

**Project Title:** Population characteristics and habitat use of ornate box turtles (*Terrapene ornata ornata*) in remnant and restored tallgrass prairie

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**Research Organization:** Cooperative Wildlife Research Laboratory, Southern Illinois University Carbondale

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**Project Objectives:**

1. Identify home-range size, movements and microhabitat using spatial distributions of *T. ornata* at the individual and population scales in remnant and restored prairie.
2. To identify critical habitat types including nesting and overwintering sites.
3. To estimate demographic parameters (sex ratio, age structure, survivorship and population size) of the *T. ornata* population on Nachusa Grasslands preserve.

**Summary of Project Accomplishments:**

Home range size, movements, and microhabitat analyses are still in progress.

Preliminary analysis of summer 2012 microhabitat data suggests shrubs are important for cover.

From June 2011-May 2014, we captured 151 individuals (48F, 58M, and 45U), 42 of which were

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transmitted. Size-class structure and sex ratio of captured animals are reported. Photographs from volunteer stewards or visitors suggest at least an additional 25 individuals are present on the preserve. Mark-recapture grids were ineffective to provide a reliable population estimate due to low recapture rates. Hatchling, juvenile, and adult turtles were found at both sites demonstrating recruitment potential. Reproductive behaviors like mating and nesting were observed on multiple occasions. Twenty-five carcasses were collected at Nachusa Grasslands from June 2011-June 2014, four of which were transmitted individuals. Turtles show a high degree of site fidelity to overwintering locations and most often use remnant prairie cover. Kimberly Schmidt, is projected to graduate in December 2014, and will send a final copy of her thesis when the project is completed.

**Introduction:**

Grasslands occur on every continent except Antarctica, occupying 40% of the Earth's surface, and qualify as repositories of biodiversity (Gibson 2009). Only 9.4% of original tallgrass prairie remains in North America (Gibson 2009). Illinois has suffered near total loss of its formerly dominant tallgrass prairie habitat. The loss of prairie occurred so rapidly that the ecological value of these grasslands was never adequately assessed. In 1978, the Illinois Natural Areas Inventory estimated that less than 0.01% (951 ha of 8.9 million ha) of original prairie survived (Critical Trends Assessment Project 1994). Over 200 imperiled species in Illinois rely on prairie habitat (Illinois Endangered Species Protection Board 2011).

The ornate box turtle (*Terrapene ornata*) is a prairie-obligate species; as grassland ecosystems become scarcer or more degraded, studies must be done to ascertain microhabitat selection to conserve the species. Large populations of *T. ornata* have disappeared across the eastern part of the turtle's range (Redder et al. 2006). Although there is an apparent gap in our

knowledge regarding population abundance and distribution of *T. ornata*, the species is not federally protected. *T. ornata* were listed in Illinois in 2009 as a state-threatened species, where they are on the northeastern edge of their distribution (Illinois Endangered Species Protection Board 2011). *T. ornata* have only recently been studied in Illinois (Philips et al. 1999, Kuo and Janzen 2003, Philips et al. 2003, Bowden et al. 2004, Kuhns 2004, Refsnider et al. 2011, Refsnider et al. 2012). The relative suitability of remnant and restored prairies for the species is unknown. Our objectives were to determine home-range size, movements, site fidelity, and microhabitat selection, as well as describe demographic characteristics (age and sex structure, density, and survivorship) of *T. ornata* in northern Illinois.

## **Materials and Methods:**

### ***Study Site***

Nachusa Grasslands (Franklin Grove, Illinois) is 1200 ha of tallgrass prairie remnants, prairie reconstructions, and degraded agricultural fields pending restoration (Figure 1). Owned and managed by The Nature Conservancy, this preserve has multiple habitat types including dry or mesic prairie, hill gravel prairie, oak savanna, woodlands, and wetlands (Taft et al. 2006). In 1986, the original purchase of hill prairie remnants with interspersed areas of row-crop fields was made by TNC. These remnants remained in higher quality because the rocky soils made them unsuitable for row crop cultivation; however, their rocky slopes did not give them protection from being periodically grazed. Soils are typified by silt-loam or sandy-loam soils where this sandstone substrate is buried more deeply by glacial outwash and loess deposits (Taft et al. 2006, Hansen 2009). Mean annual precipitation is 900–1000 mm, of which approximately 66% falls between April and September (Elmer and Zwicker 2005). Average annual temperature

is 8.4° C, with an average maximum and minimum temperature of 13.8° C and 2.9° C, respectively (Elmer and Zwicker 2005).

Research activities will be focused on 2 populations of *T. ornata* at Nachusa Grasslands. One turtle population occurs in a conservation easement on the Baumgardner residence (14.2 ha), Rolling Thunder Prairie, and the Headquarter Prairie (Figure 2). The Headquarter Prairie (HQ) is 160 ha with a mosaic of dry-mesic remnant prairie and restoration plantings. The land was grazed for decades prior to ownership by TNC. Sally Baumgardner has kept field records on *T. ornata* sightings since 1981, including locations and dates where *T. ornata* have been sighted. Dominant graminoids at Nachusa Grasslands include a number of cool-season grasses and native forbs associated with tallgrass communities. The other turtle population occurs in the Orland Tract (OT), a property purchased by TNC in 2010 near the Lost Nation residential development (Figure 2). The OT is roughly 142 ha, composed of degraded dry-mesic remnant prairie and forage fields, where extensive cattle grazing occurred for over 40 years prior to its purchase by TNC. Mike Carr, a volunteer restoration steward and resident of Lost Nation, informed us of the presence of *T. ornata* at this site. Dominant graminoids at the OT include a mixture of cool- and warm-season grasses, common pasture forbs, and weedy shrubs. Woody encroachment is present due to the lack of fire. Future restoration goals are to reduce woody encroachment by herbicide application, removal, and prescribed fire to give herbaceous plants a competitive advantage. Both HQ and OT sites are bounded by agriculture, residential housing, and roads. *T. ornata* have been sighted sporadically in other areas of NG but targeted searches have yielded few if any captures by researchers or land managers.

### ***Turtle Capture and Mark-Recapture Processing***

Turtles were captured at NG through intensive visual searching, use of detector dogs, opportunistic encounters, and road cruising from 13 June 2011 to 20 October 2013. Intensive visual searching occurred on remnant knobs and open restored habitat where turtles were easier to detect due to a relative lack of vegetation and were based on observed localities of turtles. Detector dogs (Schwartz and Schwartz 1974, Converse and Savidge 2003) were used to search for *T. ornata* for two days in fall 2011 and seven days in burned areas during spring 2012. Surveys through burned areas allowed for efficient travel for the dogs and provided accurate visual locations where turtles were retrieved. Opportunistic encounters of box turtles occurred through volunteer steward observation or by sighting new individuals while pursuing radioed individuals. Road cruising was conducted on paved roads, 2-tracks, or mowed paths before and after thunderstorms to increase the chance of an opportunistic encounter.

Volunteers carried out mark-recapture sessions twice monthly on the OT during the 2013 active season (April-June). Each sampling session consisted of two researchers searching a designated grid for two consecutive days followed by a period of twelve days without search effort (Figure 3). Transect lines were spaced no more than 2 m apart to ensure a grid was sufficiently covered. Concentrated search efforts occurred when turtles were more likely to be active and visible (Converse and Savidge 2003). Volunteers stopped searching for turtles when no turtles were found after 5 hours of searching on a given day. Each mark-recapture session was ended after approximately 20 person-hours of searching, so that monthly mark-recapture sessions had approximately equal search effort.

Previous methods described for marking turtles involved notching or drilling into the shell of the turtle to create an alphabetical or numeric code (Cagle 1939). This technique may lead to secondary infection caused by bacteria, fungi, or viral pathways. Marking animals in this

way causes unnecessary stress that changes the physiology and behavior of the turtle (J. Johnson DVM, personal communication April 2011). To avoid these potential problems, we marked animals in a less-invasive manner by labeling the carapace with a yellow paint pen and using epoxy to adhere ID tags to the shell of the turtle. Shellfish FT-LT-97 FLOY tags (Floy Tag & Mfg., Inc., Seattle, Washington) were made of flexible, strong laminated vinyl which conformed to the carapace. The marking pen and laminated tags were yellow to reduce visibility to predators and to minimize impacts on behavior (Figure 4). We implemented a secondary marking system through digital photography (Franklin and Killpack 2009, Weis 2009). Much like our own unique fingerprints, each *T. ornata* has its own unique color pattern. Using this system, handling time was reduced when recapturing individuals. Digital photographs of the turtle were taken from standard angles and were used to identifying individuals based on unique color patterns of the carapace and plastron (Figure 5).

We processed marked animals for demographic and morphometric data, namely sex, mass, carapace length, carapace width, plastron width, anterior plastron length, posterior plastron length, shell height, and annuli counts (Legler 1960). We collected one claw clipping from the front limb of *T. ornata* for future genetic analysis (Lutterschmidt et al. 2010). A general health assessment was given to each animal being processed, and researchers implemented cleaning procedures to minimize the risk of spreading disease within and among populations. We checked the clarity of eyes and nares, and quantified respiration for sign of *Mycoplasma sp.* or *Iridiovirus* infection. These conditions could have significant impacts on populations should pathogens be spread by human-handling of turtles. The lack of knowledge with respect to the precise impacts of potential infectious diseases within *T. ornata* populations and disease impacts to long-term population viability produce a major quandary for biologists, veterinarians, and

policy makers (United States Fish and Wildlife 2011). Clinical signs in *Mycoplasma sp.* infected animals include nasal discharge, epiphora, ocular edema, and conjunctival injection (Brown 1994). *Mycoplasma* or *Iridiovirus* infection contributes to upper respiratory tract disease which has negative impacts on chelonian populations (Chinchar 2002; Johnson et al. 2007 United States Fish and Wildlife 2011). Clinical signs associated with ranavirus infections are similar to that of *Mycoplasma* infection and include symptoms of upper respiratory tract disease, including respiratory distress and nasal discharge, as well as oral ulceration, cutaneous abscessation, anorexia, and lethargy (Brown et al. 1994, Dodd 2001, Johnson et al. 2007). The health assessment also noted shell condition (e.g., normal vs damaged) and cause of observed damage (e.g., infection, depredation attempt, interaction with agricultural equipment). Turtles were also searched for ectoparasites (e.g., ticks, lice). Researchers wore disposable vinyl or nitrile gloves and changed gloves before handling different animals to minimize the risk of spreading disease within and among populations (Greer et al. 2009, Green et al. 2009). After use, all sampling gear was disinfected with chlorhexidine or a 3% bleach solution (Bryan et al. 2009).

### ***Telemetry and Microhabitat Procedure***

We affixed 9-15-g radiotransmitters (SOPR-2380 -MVS radiotransmitters, Wildlife Materials, Murphysboro, IL) with a 16-month battery life to adult turtles. Transmitters were chosen uniquely for individuals so the carried load did not exceed 5% of the turtle's body mass. Transmitters were affixed to turtles with epoxy using the protocol of Boarman et al. (1998) to minimize effects on turtle behavior, physiology, reproduction success, or survival. Male turtles with transmitters were captured up to twice annually to collect repeated morphometric measurements as they emerged (March, April or May) and returned to brumation sites (September and October). Reproductive females were measured additional times during May

and June to estimate clutch size following Doroff and Keith's (1990) logic that each egg weighs approximately 10 g (Legler 1960). Abrupt reductions of body mass of > 30 g indicated females had laid their eggs. Captures did not occur if the animal was in its overwintering chamber to minimize disturbance. After initial capture, handling was minimal and done in the field.

Transmitters were removed at the end of the study.

Animals were located by homing 1-4 times weekly during the summer season (mid May-mid August) with a Model R1000 Communications Specialist (Communications Specialist Inc., Orange, CA ) Receiver and 3-5element Yagi antennas (Wildlife Materials Inc., Carbondale, IL) or a Telonics RA-2AK VHF H-directional Antenna (Telonics, Inc. Mesa, AZ). Turtles were located at randomized times to survey activity and behavior during all daylight hours. Turtles were located 1-6 times monthly during the fall (mid August-October) and spring seasons (March-mid May). When a turtle was located, the Universal Transverse Mecator (UTM) coordinates were taken with a Model PN-40 Delorme (Delorme, Yarmouth, ME) Global Positioning System GPS with a 2-3 meter maximum accuracy. When recording environmental and microhabitat data, care was taken not to disturb turtles; however, sometimes turtles were disturbed and animals moved slightly from original locations. Turtle behavior was not adversely biased.

At unique turtle occupied locations, independent variable data were collected (Table 1). Observers quantified behavior of turtles (agonistic, at rest active, basking, digging, eating, mating, moving, eating, or unknown). A compass bearing was recorded in the direction that the turtle was oriented and the slope and associated aspect was measured using a Silva Ranger CL compass (Johnson Outdoors Gear, Inc., Racine. WI). General locality data was collected to determine where an observer saw a turtle (road, two-track, mowed path, vegetation, rock, open,



burrow, form, or underground). If a burrow was visible within 1 meter of the plot, its distance was measured from the turtle in centimeters. Observers estimated the distance the turtle was from a graded road or two-track if it was under 10 meters. Geographic Information System (GIS) was used to determine the distance of a turtle to the nearest graded road, two-track, mowed path, remnant, restoration, agriculture, pasture, forest, water source, and nearest neighboring turtle.

