Inventory of Amphibians and Reptiles in the Green River Conservation Opportunity Area

Project Number T-111-R-1 Final Report

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Smooth Greensnake, *Opheodrys vernalis*, at Green River State Wildlife Area (photo by J. Vanek)

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Purposes: The overarching purposes of this project are (i) to promote the development of the Green River Conservation Opportunity Area as a model for the protection and recovery of species in greatest conservation need (SGCN) within the Grand Prairie Natural Division of Illinois and (ii) to promote Blanding's Turtle conservation state-wide.

Objectives: Objectives to achieve these purposes include the following.

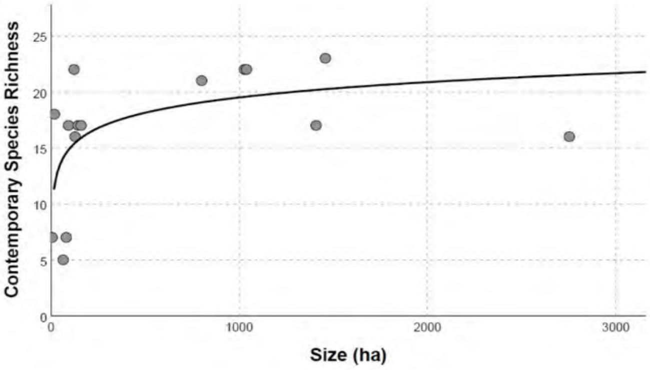
1. Conduct a comprehensive inventory of amphibians and reptiles at Green River SWA emphasizing
   a. Detection of Species in Greatest Need of Conservation and
   b. Animal health (body size, condition, and growth, disease status) of emblematic grassland species.
2. Assess changes in species composition since 1991.
3. Conduct initial inventories at and compile existing data from other sites within the Green River COA.
   a. Update maps of SGCN based on new records.
   b. Conduct conservation assessments using NatureServe Rank Calculator where possible.
4. Compare species composition and animal health between areas differing in management practices within the Green River SWA (e.g., between remnant and restored prairie, between wetland areas differing in extent of invasive vegetation).
5. Compare species composition and animal health of emblematic grassland species among regional grassland restoration sites.
6. Develop a baseline Population Viability Analysis for Blanding's turtle using new and existing data.
   a. Provide updated estimates of Blanding's turtle vital rates based on the Spring-Bluff – Chiwaukee Prairie population
   b. Compile estimates of Blanding's turtle vital rates range-wide
   c. Generate a baseline Illinois Blanding's turtle PVA
   d. Explore PVA outcomes over a range of initial population sizes and degrees of connectivity
   e. Conduct sensitivity analysis of model parameters

Report Organization: Given the broad and over-lapping nature of these objectives, this report is organized into five parts.

1. Amphibian and reptile occurrence and distribution within the Green River COA; relationships to preserve size, land cover and management (Objectives 1, 2, 3, 4, 5)
2. Historic and current status and NatureServe occurrence ranks of SGCN within the Green River COA (Objective 1, 3)
3. Amphibian and reptile disease status within the Green River COA (Objective 1, 4, 5)
4. Size, condition, and growth of emblematic snake species within the Green River COA (Objective 1, 4, 5)
5. Blanding's turtle population viability and sensitivity (Objective 6)

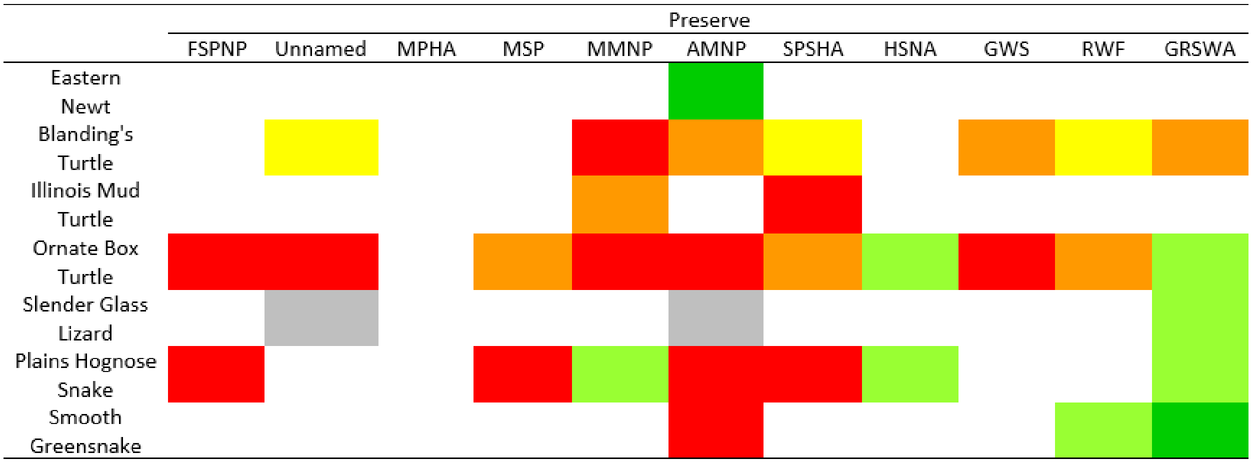
Executive Summary:

- Protected grasslands within the Green River Conservation Opportunity Area (COA) and in northern Illinois more generally provide habitat for a diverse array of amphibians and reptiles, including seven species in greatest conservation need (SGCN) (Part 1).
- Amphibian and reptile species richness exhibits a positive association with preserve size. After removing the effect of size, effects other preserve characteristics (e.g., land cover, years of protection, prior land use) on species richness were not evident (Part 1).
- Local extirpations were more frequent in small preserves than in large preserves: of 17 apparent extinction events, 14 occurred in preserves smaller than 200 ha (Part 1).



Relationship of amphibian and reptile species richness to preserve area.

- Given the abundance observed in this study, Green River SWA likely encompasses one of largest Smooth Greensnake populations in Illinois (Part 2).
- More generally, occurrences of SGCN amphibians and reptiles within the Green River COA frequently fall within fair, poor, or extirpated NatureServe categories or were undetected (yellow, orange, and red cells in the table below) (Part 2).

