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USE OF WILDLIFE DETECTOR DOGS TO STUDY EASTERN BOX TURTLE (*TERRAPENE CAROLINA CAROLINA*) POPULATIONS

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***Abstract.*—The Eastern Box Turtle (*Terrapene carolina carolina*) is listed as "vulnerable" by the IUCN, and is protected in several states within the Midwestern and Northeastern USA. Population data on this species is critical for design of effective conservation plans. Yet, population studies of box turtles can be difficult to conduct, as their cryptic coloration and behaviors make them challenging survey subjects. Survey techniques commonly employed, such as Visual Encounter Surveys (VES), vary in their effectiveness. We tested how effective wildlife detector dogs (WDD) were at surveying for box turtles. We conducted WDD surveys for 1.5 h on each of two consecutive days in the same 11 ha area. We estimated population size from survey results with a two-sample mark-recapture estimator (unbiased Lincoln-Peterson) and by comparing turtle counts to a measure of capture probability (p_i: the capture probability at sampling occasion *i*). We also used our estimates of population size to calculate number of turtles per unit area surveyed (ha). Our WDD surveys resulted in a total of 25 captured box turtles (including three recaptures on day 2). We estimated population sizes ($\hat{N}$) of 19.5 and 44.5, and determined the number of turtles per area was 1.74 to 3.97 per ha, depending on the population estimator employed. Our measures of p_i equaled 0.67 and 0.33 for each of the two survey days. Surveys with WDD were more effective than VES, which captured only 22 turtles in 316.5 h.**

Key Words.—Eastern Box Turtles; populations *Terrapene carolina carolina*; wildlife detector dogs

INTRODUCTION

More than 50% of modern turtles and tortoises are either threatened with extinction or extinct according to a recent review by the International Union for Conservation of Nature (IUCN. 2010. Turtles of the World, 2010 Update: Annotated Checklist of Taxonomy, Synonymy, Distribution, and Conservation Status. Available from http://www.iucn-tftsg.org/checklist/ [Accessed 17 February 2012]). Threats such as habitat loss and over-harvesting are acutely harmful to turtle populations, due to their low fecundity and slow maturity (Ernst and Lovich 2009). Box turtles (*Terrapene* spp.) are particularly susceptible to such anthropogenic influences (Dodd 2001). The Eastern Box Turtle (*Terrapene carolina carolina*), for example, was once common throughout much of its range (Ernst and Lovich 2009). It is currently listed as "vulnerable" by the IUCN (IUCN. 2010. IUCN Red List of Threatened Species. Version 2011.1. Available from http://www.iucnredlist.org/apps/redlist/details/21641/0 [Accessed 17 February 2012]), and is afforded legal protection within several Northeastern (Connecticut, New Hampshire, New Jersey, New York, Maine, Massachusetts, Pennsylvania, and Rhone Island) and Midwestern (Ohio, Indiana, and Michigan) states. These protections are necessary because, in addition to threats associated with habitat loss and overharvesting, several studies have documented substantial box turtle population declines. For example, declines of 50% to 75% were estimated for populations in Maryland (Stickel 1978; Hall et al. 1999). Williams and Parker (1987) also reported that the number of box turtles per ha at a site in Indiana declined from up to 5.7/ha to 2.7/ha in less than 20 years.

Given (1) the documented population declines, (2) the "vulnerable" status, and (3) the legal protection granted the Eastern Box Turtle, studies on the efficacy of survey methods for cryptic terrestrial turtle species can provide information that will improve many future conservation efforts. For example, better knowledge of effective survey techniques will allow greater ease and accuracy in conducting surveys to monitor this species, including site occupancy estimates and short-term population assessments. Unfortunately, research on box turtle populations, particularly research conducted over a short time frame, faces several obstacles. The secretive habits of box turtles make them difficult to detect during visual surveys and may substantially hamper the potential for recapture of marked individuals. Refsnider et al. (2011) recently reported that detectability of Ornate Box Turtles (*Terrapene ornata*) during Visual Encounter Surveys (VES) was low at a location in Illinois, USA. Furthermore, many studies have required multi-year, long-term, or high intensity sampling to make accurate assessments of box turtle population parameters (Stickel 1978; Williams and Parker 1987; Nazdrowicz 2003; Dodd et al. 2006; Dodd and Dreslik 2008). Developing techniques that are both efficient and effective can establish important baseline information that could be re-visited opportunistically, thus providing short- and long-term benefits toward population status and conservation of target species.