Microhabitat factors were measured at each turtle's location. We assessed percent cover of bare ground, cactus, fern, forb, fungi, grass, litter (herbaceous and woody dead vegetation), moss, rock, rush, sedge, shrub, tree, and water using a 1- x 1-m Daubenmire frame centered over the turtle (Daubenmire 1959) using a modified Daubenmire scale from Abrams and Hulbert (1987). Coverage percents did not sum to 100 percent in 2012 and were transformed to Daubenmire midpoints. Coverage points in 2013 and 2014 did not sum to 100 percent. Vegetation height was measured in centimeters at 6 points on a transect running along the left side of the frame. These 6 readings were averaged using a modified protocol from Converse and Savidge (2003). Dominant plant species were identified to genus in the plots according to Swink and Wilhelm (1994) or Mohlenbrock (1986).

Environmental factors were also obtained at turtle locations. Wind speed (actual, maximum and average), altitude, air temperature, wind chill, relative humidity, heat stress, dew point, wet bulb temperature, and barometric pressure were collected with a Model 0835 Kestrel® 3500 Pocket Wind Meter (Nielsen-Kellerman, Boothwyn, PA) approximately 40 centimeters from the ground, 2 centimeters behind the turtle. Percent cloud cover visibility was estimated by observing cloud coverage (0% clear to 100% overcast) directly above the turtle and occupied plot. Substrate temperature readings were collected with a Model Number thermometer (Taylor

Precision Products, Oak Brook, IL) just below the surface (tip of the probe) as well as 12 cm below the surface of the ground (the entire length of the probe).

Random microhabitat and environmental measurements were paired with the turtle sighting to compare habitat use (Figure 6). The second hand of analog watch was used to select the arbitrary direction to walk 5 meters. If a digital watch was worn, the observed seconds were converted to a compass bearing by multiplying the number by 6. Slope, aspect, and elevation were also collected for the random plot. To avoid pseudoreplication, turtle locations were noted to be unique or same as site (SAS). Flagging was placed above the turtle location and the date and turtle identification number was written on the flag. If a turtle was found underneath a flag in the exact same spot or within 1 m of its previous location, the data could be separated for independence.

#### ***Data Analysis for Demography and Survival and Microhabitat***

We evaluated sex ratios of each population of ornate box turtles at Nachusa Grasslands. Sex ratios were compared between populations using Chi-square tests of homogeneity. Mean size-class structures (carapace and plastron length) were determined for each population. Turtles with undetermined sex were evaluated using a modified method between Legler (1960) and Converse (1999). It was assumed that turtles were juveniles if they were smaller than 100 mm carapace length, had no obvious secondary sex characteristics, or had fewer than 13 growth rings. To construct size-class tables, recapture measurements were averaged to account for differences in observer measurements. Size classes were established every ten millimeters. To account for growth, mean measurements were compared with the last measurement taken so the animal could be placed into the correct size-class bin.

A logistic regression model was used to try to predict where turtles were more likely to be present in the landscape, using the microhabitat Daubenmire frame data (LOGISTIC, SAS Institute, Inc., Cary, NC). Dependent variables included vegetation cover variables. Response variables were binary (occupied vs. random). AIC modeling was employed for hierarchical habitat selection.

## **Results:**

### ***Demography and Survival***

From June 2011-May 2014, we captured 151 individuals (48 female (F), 58 male (M), and 45 undetermined (U)) (Figure 7 and 8, Table 2). Between sites, the Headquarter Prairie (HQ) had fewer turtle captures than the Orland Tract (OT). OT had 6 additional F, 16 additional M, and 9 additional U (Table 2). Over the duration of the study 42 (20F, 22M) individuals were transmitterd and monitored for survival (Table 3). 21 turtles (9F, 12 M) were transmitterd at the HQ and 21 individuals (11F, 10M) were transmitterd at the OT.

Mean straight carapace length (CL) size-class structure (Figure 9) and mean straight plastron length (PL) size-class structure (Figure 10) were generated at each site. Most turtles (94%, 17 of 18 at HQ and 93%, 25 of 27 at OT) with undetermined sex were considered juveniles because they had smaller than 100mm CL. At HQ, there was a gap of missing CL size-classes including the 40-49mm and 50-59mm class (Figure 9). Only 1 juvenile turtle was found in the <39 mm and 60-60 mm CL class at both sites (Figure 9). At HQ, juvenile turtles were evenly distributed in the 70-79 mm, 80-89 mm, and 90-99 mm CL size-class bin (5 individuals/bin) (Figure 9). At OT, 3 juvenile turtles were found in the 70-79 mm bin, 6 were found in the 80-89 mm bin, 12 were found in the 90-99mm bin, and 2 were found in the 100-109 mm CL size-class (Figure 9). At the HQ, there were more females in the 110-119 mm CL size-

class than in the 100-109 mm or 120-129 mm bin, whereas male turtles dominated the 100-109 mm and 120-129 mm CL size-class. At the OT, more adult males were in the 110-119 mm and 120-129 mm CL size-class bin (Figure 9). The only CL size-class bin where female turtles were greater than male turtles at the OT was the 100-109 mm bin (Figure 9). At both sites, the greatest number of adult turtles captured were in the 110-119 mm CL size-class bin (28 HQ, 39 OT), followed by the 100-109 mm bin (10 HQ, 18 OT), 90-99 mm bin (5 HQ, 13 OT), and 120-129 mm bin (4 HQ, 8 OT) (Figure 9).

Most turtles (44.4%, 8 of 18 at HQ and 40.7%, 11 of 27 at OT) with undetermined sex had PL from 90-99mm size-class. At HQ, there was a gap of missing PL size-classes including the 40-49mm, 50-59 mm and 60-69 mm size classes (Figure 10). Only 1 turtle with undetermined sex was found in the <39 m, 100-109 mm, and 110-119 mm PL size-class at HQ (Figure 10). At OT, there were no gaps in PL size-class structure (Figure 10). 1 undetermined turtle was found in the following PL size-classes <39mm, 40-49mm, and 60-69 mm at OT (Figure 10). Two turtles were found in the 50-59 mm and 70-79 mm PL size class (Figure 10). At the HQ, there were more females in the 120-129 mm PL size-class than 100-109 mm or 110-119 mm bin, whereas a greater number of female turtles at OT were found in the 110-119 mm (Figure 10). At both sites, most males were in the 110-119 mm PL size-class bin (81%, 17 of 21, HQ and 64.9%, 24 of 37, OT) (Figure 10). The only PL size-class bin where female turtles were greater than male turtles was the 130-139 mm bin (Figure 10). At both sites, turtles followed the same pattern of having the most adult turtles in the 110-119 mm PL size-class bin (24 HQ, 36 OT), followed by 120-129 mm (11 HQ, 15 OT), 100-109 mm (3 HQ, 12 OT), and having the fewest number of turtles in the 130-139 mm (2 HQ, 1 OT)(Figure 10).

Capture data between sites suggests variation in sex ratios between our two study populations. HQ was composed of an equal number of male and female turtles (21F, 21M,  $\chi^2=0.00$ ,  $p=1.000$ .) for a 1:1 sex ratio (Table 4). OT was male-biased 1.4:1 (27F, 37M,  $\chi^2=1.563$ ,  $p=0.211$ ) (Table 4). If the study populations were combined, the sex ratio would be 1.2:1 (48F, 58M,  $\chi^2=0.943$ ,  $p=0.331$ ) (Table 4) for all turtles at Nachusa Grasslands (Table 4).

Twenty-five carcasses have been collected at Nachusa Grasslands from June 2011-14, 4 of which were transmittered individuals and 1 of which was marked with a FLOY tag. Mortality resulted from unknown natural causes ( $n=8$ ), depredation ( $n=7$ ), road-kill ( $n=4$ ), overwintering ( $n=3$ ), burn damage ( $n=2$ ), heat exposure ( $n=1$ ), and disease control ( $n=1$ ) (Table 5).

Depredation occurred by mammalian (suspected coyote, raccoon or dog) and avian predators (suspected crow or raven). No transmittered turtles died during hibernation over winter 2011-2012, 1 died between 2012-2013, and 2 are suspected to have died between 2013-2014 (Table 5).

We observed 2 cases of different flesh-eating bacterial infection in the turtle populations. The first case was observed 20 April 2013, after the turtle emerged from its overwintering site in a coniferous forest underneath honeysuckle shrubs. The turtle had raw and necrotic tissue on both hind feet (Figure 11). There were no lacerations, cuts or punctures present on the limbs or shell of the turtle that would indicate a depredation attempt. The turtle (number 023, M) was removed from the HQ study area for disease control and was placed in Dr. Matt Allender's care at University of Illinois, 28 April 2013. The necropsy reports revealed microscopic diagnoses of diffuse acute heterophilic bronchopneumonia with mixed intralesional bacteria. The feet had severe locally extensive chronic ulcerative pododermatitis. The flesh-eating bacteria were in the bones of the animal. It could not be determined which infection (bacterial or fungal) were the primary or secondary source of infection.

The second case of disease was observed 24 July 2013. The tail of the female turtle was observed to be necrotic and disintegrating (Figure 11). Again, there were no lacerations, cuts or punctures present on the limbs or shell of the turtle that would indicate a depredation attempt. There was an inflamed area on the rear left leg and tail region. It appeared to be a cyst or a structure that was filled with maggots or botfly larvae. A piece of tissue with the insect larvae was stored in ethanol for further inspection. The cloaca and tail region of the turtle was swabbed and sent to Dr. Matt Allender, DVM, University of Illinois, for analysis on 18 August 2013. The microscopic culture diagnosis contained three genera of bacteria, *Peptostreptococcus sp.*, *Bacteroides sp.*, and *Fusobacterium sp.* It is unknown if any of these pathogens can cause disease on their own or just happen to be on the swabbed sample (i.e. in the dirt or urogenital tract of the turtle, but not really causing infection). Over time the inflammation of the cloaca and region of the left rear leg has resided, and healed. Little is known about the internal extent of the tissue damage or if this could negatively affect this turtle's reproductive success. The turtle survived overwintering in 2013. Observations of this animal occurred until 31 May 2014 when her transmitter was removed. The raw area had closed and appeared to have healed, but the turtle is missing a considerable portion of her tail. No other turtle on the site has shown symptoms of raw tails with disintegrating tissue, but other turtles have been observed with healed "stub" tails.

Capture-mark-recapture-grids (CMRG) were walked at the Orland Tract from 13 April-16 June 2013 to estimate population size. Data have been recorded, but not analyzed with Program MARK, due to the relatively low number of recaptures. Nineteen individual ornate box turtles and one painted turtle were observed in the CMRGs. The greatest number of turtles detected on one day was 5 turtles. In grid C, 2M, 4F, and 3U were captured 1 time, and only 1M

was recaptured. In grid D, 3M and 2U were captured 1 time, 1F was captured 2 times, 1M and 1F were captured 3 times, and 1F was captured 4 times. Three radio-transmitted turtles were detected in the CMRG's via telemetry, but were not found by grid observers on a minimum of 6 occasions. More work will be done to map all transmitted turtles on the dates of walks to determine detection probability.

Opportunistic observations due to the CMRG allowed for 3 previously radio-tagged animals to be observed live (Turtle 043, Turtle 048, and Turtle 055, all M). Transmitters were not available to be placed back on these individuals for future tracking. In fall 2012, Turtle 043 had his transmitter removed prematurely due to a break in the antenna, but he was observed twice on 4 and 5 May 2013. Turtle 048 was located only 3 times in April 2013 before transmitter malfunction, but was observed in a MCRG on 5 May 2013. It is likely the transmitter was damaged during emerging or a depredation attempt based on photographs taken by volunteer grid walkers (there was no antenna and battery corrosion and exposure was evident). Turtle 055 had his transmitter removed prematurely on 30 May 2013 because the whip antenna had become detached from the transmitter. The turtle was observed in a grid 2 June 2013.

### ***Reproductive Behavior and Nesting Sites***

Turtles were observed mating and nesting in May or June during 2012 -2014 (Table 6). Turtles were not observed mating or nesting in 2011. In 2012, one nest was found on a sand two-track (road). In 2013, four nests were detected in remnant prairie and 1 nest was detected in restored prairie. In 2014, the same individual that nested in the two-track in 2012, nested there again. Hatchlings were not observed exiting any documented nests. No above ground disturbances were observed where nesting took place. Nests were not excavated to count eggs or determine hatchling survival.

### *Overwintering Sites*

Overwintering site habitat characteristics were quantified and site fidelity was examined from 2011-2013. We affixed radiotransmitters to 37 adult turtles (19F, 18M). Turtles were tracked to their overwintering sites (8 animals in 2011, 28 animals in 2012, and 30 animals in 2013). Overwintering sites of 24 animals (13F, 11M) were compared across two years.

Emergence and submergence dates differed between years and seem to be correlated with temperature (data analysis in progress). Spring 2012 was unusually warm, which may have cued turtles to emerge in mid-March and early April. Turtles emerged during late April-May in 2013 and 2014. Turtle submergence started in mid-September and continued through October during 2012-2014. In 2011, November warm spells encouraged turtles to emerge and in some cases select a different overwintering location nearby. Tracking in November did not occur in 2012-2013.

Overwintering site fidelity was high. At HQ, 60% of turtles (3 of 5) exhibited fidelity within 5 m buffers from the previous overwintering site (2011-2012) (Figure 12). From 2012-13, fidelity within 5 m buffers increased to 83.3% (10 of 12) (Figure 12). If points were viewed along with 50 m buffers, 100% of turtles exhibited fidelity (12 of 12) (Figure 12). Overwintering locations of 5 animals (2F, 3M) were compared across three years (2011-2013), with varying patterns of site fidelity (Figure 12). At OT during 2012-2013, 75% (9 of 12) of turtles exhibited fidelity within 5 m buffers, 83.3% (10 of 12) exhibited fidelity within 10 m buffers, and 100% exhibited fidelity within a 50 m buffer (12 of 12) (Figure 13).