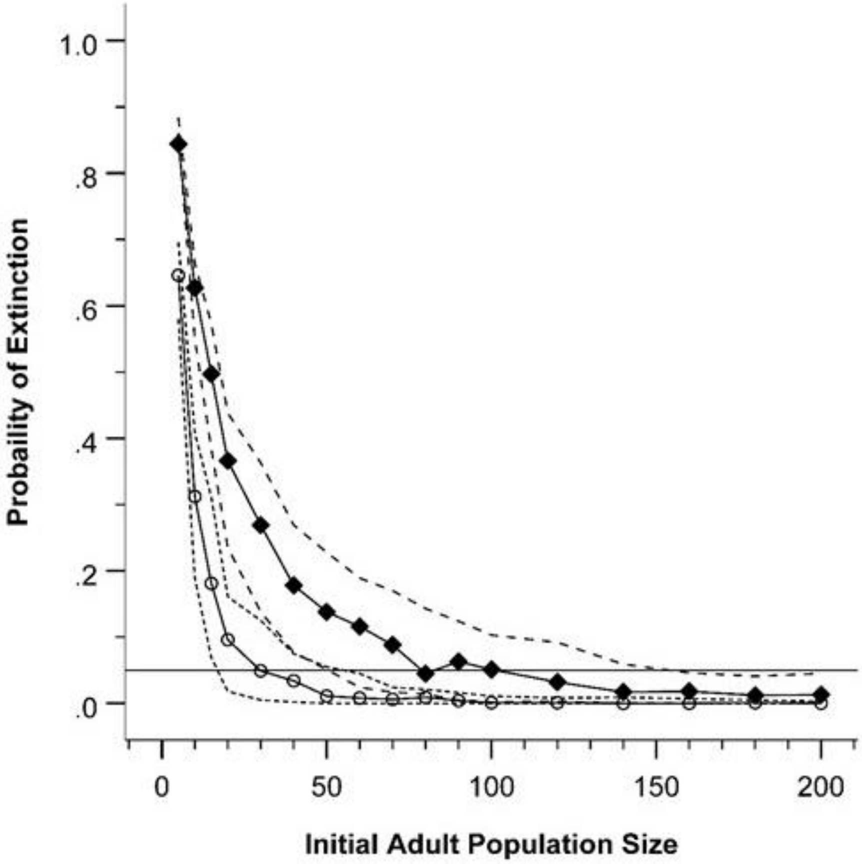


	Preserve										
	FSPNP	Unnamed	MPHA	MSP	MMNP	AMNP	SPSHA	HSNA	GWS	RWF	GRSWA
Eastern Newt						🟩					
Blanding's Turtle		🟨			🟥	🟧	🟨		🟧	🟨	🟧
Illinois Mud Turtle					🟧		🟥				
Ornate Box Turtle	🟥	🟥		🟧	🟥	🟥	🟧	🟩	🟥	🟧	🟩
Slender Glass Lizard		▨				▨					🟩
Plains Hognose Snake	🟥			🟥	🟩	🟥	🟥	🟩			🟩
Smooth Greensnake						🟥				🟩	🟩

- Threats to SGCN amphibians and reptiles within the Green River COA include incompatible land use (e.g., game fields and agricultural leases), off-property movements that may place animals in harm's way, non-native vegetation, and depredation of vulnerable life stages. Given their isolation from each other and from populations elsewhere, SGCN amphibians and reptiles are at risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes (Part 2).
- Amphibian and reptile pathogens, including *Batrachochytrium dendrobatidis* (the fungus that causes amphibian chytridiomycosis), *Ophidiomyces ophiodiicola* (responsible for snake fungal disease), *Emydoidea herpesvirus 1* and *Terrapene adenovirus* (viral pathogens of

turtles) were detected from swab samples collected within the Green River COA but there were no observations of compromised health as a consequence of these pathogens (Part 3).

- Patterns of variation in body size, condition and growth suggest only weak effects of prescribed fire, Illinois Natural Area Inventory status, or preserve on emblematic grassland snake species (Part 4).
- Population viability analysis (PVA) using demographic parameters from the Spring Bluff Chiwaukee Prairie Blanding's turtle population resulted in optimistic population projections (probability of extinction = 0% over 100 years) but results were less optimistic when catastrophes or parameter uncertainty were incorporated (probability of extinction = 3% and 16%, respectively) (Part 5).
- Uncertainty in estimates of age-specific mortality had the biggest impact on PVA outcomes but uncertainty in other parameters also contributed (Part 5).
- Blanding's turtle demography varies geographically; this variation resulted in both mortality- and fecundity-related parameters affecting PVA outcomes (Part 5).
- Projected extinction risk decreased rapidly with increasing initial population size. In the absence of catastrophes, ≥20-50 adults were necessary for extinction risk <5% over 100 yrs; when catastrophes were included, ≥50-200 adults were necessary for extinction risk <5% over 100 yrs (Part 5).



Relationship of probability of extinction to initial adult Blanding's turtle *Emydoidea blandingii* population size with (filled diamonds) and without (open circles) catastrophes in scenarios lasting 100 yr.

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Part 1. Amphibian and reptile occurrence and distribution within the Green River COA; relationships to preserve size, land cover and management

1.1 Introduction

Habitat loss and fragmentation is a significant cause of biodiversity decline (Fischer and Lindenmayer 2007; Stuart et al. 2004; Lara-Tufino et al. 2019). In areas were natural land cover has been converted to agriculture or urbanized, natural areas often occur as patches ('virtual islands') and biodiversity within these patches is influenced by colonization and extinction processes just as on real islands (Diamond 1975; Macarthur and Wilson 1967). The relative importance of colonization vs. extinction varies with patch history and species characteristics. Local extinction is expected to be the predominate in patches that remain following land cover conversion (land-bridge islands) and for species that have limited mobility whereas colonization is expected to be of greater importance in created habitat patches (restorations) and for species with high mobility.

In northern Illinois, patches of protected habitat ('preserves') mostly represent habitat islands that were once part of a larger, continuous, mostly grassland-dominated landscape. Among terrestrial vertebrates, amphibians and reptiles are poor dispersers compared to birds and mammals and so their biodiversity within preserves is likely to be more strongly influenced by local extinction than colonization (Driscoll 2004; Herkert 1994). As a consequence, amphibian and reptile biodiversity is expected to be strongly associated with preserve size (vs. distance to source populations) with extinction events occurring more frequently in smaller vs. larger preserves. Management practices within preserves and land use in areas surrounding preserves might accelerate or slow extinction, resulting in lower or higher biodiversity than expected given preserve size (Boudjemadi et al. 1999; Larson 2014).