The use of trained domestic dogs (*Canis familiaris)* to find reptiles through olfaction has shown promise as a survey technique (e.g., Cablk and Heaton 2006; Nussear et al. 2008; Schwartz and Schwartz 1991; Sammartano 1994; Stevenson et al. 2010). This method has several applications, which range from confirming presence of species to conducting mark-recapture population studies. The objectives of our study were to measure the detectability of box turtles using wildlife detector dogs (WDD) and to use the counts of turtles detected by WDD to estimate population parameters. We also compared the results of our WDD surveys to VES we conducted at the same site to determine how effective WDD are as a survey technique for Eastern Box Turtles.

MATERIALS AND METHODS

Surveys with trained wildlife detector dogs.—We conducted box turtle surveys with WDD (Boykin spaniels; n = 3) on 12 and 13 July 2011. We focused our efforts within a pre-determined 11.2-ha plot of lowland and upland deciduous hardwood forest, as well as associated riparian habitat, in Alamance County, North Carolina, USA. Lowland deciduous forest was comprised of both mesic forest and alluvial forest tree species, such as Ironwood (*Carpinus caroliniana*), Red Maple (*Acer rubrum*), and Sycamore (*Platanus occidentalis*; Spira 2011). Upland deciduous forest was dominated by oak-hickory communities, which include White Oak (*Quercus alba*), Southern Red Oak (*Q. falcata*), and Mockernut Hickory (*Carya tomentosa*; Spira 2011). Riparian habitat (terrestrial habitat within 10–15 m of stream banks) was also wooded, with vegetative species similar to those in lowland deciduous forests.

All WDDs had been professionally trained to find turtles through olfaction, and had conducted past surveys for numerous species, including *T. carolina*, *T. ornata*, and *Glyptemys muhlenbergii* (John Rucker, pers. comm.). We conducted surveys with WDD as a constrained random walk, where dogs were allowed to wander freely (i.e., not leashed) through the pre-determined survey area. Although dogs worked off-leash, they were under the direction of a handler that used auditory signals to keep them within the general vicinity of human surveyors. We followed a snaking path with WDD, back and forth across the length of the pre-determined search area. We also conducted these parallel sweeps in a manner that did not result in backtracking on areas already searched and that the search area was thoroughly covered. We repeated these sweeps in the same manner on both days.

Surveys with WDD lasted approximately 1.5 h each day, equaling nine total dog h. We initiated WDD surveys at the same time on each survey day (~1000) and the same three dogs conducted surveys on both days. Human observers present during surveys did not partake in the search for box turtles, nor were they allowed to walk in areas that the dogs had not already searched. We held the turtles found by WDD in cloth sacks in a cool location until surveys were completed to avoid accidental turtle recapture during the same survey period. We flagged the turtle capture locations so that individuals could be returned to their origin of capture.

We also conducted VES at this property over the course of 14 months preceding the WDD surveys, although we expended the greatest effort from March to November when turtles were most active. We conducted all VES randomly throughout portions of the property, and the number of surveyors varied by survey event. We attempted to standardize VES by recording the number of “person hours” spent searching (i.e., the amount of time spent searching times the number of surveyors on a given survey day). A total of 316.5 person h were completed throughout the period when VES for box turtles were conducted on-site. Visual encounter surveys were not always constrained to the exact areas as WDD surveys, although we primarily focused them in the same areas. We measured all box turtles captured during VES and WDD surveys to the nearest 0.1 mm with calipers. We gave all individuals captured a unique mark via shell-notch, as outlined by Cagle (1939). We included both adults and juveniles in our population estimates.

