Small and large-scale landscape approaches for conservation of the imperiled Blanding's
turtle, *Emys blandingii*

by

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CHAPTER 1. INTRODUCTION

Recent decades have witnessed the world-wide decline of numerous species. The World Conservation Union (IUCN) classifies, out of 29,354 evaluated animal species, 698 species as extinct in the wild, 1,549 critically endangered species, 2,284 endangered species, and 4,017 vulnerable species (IUCN Red List 2007). Plant species have also experienced sharp declines in recent years, with 87 species extinct in the wild, 1,569 critically endangered species, 2,278 endangered species and 4,600 threatened plant species; 12,043 plant species were evaluated for the 2007 listing (IUCN Red List 2007). Worldwide, reptiles have been particularly devastated by recent declines: for class Reptilia, 422 out of 1,385 (30%) reviewed species are either extinct in the wild (23 species), critically endangered (79 species), endangered (139 species) or threatened (204 species) (IUCN Red List 2007). In the United States, 32 reptile species are listed as critically endangered, endangered or vulnerable (IUCN Red List 2007). Given these recent dramatic declines, reptiles are one group, both within the U.S. and world-wide, in need of serious conservation effort.

In order to combat the current conservation crisis, conservationists, researchers and managers need to develop an understanding of the factors most responsible for species decline, and how to combat the impacts of those factors. There are numerous threats that can contribute to species decline, and these are broadly defined in two categories: 1) exogenous, or those threats originating independently of the species' biology and 2) endogenous, or those threats originating as part of the species' biology (Fischer and Lindenmayer 2007). These two broad categories are further divided into 1) deterministic threats, which predictably lead to the decline of a species and 2) stochastic threats, which are driven by chance events (Fischer and Lindenmayer 2007). Exogenous, deterministic effects are broadly defined as

habitat change, including habitat degradation, loss, subdivision, isolation and fragmentation. Disruptions to behavior, such as dispersal patterns or species biology, are considered endogenous, deterministic threats. Exogenous, stochastic threats include environmental stochasticity and natural catastrophes, whereas endogenous stochastic threats are demographic and genetic stochasticity (Fischer and Lindenmayer 2007).

However, despite the numerous threats facing species, it is often exogenous, deterministic effects (habitat loss, etc) that cause initial population declines. Indeed, some form of habitat modification, be it degradation, fragmentation or loss, has been cited as the cause of numerous species' declines (Andrn 1994, Hobbs and Yates 2003, Kerr and Currie 1995, Arajo et al. 2006, Carrete et al. 2007, Fischer and Lindenmayer 2007, McKenzie et al. 2007, Mora et al. 2007, Nichols et al. 2007). Habitat modification and degradation has been estimated to impact 86% of threatened bird species, 88% of threatened amphibian species, 86% of threatened mammal species and has also been indicated as a significant cause in the decline of marine and freshwater fish populations (IUCN Red List 2007). Habitat modifications can have numerous negative impacts on species. Habitat degradation, defined as the gradual deterioration of habitat quality, can either cause the decline of a species, lower density or impact reproductive success by reducing the probability of finding mates or suitable nesting habitat (Fischer and Lindenmayer 2007). Habitat degradation can be difficult to detect, and may be deceiving in that long-lived species may continue to be present but are unable to breed. Habitat sub-division, defined by Fischer and Lindenmayer (2007) as the breaking apart of continuous habitat into multiple patches, can cause population declines by limiting the availability of resources or increasing the isolation of remaining habitat. In turn, habitat isolation impacts day-to-day movement of individuals between nesting and

foraging reserves (Luck and Daily 2003), affects the recruitment and dispersal of individuals (Cooper and Walters 2002) and finally limits large movements, such as seasonal migrations or range shifts in response to global climate change (Araújo et al. 2006, Fischer and Lindenmayer 2007, Mora et al. 2007, Wake 2007).

Species directly and negatively affected by exogenous, deterministic threats (e.g. habitat loss) are then more susceptible to endogenous deterministic threats. Endogenous threats include disruptions and changes to dispersal patterns (Brooker and Brooker 2002, Cooper and Walters 2002, McNerny et al. 2007, Norris and Stutchbury 2001) and shifts in home ranges (Pope et al. 2004). Another endogenous threat experienced by species occupying modified habitat is changes in species interactions, such as competition, predation, parasitism and mutualism (Ryall and Fahrig 2006, Fischer and Lindenmayer 2007). Habitat fragmentation has been recently implicated in causing the collapse of pollination webs in conservation areas of insufficient size to support generalist pollinators (Pauw 2007). Also, changes in the interactions of keystone species, those species of disproportionate effect in a community, can have particularly severe effects on the overall operation of the ecosystem. Recent research has suggested Neo-tropical deforestation may cause the extinction of keystone species, such as the army ant, *Eciton burchelli*. Given the numerous impacts this species has on neo-tropical ecosystems, including promoting biodiversity by creating habitats of different successional stages, numerous biological associations and flushing out prey items, their extinction would almost certainly trigger an extinction cascade (Boswell et al. 1998).

Once species numbers have been initially decreased due to endogenous and exogenous deterministic effects, they are more susceptible to exogenous and endogenous

stochastic threats. Stochastic, exogenous threats include environmental stochasticity and natural catastrophes. Environmental stochasticity has been a major factor impacting already imperiled species or populations and has been shown to increase extinction risk (Bull et al. 2007). Additional research suggests that the threat of environmental stochasticity may increase as the range of climatic conditions experienced continue to increase in the face of global warming and poses a serious threat to global biodiversity (Saltz et al. 2006).

The final blow for many species comes in the form of endogenous, stochastic threats; demographic and genetic stochasticity. Demographic stochasticity is found in year-to-year variation in demographic characteristics, such as birth and death rates, reproductive success, etc (Ezard and Travis 2006). Genetic stochasticity is found in the random fluctuations of gene frequencies from generation to generation (genetic drift) and may lead to the fixation of deleterious alleles within populations (Ezard and Travis 2006). The only way to combat fixation is through mutation and migration; however, if populations are isolated because of habitat fragmentation, the migration of genetically distinct individuals into fixed populations may not be frequent enough to counter any negative effects of genetic drift. Even if populations are able to numerically recover from habitat loss, fragmentation or natural catastrophes, the loss of genetic diversity during a population bottleneck may not be recovered, and can pose serious new threats to the population. As an example, the lions of Ngorongoro Crater, Tanzania, have historically been a small and isolated population. In 1962, an epizootic swept through the crater and reduced the population to nine females and one male. Since then, there have only been seven immigrants into the crater, and none since 1965. By 1975, the population had recovered to historic population numbers, but all members are descended from only 15 founders. Recent work has shown that this population

shows significantly less genetic heterozygosity than the surrounding Serengeti populations (Packer et al. 1991). Male lions in the crater also have higher levels of sperm abnormality, resulting in decreased reproductive performance (Packer et al. 1991). Despite numerically recovering from the epizootic, persistent problems, as a result of the population bottleneck, plague the population.

Therefore, conservation biologists and managers are responsible for understanding and then mitigating the threats to populations on two main fronts: 1) habitat/landscape changes and 2) genetic/demographic processes. Since these two threats both contribute to species extinction, a broad, two-prong scientific approach is necessary to ensure that all threats faced by a population are combated, and that conservation efforts are focused appropriately. Information gathered from research investigating both these threats experienced by imperiled species can provide powerful insights into their conservation and management.

Numerous tools are available for conservationists and managers to assess the threats faced by species, and the resultant impact of those threats on species integrity. These tools include approaches for investigating how mobile species experience habitat and landscape changes. On the habitat scale, specific for one or several small populations, is radio-telemetry. By attaching transmitters and following individuals, it is possible to gain information on daily habitat use, seasonal differences in habitat use, and a variety of other factors relating to individuals of a specific population at a particular habitat scale. While this method provides detailed information for a specific population, it is limited in the generalization of patterns to other populations of the same species inhabiting different locations. An approach for investigating large-scale landscape responses and patterns for a

species is geographic information systems, or GIS. Increases in computing power and access to aerial images have greatly increased the use of GIS in recent years, especially with conservation applications. Some of these uses include identifying potential restoration sites and habitat suitability modeling (Stockwell and Peters 1999, Van Lonkhuyzen et al. 2004, Fernandez et al. 2006, Thompson et al. 2006) and confirming phylogeographic results (Kidd and Ritchie 2000, 2001, Ritchie et al. 2001). Using GIS and accompanying software allows for a broader investigation of how species respond to landscape changes at a much larger scale than radio-telemetry studies. By combining radio-telemetry studies with larger-scale GIS studies, much information can be gathered on not only habitat-specific characteristics, but also how species respond to landscape changes and how those changes impact daily habitat use. This information is critical for ensuring survival of populations and lowering species' extinction risk.

Other important modern tools available to conservation biologists and managers include genetic approaches, such as micro-satellites and polymerase chain reaction (PCR) technology. Micro-satellites, in many cases, are used for understanding genetic structure within and among populations, and can help researchers understand genetic threats to species (Kuo and Janzen 2004, Mockford et al. 2005, Rubin et al. 2004, Tessier et al. 2005). PCR technology, using phylogeographically informative markers, allows for investigations into a species history and distribution of genetic variation across the range and has been used in a variety of ways for not only gathering information on a species, but also informing conservation and management plans (Avice 1992, Spinks and Shaffer 2005, Templeton et al. 1995 and ref. within)

Both sets of tools provide valuable information and insight into how species are responding to changes of their surrounding habitat and landscape. Ideally, information from both sets of tools should be used in formulating conservation plans, but in the absence of any data, the first approach should probably be to employ the landscape/habitat tools. By first using radio-telemetry and GIS for highly mobile taxa, it is possible to develop hypotheses about how habitat and landscape changes have impacted and are shaping genetic structure within and among populations.

One group of organisms that has been severely impacted by anthropogenic changes is chelonians. Recent years have witnessed a rapid decline in many turtle species; in class Testudines, seven species are extinct, one species is extinct in the wild, twenty-six are critically endangered, forty-six are threatened and sixty are vulnerable (IUCN Red List 2007). There are several reasons that this group is especially susceptible to anthropogenic disturbances, including delayed sexual maturity, low survival in early life-history stages, and specific habitat preferences (Congdon et al. 1993). Also, some turtles are over-exploited in parts of the world, either over-harvested as a food source or as incidental kills in the fishing industry. While much deserving and necessary effort is being made to protect those chelonian species already listed, efforts also need to be focused on studying those species not yet identified or listed as needing special attention. Perhaps by taking a preventive approach, it will be possible to understand which species are on the verge of needed protection, and mitigate those threats before more drastic action is required. One chelonian species in need of preventative conservation effort is the Blanding's turtle, *Emys* [formerly *Emydoidea*] *blandingii*.