Wildlife conservation efforts in Illinois are delineated in the Illinois Wildlife Action Plan (IDNR 2005) and associated Implementation Guide (IDNR 2005, 2015). Among other components, these documents designate *conservation opportunity areas*, "locations with significant existing or potential wildlife and habitat resources, where partners are willing to plan, implement and evaluate conservation actions, where financial and human resources are available, and where conservation is motivated by an agreed-upon conservation philosophy and set of objectives" (IDNR 2005, p 18-19) and identify *species in greatest conservation need* (SGCN), "species with small populations, declining populations, populations dependent on rare of vulnerable habitats, and indicative of the health and diversity of the state's wildlife and habitat resources" (IDNR 2005 p 30). This study centers on amphibian and reptile biodiversity at preserves within the Green River Conservation Opportunity Area (Green River COA) and in grassland dominated portions of northern Illinois more generally. The Green River COA encompasses the Green River Lowlands, a section of Illinois' largest natural division, the Grand Prairie Natural Division (Schwegman 1973). The Green River Lowlands were once occupied by two expansive wetlands, the Great Winnebago Swamp and the Inlet Swamp, totaling ca. 25,000 ha. The area were spared conversion to agriculture until the early 1900s with the development of more advanced land drainage methods (Schanzle and Kruse 1994). Pre-settlement, the landscape was dominated by mesic and wet prairie interspersed with marshes and wetlands but by the end of the 19th century more than 99% of the Grand Prairie Natural Division had been converted to agriculture (Urban 2005).

The overarching goal of this study is to assess how preserve size, land cover, and management history have contributed to contemporary amphibian and reptile biodiversity within grassland-dominated preserves in northern Illinois. Achieving this goal is made difficult by several factors. 1) Baseline data on species occurrence prior to habitat loss and fragmentation is frequently lacking and has to be inferred from regional species lists and habitat associations. 2) Because amphibians and reptiles can be rare and cryptic, surveys are labor intensive, thus limiting the availability of contemporary assessments of biodiversity (Durso and Siegel 2015). 3) Records of management efforts are sometimes unavailable or difficult to compare across preserves. 4) Because preserves all represent formerly private lands, time under management and prior land use may have lingering effects on biodiversity. To deal with these difficulties, species occurrence data were obtained from multiple sources including site managers and stewards, unpublished reports, and field surveys conducted between 2017 and 2019. In addition, land cover, and categorical assessments of historic land use (grazing, row crop agriculture, woody encroachment, and the occurrence of grassland or savanna remnants) and management (time under management, prescribed fire frequency) were incorporated into analyses.

The a priori expectation is that natural land cover (grassland, savanna, forest, wetland) is associated with higher amphibian and reptile biodiversity whereas agriculture and urban land cover is associated with lower amphibian and reptile biodiversity than predicted from preserve size alone and that this pattern holds for land cover within preserves and in a buffer surrounding preserves. Similarly, prior land use as pasture (vs. row-crop agriculture), the absence of encroachment by woody vegetation (especially non-native woody vegetation), and the presence of patches of unplowed remnant vegetation are expected to be associated with higher amphibian and reptile biodiversity than predicted from preserve size alone (Harrison et al., 2003; Larson, 2014). Likewise, preserved that have been protected longer and that experience more frequent prescribed fire are expected to be associated with higher amphibian and reptile biodiversity than predicted from preserve size alone (Harrison et al., 2003; Larson, 2014).

1.2 Methods

1.2.1 Study Sites

Fifteen grassland-dominated preserves in Northern Illinois were identified for which amphibian and reptile occurrence data were available or collected as part of this study (Fig. 1.1, Table 1.1). Ten preserves were located within the Green River COA; five other nearby preserves were included because of habitat similarity (grassland and savanna dominance) and the availability of recent comprehensive amphibian and reptile surveys (Table 1.2). For all preserves, new and existing data were augmented with observations provided by natural history experts queried as part of this study. Preserves selected were limited to Northern Illinois within or on the margins of the Grand Prairie Natural Division to minimize differences in the candidate species pool among preserves.

1.2.2 Historic and Current Land Use and Management

Preserve age was recoded in decades based on the year of acquisition (Table 1.3, 1.4). Preserves were categorized according to historic and current land use and management practices based on current and historic aerial imagery, published and unpublished documents and information provided by site managers and stewards (Table 1.3, 1.4). Because of incomplete information, preserves were categorized dichotomously with respect to grazing (entirely ungrazed vs. portions previously grazed), ploughing (portions previously or currently used for row-crop agriculture vs. no previous or current row-crop agriculture), encroachment (encroached by woody non-native vegetation vs. little woody encroachment), use of prescribed fire (absent or infrequent vs. portions subject to frequent prescribed fire (≤ 3 growing season interval), and the presence of remnants (no or only small (<3 ha) discontinuous remnants vs. remnants ≥ 3 ha).

1.2.3 Land Cover

Land cover of preserves and associated buffers was analyzed in ArcMap 10.4.1. Preserve boundaries were obtained from land owners or georeferenced and traced. A 500 meter buffer was created around each preserve using the “buffer” tool (Fig. 1.2). The “erase” tool was then used to remove the preserve area from the buffer layer. A wetlands feature class layer was obtained from the National Wetlands Database, however, some wetlands were shifted, created, or removed based on contemporary satellite imagery (GoogleEarth) to more accurately depict current land cover. Wetlands were then clipped to preserve and buffer layers respectively. Land cover rasters were obtained from Illinois Gap Analysis Program, 1999-2000 (https://clearinghouse.isgs.illinois.edu/data/land-cover/illinois-gap-analysis-program-land-cover-classification) and the national land cover database, 2011 (https://www.mrlc.gov/data). Rasters with a cell size of 30 were clipped to preserve and buffer layers. Boundaries of land cover polygons were corrected to align with contemporary satellite imagery (GoogleEarth). To correct the land cover data the “con” tool was used to convert each land cover classification into a single layer. The “raster to polygon” tool was used to convert each land cover layer into a feature class. Land cover feature classes were combined into one of five categories (forest, savanna, grassland, crop, or urban) using the “merge” tool. After correction, the “erase” tool was used to remove wetlands from land cover categories. The “feature to raster” tool was used to convert each land cover category, including wetlands, into a raster format with a cell size of 5. “Zonal statistics” was used to calculate the area of each land cover category per preserve, which was converted into a percentage.