Detection probability at sampling occasion i (p_i).—The location of our survey was also the site of an active radiotelemetry project focused on the habitat selection and movement patterns of Eastern Box Turtles. Therefore, we accounted for detection probability as did Refsnider et al. (2011), by having a known number (n = 3) of radio-telemetered turtles within the area at the time WDD surveys were conducted. Telemetered turtles had been affixed with RI-2B, Holohil Systems, Ltd. transmitters (Carp, Ontario, Canada) over a year prior to the initiation of this study, and they were tracked with a Wildlife Materials Inc., TRX-1000S telemetry receiver (Carbondale, Illinois,USA). We determined that radio-tagged turtles were within the potential survey area immediately prior to WDD surveys, but we did not handle turtles at this time to avoid transfer of human scent. Radio-telemetered turtles did not leave the survey area between survey days one and two. We calculated p_i (or the capture probability at sampling occasion *i*) for each survey day by dividing the number of radio-telemetered turtles that the dogs discovered by the total number of radio-telemetered turtles known to be within the survey area (Lancia et al. 2005; Refsnider et al. 2011).

Population estimations (*$\hat{N}$*).—Due to the short time-period over which we conducted surveys, we only made use of techniques for estimating closed populations. We initially estimated abundance via the unbiased Lincoln-Petersen estimator, which is a simple, two-sample period estimator (Krebs 1998). We also calculated the associated population variance developed by Seber (1982) and used the method for calculating the 95% confidence interval described in Lancia et al. (2005). As with all abundance estimators, the unbiased Lincoln-Petersen has associated assumptions that should not be violated. These are that: (1) the population remains closed during the survey period; (2) all animals have an equal likelihood of capture; (3) the marks applied do not impact likelihood of capture; (4) marks cannot be lost between survey periods; and (5) it is possible to report the presence of marks during survey period two (Krebs 1998).

Although we met most of these assumptions, the assumption of equal "catchability", which is often broken in wildlife population studies (Krebs 1998), was more difficult to satisfy. It is plausible that the experience of being captured during the first survey day was an unpleasant one, which may result in captured box turtles emigrating outside of the survey area by the second survey day. We assumed this phenomenon was possible based on the actions of an individual box turtle captured in both WDD survey days. During the 24-h period between captures, this individual moved 15–25 m. Although it was recaptured, the movement occurred within the pre-determined survey area, which may not always be the case. It is possible that other individuals captured during survey day one emigrated out of the survey area due to a negative association with capture, making them "uncatchable" during survey day two. It is also possible that in response to this negative experience, some turtles burrowed below ground, making them potentially uncatchable. However, radio-telemetered turtles known in the area did not move out of the pre-determined survey site between days one and two, so individual response to capture clearly varies. Despite this, we assumed that one or more turtles captured during the first survey may have responded in a way that reduced their chance of being caught in survey day two.

In an attempt to account for violating the equal catchability assumption, we employed equation two from Lancia et al. (2005) to estimate population size ($\hat{N}$) based on box turtle captures during individual survey days:

$$\hat{N} = C/p_i$$

where C is the count of box turtles and p_i is the calculated detection probability (p) for the survey period ($_i$). We can assume p_i for turtles on-site prior to the initiation of our surveys was not influenced by the presence of dogs or humans.

As in Refsnider et al. (2011), we also incorporated Kery's (2002) method for estimating the number of unsuccessful surveys necessary to infer species absence, which can be calculated in the following fashion:

$$N\ min = \log \alpha / \log (1\text{-}p)$$

where $\alpha = 0.05$, and p is equivalent to our p_i. However, unlike Refsnider et al. (2011), we calculated this with WDD survey results. This metric provides a very general guideline for future WDD presence/absence surveys focused on Eastern Box Turtles, assuming habitat and environmental conditions are similar.

The number of individuals per unit area is a metric commonly reported by studies that investigate box turtle populations (reviewed by Dodd 2001; Ernst and Lovich 2009). Therefore, we divided our population estimates by the number of hectares surveyed to determine the number of box turtles per unit area based on WDD captures. We calculated numbers/ha associated with both the unbiased Lincoln-Petersen population estimator and the Lancia et al. (2005) estimator. The areas of all habitat types available on-site were determined via Geographical Information Systems (GIS) using ArcMap Version 9.3 (ArcMap; ESRI, Redlands, California; Table 1).