Overall, overwintering habitat varied among individuals and did not differ significantly with sex or year. Remnant prairie was the most frequently used land cover type (79.1%, 53 of 67), and agricultural fields or pasture were not used by any individuals. Most turtles (95.8%)



overwintered in the same land cover type during the duration of the study (Figure 12 and Figure 13). Only one male turtle used different habitat types between years (Turtle 018, Figure 12). In 2011, the turtle used remnant prairie, and in 2012 and 2013, he used the same uprooted tree mass in a coniferous forest. Turtles excavated their own burrows (73.1% of individuals observed) more frequently than using abandoned mammal burrows (22.4% of individuals observed) or local features (4.5% of individuals observed) (n=67). Overwintering survival was high, with only one male observed dead (2012) and 2 females suspected to have succumb in 2013. In 2012, a communal den containing 3 turtles (1F, 2M) was recorded. One male from the communal den died during the winter, but the surviving 2 animals returned to the same location in 2013.

### ***Microhabitat selection***

Preliminary microhabitat data analysis of 2012 summer field season was completed. Occupied and random points were obtained from radiolocations of 28 turtles from March-June 2012 (n=636). Independent logistic regression models were used to evaluate cover type selection (bare ground, forb, grass, litter, sedge, shrub, rush, and tree). Shrubs were highly significant habitat for turtles (<0.0001, 1.032) (Table 7). When turtles were in the same location on a consecutive sighting, data were only collected on the first sighting, because of lack of independence between consecutive sightings at the same location (Converse and Savidge, 2003). After points were removed, analyses were run with 636 observations.

Before conducting advanced analyses, data were graphed to visualize any potential trends (Figure 14 and Figure 15). We plotted a histogram of each site with the independent variables being whether the point represented an occupied or random site, month, and cover type. Our dependant variables were the percent cover of each vegetation type expressed as the average Daubenmire midpoint. Trends from the figures did not have statistical power (Figure 14 and

Figure 15). At the HQ prairie animals were monitored from March-June while animals at OT were monitored May-June. Litter declined over time and had an inverse relationship with grass and forb cover (Figure 14 and Figure 15). Cover types that were not frequently observed within occupied or random plots included moss, sedge, shrub, rush, and tree (Figure 14). Shrub and tree cover increased during late May and June (Figure 14 and 15). Water was not included in the analysis, as this cover type was not utilized or available within the study site due to the drought.

The Daubenmire frame data was analyzed with a multivariate analysis of variance (MANOVA, SAS Institute, Inc., Cary, NC) to assess habitat selection. Results were not obtained due to the complexity of the study question. Models were not built because the vegetation cover classes were correlated and dependent on one another since all vegetation cover classes added to 100%.

We ran logistic regression models to try to predict where turtles would be present in the landscape using the microhabitat vegetation cover data (LOGISTIC, SAS Institute, Inc., Cary, NC). Dependent variables included the percent cover of each vegetation type and the independent variable was the type of observation (occupied vs. random). A major issue with the analysis remained the same in that the cover classes added to 100%, which made them dependent on one another. We could not run a model framework that would test multiple cover types in the same model, so AIC hierarchical model selection were not applied to the results. To avoid erroneous conclusions, each cover class was analyzed separately. Bare ground, forb, litter, moss, sedge, and tree did not yield significant results (Table 7). Cover classes that were marginally significant were grass ( $p=0.0218$ ,  $df=1$ ) and rush ( $p=0.0179$ ,  $df=1$ ; Table 7). Shrub cover was the only highly significant cover class ( $p<.0001$ ,  $df=1$ ; Table 7).

### **Discussion:**

### *Demography and Survival*

Capture detections and sex-ratio estimates differ from the literature, which suggests female-biased populations (Legler 1960, Blair 1976, Doroff and Keith 1990, Bowden et al. 2004), and may be explained by differences in site variation and sampling methodology. Variation between sites occurs due to the different macrohabitat complexes and management histories. Turtles were detected in a variety of ways throughout the study by different observers. It is possible when detector dogs were employed in early May or late September that they were more likely to encounter male turtles that were making movements to find females during the breeding season (Figure 4). Turtles are not distributed evenly in the landscape. It is possible that areas with high female densities were not located and have yet to be discovered. Additionally, road mortality causes male-skewed adult sex ratios in freshwater turtles (Aresco 2005, Gibbs and Steen 2005, Steen et al. 2006). Female turtles are hypothesized to more likely cross roadways when they are on nesting forays that take them out of their home range, putting them at greater risk than their male counterparts (Steen et al. 2006). Finding quarter-sized hatchlings or juvenile turtles may be difficult to detect due to their small size and cryptic coloration. Little is known about the life history strategies of young animals which may explain why there is less detection of those individuals within the size-class distribution (Figure 9 and Figure 10). Another possible explanation is that turtles exhibit type III survivorship where few hatchlings survive to adulthood.

Mortality of box turtles at Nachusa Grasslands did not differ greatly from other studies set in other rural areas (Doroff and Keith 1990). Aside from natural unknown causes or depredation, the next largest factor contributing to mortality could be damage caused by vehicles or agricultural equipment. Vehicle strikes can be a significant cause of turtle mortality (Legler

1960, Doroff and Keith 1990, Dodd 2001, Franklin and Killpack 2009). Most vehicle strikes occurred in late summer through fall when turtles were traveling from summer foraging grounds to their overwintering habitat. In the spring, the male turtle that was hit was likely making exploratory movements find female turtles for mating or was returning from its overwintering site to spring foraging grounds. In late May, a female turtle was hit with a boom sprayer as it was driven through the prairie to reduce red clover cover. Cureton and Deaton (2012) found hot spots of turtle vehicular mortality coincides with the breeding season (over 98% of turtle-vehicle collisions occur during the months of May and June) and are associated with the habitat where they are commonly observed. For many terrestrial turtle species, size of an individual's home range may determine its relative vulnerability to vehicular road mortality (Stickel 1950). Failing to protect adult densities reduces the likelihood of mating encounters and reduces recruitment (Belzer 2000).

Infectious diseases in ornate box turtles are not well studied. Little work has been done to determine prevalence of bacterial or viral infection in box turtles. It is unknown whether the bacterial infection is a novel problem at the site or if turtle populations have co-evolved with these periodic infections.

### ***Reproductive Behavior and Nesting Sites***

Turtles were observed mating during May-June, which is consistent with observations from other studies (Legler 1960). Turtles at Nachusa, were found to nest in late May or the beginning of June, which is consistent with observations from Doroff and Keith (1990). Nesting at the Wisconsin site occurred from 10-26 June in 1986 and 29 May to 23 June in 1987 (Doroff and Keith 1990). Legler (1960) noted that turtles began nesting at the beginning of May and could continue nesting until mid-July. Most nests (85.7%, 6 of 7), were in open habitat on

upland prairie sites in well-drained, friable soil (Legler 1960, Doroff and Keith 1990, Converse et al. 2002) (Figure 16). One nest, however, was found on a sand two-track (road). A two-track is a sand path on the preserve that provides vehicle access to different plantings and remnants on the preserve. Due to low sample size it is difficult to know if this is an anomaly at the site.

There may be fidelity to nesting locations (n=1).

There are many potential problems with nesting on a two-track. Female turtles could be run over during the act of excavating the nest, depositing eggs, or covering the nest resulting in the loss of future recruitment potential (Steen et al., 2006). Two-tracks receive daily traffic which may increase the compaction of the soil making it difficult to excavate a nest for a female turtle or making it harder for hatchlings to dig out if the eggs survive the weight of the vehicle. If the eggs survive, hatchlings could be run over when they emerge from the nest. Predators also frequent the two-track for ease of travel and could depredate nests more easily. We observed a snapping turtle nest in the two-track on the HQ site that was depredated in less than 24 hours with 100% egg mortality in 2012.

Hatching success could not be determined due to sporadic observations in fall and spring months. It is possible that eggs hatched and hatchlings emerged in early fall or the following spring (Doroff and Keith 1990, Converse et al. 2002, Franklin and Killpack 2009) and signs of disturbance were concealed due to rain events after the emerging event. Hatchlings found in March and April on remnant hills suggest hatchlings may remain underground in the nest chamber until the following season as described in other work (Doroff and Keith 1990, Converse et al. 2002, Franklin and Killpack 2009).

### ***Overwintering Sites***

Overwintering sites are important habitat features to understand because turtles may spend more than half of their lives in an overwintering state (Ultsch 2006). Ornate box turtles range from being underground for less than 4 months in Texas (Blair 1976) to 7 months in Nebraska and Wisconsin depending on their geographic location (Converse et al. 2002; Doroff and Keith 1990). Hibernation and emergence dates vary based on latitude and climate, although it is suggested that dates are strongly correlated with soil moisture and soil temperature (Grobman 1990, Ultsch 2007). At Nachusa Grasslands turtles spent about 4.5 months overwintering in 2011-2012, 5-5.5 months in 2012-2013, and 6 months in 2013-2014.

Exact features of overwintering sites varied among animals. Turtles likely choose locations that prevent them from freezing or desiccating (Ultsch 2006). Individuals selected sites on flat land or on south/west-facing hill slopes (Dodd 2001), in open sandy areas or areas with accumulated litter (Legler 1960, Stickel 1989, Doroff and Keith 1990), in thorn-laden thickets (Carpenter 1957, Metcalf and Metcalf 1970, Metcalf and Metcalf 1979), in root tunnels or abandoned mammal burrows (Legler 1960, Trail 1995). Most turtles used remnant prairie for overwintering (n=53), followed by coniferous forest (n=8), oak savanna (n=3) and prairie restorations (n=3). All turtles that used restored prairie for overwintering sites were male. Pine stands may be used more often due to open soil available for digging. Understory and herbaceous cover is limited due to the shading of other species by the pine canopy or dense needle litter, potential allelopathy, acidification of the soil through needle decomposition, and shallow root structure making space less available for other plants. Remnant oak savanna was used by female turtles (n=3). Savanna contained sandy soils and deep 3-6 cm leaf litter. Litter likely provided the turtles with more insulation during the winter.

We also found that most animals typically overwintered alone (92.5%, 62 of 67) (Legler 1960). Animals overwintered in clusters in close proximity to each other (Figure 12 and Figure 13). Occasionally, as Carpenter (1957) found, turtles overwintered within 0.5 m of other turtles. It is likely turtles are found overwintering near each other because they are taking advantage of ideal conditions (Carpenter 1957, Metcalf and Metcalf 1970, Metcalf and Metcalf 1979, Doroff and Keith 1990, Dodd 2001).

Site fidelity of overwintering sites is common within *T. ornata* (Doroff and Keith 1990, Dodd 2001, Refsnider et al. 2012). Eight of fourteen *T. ornata* overwintered within 1 m of previously used sites in Wisconsin remnant tallgrass prairie (Doroff and Keith 1990). This phenomenon was seen at Nachusa Grasslands in 2012-2013. Ten of twelve turtles (HQ) and eight of twelve turtles were within 1m of their flagged location from the previous year. A population of ornate box turtles in northwestern Illinois also displayed site fidelity; females showed 33% stronger fidelity to overwintering sites than males (Refsnider et al. 2012).

### ***Microhabitat selection***

Trends from the preliminary cover type histograms depicting average Daubenmire midpoint through time within occupied and random plots can be explained through biology and management. In March, litter comprised a large component of the Daubenmire cover types and was severely reduced from March to April and increased from May to April (Figure 14 and Figure 15). Prescribed fire is a management tool that was used in late March which decreased litter cover within study plots. When litter was reduced, bare ground was exposed which allowed sunlight to penetrate the ground to encourage growth of herbaceous plants from April-June. In June, the preserve entered drought conditions with little rain and high daily temperatures which increased litter cover as forbs and grasses died back. When drought

conditions were apparent the data suggests that turtles used shrubs and trees during May and June (Figure 14 and Figure 15). Shrub and tree canopy cover can provide refuge from high air and substrate temperatures.

Data are currently limited because they have not been modeled with environmental covariates, however, there are several likely scenarios why turtles may selected certain cover types. Grass and rush cover were slightly significant in the logistic regression (Table 8). Dominant rushes at Nachusa Grasslands tend to be two-track edge species or wetland species. Turtles may use edge habitat for ease in moving or thermoregulation. In mesic areas, rushes could act as protection from predators and allow the turtle to cool down if overheated. Bunch grass cover may be important for turtles to use for protective cover as turtles can be completely hidden under tussocks. Temperatures under tussocks are lower and allow for higher humidity than the surrounding environment, which reduces the turtle's risk of desiccation.

Shrubs were highly significant cover habitat for turtles ( $<0.0001$ , 1.032). Shrubs are beneficial microhabitat structures for turtles because they provide shade for thermoregulation, protection from predators, and food resources (Figure 14 and Figure 15).

Data collected in June and July 2012 were collected during a drought in which the environmental conditions differed from the norm and more closely resembled climate patterns of July and August. During these summer months individuals entered a period of little to no activity because conditions were hot and dry. Box turtles can be inactive or enter aestivation for a day or weeks if environmental conditions are unfavorable (Stickel 1950, Legler 1960, Franklin and Killpack 2009). Although these turtles are prairie-obligate species, they will take advantage of shade from shrubs or lone trees for cover from excessive mid-day heat (Dodd 2001, Converse and Savidge 2003, Refsnider et al. 2012). Converse and Savidge (2003) noted turtles selected



*Yucca* (*Yucca glauca*), for thermoregulation. The yucca has a simple taproot which allows substrate to be available under the shrub for excavation of forms which can assist with thermoregulation (Converse and Savidge 2003). An additional study in Illinois noted the tendency for ornate box turtles to use Eastern red cedar (*Juniperus virginiana*) for cover during periods of high heat (Refsnider et al. 2012). Western ornate box turtles in New Mexico were observed using various shrubs for cover, including creosote bush (*Larrea divericata*), tarbush (*Flourensia cernua*), yucca (*Yucca elata*), and mesquite (*Prosopis juliflora*) (Nieuwolt 1996).

