013). However, to ameliorate the potential negative impact of removing eggs, a subset of hatchlings was released at Thomson Sand Prairie annually. Like those at Lost Mound Sand Prairie, these turtles were radio transmittered and monitored throughout their active season.

A dietary analysis of insects recovered from fecal samples of reintroduced turtles was conducted. This is the first dietary analysis of *T. ornata ornata* using non-lethal methods, which is essential for determining dietary and ecological demands in species of conservation concern. The class Insecta served as the focus of this analysis and was selected due to the indigestible nature of the chitinous exoskeleton, which enabled identification of organisms via fragmented remains. The goal of this dietary analysis was tripartite: 1) to determine if head-started turtles displayed different insect dietary composition compared to results shown in previous studies of wild-caught terrestrial *Terrapene* species, 2) identification of insect fragments to compare species composition between reintroduction sites, and 3) to determine if soft-release enclosure reintroductions had a similar diet composition to turtles hard-released without a protective fenced enclosure at Lost Mound Sand Prairie.

Materials and Methods

Study Site. Research was conducted at two units of the Upper Mississippi River National Wildlife and Fish Refuge, both of which are located 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* Michx.), with interspersed patches of prickly pear cactus (*Opuntia humifusa* Raf.), aromatic sumac (*Rhus aromatica* Ait.), and spiderwort (*Tradescantia ohiensis* Raf.). A strip approximately 10 m wide immediately bordering the river is dominated by a variety of deciduous trees, black raspberry (*Rubus occidentalis* L.), and poison ivy (*Toxicodendron radicans* L.). Isolated raspberry patches and eastern red cedar (*Juniperus virginiana* L.) are scattered throughout the study site (Bowen et al. 2004, Refsnider et al. 2012, Strickland et al. 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, which 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, Strickland et al. 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 the USFWS and Illinois Department of Natural Resources and contains sand prairie, sand dune, sand savanna, and blowout communities dominated by prairie junegrass (*Koeleria macrantha* (Ledeb.) Schult.) and little bluestem with interspersed patches of prickly pear cactus, aromatic sumac, prairie redroot (*Ceanothus herbaceous* Raf.), and spiderwort.

Methods. Seventeen ten-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 additional head-started turtles from the 2013 cohort were released in June 2014, with three added to each treatment.

Two fecal samples were collected from each turtle annually (2013–2014), when possible, from each of the head-started turtles to identify key dietary components. One turtle went undetected for a portion of the 2013 survey season and was not located for the second fecal collection. Seven 2013 reintroductions died prior to the 2014 fecal