RESULTS

Wildlife Detector Dogs encountered 25 box turtles across both days. During the first day of surveys, we captured 13 turtles (11 adults and 2 juveniles). During the second day of surveys, we captured 12 turtles (nine adults and three juveniles), three of which were recaptures from day one. The number of turtles we encountered varied based on the habitat where we conducted surveys (Table 1). In contrast, we found only 22 turtles after hundreds of hours of VES (21 adults and 1 juvenile), which included only four recaptures. During day one surveys, we captured two of three radio-telemetered box turtles using dogs ($p_i = 0.67$). On day two, only one of three available turtles was found by dogs ($p_i = 0.33$). The calculated population size ($\hat{N}$) determined from WDD captures varied by the type of estimator employed, and ranged from 19.5 to 44.5 individuals for the survey area (Table 2). We determined that 1.74 to 3.97 Box Turtles/ha were present on-site, depending on the population estimator employed (Table 2). We did not encounter nor recapture box turtles frequently enough during VES to make estimations of population size or capture probability. We determined that the number of unsuccessful WDD

TABLE 1. Habitat composition of area surveyed by trained turtle-finding dogs and the number of Eastern Box Turtles (*Terrapene c. carolina*) found in each habitat across both days (including recaptures). Alamance County, North Carolina, USA.

Habitat Type	Survey Area (ha)	Survey Area (%)	Turtles Found
Lowland Forest	4.44	39.56	7
Riparian	1.76	15.63	10
Upland Forest	5.03	44.81	8
Total	11.23	100	25

surveys necessary to infer absence of this species (*N min*) was 2.7 (rounded up to three surveys) using p_i = 0.67. General environmental conditions varied only slightly during WDD surveys. For example, air temperature ranged from 25.5–26.1 ºC (dew point: 22.2–22.7º C) for both 12 and 13 July 2011. Wind speed varied more substantially between surveys, ranging from 6–7 mph on 12 July to 3 mph on 13 July (National Oceanic and Atmospheric Administration, National Climatic Data Center Online. Available from http://www.ncdc.noaa.gov/cdo-web/search [Accessed 8 May 2012]).

DISCUSSION

Numerous past studies have estimated Eastern Box Turtle population size and numbers per area. For example, estimates of 5.25 turtles/ha (Quinlan et al. 2003/2004) and 9.9–12.4 turtles/ha (Stickel 1950) were reported for study locations in Maryland. Williams and Parker (1987) calculated 2.7–5.7 turtles/ha from 1960 to 1983 at a site in Indiana. Results of surveys at two locations in Virginia provided estimates of 23.8 turtles/ha and 16 turtles/ha (Boucher 1999; Wilson and Ernst 2005, respectively). Nazdrowicz et al. (2008) reported 0.81–3.56 turtles/ha across four study sites in Delaware. Estimates for the Florida Box Turtle (*Terrapene carolina bauri*) range from 39.9–114.4 turtles/6 ha (4.8–10.2/ha; Verdon and Donnelly, 2005) to 415–672 turtles/36.4 ha (11.4–18.5/ha; Langtimm et al. 1996). Our estimates of box turtles per area were similar to some of these past studies (Williams and Parker 1987; Quinlan et al. 2003/2004; Nazdrowicz et al. 2008), and substantially lower than others (e.g., Boucher 1999; Wilson and Ernst 2005; Langtimm et al. 1996).

After hundreds of hours of VES conducted at this same property, we obtained a similar number of box turtle captures to those that resulted from only nine h of WDD surveys. In addition, WDD captured a greater number of juvenile turtles (n = 5) than we found with VES (n = 1), which further supports the value of WDD surveys. We also found that WDD surveys require a relatively minimal survey effort to confirm species presence (*N min*). Thus, our results support that surveys with WDD have advantages over the traditional VES. They can also be conducted efficiently and a large area can be exhaustively surveyed in a short period of time.