For analysis, land cover categories were further condensed into grassland + savanna, wetland, forest, and unsuitable (crop + urban) to reduce the number of variables. Grassland + savanna land cover is characterized as largely open areas dominated by grasses and forbs with no or only sparse woody cover. Wetland land cover included areas that hold standing water into the mid-summer. Forest land cover is characterized by a closed canopy and an absence of grassland flora. Unsuitable land cover included human-modified landscapes consisting of row-crop agriculture, lawns, impermeable surfaces, and buildings.

1.2.4 Candidate Species Pool

The candidate species pool for each preserve was identified using county-level amphibian and reptile records (Phillips et al. 1999; King et al. 2020; https://www.inhs.illinois.edu/collections/herps/data/ilspecies/; https://biocoll.inhs.illinois.edu/portalx/collections/index.php; Distribution Notes from *Herpetological Review* compiled and provided by T. Anton). Because Nachusa Grasslands extends into both Lee and Ogle County, species from both counties were included in the candidate species pool for that preserve. Because county-level records appear to be incomplete for Bureau and Henry County (several common species are known from one or the other but not both), species from both counties were included as members of the candidate species pool for McCune Sand Prairie and Mineral Marsh.

1.2.5 Survey Methods

An amphibian and reptile species occurrence matrix was compiled based on field observations from 2017-2019 (Appendix 1.1), published and unpublished reports, and communication with researchers, land managers, and preserve stewards. Species records were categorized as contemporary (2002-present) or historic (before 2002). Field survey techniques included aquatic trapping (aquatic turtles; amphibian larva, metamorphs, and adults); canine-assisted searches (terrestrial turtles); artificial cover object (ACO) arrays (snakes, lizards, adult salamanders); drift fence/funnel trap arrays (snakes, lizards, adult amphibians); dip netting (amphibian larva, metamorphs, and adults); and visual encounter surveys (all taxonomic groups) although not all techniques were used at all preserves (Table 1.2).

Aquatic hoop traps included 24” X 12” hoop traps with dual 5” openings (Promar model TR-503), 30” X 54” hoop traps with a single 12” opening (Memphis Net and Twine model TN210) or 20” X 36” D-traps with a single 12 inch opening and were baited with sardines in oil. Traps were positioned so that they extended above the water line, were checked daily, and were repositioned weekly. Canine-assisted surveys occurred from June 21-23 2017, May 22-27 2018, and June 20-26 2019 during the morning hours. A team of Boykin Spaniels, ranging from 1 to 7 dogs at a time, was led by their owner through suitable Ornate Box Turtle habitat. Accompanying researchers conducted visual encounter surveys simultaneously. When turtles were found, a GPS location was recorded at the estimated site of capture. ACOs consisted of 61

X 81 cm plywood boards and recycled rubber conveyor belt. ACOs were placed at 20 m intervals within transects. Parallel transects at Green River SWA were placed 20 m apart. Parallel transects at Richardson Wildlife Foundation were placed 50 m apart. Drift fences made from prefabricated erosion mesh measured 50m with one funnel trap at each end. Funnel traps were constructed from 1/8" hardware cloth and zip-ties. Funnel traps were covered with a piece of burlap to provide shade and cover. Radio transmitters were affixed to Blanding's Turtles and Ornate box turtles opportunistically. Turtles were tracked using a hand-held receiver and antenna and geographic coordinates were recoded used a hand-held GPS receiver, providing information on movements, habitat uses, overwintering sites, and mortality. More information about field surveys is provided in Appendix 1.1.

1.2.6 Vegetation Surveys

In 2018 and 2019, vegetation surveys were conducted in early to mid-July by assessing the proportion of the ground surface occupied by bare soil, forbs, grasses, sedges, thatch, or woody vegetation within a 0.5 X 0.5 m quadrat at 10 points spaced evenly within each ACO array. Results were averaged across quadrats within ACO arrays, providing mean ground cover proportions for each array in each year. Additionally, in 2019, plant stature was assessed adjacent to each quadrate using a Robel pole; values were averaged to provide mean stature for each ACO array.

1.2.7 Statistical Analysis

Species richness – An exploratory approach to identifying variables that may influence amphibian and reptile species richness was used. Such an approach is warranted because of small sample size (15 preserves) and the possibility that there are multiple drivers of species richness. To reduce the likelihood of overlooking variables that may influence species richness (type II error), p-value less than or equal to 0.10 are highlighted.

Linear regression was used to characterize the semi-log relationship between the contemporary species richness per preserve and the log of the preserve area (Scheiner, 2003). To identify sources of remaining variation in species richness, residuals of this relationship were compared via bivariate correlation with land cover factors within preserves, land cover factors within the 500m buffer, and time under management. Independent sample t-tests were used to identify associations between residuals and the presence of remnant prairie, grazing, row-crop agriculture, encroachment and prescribed fire.

Possible local extinctions – Potential extirpations were identified by comparing contemporary and total (contemporary + historical) species lists for each preserve. A lack of prior surveys at MPHA meant that only contemporary records were available for that preserve, thus MPHA was omitted from analysis. To test whether likelihood of extirpation was independent of size, a test of independence was used to compare contemporary and historic species records within small

(<200ha) and large (>200ha) preserves. With MPHA excluded, there were 8 small preserves and 6 large preserves.

Species-specific analyses – Species-specific analyses of presence/absence were conducted for four species (Smooth Greensnake, Plains Gartersnake, Ornate Box Turtle, and Cricket Frog). These species occurred at an intermediate number of sites, facilitating analysis; other species of interest occurred at too many or too few preserves to allow for statistical testing. Variables suspected to impact species presence were history of plowing, history of grazing, woody encroachment, prescribed fire intensity, presence of remnant prairie, preserve area, years of active management, wetland area and grassland/savanna area. Species of interest were scored as 1 if contemporarily present, 0 if contemporarily absent, and blank if the species would not normally occur at a given preserve. Two-sample t-tests were performed to test for differences in preserve area, years of active management, wetland area and grassland/savanna area between preserved with and without a given species. Fisher's exact tests were used to test for independence between species presence and presence of remnant prairie, burn regime, history of plowing, history of grazing, and woody encroachment.

A separate set of analyses focused on amphibians and reptiles encountered using artificial cover object (ACO) arrays. ACO arrays provide quantitative data on the occurrence of amphibians and reptiles. Importantly, these data can be scaled by effort, thus facilitating comparisons between habitat categories or preserves. Analyses focused on the effects of fire, INAI status, and vegetation on amphibian and reptile occurrence within the Green River SWA and on differences in amphibian and reptile occurrence among those preserves within the Green River COA sampled using ACO arrays.