The Blanding's turtle, *Emys blandingii*

The Blanding's turtle, *Emys blandingii*, is a semi-aquatic, freshwater species with a range centered in the Great Lakes region of North America, extending north into southern Canada, west through Nebraska, with scattered populations along the eastern seaboard (McCoy 1973). There are several populations throughout the range that have been well studied, producing much of the life history knowledge of this species. These long-term research projects are located at Weaver Dunes and Camp Ripley, Minnesota (Pappas et al. 2000, Sajwaj et al. 1998 and refs. within), University of Michigan's E.S. George Reserve (Congdon et al. 2000 and refs. within), the sandhills of western Nebraska (Germano et al. 2000, Dinkelacker et al. 2005 and refs. within), and Kejimikujik National Park, Nova Scotia (McMaster and Herman 2000, Mockford et al. 2005 and refs. within). There have also been several smaller studies conducted on populations in Massachusetts (Graham and Doyle 1979), New York (Hartwig and Kiviat 2007), Ontario (MacCulloch and Weller 1988), Wisconsin (Ross and Anderson 1990), Indiana (Barlow 1999), Illinois (Rowe and Moll 1991) and Missouri (Kofron and Schreiber 1985).

Collectively, these studies have investigated numerous aspects of life history and ecology of Blanding's turtles, including habitat use and movement (i.e., Rowe and Moll 1991, Hartwig and Kiviat 2007), nesting behavior and ecology (i.e., Pappas et al. 2000) and population demography (i.e., Congdon et al. 1993). The combined results of these studies demonstrate that *E. blandingii* exhibits several key life history-characteristics, such as delayed sexual maturity, long distance nesting migrations and narrow habitat preferences that makes them susceptible to anthropogenic disturbances (Ross and Anderson 1990, Rowe and Moll 1991, Germano et al. 2000, Joyal et al. 2000). As a result, Blanding's turtle is listed as

endangered, threatened, of special concern or in need of conservation throughout most of its range (Rubin et al. 2004, Mockford et al. 2005) (Fig. 1).

However, despite the several large, long term studies and the numerous smaller investigations into the biology of these turtle populations, there are still several significant knowledge gaps for this species. One knowledge gap is the lack of habitat use data for populations in continuous wetland habitat; most of the previous studies have been conducted in a wetland matrix habitat. A second knowledge gap is the lack of studies investigating larger-scale habitat modifications and how those modifications impact various life history and demographic characteristics of populations. A third knowledge gap concerns the lack of published results investigating Blanding's turtles in Iowa and Illinois, a central part of the species' range.

Therefore, to further our understanding by filling knowledge gaps and to possibly prevent the federal listing of this North American species, this project investigates the Blanding's turtle at two levels. First, I used radio-telemetry technology to explore how a specific population utilizes both the aquatic and surrounding terrestrial habitat of a continuous wetland habitat, and second, I analyze how large-scale landscape changes have impacted the reproductive success of Blanding's turtle populations across the state of Iowa using GIS. The results from this study, combined with previous research, provide powerful insights for designing effective conservation strategies for this species.

Thesis Organization

Following this introduction, the second chapter investigates home range and habitat use for a population of Blanding's turtles in a human impacted area in Illinois. The third chapter assesses the impact of various landscape features and soil characteristics on the

reproductive success of Blanding's turtle populations across Iowa using a Geographic Information Systems (GIS) approach. The final chapter re-visits significant results from the two data chapters, offers some suggestions for conservation strategies of the species and ends with suggestions for future work. These chapters are then followed by several appendices, which include additional results pertinent to the research presented in two data chapters, as well as summaries of progress made on various side projects.

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Figure 1. Range map and status of Blanding's turtles in North America.

CHAPTER 2. HOME RANGE SIZE, MOVEMENT AND HABITAT USE FOR A POPULATION OF BLANDING'S TURTLES (EMYS BLANDINGII) IN A HUMAN IMPACTED AREA.

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Introduction

Recent decades have witnessed the decline of numerous species throughout the world. Of the many threats faced by these species, habitat fragmentation and alteration have been cited as the leading causes of decline (Andrén 1994, Gibbons et al. 2000, Hobbs and Yates 2003, Araújo et al. 2006, Carrete et al. 2007, McKenzie et al. 2007, Mora et al. 2007, Nichols et al. 2007). Population declines often occur initially due to habitat fragmentation and alteration. This initial decline then results in higher susceptibility to exogenous and endogenous stochastic effects, such as natural catastrophes, environmental stochasticity, and genetic and demographic stochasticity (Fischer and Lindenmayer 2007). The detrimental effects of these stochastic threats are much more pronounced in small populations, and create the situation where, even if populations are able to recover in numbers, they may persist in having lowered genetic diversity (Packer et al. 1991). Although all threats warrant detailed examination, starting with investigations into how habitat fragmentation and alteration are impacting a population has several advantages: 1) may provide information that could ultimately be used to prevent further decline (via exogenous and endogenous threats) and 2) may provide testable hypotheses for genetic studies.

Conservation biologists and managers are thus charged with not only investigating the threats faced by imperiled species, but also suggesting and implementing strategies to

mitigate damage. There are numerous tools available for this task, including landscape based approaches such as radio-telemetry or geographic information systems (GIS). Landscape tools also allow for investigation of a variety of questions, but vary in the scope.

Specifically, radio-telemetry studies allow for detailed analysis of habitat use and movement of individuals within populations. In contrast, GIS studies allow for much larger scale analysis of how landscape alteration affects populations across a wide range of habitat conditions and also provide a platform for much more powerful analyses of landscape scale data (Clark and Slusher 2000, Fernandez et al. 2006, Fischer et al. 2007, Thompson et al. 2006).

Turtles have been particularly decimated by recent habitat fragmentation and alteration because they have several life-history characteristics that make them susceptible to anthropogenic disturbances. Because of delayed sexual maturity, low survival in early life-history stages and specific habitat requirements, turtles may be unable to respond quickly to the rapidly changing habitat (Congdon et al. 1993). Therefore, to combat further declines in turtle populations, more research is needed to understand how anthropogenic habitat alterations are impacting these populations. One approach for investigating the effects of habitat alteration is radio-telemetry studies. Results from these investigations are critical in developing effective conservation plans by providing information on the minimum amount of habitat required for sustaining individuals in populations or identifying other critical areas of habitats, such as nesting or foraging grounds.

One North American chelonian threatened by habitat fragmentation is the Blanding's turtle, *Emys* [formerly *Emydoidea*] *blandingii*, a semi-aquatic, freshwater species with a range centered in the Great Lakes region of North America, extending north into southern

Canada, west through Nebraska, with scattered populations along the eastern seaboard (McCoy 1973). This wetland-dwelling species is specific in its habitat requirements, requiring slow-moving, shallow water areas and surrounding terrestrial habitat for overland migrations and nesting (Ross and Anderson 1990, Rowe and Moll 1991, Pappas et al. 2000, Piepgras and Lang 2000). Previous research shows three types of movement: 1) movements within activity centers, 2) movements among activity centers and 2) long-distance nesting migrations and terrestrial forays (Ross and Anderson 1990, Rowe and Moll 1991, Piepgras and Lang 2000, Refsnider 2005). Because of the draining of wetlands for conversion to agriculture land and other significant habitat alterations, Blanding's turtle is listed as endangered, threatened, of special concern or in need of conservation throughout most of its range (Hartwig and Kiviat 2007, Kofron and Schreiber 1985, Rubin et al. 2004, Mockford et al. 2005). In Illinois, this species is listed as threatened (Rubin et al. 2004)

The purpose of this study is two-fold. Previous radio-telemetry studies for this species have been conducted in wetland matrix (Ross and Anderson 1990, Rowe and Moll 1991, Piepgras and Lang 2000); in contrast, this study investigates Blanding's turtle movement in a continuously aquatic environment. Secondly, this study also examines this turtle's terrestrial habitat use in a human-impacted area.

Methods

Study Site

This study took place at the Thomson Causeway Recreation Area (TCRA) and Mickelson's Landing, which is 3.5 km south of the TCRA. Both these sites are part of the Upper Mississippi River National Fish and Wildlife Refuge (UMRNFWR). The TCRA consists of an island, with the main channel of the Mississippi River bordering the western

edge and a slough bordering the eastern edge (Figure 1.1). The slough was dredged in the mid to late 1990s, with water depth averaging 2 m in dredge cuts and 0.3 m outside the dredge cuts. The main vegetation in the slough includes emergent plants, such as broad-leaved and stiff arrowhead (*Sagittaria* spp.), giant bur-reed (*Sparganium eurycarpum*), softstem and river bulrush (*Scirpus* spp.), submersed plants including coontail (*Ceratophyllum demersum*), Canadian waterweed (*Elodea canadensis*), curly-leaf, leafy, small and flatstem pondweed (*Potamogeton* spp.), water stargrass (*Zosterella dubia*), and wild celery (*Vallisneria americana*). Rooted floating leaved plants include American white waterlily (*Nymphaea odorata*) and American lotus (*Nelumbo lutea*) and non-rooted floating leaved plants, such as common, greater and star duckweed (*Lemna* and *Spirodela* spp.), and Columbian watermeal (*Wolffia columbiana*). The eastern shore of the slough is bordered by a narrow wooded area, and a sand prairie upslope. This sand prairie extends 75-150 m inland from the slough, and is bounded to the east by a bike path and fence. The predominant vegetation is needlegrass (*Stipa* sp.), prickly pear cactus (*Opuntia humifusa*), skunkbrush (*Rhus aromatica*) and Ohio spiderwort (*Tradescantia ohiensis*). Several houses are also located throughout the study site, and humans are allowed to bike and walk through the sand prairie. There is also potential for human contact in the TCRA, which largely consists of a recreational vehicle (RV) park. Often, turtles have to cross roads or campsites during movements between activity centers. Boating and fishing also occurs in the slough.

The Mickelson's Landing site consists of similar vegetation and channel characteristics described above. Similar to the TCRA, the Landing is bordered by a sand prairie extending 50-75 m inland. However, unlike the TCRA, there is no RV park at this site, rather, there are permanent human establishments and a sand road along the eastern edge

of the slough (Fig. 1.2). These buildings are often used as weekend or vacation houses, but there is still a significant human presence at this site, as boating and fishing also occur in this area.

Trapping and Transmitter Attachment

Blanding's turtles were either hand-captured during terrestrial encounters or trapped aquatically using baited lobster traps. There were two main trapping locations: 1) in the slough just east of the TCRA or 2) Mickelson's Landing, which is 3.5 km south of the TCRA but still part of the UMRNFWR. Traps were checked twice daily and were baited using carp, sturgeon and catfish heads, and beef liver. Both sites were trapped in May and June of 2005 and 2006.