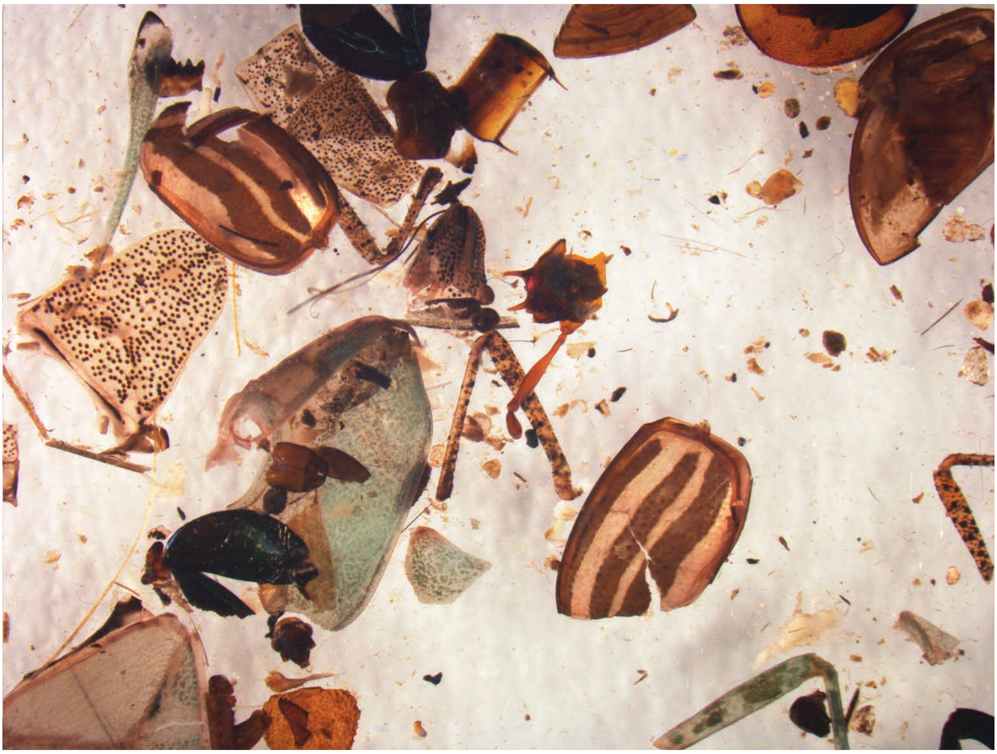

Figure 1. Single sample of insect specimens to be identified after fecal sample collection and sorting. Samples were collected from head-started ornate box turtles at Thomson Sand Prairie and Lost Mound Sand Prairie in northwestern Illinois.

analysis sampling, while two additional individuals succumbed to illness and predation during the 2014 surveying season prior to obtaining second fecal samples. 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 of water. All turtles defecated during the allotted time. The following morning, the contents of the bucket were filtered through a 250-µm wire sieve and stored in 70% alcohol for later identification. Arthropods collected, concurrently, from pitfall traps served as reference samples to aid in identification of arthropod remains from fecal material. Pitfall traps consisted of 85-mL plastic cups containing propylene glycol. Each cup was buried with the rim flush with the ground surface. Ten traps were placed in a transect and spaced 50 m apart at each release site.

Insect fragments from fecal samples were examined using a Nikon SMZ645 dissecting scope with Fisher Scientific LED gooseneck illuminator. All measurements were made using a Wild M5 stereomicroscope with a Wild MMS235 digital length measuring set. The quantification of insects in each sample was often not feasible due to the majority of material being extensively fragmented (Fig. 1). Historically, quantification of identified material has been presented as frequency of occurrence across the total number of turtles sampled (Surface 1908, Klimstra & Newsome 1960, Legler 1960). Platt et al. (2009) presented their findings as percent occurrence, which they considered a more appropriate calculation when individual food item quantification was not feasible. We have elected to quantify insect presence as the number of samples in which the food item occurred divided by the total number of samples (n).

A two-way ANOVA was conducted to examine relationships between diversity of species consumed, years, and three release locations. The statistical computing program R (2013) was used for data analysis.