For species encountered at most or all ACO arrays in all three years (Smooth Greensnake, Dekay's Brownsnake, Common Gartersnake), Anova was used to test for effects of burn and INAI status on the number of captures per 100 ACO checks. Year and ACO array (nested within INAI status) were included as additional sources of variation, thus controlling for variation among years and arrays. Lack of replication (areas containing ACO arrays were burned only once during the study) precluded tests of interactions. Follow-up analyses included Shapiro-Wilkes t-tests (allowing for unequal variances) for differences in vegetation between burn and INAI categories and correlation tests between captures and vegetation.

For species encountered in only a subset of arrays (Tiger Salamanders, Six-lined Racerunners, Eastern Foxsnakes, and Plains Gartersnakes), Fisher's exact tests were used to assess whether occurrence (present, absent) differed by burn and INAI status. Follow-up analyses included Shapiro-Wilks t-tests for differences in vegetation between occurrence categories (this modification of the familiar two-sample t-tests allows for unequal variances between samples). Two species encountered only infrequently (Eastern Hognosed Snake and Plains Hognosed Snake, each with six encounters in four arrays) were excluded from ACO data analyses.

In addition to the Green River SWA, ACO arrays were deployed at five other sites, Foley Sand Prairie Nature Preserve, Maytown Pheasant Habitat Area, Sand Prairie State Habitat Area, Gremel Wildlife Sanctuary, and Richardson Wildlife Foundation (Table 1.5). To control for variation in effort among sites, captures per 100 ACO checks was used as metric for abundance.

1.3 Results

1.3.1 Preserve Characteristics

Preserves were acquired between 0 and 7 decades ago and currently vary in size from 6 to more than 2,700 ha (Table 1.4). Roughly equal numbers of preserves fall into dichotomous categories related to the occurrence of grazing, plowing, encroachment, remnant vegetation and prescribed fire (Table 1.4).

Grassland and savanna was the predominant land cover (mean = 60%, range = 49-98%) within all but two preserves (AMNP and GWS) where forest predominated (56 and 71%). Across all preserves, forest land cover averaged 21% (range = 0-71%) and wetland averaged 12% (range - 9-33%). Land cover attributable to agriculture accounted for 14-34% in five preserves. This included land being farmed prior to restoration (FNAL, NG), game fields (e.g., and RWF, GRSWA, SPSHA), or leased for haying (GRSWA). Urban land cover occupied 7% of FNAL and less than 0.6% of other preserves. Wetland land cover had a mean coverage of 11.7% with a range of 0.0-33.2%. When agricultural and urban lands were combined into a single unsuitable land cover category, it accounted for an average of 7% (range = 0-418%; Fig. 1.3, Table 1.6).

Agriculture was the predominant land cover within buffer areas of most preserves (mean = 50%, range = 6-87%). Urban land cover was generally low (≤ 6%) in the buffers of most preserved but accounted for the majority of buffer area (60-65%) around three DuPage County preserves (FNAL, PWWFP, WCPFP). Wetland averaged 6% (range = 1-38%), grassland and savanna averaged 16% (range = 6-36%), forest averaged 13% (range = 2-30%) of buffer land cover (Fig. 1.3, Table 1.6).

1.3.2 Amphibian and Reptile Biodiversity

In total, 46 species of amphibians and reptiles have been documented from the six counties included in our study area, with 27-32 documented per county (Table 1.7). The number of candidate species per preserve was 32 at all preserves except Goose Lake (30) and Nachusa Grasslands (34).

Thirty-two species (8 anurans, 2 salamanders, 6 turtles, 2 lizards, and 14 snakes) were documented across the fifteen preserves included in this study (Table 1.8, Appendix 1.1). On average, 16.5 amphibian and reptile species were found in each preserve since 2002 (range = 6-23). Including historic records increased the mean to 17.7 (range = 6-25; Table 1.8). Among species found in the preserves included in this study are seven species in greatest conservation need, Eastern Newt, Blanding’s Turtle, Yellow Mud Turtle, Ornate Box Turtle, Slender Glass

Lizard, Smooth Greensnake, and Plains Hog-nosed snake. Thirteen species present in the candidate species pool were not recorded in any of the preserves included in this study, including the Fowler's Toad, Plains Leopard Frog, Blue-spotted Salamander, Smallmouth Salamander, Four-toed Salamander, Mudpuppy, Common Map Turtle, Eastern Musk Turtle, Eastern Box Turtle, Red-eared Slider, Kirtland's Snake, Queen Snake, and Eastern Massasauga (Table 1.7). Thirteen species were widely distributed, occurring in 13 or more preserves, including the American Toad, Gray Treefrog, Northern Leopard Frog, Bullfrog, Green Frog, Tiger Salamander, Painted Turtle, Snapping Turtle, Blanding's Turtle, Eastern Foxsnake, Dekay's Brownsnake, and Common Gartersnake (Table 1.8). Another eight species were narrowly distributed, occurring in 3 or fewer preserves, including the Red Spotted Newt, Spring Peeper, Yellow Mud Turtle, Slender Glass Lizard, Milksnake, Grahams Crayfish Snake, Redbellied Snake, and Western Ribbon Snake (Table 1.8).

Contemporary richness was positively correlated with preserve area (species richness = 5.89+1.97*ln(preserve area), R = 0.60, P = 0.019; Fig. 1.4). Residuals from this regression were generally uncorrelated with land cover within preserves or within a 500 m buffer around preserves (Table 1.9). Residual species richness did show a marginally significant positive correlation with buffer forest land cover (R = 0.50, P = 0.056). Age since management showed no correlation with the residuals. Residual species richness did not differ between preserves with or without a history of grazing, with or without a history of or current occurrence of row-crop agriculture, with or without woody encroachment, with or without presence of remnant prairie, or with or without a fire regime of greater than 4 growing seasons (Table 1.9).

Small preserves (<200 ha, n = 8, MPHA omitted due to a lack of historic data) had 14 apparent extinction events, while large preserves (>200 ha, n = 6) only had 3 (Table 1.8). Conversely, species persisted in small on 121 occasions and in large preserves on 120 occasions. Outcome (persistence vs. extinction) was dependent of preserve size (small, large); local extinctions were more frequent in small than in large preserves (χ_c^2 = 5.35, P = 0.021). Apparent extinctions included Blanding's turtle (n = 1 instance), Yellow mud turtle (n = 1), Ornate box turtle (n = 5), Six-lined racerunner (n = 1), Blue racer (n = 2), Plains hog-nosed snake (n = 2), Northern watersnake (n = 1), Bull snake (n = 3), and Graham's crayfish snake (n = 1).