Once captured, turtles were marked with notches in marginal scutes (Cagle 1939) and measured, and blood or tail clips were collected for use in future genetic studies. Females were palpated in the inguinal region for the presence of shelled eggs to determine reproductive status. Transmitters (Model #R1930, Advanced Telemetry Systems) were attached to turtles using superglue and quick-drying epoxy. Turtles were kept for 24 hours to ensure transmitter attachment and released at the site of capture.

Radio-telemetry

Once released, females were located everyday and males were located every other day in May and June of 2005 and 2006. Radio-telemetry was conducted using hand-held antennae. I used Location of a Signal (LOAS) 4.0.2.0 Beta (Biotas) and Global Positioning System (GPS, Garmin eTrex Legend and Trimble GeoXM) to triangulate turtle locations using ≥ 2 bearings taken within ten minutes of one another. I used the maximum likelihood algorithm in LOAS to estimate locations, which estimates a point location even if the

bearings do not intersect (Millspaugh and Marzluff 2001). The program also estimates error ellipses associated with a particular point estimate. Some points were excluded from further analysis if 1) error ellipses were too big (>1000 m) or 2) if estimated point locations were clearly outside the study area.

Home Range Estimation

Home range estimates were obtained by 1) minimum convex polygon (MCP) (Hayne 1949) and 2) fixed kernel density (Worton 1989). Minimum convex polygon is simply the smallest possible convex polygon to encompass all the known locations of a given individual (Hayne 1949). However, despite its simplicity and wide use, this estimator provides only a crude outline of the animal's home range and often includes areas the animal may never use (Powell 2000). Kernel estimators are used to examine the quantity of habitat use, and are the most consistent and accurate estimators available (Worton 1989). One downside of using this method is that the investigator must set several parameters, including the smoothing factor, h . Small changes in the smoothing factor can have large impacts on the home range size estimate (Kazmaier et al. 2002, Hemson et al. 2005). The least-square cross-validation method is often used to estimate the value of h that produces the minimum estimate error (Worton 1989, Millspaugh and Marzluff 2001), but problems with this estimation method arise when data are highly auto-correlated, as is often the case with herpetofauna radio-telemetry data (Row and Blouin-Demers 2006). I used both these methods (MCP and Kernel) to better understand Blanding's turtle home range estimates for this population.

I used ArcGIS 9.1 (ESRI) with the Hawth's Tools extension (Beyer 2004) to calculate minimum convex polygons and ArcView 3.3 (ESRI) Animal Movements v_2 extension (Hooge et al. 1999) to calculate 50% (core) and 95% (home range) kernels. These core areas

correspond to the activity center(s) for these turtles. The student's t-test was used to test for differences in home range sizes between the sexes and between gravid and non gravid females. I also calculated daily (or averaged) straight-line minimum, maximum, sum, average and the standard deviation for distances. All statistical analyses were performed using JMP (SAS Institute 2007).

Habitat Analysis

I performed compositional analysis (Aebischer et al. 1993) to investigate habitat use at two levels: 1) home range use within the defined study area and 2) habitat use within the home range. For the first level of comparison, I defined habitat use as the area of the home range polygon (95% kernel estimator) and defined habitat availability using a 1000 m buffer from the centroid of the home range polygon. This distance was selected because 1000 m is the maximum distance traveled by these turtles, and straight-line distances of 500 m or more are not uncommon for this species (Ross and Anderson 1990, Rowe and Moll 1991, Piepgras and Lang 2000). To determine habitat availability, I downloaded 2005 NAPP Digital Orthophoto Quarter Quadrangles from the Illinois Natural Resource Geospatial Data Clearinghouse. Using ArcGIS 9.1 (ESRI), home range polygons were used to clip the aerial photo, resulting in an aerial photo of just the home range for a specific turtle. Polygons were then created to classify the aerial photo into seven broad habitat types: 1) built (consisting of campground, roads and crops), 2) water (slough), 3) forest, 4) marsh, 5) sand prairie, and 6) river. The Xtools Pro 3.0 (Data East) extension was used to estimate the area of the created polygons. A similar procedure was used to determine habitat availability. Once the areas of the polygons were determined, the area for a particular habitat type across individual

polygons was summed and then divided by the total area of the home range or habitat availability. This procedure yielded the proportion habitat type to total area.

For the analysis of habitat use within the home range, the number of estimated locations within each habitat type was divided by the total number of estimated points, defining the habitat use for an individual turtle. Habitat availability was defined as the 95% fixed kernel home range estimate (Figure 4). The program Resource Selection for Windows was used to perform the compositional analysis (Leban 1999).

Results

In 2005, nine females, four males and one juvenile were caught in the TCRA and fitted with transmitters; of these, eight were trapped aquatically and six were collected on land, either crossing a road or walking in the sand prairie. In 2005, no turtles were caught in the Mickelson's Landing area. In 2006, three additional turtles (two females, one male) from the Mickelson's Landing area were fitted with transmitters. These three turtles were trapped aquatically; however, there were terrestrial encounters with three additional turtles (all females), none of which were fitted with transmitters. No additional turtles from the TCRA site were fitted with transmitters in 2006. Four turtles with transmitters attached in 2005 were caught in 2006 without the transmitters still attached. Transmitter failure was assumed for at least one other turtle because the turtle was observed with the transmitter still attached but I was unable to detect signal. Transmitter loss/failure occurred during the winter from 2005 to 2006. Therefore, for 14 turtles (14), only one year of data was used to calculate home ranges and habitat use, whereas three turtles were successfully tracked for two years. For these turtles, 2005 and 2006 point locations were combined for all subsequent analyses.

Home Range Analysis

Two methods were used to estimate home range sizes; minimum convex polygon (Hayne 1949) and fixed kernel estimate (Worton 1989). The second method allows for estimation of core (50% use) and home range (95% use) areas. A total of 322 points were estimated; 35 points were excluded, resulting in 287 points locations across all 17 Blanding's turtles. The number of estimated point locations per turtle ranged from 6 to 35 (mean = 19). All turtles were included in subsequent analyses; however, since only one juvenile was radio-tracked during this study, it was not possible to statistically compare home range sizes with other groups.

Home Range and Movement Patterns

Home range sizes varied widely (Table 1). Among all males (n=5), the average home range size (95%) using the fixed kernel approach, was 48.94 ± 37.04 ha, with core size estimate of 9.17 ± 8.71 ha. Among all females (n=11), the average home range size (95%) was 56.45 ± 45.68 ha, with core size (50%) estimate of 9.58 ± 6.78 ha. Among gravid females (n=2), the average home range size (95%), using the fixed kernel approach, was 44.78 ± 27.54 ha, with core size estimate of 7.29 ± 6.82 ha. Among non-gravid females (n=9), the average home range size (95%) was 59.04 ± 49.71 ha, with core size (50%) estimate of 10.08 ± 7.08 ha. Using the fixed kernel estimator, the average home range size for the Mickelson's Landing turtles was 29.11 ± 4.84 ha with core average of 7.19 ± 2.99 ha.

There was no significant difference in home range sizes between males and females or between gravid and non-gravid females, regardless of method used ($P > 0.19$ in all six comparisons) (Figure 2). However, home range sizes (95%) of the TCRA turtles were significantly larger than home ranges (95%) of the Mickelson Landing turtles by both the MCP and kernel estimator (MCP, $t=4.04$, $P=.0012$; Kernel $t=2.41$, $P=0.0314$). This

difference may be a product of the number of locations used to estimate home range size and the smoothing factor used in kernel estimation, as core sizes did not differ statistically between these two groups ($t=1.04$, $P=0.32$).

Movement patterns for females and males reveal that females were more likely to make long-distance movements, possibly associated with nesting forays (Figure 3). The frequency distribution of distance traveled between successive points reveals that the most frequent distance traveled was less than 200 m. Although it is not possible to perform a more quantitative analysis, preliminary results show that the average distance traveled by a female turtle between successive point locations was 280.6 m; the average distance traveled between successive points for males is 332.3 m. There is no statistically significant difference between these values ($t=1.25$, $P=0.21$).

Habitat Use

To determine habitat availability, an aerial photo and a 1000m buffer from the home polygon centroid was used to classify the habitat into six broad categories; water (slough), forest, swamp, built (campground, roads, cropland), river and sand prairie. For 12 turtles in the TCRA, the habitat polygons largely overlapped, and only one habitat availability polygon was created for these turtles. Since one female was always found north of the TCRA, and another was continuously located south of the TCRA, individual habitat availability polygons were created for these two females. For the Mickelson's Landing group, because of the significant overlap, only one habitat availability polygon was used in subsequent analysis. I divided the proportion of a particular habitat by the total area for the larger home range or habitat availability polygon to determine the percentage area. The percentages of habitat

within a larger polygon vary greatly (Table 2). Since this analysis did not investigate differences between sexes, data for the juvenile turtle were included.

Compositional analysis at the first level revealed the home ranges are not selected randomly within the study area ($\Lambda = 0.1761$, $P < 0.0001$), and revealed distinct habitat selection patterns within the study area. Results indicate that the water (slough) habitat was selected over all other habitats (Table 3.1). Forested was selected over sand prairie, swamp and river; sand prairie was selected over river. Surprisingly, built habitat was selected over swamp and river. The swamp area of the TCRA is ephemeral in nature, and the area increases with the amount of rainfall. This selection pattern may be deceptive, though, because at the time the aerial photo was taken, the swamp may have been smaller, thus underestimating the area of the swamp during this study. It is not possible to determine from these data any seasonal changes in habitat selection; however, it may be possible that the swamp is used more in early spring when it is larger, and use may decrease as it dries up. This result could also be an artifact of the semi-arbitrary definition of the habitat availability.

Compositional analysis also indicates that habitat is used non-randomly within the home range ($\Lambda = 0.0822$, $P < 0.0001$). Once again, water was selected over all other habitat types (Table 3.2). Forested was ranked second, followed by sand prairie and then swamp. At this level of analysis, built habitat was ranked as the least used habitat, suggesting that these turtles are only located in this habitat when moving between activity centers or on long-distance nesting migrations.

Discussion

Given the recent, rapid declines observed in many reptilian species, more data are needed in order to develop effective conservation strategies for these species. This is

especially true for chelonian species because of their reliance on both terrestrial and aquatic habitats throughout their life (Bodie and Semlitsch 2000). Therefore, in order to design effective conservation strategies for these turtle populations, basic data on home range, movement and habitat use are needed. This is especially true for Blanding's turtles because unique life-history traits, such as delayed sexual maturity and specific habitat preferences, make them highly susceptible to anthropogenic disturbances (Congdon et al. 1993).