Dominant shrubs or trees at the preserve include: American Plum (*Prunus americana*), black raspberry (*Rubus occidentalis*), Allegheny blackberry (*Rubus allegheniensis*), gooseberry (*Ribes sp.*), Autumn Olive (*Elaeagnus umbellate*), Amur honeysuckle (*Lonicera maackii*), Morrow's honeysuckle (*Lonicera morrowii*), apple tree (*Malus domestica*), black locust (*Robinia pseudoacacia*), honey locust (*Gleditsia triacanthos*), and multiflora rose (*Rosa multiflora*) (K. E. Schmidt, unpublished data). Many of the shrubs available for turtles to use as shelter have spines or thorns and grow in dense thickets (Figure 17). This can provide turtles with protection from predators (e.g. coyotes, raccoons). In addition, shrub cover may have been used for a preferred food source. *T. ornata* are opportunistic omnivores feeding on a varied food items including arthropods, annelids, gastropods, amphibian metamorphs, bird eggs, nestlings, cactus, fruit, and carrion (Legler 1960, Metcalf and Metcalf 1970, Blair 1976). Shrubs with ripe fruit during the months of May and June include Allegheny blackberry, Amur honeysuckle, black raspberry, and gooseberry. Shrubs that were not fruiting may provide habitat for the other prey items that ornate box turtles include in their diet. Additionally, turtles take similar pathways to seasonal food sources (Metcalf and Metcalf 1970). The turtles may have been strategically positioning themselves underneath shrubs that should have been fruiting, but did not due to the drought.

Limitations of the analysis include lack of predictive power and AIC modeling. The model used a subset of data and was not run with covariates to isolate season or time of day effects. We are building an improved logistic regression model, MANOVA model, and compositional analysis to describe macro- and microhabitat.

### **Management Implications Summary:**

Slower speeds or short-term road closures may prevent fall and spring migration mortality from vehicle strikes. Herbicide application on remnant prairie should be avoided during early mornings in May and June to minimize the disturbance of nesting turtles. Potential road closures would minimize risk of female nesting or hatchling emergence mortality on two-tracks. Removal of invasive shrubs should be followed by the replacement of native shrubs to provide key cover components. Broadcast seeding of native shrub plant would not be sufficient to provide the structure cover lost during removal of the invasive shrubs. Results suggest turtles display overwintering site fidelity. To prevent mortality prior to submergence or emergence, prescribed fire should be timed when turtles are underground.

### **Prospectus work:**

#### ***Survival Analysis and Population Estimate***

We will use a modified Kaplan-Meier estimator (Kaplan and Meier 1958, Pollock et al. 1989, Lebreton et al. 1992, Hellgren et al. 2000, Currylow et al. 2011) to estimate annual survival rates of telemetered adult *T. ornata* in Nachusa Grasslands with the known-fates model in program MARK (White and Burnham 1999). The effects of number of years burned, years since last burned, and prairie type (restored vs. remnant) on survival will be assessed via covariate analysis in MARK. If sufficient data are collected from the mark-recapture sessions,

we plan to estimate population size using a Jolly-Seber open population model (Jolly 1965, Seber 1965, Lebreton et al. 1992) in program MARK (White and Burnham 1999).

### ***Home Range Estimators***

Monthly, annual, and overall home ranges of ornate box turtles will be estimated by using a Geographic Information System (GIS). We will estimate turtle home range using several methods to be able to make comparisons with other studies. Spatial analyses will include 100% minimum convex polygon (MCP), fixed kernel 95% (FK) (Worton 1989), and harmonic mean (HM) (Dixon and Chapman 1980).

### ***Movement***

Other movement and activity measures that will be calculated include total distance traveled (total sequential linear distance in meters between all locations), maximum distance traveled (maximum distance between locations), mean distance traveled, mean daily speed (mean number of meters traveled per day; total distance traveled / total days tracked), maximum daily speed (maximum number of meters traveled per day), and range length (maximum linear distance between two locations in a dataset). For all movement analyses, if a turtle was located in the same site as the previous observation, the previous observation was not counted as a movement.

### ***Macrohabitat Selection***

We will assess second- and third-order habitat selection (Johnson 1980) using compositional analysis (Kazmaier et al. 2001, Aebischer et al. 1993), which allows for the testing of differential selection between effects (e.g., treatment or sex) using turtles as replicates. Second-order selection compares the habitat composition within a turtle's home range to the habitat composition of the study area. Third-order selection compared habitat composition of an

individual turtle's radiolocations with the habitat composition of its home range (Kazmaier et al. 2001, Johnson 1980). As in Kazmaier et al. (2001), main effects (sex or treatment), habitat selection, and the interaction of each main effect and selection will be tested using MANOVA in SAS 9.3 (SAS Institute, Inc., Cary, NC). Selection will be considered to occur if use is greater than availability, whereas avoidance will be considered to occur if use is less than availability. Analyses will be considered significant at  $P < 0.05$ .

### ***Microhabitat Selection***

Microhabitat analysis will be modeled after Burrow et al. (2001). We will analyze Daubenmire frame data with a multivariate analysis of variance (MANOVA, SAS Institute, Inc., Cary, NC) to assess habitat selection. Dependent variables will include vegetation cover variables, vegetation height, and environmental parameters. Independent variables will be type of location (occupied vs. random), time of day, season (spring: March-April; summer: May-July; fall: August-October), year, sex, and management treatment. All two-way interactions will be included in the model. If the MANOVA is significant, individual ANOVA tests will be conducted to isolate which dependent variables are important to habitat selection. If a habitat feature was used more than its availability, it will be considered selected; whereas if it was used less than its availability, it will be considered avoided. Interactions will be examined to determine if selection or avoidance is consistent across the independent variables.

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Table 1. Methods by which independent variables were measured at Nachusa Grasslands 2011-2014.

Independent Variable	Method
planting age	Nachusa Grasslands management records
number of prescribed burns	Nachusa Grasslands management records
burn frequency	Nachusa Grasslands management records
time since last burn (growing season)	Nachusa Grasslands management records
distance to nearest remnant (m)	GIS
size of nearest remnant (ha)	GIS
distance to nearest road (m)	GIS
distance to nearest two-track (m)	GIS
distance to nearest water source (m)	GIS
distance to nearest burrow (m)	GIS
soil drainage	county soil survey
soil texture	county soil survey
slope	county soil survey
micro-slope	field clinometer on Silva compass
wind speed	Model 0835 Kestrel® 3500 Pocket Wind Meter
wind speed maximum	Model 0835 Kestrel® 3500 Pocket Wind Meter
wind speed average	Model 0835 Kestrel® 3500 Pocket Wind Meter
air temperature	Model 0835 Kestrel® 3500 Pocket Wind Meter
wind chill	Model 0835 Kestrel® 3500 Pocket Wind Meter
Independent Variable	Method

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relative humidity	Model 0835 Kestrel® 3500 Pocket Wind Meter
heat stress/heat index	Model 0835 Kestrel® 3500 Pocket Wind Meter
dew point	Model 0835 Kestrel® 3500 Pocket Wind Meter
wet bulb temperature	Model 0835 Kestrel® 3500 Pocket Wind Meter
barometric pressure	Model 0835 Kestrel® 3500 Pocket Wind Meter
surface substrate temperature	Taylor Precision Products Thermometer
substrate temperature 12 cm in ground	Taylor Precision Products Thermometer
cloud cover	Field
bare ground percent cover	Field
cactus grass percent cover	Field
fern percent cover	Field
forb percent cover	Field
grass percent cover	Field

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Table 2. Total turtle captures by study site (Headquarter Prairie (HQ) and Orland Tract (OT)) at Nachusa Grasslands, June 2011-June 2014 (n=151).

Site	Female (F)	Male (M)	Undetermined (U)
HQ	21	21	18
OT	27	37	27
Total	48	58	45

Table 3. Status of 42 turtles affixed with radiotransmitters at Headquarter Prairie (HQ) and Orland Tract (OT) study sites at Nachusa Grasslands, Illinois, as of 3 June, 2014.

Date Attached	Actual or Scheduled Date for Removal	ID	Site	Sex	Status at Current Point in the Study, Transmitter Removed (TR) Transmitter Failure (TF)
3/25/2012	5/31/2014	001	HQ	F	Live, TR
5/5/2012	6/22/2014	004	HQ	F	Live
9/17/2011	5/3/2012	005	HQ	M	Unknown. Transmitter popped off underground. No sign of depredation.
3/25/2012	5/31/2014	007	HQ	M	Live, TR
7/28/2011	10/19/2013	008	HQ	M	Live, TR
7/29/2011	4/26/2014	009	HQ	F	Live, TR
9/23/2011	5/5/2012	011	HQ	M	Unknown. Transmitter popped off underground. No sign of depredation.
9/23/2011	Not salvaged	012	HQ	F	Unknown. Turtle did not emerge from overwintering site 2013, likely dead.
9/23/2011	6/23/2012	013	HQ	F	Died. Run over by boom sprayer.
9/24/2011	5/3/2014	018	HQ	M	Live, TR
9/25/2011	10/19/2013	020	HQ	M	Live, TR
3/24/2012	6/1/2014	022	HQ	F	Live, TR
3/24/2012	5/13/2013	023	HQ	M	Died. Euthanized for disease control.
3/24/2012	6/22/2014	024	HQ	M	Live



Date	Actual or Scheduled	ID	Site	Sex	Status at Current Point in the Study,
Attached	Date for Removal				Transmitter Removed (TR) Transmitter Failure (TF)
3/25/2012	5/18/2014	026	HQ	M	Died. Died overwintering 2012-13
3/25/2012	5/31/2014	027	HQ	F	Live, TR
4/22/2012	Not salvaged	029	OP	M	Unknown fate with telemetry, TF. Recaptured in 2012 and 2013.
4/22/2012	5/31/2014	030	OP	F	Live, TR
4/22/2012	6/22/2014	033	OP	F	Live
4/22/2012	7/13/2014	035	OP	M	Live
5/2/2012	5/31/2014	036	HQ	M	Live, TR
5/2/2012	5/31/2014	038	OP	M	Live, TR
5/2/2012	5/31/2014	040	OP	M	Live, TR
5/2/2012	6/22/2014	042	OP	F	Live
5/2/2012	9/2/2012	043	OP	M	Live. Recaptured in 2013 and 2014.
5/2/2012	Not salvaged	044	OP	M	Unknown, TF
5/3/2012	5/31/2014	046	OP	F	Live, TR
5/3/2012	Not salvaged	048	OP	M	Unknown, TF. Recaptured in May 2013.
5/3/2012	7/13/2014	049	OP	F	Live
5/3/2012	5/30/2013	055	OP	M	Live, TR
5/3/2012	7/13/2014	056	OP	M	Live
5/3/2012	7/13/2014	057	OP	M	Live

Date	Actual or Scheduled Date for Removal	ID	Site	Sex	Status at Current Point in the Study, Transmitter Removed (TR) Transmitter Failure (TF)
6/12/2013	10/7/2014	081	OP	F	Live
5/13/2013	6/22/2014	082	HQ	F	Live
9/19/2012	Not salvaged	090	OP	F	Unknown. Turtle did not emerge from overwintering site 2013, likely dead.
9/19/2012	5/27/2014	097	OP	F	Live R, TF
4/20/2013	5/31/2014	116	OP	F	Live R, TF
6/14/2014	5/31/2014	135	HQ	M	Live R, TF
6/11/2013	10/7/2014	138	OP	F	Live
6/13/2013	Not salvaged	140	HQ	M	Unknown, TF
6/13/2013	8/15/2014	141	OT	F	Live
7/3/2013	10/3/2013	145	HQ	F	Died. Run over by vehicle on 2-track.

Table 4. Sex ratio of ornate box turtles from all captures over the course of the study (June 2011-June 2014) for Nachusa Grasslands Preserve, Headquarter Prairie (HQ), and Orland Tract (OT).

	Males	Females	Sex Ratio <sup>1</sup>	$\chi^2$	p
Nachusa Grasslands	58	48	1.2	0.943	0.331
HQ	21	21	1.0	0	1
OT	37	27	1.4	1.563	0.211

<sup>1</sup>Number of males per female.

Table 5. Summary of ornate box turtle mortalities recorded at Headquarter Prairie (HQ) and Orland Tract (OT) study sites at The Nachusa Grasslands Preserve, June 2011-June 2014.

Date Carcass Collected	Study Site	Cause of Mortality	Age Class <sup>1</sup>	Turtle History on Study Site
6/14/2011	HQ	Natural <sup>2</sup>	M	not marked
6/26/2012	OT	Burn <sup>5</sup>	F	not marked
7/5/2012	OT	Depredation	A	not marked
8/27/2012	HQ	Vehicle	M	not marked
9/15/2012	HQ	Vehicle	F	not marked
9/24/2012	OT	Depredation <sup>6</sup>	A	not marked
9/24/2012	OT	Depredation <sup>7</sup>	H	not marked
9/29/2012	OT	Depredation <sup>6</sup>	H	not marked
10/6/2012	OT	Depredation <sup>6</sup>	H	not marked
4/27/2013	OT	Natural <sup>4</sup>	J	marked
4/21/2013	OT	Natural <sup>3</sup>	U	not marked
4/21/2013	OT	Natural <sup>3</sup>	U	not marked
5/11/2013	OT	Burn <sup>5</sup>	F	not marked
5/13/2012	HQ	Euthanized <sup>8</sup>	M	radio-transmitted
5/19/2013	HQ	Boom Sprayer <sup>9</sup>	F	radio-transmitted
5/19/2013	HQ	Natural <sup>2</sup>	U	not marked
5/21/2013	HQ	Depredation <sup>6</sup>	J	not marked
5/29/2013	HQ	Vehicle	F	not marked
7/31/2013	OT	Natural <sup>2</sup>	M	not marked

Date Carcass Collected	Study Site	Cause of Mortality	Age Class <sup>1</sup>	Turtle History on Study Site
10/20/2013	OT	Depredation <sup>6</sup>	J	not marked
11/24/2013	HQ	Vehicle	F	radio-transmitted
4/15/2014	OT	Natural <sup>2</sup>	J	not marked
5/3/2014	HQ	Natural <sup>3</sup>	U	not marked
5/4/2014	OT	Natural <sup>3</sup>	U	not marked
5/18/2014	HQ	Overwintering <sup>10</sup>	M	radio-transmitted
6/3/2014	HQ	Overwintering <sup>11</sup>	F	radio-transmitted
6/3/2014	OT	Overwintering <sup>11</sup>	F	radio-transmitted

<sup>1</sup>Age Class: A= adult unknown M = adult male, F = adult female, J = juvenile, H = hatchling U = unknown.