Data on historic species occurrences are especially complete for Green River SWA as a result of surveys conducted there in 1991 (Redmer 1991). Of 23 species present in 1991, 21 were confirmed as persisting at the site in this study. However, neither the Blue Racer (one observed in 1991) nor the Northern Watersnake (two observed in 1991) were encountered during this study.

Four species, the Northern cricket frog, Smooth green snake, Ornate box turtle, and Plains Gartersnake, occurred at an intermediate number of sites, allowing for tests of association between status (present, absent) and site characteristics. Sites where each of these species were present were larger, and grassland/savanna area was greater, than sites where each was absent (Table 1.10). Wetland area and years of management were greater at sites with Smooth Greensnakes and with Plains Gartersnakes than at sites where these species were absent (Table

1.10). Plains Gartersnake presence was positively associated with sites having remnant prairie (Table 1.10).

Note: Analyses presented here are based on field efforts through 2019. In 2020, Natural Heritage Biologist Russ Blogg recorded the presence of an Ornate Box Turtle at Sand Prairie State Habitat Area. This observation is unlikely to significantly alter the results and conclusions reported here.

1.3.3 Effects of fire, INAI status, and vegetation on amphibian and reptile occurrence within the Green River SWA

Over the three years of this study, areas surrounding ACO arrays were each burned once either in fall or spring prior to the onset of field work. Additionally, six arrays were located within and six arrays were located outside of INAI designated areas (Table 1.11).

Burn status had a significant effect on Smooth Greensnake and Dekay's Brownsnake captures (Table 1.12.A, B). More captures occurred following fire (Smooth Greensnakes: 1.94 vs. 0.75 captures per 100 ACO checks; Dekay's Brownsnakes: 2.87 vs. 1.78 captures per 100 ACO checks; Table 1.12.A). INAI status had a significant effect on Smooth Greensnake captures (Table 1.13.A). Fewer captures occurred within INAI-designated areas (0.84 vs. 1.45 captures per 100 ACO checks; Table 1.13.B).

Overall, thatch accounted for the largest proportion of ground cover within GRSWA (Fig. 1.5). However, thatch changed dramatically, depending on prescribed fire usage (Table 1.11, Fig. 1.5).

Across arrays and years, proportion of ground cover categorized as bare soil was negatively correlated with thatch ($r = -0.865$, $p < 0.001$); other correlations among vegetation classifications were nonsignificant (Table 1.14). Proportion of ground cover categorized as bare soil was significantly higher and thatch was significantly lower following fire (bare soil = 0.368 vs. 0.051, $P < 0.001$; thatch = 0.098 vs. 0.917, $P < 0.001$; Table 1.15). Proportion of ground cover categorized as sedge was significantly lower within INAI areas (0.013 vs. 0.130, $P = 0.027$; Table 1.15). Consistent with these differences, Smooth Greensnake abundance was positively correlated with bare ground ($r = 0.582$, $P = 0.003$) and negatively correlated with thatch ($r = -0.684$, $p < 0.001$; Table 1.16). Dekay's Brownsnake and Common Gartersnake abundance was positively correlated with sedges (Brownsnakes: $r = 0.625$, $p = 0.001$, Gartersnakes: $r = 0.517$, $p = 0.010$; Table 1.16).

Six-lined racerunners were more likely to be present within INAI-designated areas but the occurrence of racerunners did not differ with fire status (Table 1.17). Tiger salamander, Eastern Foxsnake, and Plains Gartersnake occurrence did not differ with fire or INAI status (Table 1.17).

Six-lined racerunner occurrence was associated with greater woody vegetation (Table 1.18.B). Plains Gartersnake occurrence was associated with greater sedge cover and taller stature vegetation (Table 1.18.D). Tiger salamander and Eastern Foxsnake occurrence were unassociated with vegetation (Table 1.18.A, C).

1.3.4 Variation in amphibian and reptile occurrence among sites within the Green River COA

Four species were ubiquitous, occurring at ACO arrays at all (Dekay's Brownsnakes, Common Gartersnakes) or nearly all (Eastern Foxsnakes) preserves surveyed (Table 1.19). Other species occurred at ACO arrays at just one (Plains hognosed snakes), two (Six lined racerunners, Eastern hognosed snakes, Smooth Greensnakes, Plains gartersnakes), or three (Tiger salamanders) preserves. Capture rates varied by 10-fold or more among preserves for Foxsnakes (common at Richardson Wildlife Foundation), Dekay's Brownsnakes (common at Gremel Wildlife Sanctuary and Maytown Pheasant Habitat Area), rare at Foley Sand Prairie Nature Preserve and Sand Prairie State Habitat Area), common Gartersnakes (common at Richards on Wildlife Foundation, rare at Foley Sand Prairie Nature Preserve). Ground cover attributable to different categories was also variable (Table 1.20).

1.4 Discussion

The preserves included in this analysis are inhabited by a diverse array of amphibians and reptiles, including seven species in greatest conservation need. Amphibian and reptile species richness increased with increasing preserve size but neither land cover within or immediately surrounding preserves, nor preserve age, were correlated with species richness after controlling for preserve size. Likewise, neither grazing history, historic or current occurrence of row-crop agriculture, extent of woody encroachment, presence of remnant prairie, nor prescribed fire frequency were associated with species richness after controlling for preserve size. Admittedly, given the limited number of preserves included in these analyses (15) and the need to categorize many preserve characteristics on a dichotomous scale (e.g., grazing vs. no grazing), such effects would need to be strong to be detected and more subtle effects may have been overlooked.

A comparison of species known to be present historically with those documented during recent (since 2002) surveys suggests the occurrence of local extirpations, particularly in smaller preserves. Of nine species not currently found in one or preserves in which they had been documented in the past, four are species in greatest conservation need (Blanding's Turtle, Yellow Mud Turtle, Ornate Box Turtle, Plains hog-nosed snakes). Knowledge of local extirpations is imperfect. Some apparent extirpations may represent cases where a species went undetected during recent field work and some species absences from both historic and current inventories may represent undocumented extirpations due to incomplete historic records.