Results of home range and core (activity center) estimation from this study are one to several orders of magnitude larger than home range estimates from some of the other studies investigating Blanding's turtle habitat use and movement (Ross and Anderson 1990, Rowe and Moll 1991, Piepgras and Lang 2000). However, the home range estimates obtained from the TCRA are roughly equal to home range size estimates from a radio-telemetric study conducted in Weaver Dunes, Minnesota (Hamernick 2001). The difference is not attributable to the different calculation methods, but rather to the numerous biotic and abiotic factors associated with home range size. In turtles, home range size can be influenced by ecological factors, such as population density, carrying capacity, habitat composition and resource availability (Piepgras and Lang 2000). Although impossible to quantify, it is unlikely that any of the above factors are driving the large home ranges documented in this study. Resource availability is high in the slough, as evidenced by the large number of individuals, including other chelonian species, supported in the TCRA and surrounding environment (pers. obs.). I do not currently have an estimate of population density, but a separate mark-recapture effort conducted in the TCRA has revealed that 53 Blanding's turtles (31 females, 18 males and 4 juveniles) have been recaptured 99 times (unpl. results, see Appendix B). Therefore, the factor that is thought to be driving the size of the home range is the continuity

and availability of aquatic habitat (Ross and Anderson 1990); that is, home ranges for this population are larger because continuously aquatic habitat is available. Despite the larger home range sizes, there is no difference in home range size between the sexes, a result that is consistent with previously published results (Hamernick 2001, Piepgras and Lang 2000, Ross and Anderson 1990, Rowe and Moll 1991).

The lack of difference in home range sizes between the sexes has interesting implications for the survival of adult turtles in this population. Despite being located in a wildlife refuge, there is still the threat of mortality and damage caused by anthropogenic activities. One adult Blanding's turtle was found dead in the sand prairie because it had been run over by heavy machinery during construction of a bike path (F. Janzen, pers. comm.). There are several incidences where turtles have been caught crossing a road, and although this species has never been observed dead on the road, several painted turtles (*Chrysemys picta*) and common snapping turtles (*Chelydra serpentina*) have been run over by vehicles at the TCRA and Mickelson's Landing (pers. obs). An adult male Blanding's turtle was observed tangled in fishing line in the slough, and at least 11 adult turtles have been captured with damage to the carapace (Janzen, unpub. results), most likely the result of run-ins with outboard boat motors.

Also, adult female turtles are thought to have higher mortality risk because of terrestrial movement to nesting locations (Aresco 2005). However, the similarity in home range sizes and the lack of difference between average movement distances suggest that this is not the case for adult Blanding's turtles. Indeed, preliminary analysis of mark-recapture data suggests that survival is not influenced by sex of the individual (Kasuga, unpub. results).

This, in turn, implies that the sex ratio of the population will not become male-biased as a result of differential adult survival between the sexes.

Habitat selection

Compositional analysis reveals that water, in this case the slough, is selected over all other habitat types at both levels of analysis. In comparison, turtles were never tracked into the main channel of the Mississippi River, despite the fact that this habitat is within reachable distances for these turtles. However, their primary food sources of crustaceans, snails, insects, frogs and fishes may not be readily available in the main river channel. The channel is devoid of nearly all vegetation, and is probably too fast for this wetland-dwelling species.

These results also documented significant use of terrestrial habitat, as indicated by forested areas being selected over sand prairie, human, swamp and slough habitat types. There are several reasons for terrestrial forays, including foraging, basking and movement between activity centers. Blanding's turtles are unique in that they can swallow food in and out of water, and while on land, may consume earthworms, slugs, grasses, berries and vegetation (Ernst et al. 1994).

Sand prairie habitat, in this population, is likely potential nesting ground. Indeed, depredated Blanding's turtle nests have been observed in the sand prairie (pers. obs.) and hatchlings have been captured in drift fences (Kolbe and Janzen 2002). Also, during the period of study, four gravid females were observed or captured in the sand prairie during transect walks of the site. At Mickelson's Landing, an adult female was observed to cross the sand road, and then enter the sand prairie. Once in the prairie, the female dug a burrow in the vegetation and proceeded to spend some time inhabiting the burrow (pers. obs.). There are several possible reasons for this behavior, including terrestrial foraging, escape,

thermoregulation of body temperature or nesting foray. It is most likely then that this habitat type is used seasonally by females for nesting or foraging activities, as no males were ever encountered in the sand prairie.

An unexpected result is the selection of the built type habitat, in this case including roads, bike paths, campgrounds and cropland, over swamp habitat at the first level of comparison. However, this result could simply reflect turtles passing through this habitat when moving from one activity center to another. The interior swamps of the TCRA, as mentioned previously, are ephemeral in nature, and may be utilized seasonally depending on the amount of rainfall and the size and depth of the area.

This study provides important preliminary data on home range, movements and habitat selection for a population of Blanding's turtles in a protected but still substantially human impacted area. Significant avenues for future study included additional telemetry experiments to specifically test hypotheses regarding nesting movements or differences in movement between the sexes. Also, genetic material has been collected from turtles captured since 1997, and this study provides context for developing testable genetic hypotheses. Some genetic investigations of Blanding's turtles in Nova Scotia have revealed genetic structuring over short geographic distances (Mockford et al. 2005); however, more recent work demonstrates that the Nova Scotia population may be the exception to the rule (Mockford et al. 2007) of relatively little genetic structuring in populations over larger geographic distances. Given the large home ranges and movement patterns, we expect similar results found by Mockford et al. (2007). Regardless, the movement and habitat use results described herein already provide important information for the long-term management and survival of Blanding's turtles in the UMRNFWR, and suggest several key foci for

conservation efforts: 1) any areas set aside for conservation should include continuously aquatic habitat, with easily accessible and continuous terrestrial areas and 2) lowering adult mortality risk is important for ensuring long-term survival of Blanding's turtle populations.

Radio-telemetry studies are important for gathering basic information on home range, movements and habitat use for populations and can help develop effective conservation strategies for imperiled chelonians.

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Figure 1. Study sites

Figure 1.1. Aerial photo of the Thomson Causeway Recreation Area (TCRA) study site. The purple circle indicates the area of trapping effort.

Figure 1.2. Aerial Photo of the Mickelson's Landing study site. The purple circle indicates the area of trapping effort

Figure 2. Map of study area depicting home range for the 12 turtles of the TCRA (2.1), the three turtles of Mickelson's Landing (2.2), for one male (2.3), one non-gravid female (2.4), one gravid female (2.5) and juvenile (2.6). The polygons shown are the estimates for MCP and the fixed kernel density approach, and depict home range (95%) and core (50%) estimates.

Figure 2.1.

MCP and Kernel Home Range Estimate for the Blanding's Turtles
of the TCRA

Figure 2.2.

MCP and Kernel Home Range Estimate for the Blanding's Turtles
of Mickelson's Landing

Figure 2.3.

Home Range for Adult Male

Figure 2.4.

Home Range for Non-gravid Female

Figure 2.5.

Home Range for Gravid Female

Figure 2.6.

Home Range for Juvenile

Figure 3. Map of study area depicting straight line movement for one female (Figure 3.1), one male (Figure 3.2). Figure 3.3 is a graph depicting frequency distribution of straight-line distance movements.

Figure 3.1.

Straight-line Movement for Adult Female

Figure 3.2.

Straight-line Movement for Adult Male

Figure 3.

Figure 4. Example of habitat availability and habitat use for the two levels of compositional analysis. For the first level of analysis, Figure 4.1 depicts the study area and figure 4.2 is an example of habitat classification of the 95% fixed kernel density estimator home range. Figure 4.3 depicts habitat availability (95% fixed kernel density estimator home range) and habitat use (estimated point locations)

Table 1. Table of home range size estimates using the two methods (MCP and Kernel) for all turtles. There are two columns for the kernel estimator, one depicting the 95% estimate and the other the 50% core estimate. In the location column, the abbreviation TCRA indicates the Thomson Causeway Recreation Area, and ML indicated Mickelson's Landing. For females, NG indicates not gravid, or without eggs, and G indicated gravid, or with eggs, at the time of capture. The unit on all size estimates is hectares.

Males

Turtle ID	Sex	Location	MCP	Kernel	Core
Big Bad John	M	TCRA	22.00	42.51	5.01
Edmund Fitzgerald	M	TCRA	19.98	27.63	2.84
Fernando	M	TCRA	105.66	114.37	24.42
Leroy Brown	M	TCRA	13.15	30.31	7.92
Mean			32.96	43.76	8.84
Bobby McGee	M	ML	1.85	29.86	5.68

Females

Turtle ID	Sex	Gravid	Location	MCP	Kernel	Core
Brandy	F	NG	TCRA	28.61	17.58	2.20
Cecilia	F	NG	TCRA	70.59	44.55	6.49
Janie	F	G	TCRA	24.29	25.32	2.46
Layla	F	NG	TCRA	129.01	93.28	14.12
Lola	F	NG	TCRA	40.58	41.11	7.92
Mustang Sally	F	NG	TCRA	50.15	177.38	26.56
Roxanne	F	NG	TCRA	52.44	62.20	10.69
Sweet Caroline	F	G	TCRA	34.22	64.28	12.12
Sweet Jane	F	NG	TCRA	40.70	37.81	7.16
Mean				47.96	57.25	9.87
Scarlet O'Hara	F	NG	ML	2.00	23.94	5.09
Clementine	F	NG	ML	14.64	33.53	10.55
Mean				6.22	19.82	5.88
Fred	J		TCRA	1.27	18.96	4.06

Table 2. Home range use and availability (as percentage) for all turtles at the first level of compositional analysis. For this analysis, Habitat Use is defined the 95% fixed kernel density home range estimate, and Habitat Availability is defined as a 1000m buffer from the centroid of the home range polygon. Habitat Availability, because of the overlap, was defined as the same for the twelve turtles (Big, Bad John, Brandy, Cecilia, Edmund Fitzgerald, Fernando, Fred, Janie, Layla, Leroy Brown, Lola, Mustang Sally and Sweet Jane) of the TCRA. Likewise, the Habitat Availability was defined to be the same for the three turtles (Scarlet O'Hara, Clementine and Bobby McGee) of Mickelson's Landing area.