<sup>2</sup>Natural cause. Skeletal remains of carapace and sometimes plastron.

<sup>3</sup>Natural cause. Bone fragments only.

<sup>4</sup>Natural cause. Turtle was found dead upside-down and presumably died from heat exposure.

<sup>5</sup>Burn. Skeletal remains found charred. Unsure if burn damage occurred prior to death.

<sup>6</sup>Depredation. Mammalian Predator.

<sup>7</sup>Depredation. Avian Predator.

<sup>8</sup>Euthanized. Disease control (flesh-eating bacterial infection).

<sup>9</sup>Boom Sprayer. Turtle died in late May or early June 2012. Body was recovered in 2013.

<sup>10</sup>Overwintering. Turtle died underground winter 2012-13.

<sup>11</sup>Overwintering. Turtle suspected to have died underground during winter 2013-14, carcasses not yet collected.

Table 6. Date, location, time of day and reproductive behavior of turtles at Nachusa Grasslands, Illinois, 2012-2014.

Date	Site	Time of Day	Behavior	Individuals
5/2/2012	OT	7:30 PM	Mating	042 and 043
5/18/2012	HQ	8:49 PM	Excavating nest	022
6/13/2012	OT	1:25 PM	Mating	046 and 086
5/5/2013	HQ	12:50 PM	Mating	001 and 007
5/16/2013	OT	9:45 AM	Mating	097 and 118
5/16/2013	OT	2:17 PM	Mating	124 and 056
5/19/2013	OT	11:00 AM	Mating	unmarked and 055
6/5/2013	HQ	11:00 AM	Stomping on nest	082
6/8/2013	OT	10:24 AM	Excavating nest	049
6/9/2013	HQ	10:04 AM	Stomping on nest	091
6/12/2013	HQ	10:30 AM	Stomping on nest	009
6/16/2013	OT	9:41 AM	Nesting inferred <sup>1</sup>	042
5/18/2014	HQ	4:30 PM	Mating	unmarked and 024
6/4/2014	HQ	12:14 PM	Excavating nest	022

<sup>1</sup> Turtle found struggling to leave site because antenna was buried. Weight indicated eggs could have been deposited.

Table 7. Logistic regression p-value and odds ratio estimates for single cover type with  $df=1$  for all tests.

Cover Type	Pr>Chi-Square	Odds Ratio Estimate
Bare ground	0.1650	0.994
Forb	0.3488	0.997
Grass	0.0218	0.991
Litter	0.5366	0.997
Moss	0.3359	1.153
Sedge	0.9755	1.000
Shrub	<.0001	1.032
Rush	0.0179	0.845
Tree	0.1065	1.026

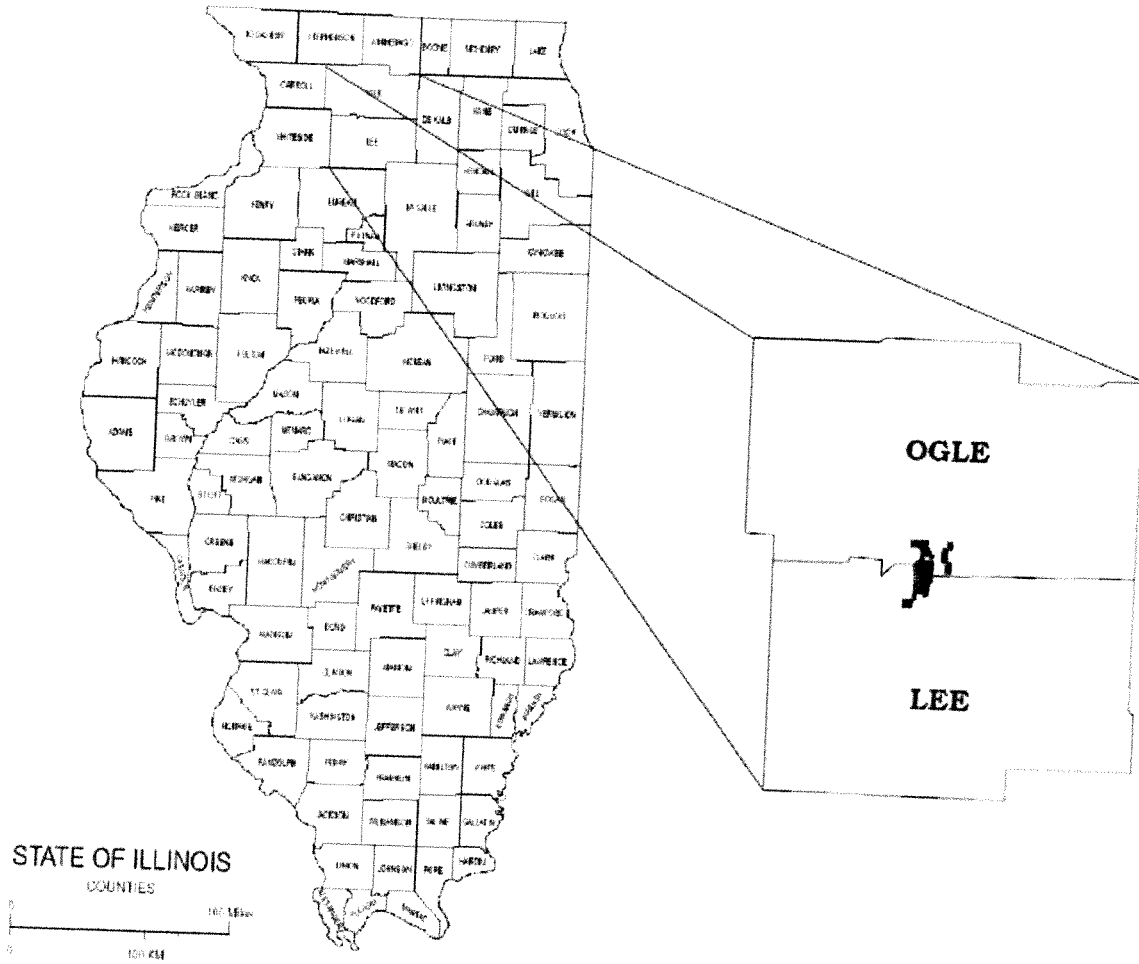


Figure 1. Location of field site at Nachusa Grasslands, The Nature Conservancy, centrally located between Lee and Ogle Counties, Illinois, USA. From Hansen 2009.



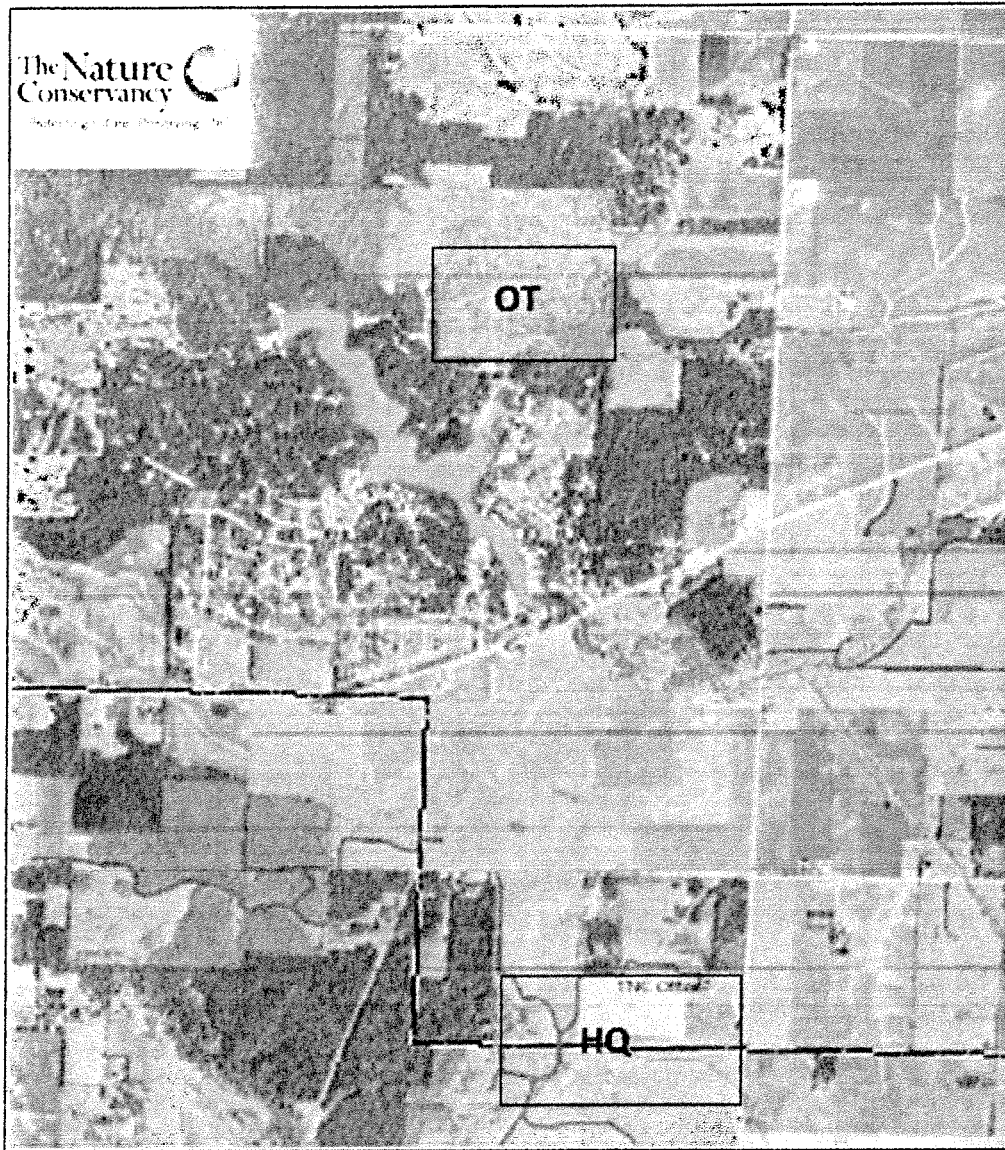


Figure 2. Northwestern segment of Nachusa Grasslands Preserve (green shading) and study sites (HQ = Headquarter's Prairie and Baumgardner Conservation Easement, OT = Orland Tract)  
Figure courtesy of Cody Considine, The Nature Conservancy 2010, Franklin Grove, Illinois.