Species specific patterns of occurrence in four species for which such analyses were possible reinforced the association of amphibian and reptile biodiversity to preserve size. In addition, area of grassland and savanna was associated with the occurrence of all four species (a pattern also seen in grassland birds, Walk and Warner 1999) and wetland area was associated with Smooth Greensnake and Plains Gartersnake occurrence. Perhaps surprisingly, wetland area was not associated with cricket frog occurrence. However, the presence of a full complement of amphibian species at most preserves suggests that even small amounts of wetland are sufficient for species persistence. This result aligns with the findings of Quesnelle et al. (2015) who report that non-wetland land cover was more important than wetland land cover to amphibian population abundance.

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Table 1.1. Preserve history and characteristics. Preserves are ordered by size. Abbreviations: FSPNP – Foley Sand Prairie Nature Preserve, MPHA – Maytown Pheasant Habitat Area, MSP – McCune Sand Prairie Land and Water Reserve, MMNP – Mineral Marsh Nature Preserve, AMNP – Amboy Marsh Nature Preserve, SPSHA – Sand Prairie State Habitat Area, WCPFP – West Chicago Prairie Forest Preserve, GWS – Gremel Wildlife Sanctuary, RWF – Richardson Wildlife Foundation, GLPSNA – Goose Lake Prairie State Natural Area, GRSWA – Green River State Wildlife Area, WWFP – Pratt's Wayne Woods Forest Preserve, NG – Nachusa Grasslands, FNLA – Fermi National Laboratory (one preserve is left unnamed at the request of stewards and preserve managers).

Preserve	Current Area (ha)	Date of Acquisition	Ownership, Preserve History and Characteristics
FSPSNP	6	1988	• Illinois Department of Natural Resources • Partially plowed for 2 consecutive years prior to acquisition
Unnamed	X	X	• Description not provided at request of stewards and preserve managers
MPHA	65	1999	• Illinois Department of Natural Resources • Little management • Public hunting area
MSP	81	ca. 1980	• Soil and Water Conservation District of Bureau County • Little management, extensive woody succession • Grazed historically • Lots of prickly pear
MMNP	93	1999	• Illinois Department of Natural Resources • Expansive sand prairie/savanna • Grazed prior to acquisition
AMNP	122	2013	• Illinois Audubon Society • Wetland restoration and active management since acquisition
SPSHA	128		• Illinois Department of Natural Resources • Row crop game fields • Modified sandy bottom wetlands • Public hunting area
WCPFP	145	1979	• Forest Preserve District of DuPage County • Originally purchased by railroad for use as cattle yard • Adjacent to active stock yard

Preserve	Current Area (ha)	Date of Acquisition	Ownership, Preserve History and Characteristics
GWS	160	2017	• Illinois Audubon Society • Formerly used as hunt club, church camp, and university field station • Expansive pine plantings and woody succession • Active management since acquisition
RWF	800	1989	• Privately owned wildlife foundation • Remnant prairies and wetlands • Active prairie restoration and wetland creation • Pine plantings • Private hunting area
GLPSNA	1027	1969	• Illinois Department of Natural Resources • Extensive remnant grassland • Parts formerly used for coal mining • Reservoir created to the north • Public hunting area
GRSWA	1038	1943	• Illinois Department of Natural Resources • Extensive remnant grassland • Row crop game fields and hay leases • Small abandoned sand mining operation • Public hunting area
PWWFP	1407	1965	• Forest Preserve District of DuPage County • Highly fragmented by busy roads and railroads
NG	1457	1985	• The Nature Conservancy • Large-scale restoration project • Initial 110 ha of remnant has expanded to 1460 ha
FNAL	2752	1975	• U.S. Department of Energy • Large-scale restoration project • Initial 3 ha prairie restoration in 1975 increased to 400 ha by 2000

Table 1.2. Survey methods and sources of information on amphibian and reptile species richness. See Table 1.1 for Preserve abbreviations. Survey Method abbreviations: ACO – Artificial Cover Objects, AT – Aquatic Trapping, DF – Drift Fences, CAS – Canine Assisted Surveys, VES – Visual Encounter Surveys, TC – Trail Cameras.

	Survey Method						
Preserve	ACO	AT	DF	CAS	VES	TC	Data Sources
FSPSNP	●			●	●		R. Nyboer, R. Blogg; this study
Unnamed					●	●	Nelson et al. 2017; D. Carey; W. Rogers
MPHA	●				●		This study
MSP					●		Horger 2006; Lerczak 2008; R. Blogg; this study
MMNP		●	●	●	●		Horger 2005, Tuma 1993, 2006; R. Blogg, this study
AMNP		●			●	●	Nelson et al 2015; Phillips (2014); D. Carey; W. Rogers; this study
SPSHA	●	●	●		●		R. Blogg, this study
WCPFP	●	●			●		D. Thompson; J. Vecchiet
GWS	●	●			●		Vecchiet 2017; D. Carey; S. Hager; W. Rogers; this study
RWF	●	●		●	●		J. B. Towey; this study
GLPSNA	●	●			●		King and Sacerdote 2014
GRSWA	●	●	●	●	●		Redmer 1991; R. Blogg; this study
PWWFP	●	●			●		D. Thompson; J. Vecchiet
NG	●	●		●	●		Anton et al. 2013; King and Vanek 2019; this study
FNAL	●	●			●		Schramer and Anton 2018

Table 1.3. Historic and current land use and management scoring system. Higher scores are expected to promote amphibian and reptile species persistence.

Variable	Score	Description
Age	0-7	Preserve age in decades
Grazing	0, 1	Entirely ungrazed vs. portions previously grazed
Unplowed	0, 1	Portions previously or currently used for row-crop agriculture vs. no previous or current row-crop agriculture
Woody Encroachment	0, 1	Grassland areas encroached by woody non-native vegetation vs. little woody encroachment
Prescribed Fire	0, 1	Use of prescribed fire absent or infrequent vs. portions subject to frequent prescribed fire (≤ 3 growing season interval)
Remnant Grassland	0, 1	No or small (<3 ha) discontinuous remnants vs. remnants ≥ 3 ha

Table 1.4. Preserve management and history characteristics. Grazed: 0 = not grazed, 1 = grazed. Plowed: 0 = plowed, 1 = not plowed, Woody: 0 = major woody encroachment, 1 = minimal woody encroachment. Fire: 1 = 1-3 growing seasons, 0 = > 4 growing seasons. Remnant: 0 = absent or less than 6 ha present, 1 = greater than 6 ha present. See Table 1.1 for Preserve abbreviations.