Turtle ID	Water	Forested	Sand Prairie	Built	Swamp	River
Habitat Use						
Big Bad John	34.24	52.10	3.48	7.76	2.32	0.00
Brandy	52.59	42.69	1.39	3.33	0.00	0.00
Cecilia	51.64	44.11	1.15	3.10	0.00	0.00
Edmund Fitzgerald	53.64	44.54	1.81	0.00	0.00	0.00
Fernando	35.95	50.07	0.00	11.16	2.83	0.00
Fred	53.83	34.34	0.00	11.83	0.00	0.00
Janie	62.13	34.34	0.00	3.53	0.00	0.00
Layla	46.71	37.37	4.21	8.11	3.61	0.00
Leroy Brown	58.43	39.04	2.15	0.38	0.00	0.00
Lola	59.02	37.57	0.00	3.41	0.00	0.00
Mustang Sally	39.62	50.23	2.87	7.29	0.00	0.00
Sweet Jane	60.25	33.26	0.00	6.50	0.00	0.00
Roxanne	28.87	43.73	14.63	4.10	0.00	8.66
Sweet Caroline	81.56	0.00	18.44	0.00	0.00	0.00
Scarlet O'Hara	56.19	25.07	6.74	12.00	0.00	0.00
Clementine	18.88	13.22	59.76	8.14	0.00	0.00
Bobby McGee	61.22	26.94	0.00	11.84	0.00	0.00
Habitat Availability						
TCRA	35.81	47.01	6.65	7.39	2.07	1.08
Roxanne	2.33	14.71	3.28	9.65	0.00	41.45
Sweet Caroline	14.81	34.35	27.98	9.29	0.00	0.00
Mickelson's Landing	36.28	31.46	14.83	16.42	0.00	0.00

Table 3. Results of compositional analysis for home range selection with study area and habitat use within home range.

Table 3.1. Table depicting compositional analysis results and interpretation for compositional analysis of home range use within study area.

Water vs. all other types	all $p < 0.037$	Water ranked over Forested, Sand Prairie, Built, River and Swamp
Forested vs. Sand Prairie, Swamp and River	all $p < 0.0044$	Forested ranked over Sand Prairie, Swamp and Built
Sand Prairie vs. River	0.039	Sand Prairie ranked over River
Built vs. Swamp and River	all $p < 0.04$	Built ranked over Swamp

Table 3.2. Table depicting compositional analysis results and interpretation for compositional analysis of habitat use within home range.

Water vs. all other types	all $p < 0.0176$	Water ranked over Sand Prairie, Built, and Swamp
Forested vs. Sand Prairie, Swamp and Built	all $p < 0.0203$	Forested ranked over Swamp and Built
Sand Prairie vs. Swamp	0.0032	Sand Prairie ranked over Swamp
Swamp vs. Built	0.0079	Swamp ranked over Built

**A GEOGRAPHIC INFORMATION SYSTEMS (GIS) APPROACH FOR
ANALYZING THE EFFECTS OF LANDSCAPE COMPOSITION ON
REPRODUCTIVE SUCCESS OF THE BLANDING'S TURTLE, EMYS
BLANDINGII IN IOWA**

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Introduction

Anthropogenic alterations of critical habitat are one of the leading causes of species decline (Fischer and Lindenmayer 2007). These modifications include deforestation, urbanization, and draining and tiling wetlands for conversion to agricultural land. These changes impact species adapted for survival in such habitats by influencing a variety of life history and demographic factors, including home range size, interactions with other species and resource availability. To ensure the long-term survival of species, conservation biologists are charged with not only understanding how individual species utilize habitat, but also how habitat and landscape changes impact demographic and genetic characteristics of populations.

One important demographic consideration for conservation biologists is the impact of significant habitat modifications on the viability and survival of species and populations of concern. Anthropogenic alterations over the past 300 years have significantly changed the structure and function of communities (Fischer and Lindenmayer 2007). For instance, constructing dense road networks, draining and tiling wetlands for conversion to agricultural land, clearing forests, adding nutrients via fertilizer and applying pesticides have all documentably impacted the quality and integrity of surrounding ecosystems and communities (Rohr and Crumrine 2005). The anthropogenic modifications and addition of nutrients can

have significant impacts on eutrophication and hypoxia in lakes (Allan and Flecker 1993, Rabalais et al. 2002), organismal growth and development (Allran and Karasov 2001, Gammon et al. 2005, Wilhelms et al. 2005) and community structure (Rohr and Crumrine 2005). An additional consequence of these activities is turn-over in the species composition of habitats, often resulting in one or two species overwhelming a particular habitat (Kostecke et al. 2004). These changes in community structure have significant impacts on the survival and reproduction of species adapted to those communities.

Habitat specialists are especially vulnerable to anthropogenic habitat alterations. These species often exhibit morphological, behavioral or physiological, adaptations, indicating long evolutionary associations between the specialists and their habitats (Futuyma and Moreno 1998, Attum et al. 2006, Polus et al. 2007). Habitat specialists also have higher site fidelity and lowered tolerance for disturbance (Pimm et al. 1998, Henle et al. 2004). In contrast, habitat generalists have fewer adaptations to a specific habitat, and are less exacting in habitat preference (Futuyma and Moreno 1998, Foufopoulos and Ives 1999). Recently, much research has been focused on predicting extinction risk for species (Gaston and Blackburn 1995, Gaston 1996, Foufopoulos and Ives 1999). Specifically, research investigating reptilian extinction risks has demonstrated that both abundance and habitat specialization are significant predictors of extinction risk, whereas body mass and longevity are not (Foufopoulos and Ives 1999).

One reptilian habitat specialist in need of conservation is the Blanding's turtle, *Emys blandingii*. This turtle is well adapted to wetland systems characterized by slow current, shallow water, and vegetation cover consisting of coontail (*Ceratophyllum demersum*), speckled adder (*Alnus rugosa*), pondweed (*Potamogeton pectinatus*) and other wetland

vegetation (Pappas et al. 2000). In states such as Iowa, however, human modifications over the past 150 years have drastically altered wetland habitat availability and suitability.

Massive efforts to tile and drain wetlands have reduced the amount of wetland by greater than 80% (Zedler 2004). More recently, herbicides have significantly altered the vegetation composition of these habitats, resulting in wetlands choked by the nitrogen-phillic cattail (*Typha latifolia*). Cattails may impact the breeding success of *E. blandingii* because these plants choke the shallow wetland habitat and prohibit proper thermoregulation for completing egg production (Ross and Anderson 1990).

This project used a Geographic Information System (GIS) based approach to understand how characteristics of the Iowa landscape may have impacted the breeding success of populations of the habitat specialist *E. blandingii* across the state. Specifically, we assessed how the amount of agriculture, suitable nesting locations and amount of wetland within biologically relevant buffers may be linked to reproductive success. By understanding how these landscape changes impact breeding success, mitigation steps can be taken to prevent actively reproducing populations from declining, and, combined with more data, will help predict extinction risks for these populations.

Methods

Reproductive Success

Aquatic trapping, using net hoop traps, of Blanding's turtles was conducted at 21 localities distributed throughout the range of the species in Iowa during the summers of 1997 and 1999 by Christiansen and VanDeWalle (Figure 1). Turtles were also collected during walking and road-driving surveys. These localities were chosen from known or suspected Blanding's turtle populations. A minimum of 20 trap nights were spent at each locality with

traps being set in a variety of wetlands habitats, including shallow vegetated areas, transitions between shallow and deep water, and at the aquatic edge of cattail rings, to promote an equal likelihood of catching both adult and juvenile turtles. Additional information regarding overall wetland characteristics, including temperature and status of vegetation, were recorded (Christiansen and VanDeWalle 1999). Based on the reproductive status of Blanding's turtle caught, populations were then assigned to one of three categories: 1) Present and Actively Reproducing (if adults and juveniles were caught), 2) Present but Not Actively Reproducing (if only a few adults were caught and no juveniles were caught), and 3) Not Present (if no individuals were caught) (Figure 1).

Landscape Classification

In order to describe and classify the habitat at the 21 trapping sites, we downloaded aerial photos [from the Iowa State University GIS server (gis.iastate.edu) and the Iowa Department of Natural Resources NRGIS Library (www.igsb.uiowa.edu/nrgislibx/)] taken in the early to mid-1990's (see Table 1 for specific photo dates). These photos, along with several other data including the National Wetland Inventory (NWI), Roads, Designated Lakes, DNR Lands, Public Prairies, Public Land Survey System (PLSS) and Common Land Units (CLU), were downloaded on a per county basis to aid in landscape classification.

We then imported these aerial photos into ArcGIS 9.1 (ESRI) to identify and locate the trapping locations. In most cases, the wetlands were listed in the NWI layer for easy identification. In some cases, the PLSS layer, in combination with the Public Prairies and NWI, had to be used to identify sites. We created a polygon layer for each wetland trapping location once all the trapping localities were correctly identified.

In order to assess how landscape composition might be impacting reproductive success, we used the biologically relevant distances of 100 m, 200 m, 500 m and 1000 m to create buffers around the trapped locations and clipped the aerial photo, resulting in four photos per location. The 100 and 200 m buffers were selected because previous telemetry studies indicate that these straight-line distances are the most frequently documented movements (Ross and Anderson 1990, Rowe and Moll 1991, Piepgras and Lang 2000, Refsnider 2005, Kasuga, unpub.). Straight-line distances up to 500 m are not uncommon, however, for many Blanding's turtles, the maximum straight-line distance traveled is 1000 m (Kasuga, unpub.).

Once the aerial photos had been clipped, the CLU polygon layer was used to classify the habitat features of the landscape. However, since the CLU layers were created after the aerial photos were taken, modifications and edits were made to the CLU layer to depict the land units at the time the aerial photos were taken. Habitat was classified into six broad categories, including Forest, Wetland, Agriculture, Built, Prairie and Road. We further classified Forest into Forest/Prairie and Forest/Riparian Corridor sub-categories. Wetlands were further classified into Open, Vegetated Forest, Vegetated Submergent, and Vegetated Grass. Once all the polygons had been classified, the XTools Pro 3.0 extension (Data East) was used to calculate the Perimeter (m) and Area (m², acres and hectares) for each polygon. These values were then summed across the broad habitat types to obtain the total for a particular habitat and buffer. Proportions of each broad habitat classification were then created by dividing the total for one habitat type by the total area within the buffer. These tables were then exported for further data analysis (see Figure 2 for a habitat classification example at one site).

In order to assess whether the amount of available nesting area impacted reproductive success, we used soil classification as a surrogate for suitable nesting site. Specifically, research has shown that female Blanding's turtles west of the Mississippi River prefer sandy soils in which to nest (Sajwaj et al. 1998, Rowe 1992). To address how the amount of suitable nesting sites may be impacting reproductive success, we downloaded the soils raster data layer from the Iowa Geographic Map Server. This download also included the Iowa Soil Properties and Interpretations Database (ISPAID 7.0), which contains information about soils throughout Iowa. Once imported into ArcGIS 9.1, the soil raster data were joined to the ISPAID table. The soil raster was clipped using the same biologically relevant buffers described above and was then converted to a soil polygon layer in order to determine the area. We used the Texture (Surface Horizon) to classify the soils into four main categories: Clay, Sand, Loam, and Muck (see Figure 2 for soil type classification example at one site). All data tables were exported for further analysis.