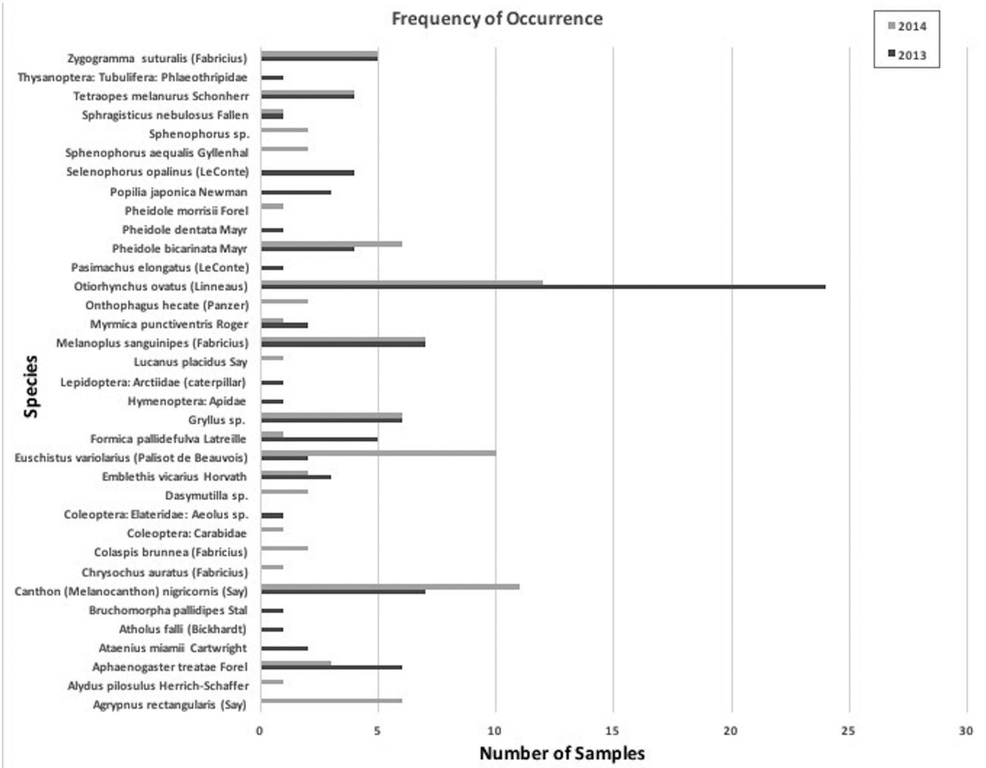

Figure 2. Frequency of occurrence in 2013 (n=31) and 2014 (n=24) of insects found in fecal samples of reintroduced ornate box turtles at Thomson Sand Prairie and Lost Mound Sand Prairie.

Results

Insects were found in 31 of 33 (94%) fecal samples collected in 2013, and 24 of 25 (96%) in 2014. Coleoptera were recovered from 27 of 31 fecal samples, Orthoptera in 9 of 31 samples, Hymenoptera in 13 of 31 samples, and Hemiptera in 6 of 31 samples in 2013. In 2014, Coleoptera were found in 19 of 24 fecal samples, Orthoptera in 13 of 24 samples, Hymenoptera in 10 of 24 samples, and Hemiptera in 13 of 24 samples. In 2013, the orders Lepidoptera and Thysanoptera were each represented in single samples. Acrididae, Curculionidae, and Formicidae were recovered from samples in both years from all three sites. Six families occurred once each in fecal samples: Histeridae (*Atholus falli* (Bickhardt)), Mutillidae (*Dasymutilla* sp.), Apidae, Lucanidae (*Lucanus placidus* Say), Alydidae (*Alydus pilosulus* Herrich-Schäffer), and Caliscelidae (*Bruchomorpha pallidipes* Stål) (Fig. 2). *Otiorhynchus ovatus* (Linnaeus) and *Melanoplus sanguinipes* (Fabricius) were the only two species that occurred at all three sites in both years of the study. The insect most commonly encountered was *O. ovatus*, which occurred in 73% of samples in 2013 and 48% of samples in 2014. *Aphaenogaster treatae* Forel (Formicidae) was commonly collected in both Lost Mound sites, but was entirely absent from Thomson Sand Prairie. Formicidae had the greatest diversity with six species across four genera; Scarabaeidae, also represented by four genera, was limited to only four species. Eight families of Coleoptera were represented in the samples, the greatest familial diversity of the six sampled orders.

Turtles released into the LM IN enclosure had insect remnants in 7 of 9 samples in 2013, while insect fragments were present in 13 of 13 samples from LM OUT. There was no significant difference in the number of species consumed between the three release sites ($F = 1.6044$, df = 2, 49, $P = 0.2114$). No significant difference in the number of species consumed between the two years was apparent ($F = 2.2246$, df = 1, 49, $P = 0.1422$). No significant interaction of site and year for number of species recovered from fecal material was apparent ($F = 2.0350$, df = 2, 49, $P = 0.1416$).

Insect composition was the focus of this study, although identifiable plants, fungi, and other invertebrates were recorded. Gastropod shells were recorded in three total samples from 2013 and 2014. Fungi were recorded in two samples in 2014 and one sample from 2013. Plant matter was found in 27 of the 33 samples collected in 2013, and 24 of the 25 samples in 2014. The majority of plant material was unidentifiable, although seeds, stems, glochidia, and inflorescences were used to identify several plants. *Equisetum* L., *Acer* L., *Rhamnus* L., *Carex* L., *Selaginella* P. Beauv., *Celtis occidentalis* L., *Rubus* L., *O. humifusa, Callirhoe* Nutt., Bryophyta, *Lithospermum canescens* (Michx.) Lehm., and *Morus rubra* L. were recovered from turtle fecal samples during this study.