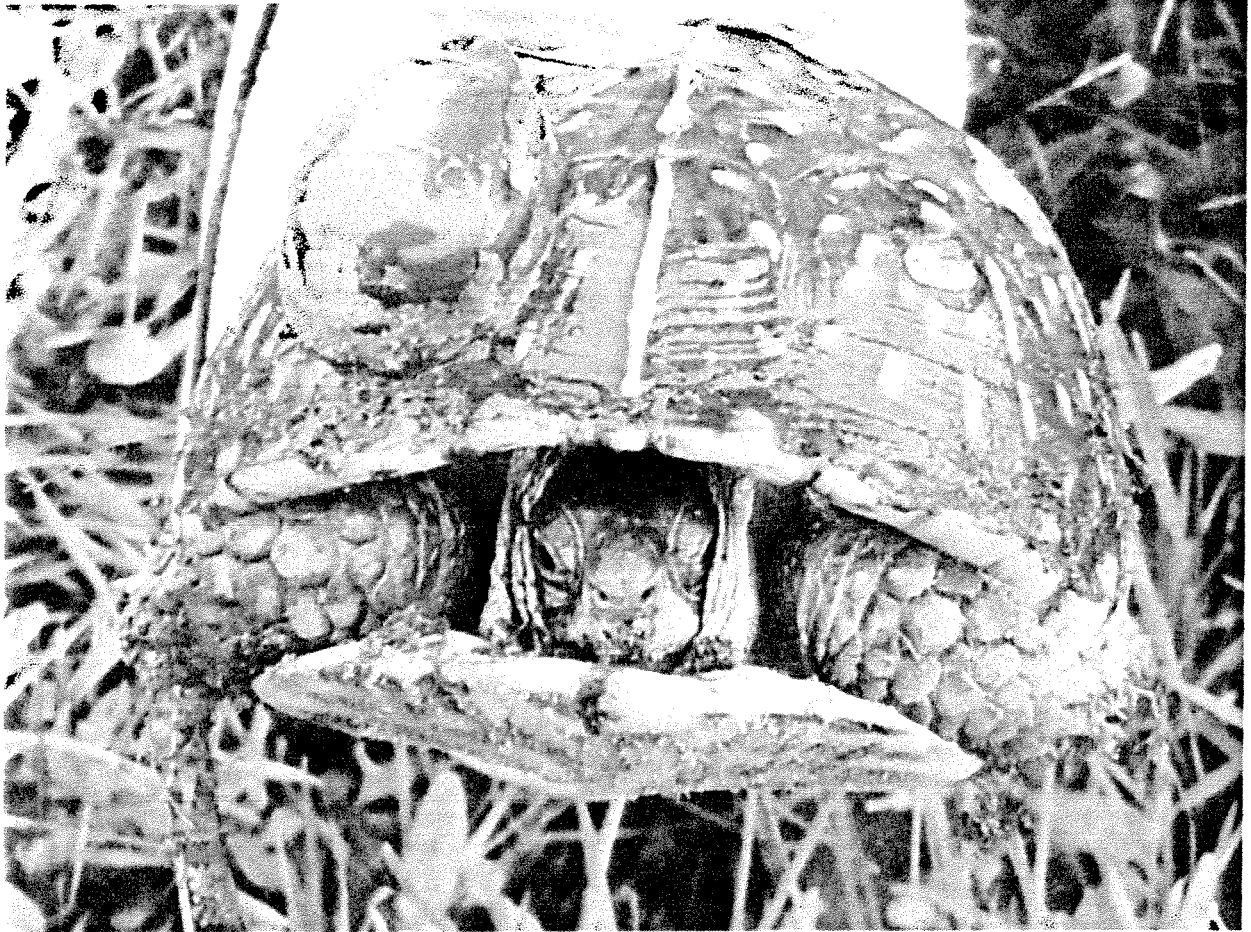


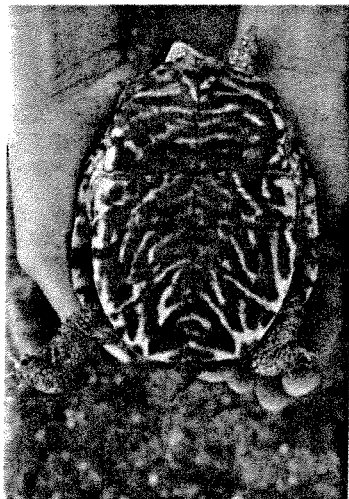
Figure 4. Shellfish FT-LT-97 FLOY tags (Floy Tag & Mfg., Inc., Seattle, Washington) were used as a primary marking system instead of notching or drilling.



June 9, 2010; Bush Clover Rise/LES LEP



September, 2011; Between Sally's and Earls



August 27, 2010; Crossing Lowden Rd



August 9, 2011; Headquarter's Prairie

Figure 5. Digital photographs of turtle plastrons (top: turtle 012; bottom: turtle 009) exemplifying identification of based on unique color patterns. This technique can be used with dorsal carapace and lateral photos.

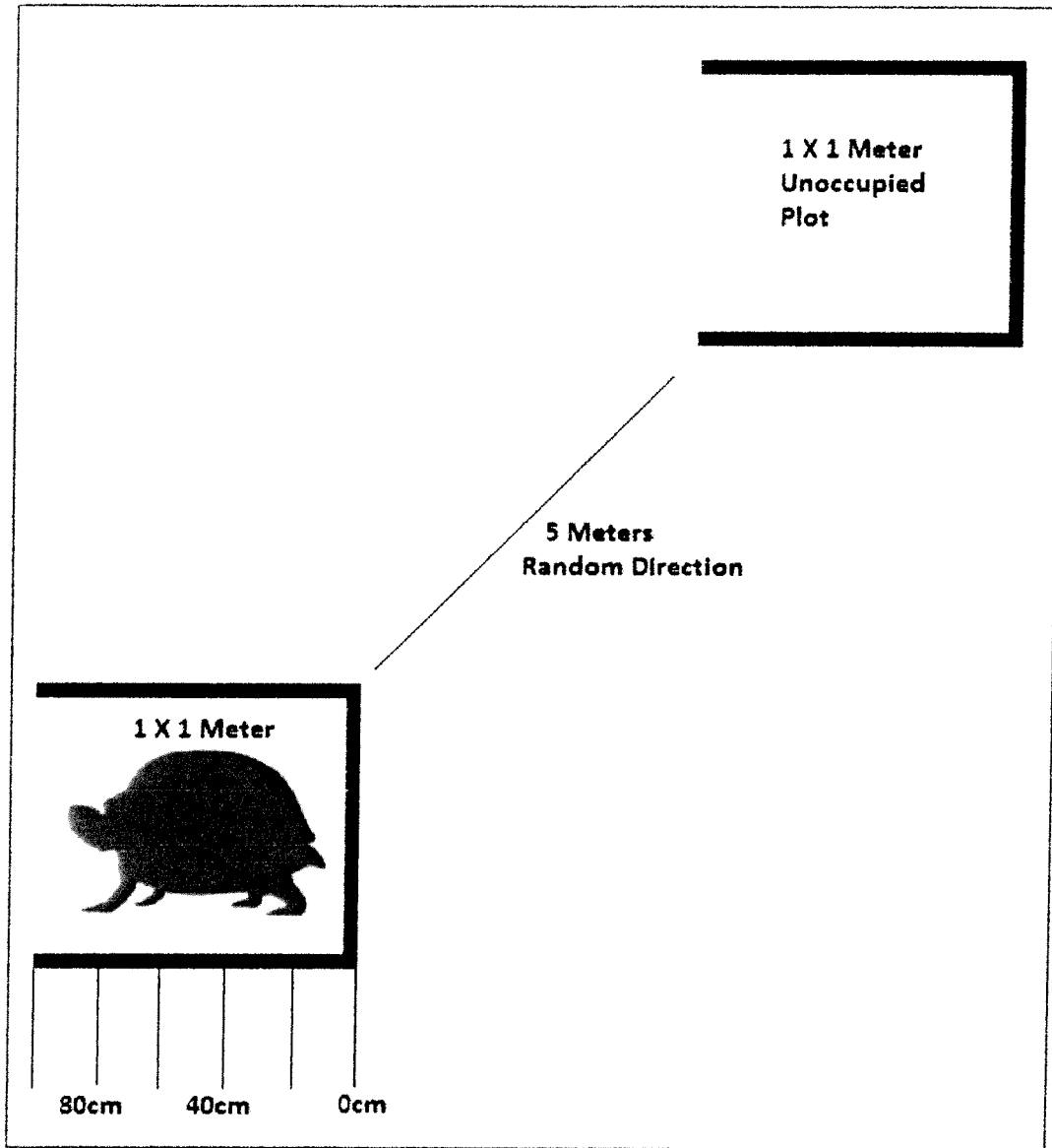


Figure 6. Schematic of paired (occupied, unoccupied) sampling sites for microhabitat selection by ornate box turtles at Nachusa grasslands, 2011-2013. Vegetation measurements are collected within the paired plots, and vegetation height is measured along the left-hand axis of the frame every 20 cm (n = 6).

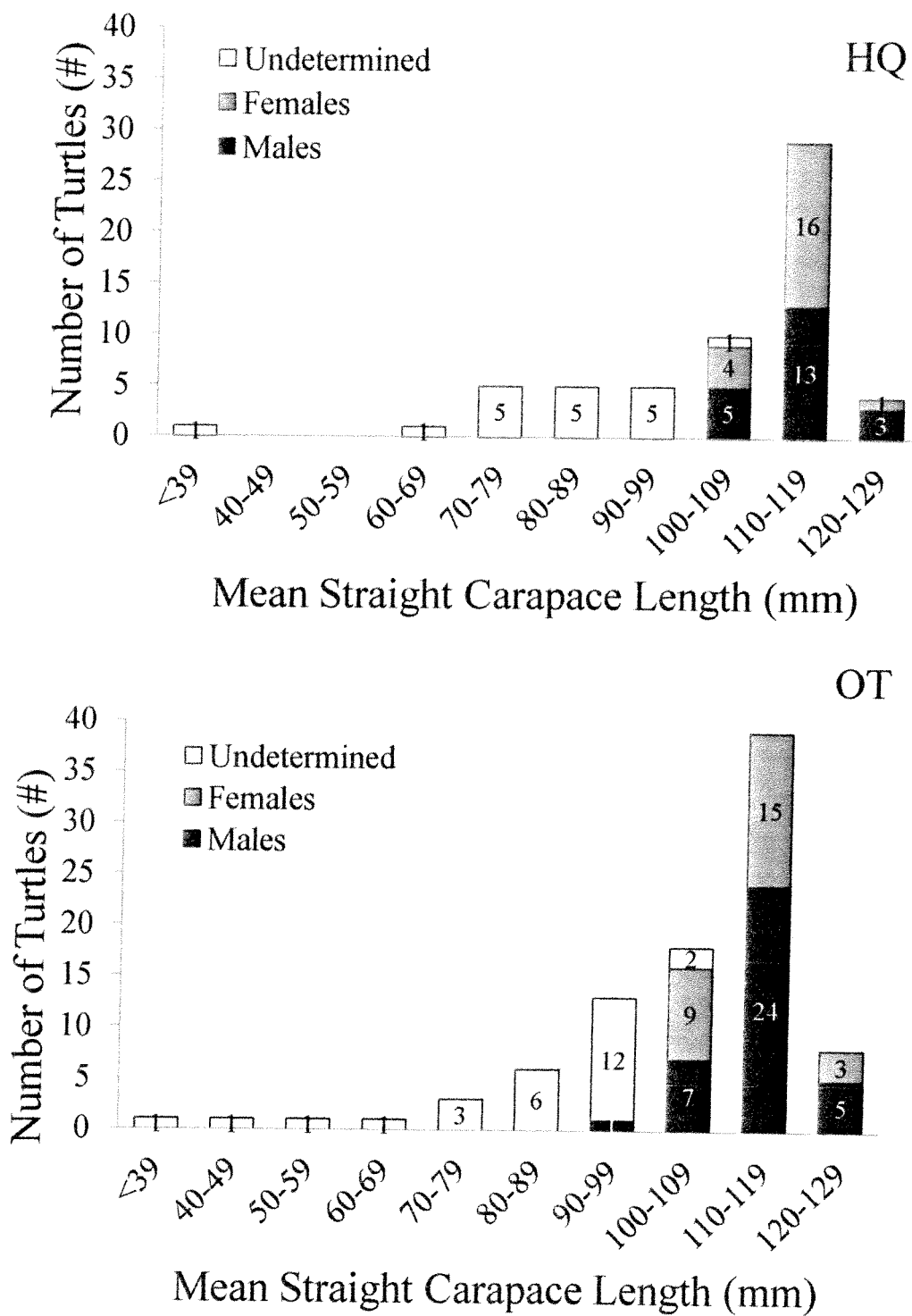


Figure 9. Mean carapace size-class population structures of the Headquarter Prairie (HQ) and Orland Tract (OT), Nachusa Grasslands, Illinois, 2011-2014 (n=151).

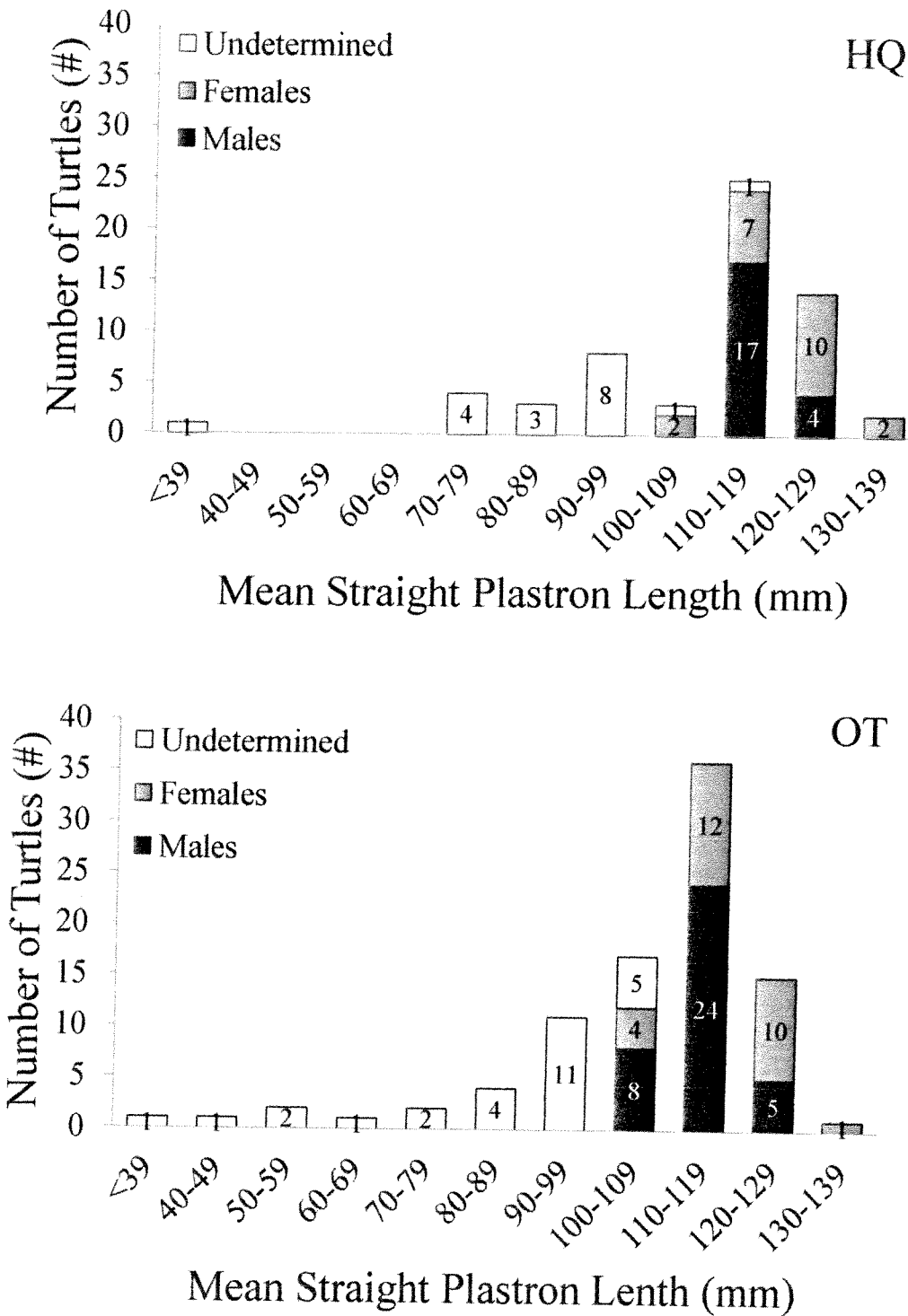


Figure 10. Mean plastron size-class population structures of the Headquarter Prairie (HQ) and Orland Tract (OT), Nachusa Grasslands, Illinois, 2011-2014 (n=151).