We used logistic regression to test for relationships between habitat composition and presence/absence of Blanding's turtles and reproductive status of populations. Logistic regression was also used to test for relationships between soil type and presence/absence of Blanding's turtles and reproductive status of populations.. All statistical analyses were performed using JMP (SAS Institute 2007).

Results

A total of 21 trapping localities with known or suspected Blanding's turtle populations were included in this study. No Blanding's turtles were caught at 10 sites; these sites were classified as Not Present. Four sites were classified as Present and but Not Actively Reproducing as only adult Blanding's turtles were caught. Seven sites were

classified as Present and Actively Reproducing because both adults and juveniles were trapped at those sites.

Logistic regression revealed no significant relationship between any of the broad habitat classifications (Forest, Wetland, Agriculture, Built, Prairie and Road) and presence/absence of Blanding's turtles at any buffer range (all $p > 0.2$). Likewise, there was no significant relationship between the reproductive classifications (Present and Actively Reproducing, Present but Not Actively Reproducing and Not Present) and any broad landscape variable at any buffer distance (all $p > 0.12$). There was also no significant relationship between any of the wetland sub-categories of Open, Vegetated Forest, Vegetated Submergent, and Vegetated Grass and presence/absence or reproductive success (all $p > 0.2$). Surprisingly, Wetland Vegetated Submergent was negatively correlated with presence/absence, but not significantly so ($p = 0.26$)

Logistic analysis revealed that clay, loam and muck were not significant predictors of presence/absence or reproductive success of Blanding's turtles at any buffer distance. However, the proportion of sand within the 100 m buffer significantly predicts presence/absence ($p = 0.039$) (Figure 3), but not the reproductive success ($p = 0.11$) of Blanding's turtles. In other words, a wetland was more likely to contain Blanding's turtles when near-shore soils were comprised of substantial quantities of sand.

Discussion

Recent (last 150 years) anthropogenic changes to the Iowa landscape have stressed habitat specialist species (Futuyma and Moreno 1998, Leinert and Fischer 2003, Attum et al. 2006, Polus et al. 2007). In particular, because wetlands have been drained and tilled for conversion to agricultural land, wetland habitat specialists have experienced dramatic

decreases in habitat availability and suitability. This study aimed to investigate how habitat composition might impact reproductive success of Blanding's turtle populations across Iowa.

The proportion of sandy soil within a 100 m buffer around the wetland habitat was a statistically significant predictor of presence/absence of Blanding's turtles. Although it may be the case that the adults utilize this habitat for a variety of reasons, it is most likely that these soils serve as nesting areas. As in many turtle species, the environment experienced by the embryo during development has significant implications for survival, rate of development, and sex in turtles with temperature-dependent sex determination (TSD) (Kolbe and Janzen 2002). There are numerous reasons for nest failure, such as flooding, predation and other causes (Standing et al. 2000). Research into survival of Blanding's nests showed that out of 124 nests, nearly half (50 out of 124, 40%) failed due to predation (16), vandalism (1), flooding (15) and other causes (18) (Standing et al. 2000), suggesting that nest location is critical for survival. For populations in Iowa, nests laid in inappropriate soils may not be able to successfully produce hatchlings. The results from this study suggest the importance of nesting in appropriate soil; for this region of the species' range, research suggests that soil is sand (Sajwaj et al. 1998, Rowe 1992). Sites lacking sufficient amounts of sandy soil may not be able to successfully complete development of embryos to hatching and would eventually result in the extirpation of the population from the area (Gutzke and Packard 1987).

The logistic regression of Wetland Vegetated Submergent against presence/absence of Blanding's turtles was negatively correlated. This result is surprising because this wetland classification is the preferred habitat of these turtles (Piepgras and Lang 2000). It could be the case that in this portion of the species' range, the preferred wetland habitat is different;

however, we feel this is more likely explained by population extirpation from these areas. That is, some sites once contained active populations of Blanding's turtles, but by either natural processes or anthropogenic activities, these populations have been removed. Investigation of historical trapping and occurrence records, as well as additional trapping in these sites, will help clarify local patterns of extinction in this portion of the range.

Although not significant, there are several suggestive results ($p < 0.15$) from the logistic regressions of soil and habitat type against presence/absence of Blanding's turtles. The proportion of the soil type muck within a 100 m buffer did appear to be suggestively predictive of presence ($p=0.12$). This is not surprising, as research shows that these turtles prefer wetland habitat with muck/silt bottoms (Standing et al. 2000). Also, the amount of prairie within a 100 m buffer was suggestively predictive of presence/absence, but not significantly so. This may be the case because adult turtles do spend some time in terrestrial habitats, possibly foraging or thermoregulating. Additional data are needed to fully appreciate the impact of the muck soil type and prairie landscape type on presence/absence of these turtle populations.

Logistic regression revealed that the broad habitat classifications of Forest, Wetland, Agriculture, Built, Prairie and Road are not significantly correlated with the presence/absence or reproductive success of Blanding's turtle populations across Iowa. This does not mean, however, that there are no landscape features influencing reproductive success. Rather, it may be the case that we have not yet identified the appropriate landscape features or analyzed these classifications at the appropriate level of detail. It may also be the case that we do not have enough statistical power to detect any differences or patterns in how landscape classification may be impacting reproductive success. This analysis focused on 21

sites, but there are more sites within Iowa that have been trapped for Blanding's turtles. Trapping efforts at these additional sites would result in more data and possibly help tease apart relationships between landscape composition and Blanding's turtle populations. Finally, it may be the case that the statistical analysis used (logistic regression) may not be the most appropriate for testing these relationships. Future work includes using ordination methods to analysis these data.

GIS based approaches provide a powerful platform for analyzing large-scale landscape impacts in a variety of situations (Foody 2007, Flemons et al. 2007). This is evidenced by the recent use of GIS to address a wide variety of biologically related issues, including identifying potential restoration sites and habitat suitability modeling (Stockwell and Peters 1999, Van Lonkhuyzen et al. 2004, Fernandez et al. 2006, Thompson et al. 2006) and confirming phylogeographic results (Kidd and Ritchie 2000, 2001, Ritchie et al. 2001). However, despite the power and advantages this approach, there are several problems associated with using GIS. For this analysis, data collection relied on the investigator's identification of landscape type from aerial photos. In some habitat types, such as agriculture, forests and human/artificial, identification is fairly straightforward. In other situations, the habitat type may not be as obvious. Moreover, habitat identification involves a combination of experience and other data layers. It is possible that some habitat areas may be incorrectly identified, but given the additional data used to aid in identification, we do not feel misclassification is an issue for this particular study.

Another caution when using GIS based approaches concerns the quality of data. The data for this analysis were downloaded from a variety of GIS web servers. In most cases, the data were complete; however, the soil data layer had a large fraction of areas unidentified to

soil type. In some cases, unidentified soil was greater than 80% of the biologically relevant buffer. It may be possible, using aerial photos and other layers, to fill in the missing data. However, the ISAPD database contains a lot of information about Iowa soils, and another classification scheme may contain the same data but could be more complete. Re-analyzing the soil data using a more complete classification scheme would correct the problem discussed above, and may help tease apart the relationship between soil types and presence/absence of Blanding's turtles.

However, despite the issues associated with the methods, this study demonstrates the power and ability of a GIS based approach to answer large-scale landscape questions addressing conservation efforts for imperiled species. With respect to the Blanding's turtles, this study provides a few more pieces of the puzzle for conservation of this species in Iowa, and combined with other data, these results will provide powerful insights for designing and implementing effective conservation strategies. As an example, any habitat or soil classification correlated with either presence/absence or reproductive would need to be included in defining critical habitat for this species. This research, as well as previous published research (Piepgras and Lang 2000, and refs. within), suggest that this species does spend some time in wetland areas, but they also utilize the surrounding terrestrial habitat, possibly for nesting, foraging or thermoregulating. Areas designed to protect this species must include the necessary terrestrial components, in addition to continuous wetland habitat.

There are several avenues for continued research and analysis of this project. For some counties, historic (1930's) aerial photos are available, as are much more recent (2005) aerial photos. Repeating the methods described above for these additional photos may yield insights into how changes in landscape composition are influencing reproductive success. It

may also be appropriate to view and analyze the broad habitat classifications, particularly wetlands in more detail. Also, genetic samples were collected from individuals during this study, and genetic analysis, combined with these results, would suggest a variety of important conservation strategies for this species.

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Table 1. Table of county, site, status and photo dates for the eighteen trapping locations included in this analysis. Under status, NP means not present, P means present (* indicates a Blanding's turtle was seen in vicinity, but not aquatically trapped) and NPR indicates present but no reproduction. Under the photo date categories, NA means the metadata is not available, and a * indicates that only the year was given, not a specific date.

County	Sites	Status	Photo Date Start	Photo Date End
Emmet	Cheever Lake	NP	4/13/1990	6/26/1997
	West Swan Lake	NP	4/13/1990	6/26/1997
Osceola	Ocheyedan Pits	NP	3/12/1990	6/26/1997
Palo Alto	Lost Island Marsh	NP	1/1/1990*	12/31/1994*
	Mud Lake	P*	1/1/1990*	12/31/1994*
Clay	Kirshner Prairie	NP	NA	NA
Sac	Kiowa Marsh	NP	1/1/1990*	12/31/1994*
Greene	Dunbar Slough	PNR	1/1/1990*	12/31/1994*
Guthrie	Bay's Branch	PNR	1/1/1990*	12/31/1994*
Worth	Elk Creek Marsh	PR	4/17/1991	6/30/1994
Winneshiek	Cardinal Marsh	NP	4/10/1991	5/19/1994
Tama	Otter Creek Marsh	NP	4/22/1994	4/23/1994
Bremer	Sweet Marsh	PR	NA	NA
	Aldo Leopold Complex	PR	NA	NA
Wright	Big Wall Lake	PNR	1/1/1990*	12/31/1994*
	Elm Lake	NP	1/1/1990*	12/31/1994*
Butler	Big Marsh	P*	NA	NA
Jones	Muskrat Slough	PR	1/1/1990*	12/31/1994*
Henry	Gibson Recreation Area	NP	1/1/1994*	12/31/2000*
Wapello	Eddyville	PNR	1/1/1990*	12/31/1994*
Muscatine	Wiese Slough	PR	3/22/1994	6/15/1994

Figure 1. Trapping sites and reproductive status from 21 trapping sites across Iowa.

Figure 2. Landscape composition and soil type classification for one actively reproducing population of Blanding's turtles.

Figure 3. Logistic regression for sand (in the 100m buffer) and Blanding's turtle presence/absence. P means present and NP means not present.