Calculating the probability that individuals will be detected during surveys is an important component of wildlife population studies (Armstrup et al. 2005; Lancia et al. 2005). Despite this, many past population studies focused on amphibians and reptiles have failed to include some measure of detection probability (Mazerolle et al. 2007), and currently very few studies focused on box turtles have included such measures (e.g., Dodd et al. 2006; Refsnider et al. 2011). Our findings support that higher measures of p_i can be obtained with WDD, and that this method was more effective than VES for locating Eastern Box Turtles at our study site. Refsnider et al. (2011) discuss the problems associated with use of VES alone for box turtles, which they found resulted in low estimates of p_i (0.03). They suggest the use of WDD as a possible option for box turtle surveys. Our experience with conducting VES for *T. c. carolina* at our study location supports this.

Our results differ from research that has found WDD and humans to be equally effective during surveys for other turtle species (e.g., Desert Tortoises, *Gopherus agassizii*; p = 0.70 for both WDD and VES; Nussear et al. 2008). However, when Nussear et al. (2008) examined detection probabilities by microhabitat, they determined that WDD were more effective at finding Desert Tortoises concealed in vegetation than humans. As a woodland species, the Eastern Box Turtle prefers microhabitats with dense vegetation or surface debris for concealment. Desert Tortoises, on the other hand, are found in more open and arid habitats with less surface debris than deciduous hardwood forests. This may explain the typically low measures of p_i reported for Eastern Box Turtles as a result of VES and further support that WDD are appropriate to implement for Eastern Box Turtle surveys. Furthermore, some research projects are forced to deal with restricted time frames and budgets, which can limit sampling intensity. Yet, we believe such studies can still collect useful population data on Box Turtles if they incorporate short-term WDD surveys.

Although WDD surveys have obvious advantages over VES, they also present challenges. A substantial amount of time and funding may be required to train dogs so that they are effective at finding turtles. As such, it is probably not practical for studies with limited timeframes to expect to train WDD and put them to use in a timely fashion. As an alternative, dog teams already trained to find turtles can be hired, but this also incurs cost. There is the potential for great variation in WDD

TABLE 2. Eastern Box Turtle (*Terrapene c. carolina*) population sizes (including measures of variance and confidence intervals where possible) and turtles/ha calculated using two estimators for the 11.2 ha of area surveyed with WDD, Alamance County, NC, USA. Variance measured using methodology in Seber (1982) and confidence interval measured as described in Lancia et al. (2005).

	Population Estimate	Turtles/ha	Detection Probability	Population Variance	95% Confidence Interval
Unbiased Lincoln-Petersen	44.5	3.97	--	204.75	16.3, 72.6
Lancia et al. (2005)					
Day 1 Surveys	19.5	1.74	0.667	--	--
Day 2 Surveys	36	3.21	0.333	--	--

effectiveness based on factors associated with dog training and implementation. These factors include, but are not limited to: (1) how dogs are trained, such as the reward-system used by trainers, and whether dogs physically retrieve turtles (and the possible injury or spread of disease among turtles in such cases); (2) what dog breeds are employed; (3) the number of dogs employed/survey; and (4) the time spent surveying/unit area. If WDD are to be incorporated more frequently in turtle surveys, and maximum comparability among studies is the goal, we suggest that a set of professional standards be designed (for both the training of WDD and their implementation in field surveys). However, if the goal of WDD surveys is simply to capture individuals for affixing radio transmitters (for example), less standardization is necessary.

Local environmental conditions may further influence how effective WDD are at finding box turtles, although past work has found this to be less significant in regards to Desert Tortoise surveys (Nussear et al. 2008). The day after our surveys concluded, the same group of dogs were used to search for box turtles at a site within 8 km of our study location, which contained similar habitat characteristics. Success at this second site was minimal (one turtle captured in several hours of WDD surveys). Eastern Box Turtles had been confirmed on-site previously, and past surveys with WDD had been highly successful (pers. obs.). The only major difference that we can determine was that surveys at this second property were initiated at approx. 1130, which was 1.5 h after we initiated surveys at our study location on the previous day. Although local environmental conditions were not recorded at this location during WDD surveys, regional average daily air temperature was similar to previous days (22.7–25 ºC; dewpoint 16.1–16.6 ºC; National Oceanic and Atmospheric Administration, National Climatic Data Center Online. Available from http://www. ncdc.noaa.gov/cdo-web/search [Accessed 8 May 2012]). Wind speed during the time of these surveys was reportedly greater than surveys on previous days (9–13 mph).