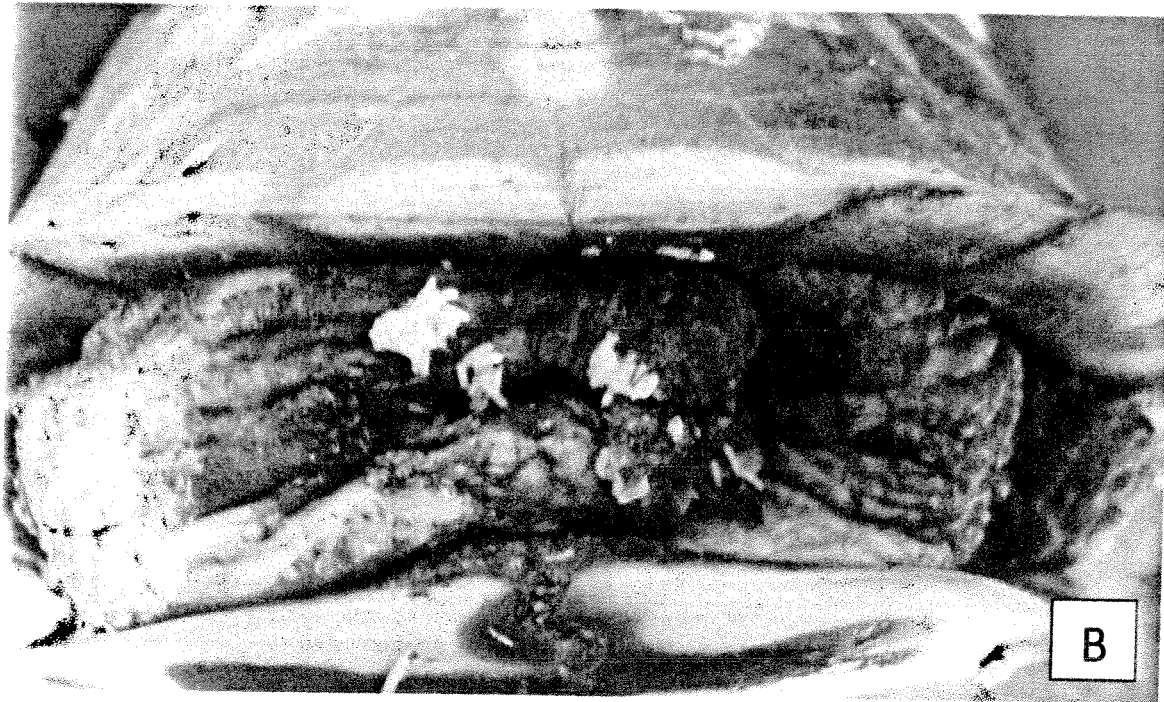
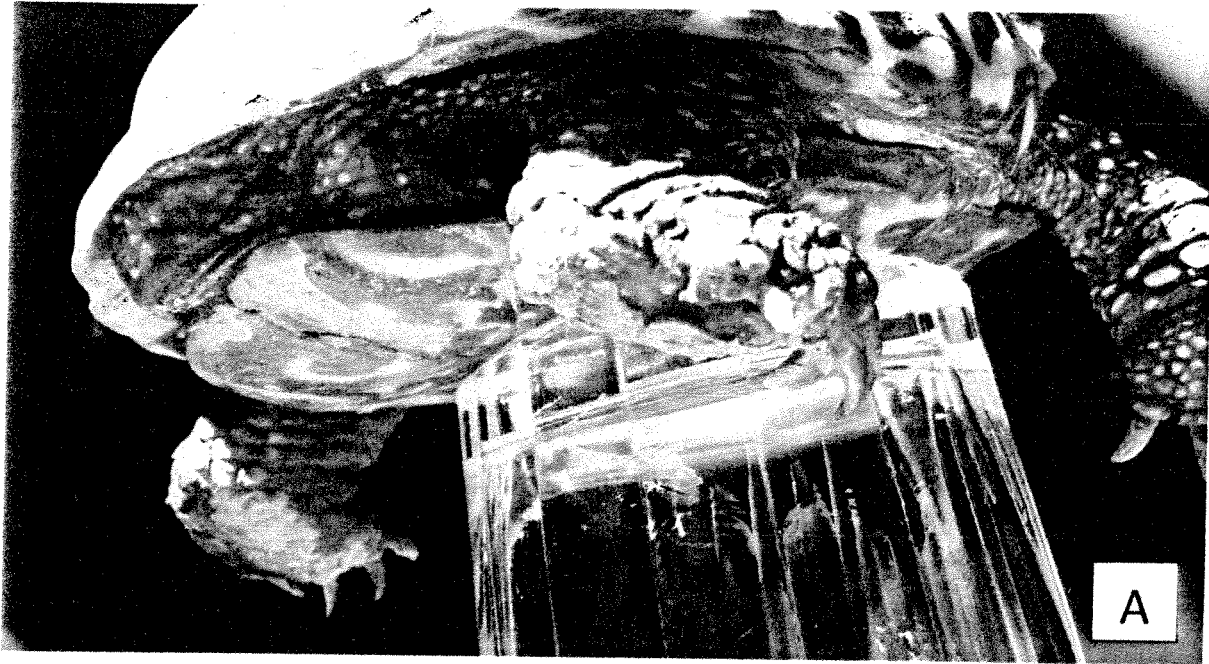


Figure 11. A. Raw and necrotic tissue on the rear feet of male turtle 023, Headquarter Prairie, April 20, 2013. B. Missing tail and maggots on female turtle 030, Orland Tract, 24 July 2013.

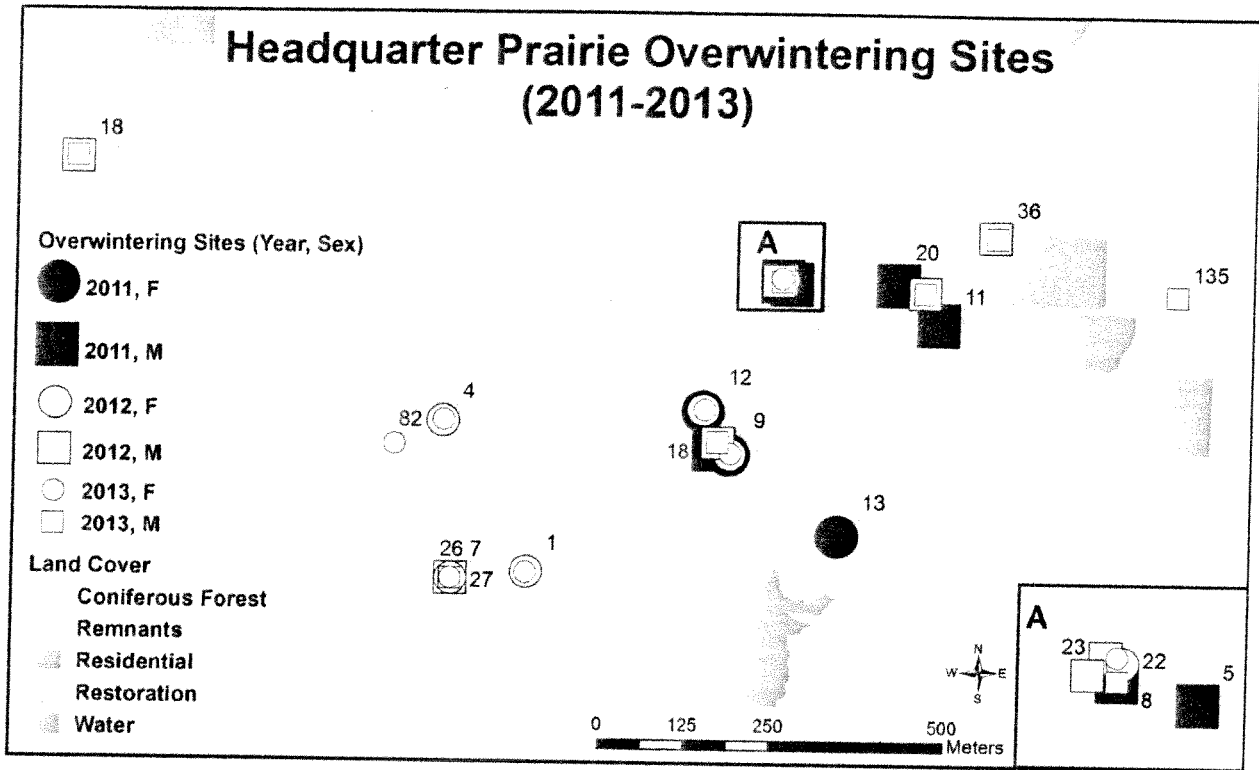


Figure 12. Site fidelity and land cover types used by ornate box turtles at the Headquarter Prairie Site, Nachusa Grasslands for overwintering 2011-2013. Year is indicated by color: black (2011), yellow (2012), and white (2013). Sex is indicated by circle (female) and squares (male). Inset A contains 4 individuals.



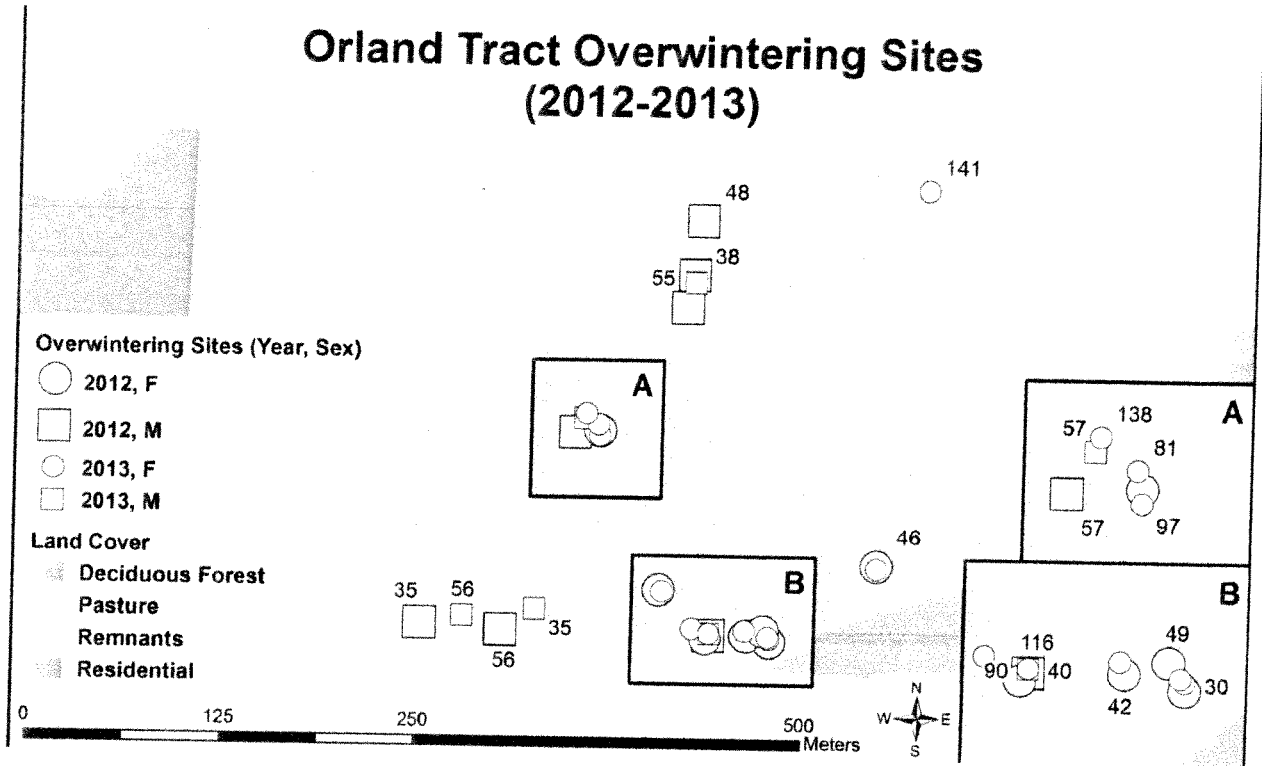


Figure 13. Site fidelity and land cover types used by ornate box turtles at the Orland Tract Site, Nachusa Grasslands for overwintering 2012-2013. Year is indicated by color: yellow (2012) and white (2013). Sex is indicated by circle (female) and squares (male). Inset A contains 4 individuals. Inset A contains 5 individuals. Inset B contains 6 individuals.