CHAPTER 4: CONCLUSION

Reptilian species, especially chelonian species, have experienced dramatic declines in both population abundance and distribution in recent years. Biologists and managers charged with the conservation of these species are challenged on several fronts, but perhaps the most daunting is the lack of basic and complete data for many of these species. The World Conservation Union, which publishes the IUCN Red List, was only able to evaluate 1,385 reptile species out of 8,240 described reptile species. Lack of data on species poses a serious problem for conservation efforts, and collection of basic data is important for ensuring long-term survival.

The Blanding's turtle, *Emys blandingii*, is one species for which there are several knowledge gaps. Indeed, the last time this turtle was evaluated by the IUCN was 1994, and was classified as Lower Risk/near threatened. In an effort to both fill knowledge gaps and potentially aid in future evaluation of this species, this project analyzed the Blanding's turtle at several levels, with the ultimate goal of aiding in the design of conservation strategies for this species.

The first project analyzed home range, movement and habitat composition for a population of Blanding's turtles in a continuous wetland habitat. Results of home range size and movement were consistent with previously published results (Hamernick 2001), and habitat composition analysis revealed an interesting pattern of selection of home ranges within the study sites. Although water and forested types were selected preferentially over all other habitat types, the human area was then selected preferentially over all remaining types. And although this result may be a product of the methods, it may also be the case that these turtles truly are selecting for human areas. This has several significant conservation

implications for this population, mainly in the increased exposure to mortality threats from humans. Since turtle populations rely on high survival of adults for persistence (Congdon et al. 1993), an increase in adult mortality would likely result in the extirpation of this population. The lack of difference in home range sizes between the sexes suggests that this mortality threat would be faced equally by both sexes. The apparent selection of human habitat over sand prairie and swamp habitat disappeared at the second level of analysis, indicating that within home ranges, this is the least preferred habitat. However, as Blanding's turtles are often observed passing through human areas, the mortality threat remains.

The second project analyzed how broad-scale landscape composition, using a GIS based approach, influences the reproductive success of Blanding's turtle populations across Iowa. Although there was only one significant indicator of presence/absence (proportion sand in the 100 m buffer) and no significant indicators of reproductive success, there were several suggestive results. Both the amount of prairie within the 100 m buffer and the proportion of muck soil type showed almost statistically significant relationships with reproductive success, suggesting an influence of these variables on reproductive success. In addition, the lack of significant results may be an indication of the lack of data, and not of a lack of correlation between landscape or soil variables and reproductive success. Several additional analyses of these data include adding several other sites and exploring several landscape characteristics, such as wetland composition, in more detail. Other avenues of future work include investigating how changes in the Iowa landscape (from 1930s to present) may be influencing reproductive success. These results certainly suggest several conservation strategies for this species, reiterating the multiple habitat requirements. This

suggests that the critical habitat for this species is not limited to water areas, and any listing efforts should include all aspects of necessary habitat.

However, despite the usefulness of these approaches for informing conservation management of this species, there are other tools that, in conjunction with landscape approaches, can provide much more powerful insight for designing effective conservation strategies. These other tools are genetic approaches, which are used to address a whole different suite of issues such as population structuring, movement and assignment of individuals and phylogeographic analysis.

One potential avenue for continued work on this species is to use micro-satellites to investigate population structuring at various geographic scales throughout the range. Tissue samples were collected from individuals in the Upper Mississippi River National Fish and Wildlife Refuge, as well as the populations throughout Iowa. The landscape results discussed above provide a basis for testable genetic hypotheses for these populations, and can lend insight into how various geographic regions have been colonized. Ideally, this research would also use additional tissue samples collected from other populations throughout the North America. Although there has been some published work on genetics of Blanding's turtle populations (Rubin et al. 2004, Mockford et al. 2005), these studies focused on structuring of populations over short geographical distances and the results obtained may not be applicable to other populations throughout the range. More recently, research has been published using micro-satellites to determine evolutionary significant units for this species; however, this study did not include populations from the entire range (Mockford et al. 2007). The addition of more populations to these analyses will increase the understanding of genetic

structuring within this species, and will certainly prove useful for designing effective conservation strategies.

Another genetic approach is to use nuclear or mitochondrial DNA to perform phylogeographic analysis. In recent years, phylogeographic studies have become increasingly popular because of the power of these investigations to resolve issues that may have been historically problematic. Recent investigations have included identifying cryptic variation within a species (Spinks and Shaffer 2005), resolving species' relationships (Feldman and Parham 2002, Krenz et al. 2005), understanding post-glacial colonization patterns over broad geographic ranges, including responses to climate change (Starkey et al. 2003), deciphering population history, genetics and historical gene flow (Templeton et al. 1995) and finally helping to identify conservation units (Davis et al. 2006). This approach would be especially useful for several reasons because 1) no such study has been performed on Blanding's turtles, 2) this approach may identify any cryptic variation within the species and 3) would help with the identification of evolutionary significant units. Again, this information would aid in designing effective conservation strategies for this species.

In face of the current conservation crisis, conservation managers and planners are charged with species protection in the face of numerous challenges. This project aimed to help the conservation of the imperiled chelonian *Emys blandingii* by using landscape based approaches to analyze various characteristics of populations. This study provided new insights into populations of this species and will ultimately, with additional data collection and analyses, aid in ensuring the long-term survival of this species.

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**APPENDIX A. HOME RANGE AND HABITAT COMPOSITION MAPS FOR ALL
17 RADIO-TRACKED TURTLES IN THE UPPER MISSISSIPPI RIVER NATIONAL
FISH AND WILDLIFE REFUGE**

Home Range and Habitat Composition for Big Bad John

Home Range and Habitat Composition for Bobby McGee

Home Range and Habitat Composition for Cecilia

Home Range and Habitat Composition For Clementine

Home Range and Habitat Composition for Mustang Sally

APPENDIX B. PRELIMINARY MARK-RECAPTURE ANALYSIS OF BLANDING'S TURTLES IN THE THOMSON CAUSEWAY RECREATION AREA

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Introduction

Mark-recapture studies provide a method for investigating various aspects of population demography, including survival (Φ), recruitment (f) and the population growth rate (Λ) (Lebreton et al. 1992). These studies have been performed on a variety of animals, including mammals (Wilson et al. 2007), birds (Sidhu et al. 2007) and even reptiles (Bowen et al. 2004) and offer insights into the demography of these populations. Mark-recapture studies may be especially useful in reptile species, as understanding survival and recruitment in populations is critical for ensuring long-term survival (Congdon et al. 1993). This analysis offers a preliminary investigation of survival (Φ) and recruitment (f) for the population of Blanding's turtles in the Thomson Causeway Recreation Area (TCRA), part of the larger Upper Mississippi River National Fish and Wildlife Refuge (UMRNFWR).

Methods

Trapping efforts have been conducted in the TCRA beginning in 1997 and continuing through the present. A variety of aquatic traps, including fyke and lobster traps, were placed in the slough just east of the TCRA (for a more detailed description of the trapping area, see Methods section of Chapter 2). Blanding's turtles were also encountered and caught terrestrially. Once captured, turtles were uniquely marked by filing notches in the marginal scutes (Cagle 1939). Various measurements, such as carapace length, sex and reproductive status were collected on each turtle. Tissue samples were collected for genetic analysis, and turtles were released at the site of capture. Mark-recapture data were analyzed using Pradel's

reverse time model, which allows for estimation of survival (Φ) and recruitment (f) parameters (Pradel 1996), using the program MARK (White and Burnham 1999)

Results

Between 1997 and 2007, 56 turtles (18 males, 29 females, 4 juveniles and 5 of undetermined sex) were capture 99 times. Within the 10 years, 36 individuals were captured only once, 7 turtles were captured twice, 6 turtles were captured 3 times, 5 turtles were capture 4 times, 1 turtle was capture five times and one other turtle was capture six times. Below is a table depicting the number of new captures (never captured before) and recaptures (captured and marked in a previous year) by sex across years (Table B.1).

Before running any additional analyses in program MARK (White and Burnham 1999), program RELEASE GOF (goodness-of-fit) tests were run. Goodness-of-fit tests several of the key assumptions, such as equal probability of capture and survival among individuals from time t to time $t+1$, which must be met in order to accurately estimate parameters of interest. However, using these data, the goodness-of-fit test fails, indicating that the key assumptions of equal probability and equal survival of individuals from time t to time $t+1$ are not met. For a variety of reasons discussed above (see Chapter 2), it is unlikely that there are differences in probability of survival or capture. The more likely case is that the data are over-dispersed, or we have not yet collected enough data to accurately obtain estimates of survival and recruitment for this population of Blanding's turtles. In long-lived species, such as the Blanding's turtle, long-term data are needed to accurately estimate population parameters of interest and although this work has been conducted at the TCRA for the past ten years, more data are needed. It is possible to perform analyses to obtain estimates of population size; however, this requires the assumption that populations are

closed, or there is no migration in or out of the population, during the study. Given the number of individuals that are only captured once and never seen again, we do not feel that this is a valid assumption for this population.

For some species, mark-recapture analyses provide one method for estimating key population parameters, such as survival and recruitment. However, in long-lived species, such as the Blanding's turtle, long-term studies are needed to accurately estimate parameters of interest. With additional data collection, mark-recapture analyses can be used to understand how anthropogenic activities are impacting survival and recruitment for the population of Blanding's turtles in the TCRA.

Table B.1. Table depicting number of new capture (never captured before) and recaptures (captured and marked in previous year) by sex across all ten years.

Year	Number of Captures	Number of Recaptures by Sex				Number of New Captures by Sex			
		Males	Females	Juveniles	Unknown	Males	Females	Juveniles	Unknown
1997	2	0	0	0	0	1	0	0	1
1998	7	0	0	0	0	2	3	0	2
1999	9	0	1	0	0	2	3	0	1
2000	15	3	4	0	0	4	4	0	0
2001	5	3	1	0	0	0	1	0	0
2002	12	4	2	0	0	2	2	1	1
2003	6	0	2	0	0	1	3	0	0
2004	3	1	1	0	0	0	1	0	0
2005	14	3	4	0	0	1	4	2	0
2006	15	3	5	1	0	2	4	0	0
2007	12	3	1	0	0	3	4	1	0

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APPENDIX C. CANOPY COVER, SOLAR RADIATION, AND TEMPERATURE DEPENDENT SEX DETERMINATION: A PREDICTIVE MODEL

A paper to be submitted to *Journal of Herpetology*

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Introduction

The location of reptilian nests greatly influences incubation temperature and other environmental factors, which have significant impacts a wide variety of embryonic and offspring traits (Janzen 1994, Weisrock and Janzen 1999, Kolbe and Janzen 2002, Shine et al. 2002). One trait that is greatly influenced by temperature in many reptiles and has significant impacts on life history is sex. Therefore, quantifying nest-site choice by females and evaluating its impact on the offspring is essential for a proper understanding of reptile population biology, specifically with regard to understanding temperature-dependent sex determination and how changes in nest temperature impact the sex of the developing embryo.