Discussion

Head-starting conservation programs have brought concerns and criticism regarding the spread of disease to wild populations, loss of fear to potentially harmful organisms and, important to this study, the adjustment to natural food resources after prolonged captivity (Dodd Jr. and Seigel 1991, Berry and Christopher 2001, Smith 2015). Head-started turtles, in this study, displayed insect consumption at percentages comparable to or even greater than those reported in previous studies on wild populations of midwestern *Terrapene* species (Cahn 1937, Klimstra and Newsome 1960, Legler 1960). Successful feeding behavior could be a result of innate predatory behavior and general omnivorous tendencies of the species.

Enclosures can be beneficial for monitoring released organisms by increasing site fidelity and providing protection from predators, but can lead to unsafe behaviors such as frequent walking along the enclosure barriers (presumably in an effort to move beyond the confines of the enclosure), targeting by predators, or limited nutrient availability. Benefits may be offset by the often high cost associated with constructing and maintaining an enclosure. Analysis of the diversity of insect species consumed was not significantly different between enclosure or open release areas, suggesting that foraging behavior for insects is not compromised by enclosures. The lack of differences in insect consumption between soft and hard release approaches may be due partly to the enclosed area being similar in size and habitat to other release sites without an enclosure. While insects are mobile and capable of moving through these enclosures, the same is not true for sessile organisms that turtles frequently eat and that are less likely to be repopulated from outside the enclosure. To address this concern, future studies may assess whether enclosures significantly impact patterns of turtle foraging on plants and fungi. While insect consumption appears unaffected by enclosures, additional factors such as predation, access to suitable overwintering sites, and limited breeding potential should be measured to determine the benefits and efficacy of using soft-release enclosures.

Diet was similarly diverse among all three treatments. Insect prey selection by *T. ornata ornata* appears indiscriminate. The classification of *T. ornata ornata* as an opportunistic omnivore is supported by a wide diversity of insect fragments recovered from fecal material.

Diverse invertebrate populations are likely an important continuous food source, as insects were a key dietary element at all three release sites. The abundance of the flightless weevil, *O. ovatus*, seen in over half of the samples, is likely attributable to the high density of its host, *Rubus* L., at all three sites. Ingested food items that lack rigid structures, such as earthworms and caterpillars (Metcalf and Metcalf 1970), were often not identifiable in the fecal samples and may have led to results that are biased towards insects with heavily sclerotized exoskeletons and highly fibrous plants and seeds.

We assumed *T. ornata ornata* preference for highly productive habitats would increase the likelihood of predator interactions; however, only one of 26 turtles was predated during this two-year study. In heterogeneous habitats, predation rates on *T. ornata ornata* nests increases near ecological edges (Temple 1987); this trend was documented at TSP where mesopredators were extremely effective at raiding nests along riparian areas. Head-starting is often most successful in populations where juvenile and adult survival rates are stable and high (Heppell et al. 1996); high juvenile and adult survival rates are indicative of populations experiencing low to moderate predation and high resource availability. These factors are generally in congruence with areas selected for head-start or reintroduction programs.

In instances where constant monitoring of target individuals in reintroduction programs is not feasible, analysis of feces may anecdotally indicate where individuals forage, especially in heterogeneous habitats where food resources are patchy. The ability to clearly predict microhabitat preference is reduced by the mobility of the turtle and the high mobility of insects they consume. Future research on predation of adult and neonate turtles, and turtle nests in highly productive habitats is necessary to evaluate the cost-benefit of head-start release into areas with higher resource availability.

Success in reintroduction programs may be facilitated by avoiding or limiting release into habitats where individuals do not spend a majority of their time foraging.

Perhaps the most notable insect species encountered during this study was ***Cyclocephala longula*** **LeConte**-[IL: Carroll Co./Thomson Sand Prairie/REF pitfall/15 July 2013/E. Sievers]. Two males were collected from a pitfall trap in Thomson Sand Prairie, Carroll County, Illinois. *Cyclocephala longula* is known from Oregon south to Mexico and as far east as Kansas. The species' occurrence is surprising, but the habitat coincides with that experienced in the species' previously known range. These individuals represent a new state record for Illinois. The presence of *C. longula* in Illinois adds validity to Endrödi's (1985) record of the species from Wisconsin, which was at the time deemed questionable due to the distance from the species' known range (Saylor 1945).

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