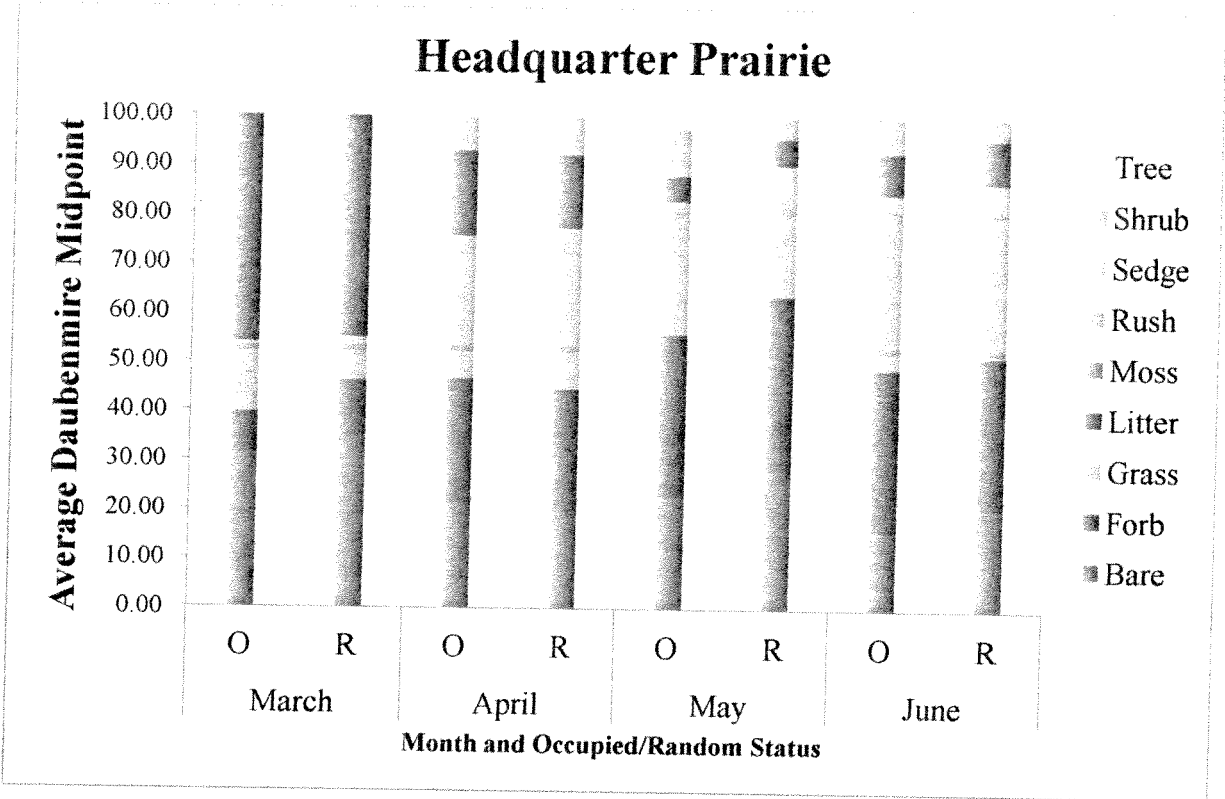


Figure 14. Preliminary cover types used by ornate box turtles at the Headquarter Prairie Site, Nachusa Grasslands March-June 2012.

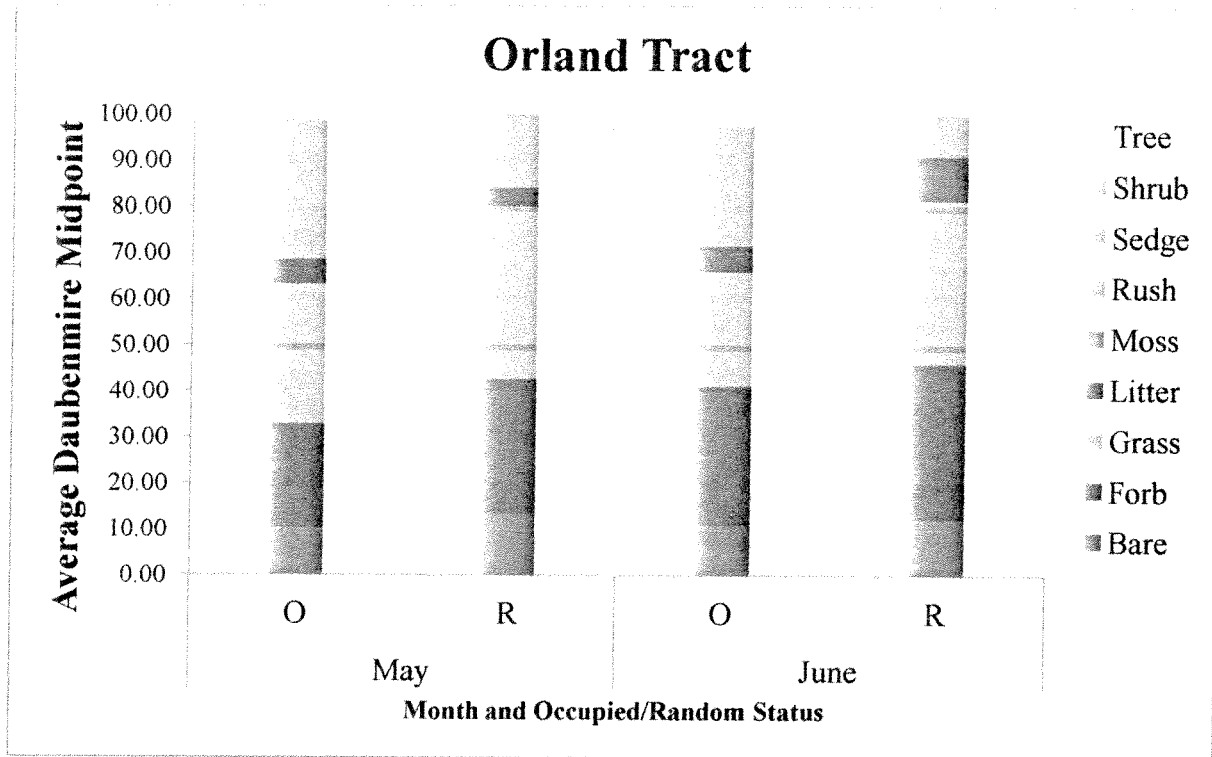


Figure 15. Preliminary cover types used by ornate box turtles at the Orland Tract, Nachusa Grasslands May-June 2012.



Figure 16. 12 June 2013, Turtle 009 observed covering a nest at Headquarter Prairie site, Nachusa Grasslands Preserve, Lee County, Illinois. Vegetation in photo June grass, Kentucky bluegrass, little bluestem, sheep sorrel, Photo courtesy of Tyler Berndt.



Figure 17. Female turtle 116 using thorny thickets for summer habitat use, Orland Tract, Nachusa Grasslands July 2013.



Figure 18. Poster displayed at Nachusa Grassland's Autumn on the Prairie event.

Select Photographs from 2012-2013 field season: [Upon the completion of the project copies of all photos taken or submitted to researchers will be sent to funding agency]



**17 March 2012. An unusually warm winter triggers early emergence of ornate box turtles at Nachusa Grasslands Preserve, Lee and Ogle Counties, Illinois. Photo courtesy of Heather Baker Marshall.**



**22 April 2012. Systematic search for turtles at the Orland Tract site, Nachusa Grasslands Preserve, Ogle County, Illinois. Photo courtesy of Amy Santee.**





**5 May 2012. Kevin Rohling and John Rucker's detector dogs searching for ornate box turtles at the Orland Tract site, Nachusa Grasslands Preserve, Ogle County, Illinois. Photo courtesy of Audra Jervey.**



**13 June 2012. Turtle in a deep, sandy form escaping the drought at the Orland Tract site, Nachusa Grasslands, Ogle County, Illinois. Typically, only the antenna was visible when tracking turtles in June and July. Photo courtesy of Ashley Staat.**



**8 September 2012. Adult male ornate box turtle fitted with a FLOY tag and transmitter.**

**Photo courtesy of Emily Berg.**



**15 September 2012. Kimberly Schmidt giving Autumn on the Prairie visitors a chance to go on a turtle hike and learn about wild ornate box turtles at Nachusa Grasslands Preserve, Lee County, Illinois. Photo courtesy of Lisa Lanz.**



**7 April 2013. Setting up mark-recapture grids Cassandra Wilcoxon and Timothy Stedman came across an eastern hognose snake at the Orland Tract site, Nachusa Grasslands Preserve, Ogle County, Illinois. Photo courtesy of Cassandra Wilcoxon.**



**22 May 2013. Nick Buss, Tyler Berndt, and Kimberly Schmidt collect microhabitat data for a telemetered turtle at Orland Tract site, Nachusa Grasslands Preserve, Ogle County, Illinois. Photo courtesy of Chad Harrision.**

**Education of the general public:**

The ornate box turtle was the wildlife study subject that benefitted from the project. Declines among reptile populations worldwide represent a conservation crisis (Gibbons et al., 2000). There is a limited understanding of microhabitat selection of herpetofauna in tallgrass prairie. Our study will determine the relative suitability of remnant and restored prairies for *T. ornata*, which is unknown. With remnant prairies being small and fragmented, there is a priority to restore land. If box turtles are not using restored prairie, it is crucial to protect remnant prairies as well as determine what restoration practices are needed to improve restorations to act as better surrogate habitat. Results from our study will spotlight preferred microhabitat types for the persistence of a healthy ornate box turtle population. Land managers will use the microclimate, cover, and community data we collect to maintain and restore suitable habitat across their range. Our research advances herpetology because my data are described in greater detail. The only other study addressing the microhabitat selection of *T. ornata* was in Nebraskan shortgrass prairies and eluded that turtles did not use tallgrass prairie (Converse and Savidge, 2003). On the Northeastern fringe of their range turtles use tallgrass prairie, so our data are crucial to the retention of the species. Converse and Savidge (2003) aggregated vegetation cover classes which may reveal incomplete conclusions regarding habitat selection

Kim offered environmental education outreach by research by taking school groups of all ages out on turtle tracking tours when she was available on site. Nachusa Grasslands hosts an annual Autumn on the Prairie event where people of all ages were able to learn about ornate box turtle natural history through a poster (Figure 18), turtle matching game, trapping/tracking informative talk, and educational hikes. Although pre- and post-tests were not created to measure how much the public learned or retained about box turtle natural history, visitors were

informed on a number of topics. Many visitors are unfamiliar with ornate box turtles and are amazed to learn that there are land-dwelling turtles in Illinois. Tours at Autumn on the Prairie that take visitors in the field to see turtles are always full to capacity and are limited to the number of trucks that can run people back and forth to the site. On average, ~30-40 people go on this event to help Kim search for ornate box turtles, see how radio-telemetry works, and listen to a 15 minute talk about natural history over the ornate box turtle. After interacting with Kim, several visitors have e-mailed us photographs of animals they had seen at Nachusa that day or on previous visits. Kim also gained a few volunteers from these groups who have gone onto collect microhabitat data in the summer.



**Total Project Expenditures**

Name and Address of Vendor	Item Description	Quantity Purchased	Date Item Purchased	Cost
Wildlife Materials, Inc., 1202 Walnut Street, Murphysboro, Illinois 62966 Invoice 107042            9/26/2012	refurbish transmitters	4 units repaired	10/17/2012	\$214.50
Wildlife Materials, Inc., 1202 Walnut Street, Murphysboro, Illinois 62966 Invoice 107590            1/23/2013	refurbish transmitters	5 units repaired	2/8/2013	\$252.00
Wildlife Materials, Inc., 1202 Walnut Street, Murphysboro, Illinois 62966 Invoice 110315            4/25/2013	refurbish transmitters	4 units repaired	5/10/2013	\$200.00
Wildlife Materials, Inc., 1202 Walnut Street, Murphysboro, Illinois 62966 Invoice 110368            5/3/2013	refurbish transmitters	5 units repaired	5/22/2013	\$267.00
Southern Illinois University Carbondale, Carbondale, IL 62901	travel to field site	1/2 trip	8/17/2012	\$151.90
Southern Illinois University Carbondale, Carbondale, IL 62901	travel from field site	1/2 trip	8/19/2012	\$151.90
Southern Illinois University Carbondale, Carbondale, IL 62901	travel to field site	1/2 trip	8/24/2012	\$147.84
Southern Illinois University Carbondale, Carbondale, IL 62901	travel from field site	1/2 trip	8/26/2012	\$147.84

Name and Address of Vendor	Item Description	Quantity Purchased	Date Item Purchased	Cost
Wildlife Materials, Inc., 1202 Walnut Street, Murphysboro, Illinois 62966	co-axial cable; commodities	1 cable	6/18/2013	\$15.00
Office Max Online, 6585 N. Illinois St. Fairview Heights, IL 62208	AA batteries; commodities	1, 24 pack	6/20/2013	\$12.38
Office Max Online, 6585 N. Illinois St. Fairview Heights, IL 62208	AA batteries; commodities	1, 10 pack	11/22/2013	\$8.92
Project Cost for IWPF				<b>\$1,569.28</b>
Indirect Project Cost				<b>\$156.93</b>
Total Project Cost				<b>\$1726.21</b>

**Project Expenditures Paid by Other Funds**

Grant Provided By	Awarded	Description of use
Friends of Nachusa Grasslands Grant 2012	\$2000	New transmitters, refurbished transmitters, fuel, GPS units, batteries, and processing gear.
Sigma Xi Grants-in Aid of Research	\$500	Travel to/from the field site.
Chicago Herpetological Society Grant 2013	\$1000	New transmitters, travel, and thermometers
Friends of Nachusa Grasslands Grant 2013	\$1125	New receiver, new antenna, travel, and processing gear.
Friends of Nachusa Grassland Grant 2014	\$800	Travel to/from the field site.
<b>Total</b>	<b>\$5425</b>	