Accurate characterization of the radiation regime beneath a forest canopy and in a nest is important for validation of physically based canopy models, assessment of variability of both solar and longwave radiative components, and input to energy-balance models concerned with the snow cover or soil conditions beneath a canopy. The forest canopy architecture (tree elements and gaps) strongly influences solar radiation interception and transmittance and varies with space and time, and depends on tree species, size and location of canopy gaps, and on the angle of solar incidence. Energy balance modeling at the stand scale presents challenges to account for these large variations of solar radiation incident on the forest floor. It is precisely these factors that may strongly influence nest site selection in species with TSD that oviposit in shallow nests beneath the forest floor.

Historically, some aspects of nest site choice have been difficult to quantify. Traditional techniques, such as the spherical densiometer, used to quantify canopy cover of nest site locations are circuitous at best and evidence suggests high observer bias, unpredictable variance and overestimation of canopy cover (Cook et al. 1995). In addition, variables of interest, such as site openness, are not direct measures of incident solar radiation,

which may be a significant contributor to the temperature experienced by the developing embryo in the nest (Doody et al. 2006). In response to these criticisms, recent developments, such as the use of hemispherical photography (HP) and the gap light analysis (GLA) program (Frazer et al. 1999, Frazer et al. 2000), have allowed for direct quantification of site openness and measurements of various radiation related variables as well as addresses issues of observer bias and unpredictable variance (Doody et al. 2006). This method is thought reduces observer biases in quantifying nest site selection, and may help in developing a model to predict nest thermal regimes based on the previously mentioned radiation related variables (Doody et al. 2006). However, although the HP and GLA techniques has been in use in forestry for a much longer period of time (Englund et al. 2000, Hale and Edwards 2002, Hardy et al. 2004), they have only recently been adapted for use in quantifying nest site selection in reptiles (Shine et al. 2002, Doody et al. 2006) and other vertebrate species (Wachob 1996).

Before the advent of HP and GLA, spherical densimeters had been the most widely used method for quantifying nest site selection by measuring canopy cover above the nest in the four cardinal directions (Lemmon 1956, Janzen 1994). Work in forestry has demonstrated a strong correlation between spherical densitometer readings and measurements obtained from HP above the area of interest in thick forest canopies (Englund et al. 2000), but this correlation has yet to be tested in areas of limited canopy structure. In this study we determined the relationship between spherical densitometer readings and hemispherical photograph analyses, as well as determined whether sex ratio of a turtle nest could be determined using HP analyses. We quantified canopy cover of nests of the painted turtle, *Chrysemys picta*, at the Thomson Causeway Recreation Area (TCRA) using both techniques. Research regarding turtle nesting behavior at this site has been on-going since 1988, and canopy cover measurements have been obtained using spherical densimeters for all nests since 1989. These readings, particularly south + west vegetation cover, have been shown to accurately predict hatchling sex ratio (Janzen 1994).

Methods

We estimated canopy cover over nests using spherical densitometer readings and hemispherical photography. Spherical densitometers readings were obtained using previously established methods (see Janzen 1994).

Hemispherical photos were taken approximately 25 cm above each nest using a Pentax MZ-5N camera fitted with a pentax 16-45mm lens equipped with a Fisheye Converter. Images were saved in JPEG format and according to Frazer et al. (2001), the image compromise attributable to compression was negligible. We used the scientific image processing software, Gap Light Analyzer (GLA) Version 2.0 developed by Frazer et al. (1999, 2000), to process and analyze the digital hemispheric canopy images. The software extracts canopy structure data (gap fraction, canopy openness, effective LAI) and gap light transmission indices based on user-specified parameters. The simplified radiation model within GLA assumes that when the sun position is obstructed by the canopy, the direct radiation is zero, and when unobstructed, direct radiation is equal to the above-canopy value (Frazer et al., 1999). Beam enrichment by scattered and reflected radiation is not considered. Frazer et al. (1999) used the gap light index (GLI) developed by Canham (1995).

General description of user-input variables is from the GLA Version 2.0 User Manual and Program Documentation. The cloudiness index is a site-specific measure of cloudiness and ranges from 0 to 1. The spectral fraction is the ratio of solar energy that falls within a limited range of the electromagnetic spectrum to the total shortwave radiation contributed by all wavelengths (0.25–25 μm). The beam fraction is the ratio of direct to global spectral radiation incident on a horizontal surface. The clear-sky transmission coefficient is a factor that describes the regional clarity of the atmosphere with respect to instantaneous transmission of direct radiation (approximately 0.3–3 μm) and ranges from 0.4 to 0.8. The solar constant is the total radiant flux of the sun outside the Earth's atmosphere. Additional input variables include image orientation, site location, time step, sky regions, and dates of interest. The maximum zenith angle of the mid-day sun path was calculated from June 1st-August 31st, which encompasses the incubation period during which sex is determined for most *C. picta* at TCRA (Janzen 1994). We designated an intensity threshold between 122-128 which best corresponded with the registered image, the software then creates binary classes of sky (white) and canopy (black). Using these parameters we used GLA to calculate

total solar radiation ($\text{MJ}/\text{m}^2/\text{day}$) at each nest over the incubation period, as well as site openness (canopy cover)

Densitometer readings and hemispherical photographs were taken at 50 nests at TCRA in 2004. The sex of hatchlings from nests that survived the incubation period were also determined (Janzen 1994). Hemispherical photographs were also taken from 50 random points throughout TCRA to determine nest site preference in relation to site openness. We used linear regression techniques to determine if densitometer parameters correspond to analyses from GLA (eg. Site openness and total solar radiation) and Chi-squared analyses was used to determine if canopy cover (or site openness) of nest sites differed significantly from the general canopy cover available at the site. Nonlinear (sigmoidal and logistic) regression techniques were used to determine if sex ratio of a nest was related total solar radiation or site openness.

Results and Discussion

Analyses indicate a strong linear relationship between south + west densitometer readings and total transmitted solar radiation ($R^2 = 0.72$ - Fig. 1). Analyses also indicated a linear relationship ($R^2 = 0.62$) between total densitometer readings (north + south + east + west) and site openness, or the percentage of open sky beneath the canopy (Fig. 2).

Using the equation given from this regression, it is possible to convert the south + west densitometer readings from previous years (1989-2002) to total transmitted solar radiation levels. This step is crucial for using this information to develop explicit models relating total transmitted solar radiation to nest temperature. In turn, understanding the temperature experienced by developing embryos is critical for unveiling the phenomenon of temperature dependent sex determination. Densitometer S+W totals from densitometer readings have been shown to be a relatively accurate predictor of sex ratio (Janzen 1994). This study shows that there is a strong linear relationship between S+W reading totals and calculated solar transmitted radiation, which provides the majority of energy or warmth in a shallow nest (Janzen 1994). The rays of the summer sun, high in the sky, arrive at a steep angle and heat the land much more than those of the winter sun, which hit at a shallow angle. Essentially, sunlight spreads itself over a smaller area of ground than it does in winter, and thereby heats the ground more efficiently, yielding more heat. The sun is most efficient when

it is highest in its ecliptical path; in the early afternoon when it is mainly contained in the South and West quadrants (Fig. 3). Hence it is not surprising that (a) nest temperature or sex of *C. picta* is related to densitometer readings from the south and west quadrants (Janzen 1994) and (b) solar radiation is correlated with south and west densitometer readings (Fig. 1).

Figure 1: Relationship between total transmitted solar radiation and total south + west densitometer readings (S + W). The equation of the line is: $y = -0.46x + 104$

But turtles may in fact be using simpler cues than total solar radiance over summer to select nest positions. A relatively easy parameter to assess is site openness, which we have found to also be a reasonable predictor of S+W densitometer readings (Fig. 2, Janzen 1994).

Basically, the amount of light penetrating through the canopy may prove a more obvious cue to a nesting female about incubating conditions of a potential nest location. Nest site choice of *C. picta* is highly repeatable and heritable at TCRA (Janzen and Morjan 2001).

Figure 2. Relationship between site openness and total ($R^2=0.63$) and south + west ($R^2=0.49$) densimeter readings (S + W).

Figure 3. The apparent path of the Sun across the sky.

In our study, we found that a disproportionate number of turtles nested in areas that were between 50-80% open (Fig. 4), and generally avoided areas that had large canopy cover despite the relatively high occurrences of these areas at TCRA (Fig. 4). Janzen and Morjan (2001) also found that *C. picta* at TCRA tended to nest disproportionately in relatively open microsites and clearly avoided the most heavily shaded areas. *Chrysemys picta* also exhibit field repeatability for overstory vegetation cover at oviposition, a trait that is correlated inversely with nest temperature during embryonic sex determination and positively with offspring sex ratio (Janzen and Morjan 2001).

Figure 4. Histogram comparing site openness (canopy cover) calculations of 100 random points at TCRA with nests created at TCRA in 2004.

Despite preference for relatively open conditions for nest locations, site openness *per se* was not a good predictor of the sex ratio of the nest, except for nests that were >75% open which produced 100% female nests. On the other hand, total solar radiation was an excellent predictor of sex ratio (sigmoidal- $F_{2,12}=9.9$ $p=0.02$; $R^2=0.63$) with nest sites receiving less than 60-65 MJ/m²/day predicted to be 100% male. Nest locations receiving 75-90 MJ/m²/day produced mixed sex ratios and nests receiving >90 MJ/m²/day produced 100% females (Fig. 5). Total solar radiation takes into account the sun's path during the summer months, which is

a more realistic predictor of incubation thermal regime than site openness which assesses the total amount of light penetrating the canopy, independent of the sun's path.

Figure 5. Relationship between solar radiation on a nest over summer and sex ratio. 3-parameter sigmoidal curve best describes the relationship ($R^2=0.63$)

There are several criticisms against using spherical densimeters to measure canopy cover, namely observer bias (Cook et al. 2005), however our work suggests that these instruments are capable of accurately capturing parameters (south + west canopy cover) that are most strongly associated with important variables such as total transmitted solar radiation and site openness. Hemispherical photography reduces observer bias and provides a good predictor of nest sex ratio, but when observer bias is controlled for densimeter readings also provide an accurate predictor of thermal conditions of a nest and sex ratio (Janzen 1994). However, despite the criticisms of this spherical densimeters, our study demonstrates these tools, combined with more powerful technology, provide accurate means for helping to understand temperature-dependent sex determination.

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