Time from the last precipitation event may also influence humidity levels at survey sites and survey effectiveness. Microhabitat quality at a given site may influence ambient air temperatures and concomitant efficacy of WDD (e.g., recently disturbed sites, with more open tree canopies may experience higher temperatures and lower moisture levels). We recommend that standardized survey guidelines be created that outline the best conditions (ambient air temperature, wind speed, etc.) under which turtle surveys with WDD be conducted for maximum effectiveness. Such recommendations could be created based on region, habitat, and target species, although further experimental work would be necessary to accomplish this. It is, unfortunately, difficult to plan WDD surveys around suitable environmental conditions, particularly if dogs must be scheduled for surveys in advance and must travel from some distance to conduct surveys. The level of uncertainty associated with environmental conditions and planning box turtle surveys may be particularly problematic for studies with short timeframes or limited resources.

An alternative method to using radio-telemetered turtles for calculating capture probabilities would be to conduct a "preemptive" WDD search of the survey area with dogs that will not be used in later surveys. All turtles captured during preemptive surveys can be marked so that a number of known, identified animals exist within the survey area for calculating p_i during later WDD surveys. During later surveys, a new set of survey dogs can then search the survey area (so that a potential "memory" of where turtles were found in preemptive surveys does not confound the outcome of later surveys). The ratio of marked turtles captured during later WDD surveys to the number of turtles marked during preemptive surveys would result in a measure of p_i. If later surveys are conducted immediately after preemptive surveys, then turtles that would migrate out of the survey area due to the negative experience of capture will hopefully not have had enough time to leave the survey site. Care must be taken to ensure that dogs in later surveys do not find turtles by following the scent trails of dogs used in preemptive surveys, as this could also confound the results. More research on this topic is necessary, which includes investigation into whether p_i consistently decreases over multiple WDD survey periods because of a negative behavioral response by turtles.

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JOSHUA M. KAPFER (holding turtle), (pictured with Boykin Spaniel owner, John Rucker, and two Eastern Box Turtles, *Terrapene carolina carolina*) received his Ph.D. in Ecology and Evolution from the University of Wisconsin-Milwaukee in 2007. He is broadly interested in vertebrate ecology and conservation, including wildlife habitat preferences, spatial ecology, population biology and community ecology. Josh is currently an Assistant Professor in the Department of Biological Sciences at the University of Wisconsin-Whitewater (Wisconsin, USA), and is an Associate Researcher at the University of Wisconsin-Milwaukee Field Station (USA). (Photographed by T. Tomasek).

DAVID J. MUÑOZ (pictured here holding an Eastern Ratsnake, *Pantherophis alleghaniensis*, captured in a drift fence) completed his B.S. in Environmental and Ecological Science at Elon University (North Carolina, USA) in Spring 2012. He began participating in herpetofaunal and small mammal surveys during the second year of his undergraduate degree and has become involved in a variety of field-based ecological studies since. During the summer of 2012, David worked as a Biological Intern for the U.S. Fish and Wildlife Service at the Wallkill River National Wildlife Refuge. He will then pursue future graduate studies in wildlife ecology and conservation biology. (Photographed by J. Kapfer).

TERRY M. TOMASEK (left) (pictured [with hat] teaching a group of students) received her Ph.D. (2006) in Science Education from the University of North Carolina at Greensboro. She is broadly interested in herpetology and engaging high school students in various scientific investigations. Terry is currently an Associate Professor in the Department of Teacher Education at Elon University (North Carolina, USA). (Photographed by C. Matthews).

Errata: Some minor corrections to the text were made by the author 22 September 2012. These did not change the context or outcomes of the study.