Preserve	Area (ha)	Decades Managed	Grazed	Plowed	Woody	Fire	Remnant
FSPSNP	6.1	3	0	0	1	1	1
Unnamed	18.2	1	1	1	0	1	0
MPHA	64.7	2	1	0	0	0	0
MSP	80.9	3	1	1	0	0	1
MMNP	93.1	2	1	1	1	1	1
AMNP	122.2	0	1	1	0	0	0
SPSHA	127.9	2	1	0	1	1	0
WCPFP	144.9	3	1	0	0	0	1
GWS	159.9	0	0	1	0	0	0
RWF	799.7	3	0	0	1	1	1
GLPSNA	1026.7	5	0	0	1	0	1
GRSWA	1038.0	7	0	0	0	1	1
PWWFP	1407.5	4	0	0	0	0	0
NG	1456.9	2	1	0	0	1	1
FNAL	2751.9	4	0	0	1	1	0

Table 1.5. Number of ACO arrays, number of AC0s per array, survey years, and number of ACO checks at preserves within the Green River COA (see Table 1.1 for preserve abbreviations).

Preserve	# of Arrays	ACO per array	Years	Total ACO Checks	Mean ACO checks per array (standard deviation)
FSPNP	2	8	2018-2019	272	68.0 (60.0)
MPHA	2	8	2018-2019	224	56.0 (18.5)
SPSHA	5	8	2017-2019	1,104	73.6 (40.6)
GWS	1	32	2017	192	192.0 (-)
RWF	7	16	2018-2019	1,904	146.5 (56.1)
GRSWA	12	32	2017-2019	18,592	516.4 (126.2)
Total	26	600		22,288	305.3 (231.4)

Table 1.6. Percentage of land cover type within the preserve (A) and within the buffer (B). See Table 1.1 for preserve abbreviations.

	Ground Cover			
	Wetland	Grassland/ Savanna	Forest	Unsuitable
A. Preserve				
FSPSNP	0.0	97.8	0.0	0.0
Unnamed	9.3	75.0	15.0	0.0
MPHA	0.8	80.5	18.4	0.0
MSP	0.0	66.6	33.2	0.0
MMNP	1.1	96.9	1.8	0.0
AMNP	20.6	23.3	55.5	0.0
SPSHA	7.2	67.2	0.0	25.3
WCPFP	20.3	48.8	30.0	0.6
GWS	17.3	11.3	70.7	0.1
RWF	8.5	57.2	25.6	8.5
GLPSNA	33.2	61.7	4.5	0.6
GRSWA	17.9	52.8	13.5	15.7
PWWFP	25.7	58.9	14.1	0.9
NG	2.0	68.9	13.9	14.7
FNAL	11.3	36.7	11.2	40.8
B. Buffer				
FSPSNP	0.7	7.5	2.2	88.1
Unnamed	2.7	6.0	12.4	78.8
MPHA	0.6	24.3	7.3	67.5
MSP	1.0	28.1	8.3	62.5
MMNP	3.0	35.5	5.7	55.4
AMNP	6.1	12.8	39.5	41.5
SPSHA	1.2	18.9	4.5	75.0
WCPFP	5.9	9.6	14.4	69.2
GWS	4.5	15.5	27.3	52.2
RWF	2.0	7.1	6.7	83.8
GLPSNA	38.4	26.1	9.1	25.5
GRSWA	4.1	7.6	11.4	76.2
PWWFP	6.1	14.6	6.6	71.9
NG	4.1	14.2	30.3	50.6
FNAL	5.5	15.5	8.1	70.2

Table 1.7. Amphibian and reptile species documented to occur within Illinois counties surrounding preserves included in this study. Species not found in the preserves included in this study are marked with asterisks. County records were compiled from Phillips et al. 1999; King et al. 2020; https://www.inhs.illinois.edu/collections/herps/data/ilspecies/; https://biocoll.inhs.illinois.edu/portalx/collections/index.php; Distribution Notes from *Herpetological Review* compiled and provided by T. Anton.

Scientific Name	Common Name	County					
		Lee	Ogle	Henry	Bureau	DuPage	Grundy
Frogs and Toads							
Anaxyrus americanus	American toad	+	+	+	+	+	+
*Anaxyrus fowleri**	Fowler's toad				+		+
Acris crepitans	Northern cricket frog	+	+	+	+	+	+
Hyla veriscolor/chysoscelis	Gray treefrog complex	+	+	+	+	+	+
*Lithobates blairi**	Plains leopard frog			+			+
Lithobated catesbeianus	Bullfrog	+	+	+	+	+	+
Lithobates clamitans	Green frog	+	+	+	+	+	+
Lithobates palustris	Pickerel frog	+	+				
Lithobates pipiens	Northern leopard frog	+	+	+	+	+	+
Pseudacris crucifer	Spring peeper	+	+		+	+	+
Pseudacris maculata	Chorus frog	+	+	+	+	+	+
Salamanders							
*Ambystoma laterale**	Blue-spotted salamander					+	
*Ambystoma texanum**	Smallmouth salamander			+	+		
Ambystoma tigrinum	Tiger salamander	+	+	+	+	+	+
*Hyla scutatum**	Four-toed salamander		+				
*Necturus maculosus**	Mudpuppy					+	
Notophthalmus viridescens	Eastern newt	+				+	
Turtles							
Apalone spinifera	Spiny softshell	+	+	+	+	+	+

Scientific Name	Common Name	County					
		Lee	Ogle	Henry	Bureau	DuPage	Grundy
Chelydra serpentina	Common snapping turtle	+	+	+		+	+
Chrysemys picta	Painted turtle	+	+	+	+	+	+
Emydoidea blandingii	Blanding's turtle	+	+	+	+	+	+
*Graptemys geographica**	Common map turtle					+	+
Kinosternon flavescens	Illinois mud turtle	+		+			
*Sternotherus odoratus**	Eastern musk turtle					+	
*Terrapene carolina**	Eastern box turtle						+
Terrapene ornata	Ornate box turtle	+	+	+	+		
*Trachemys scripta**	Red-eared slider	+		+	+	+	
Lizards							
Aspidoscelis sexlineata	Six-lined racerunner	+		+	+		+
Ophisaurus attenuatus	Slender glass lizard	+					+
Snakes							
*Clonophis kirtlandii**	Kirtland's snake					+	
Coluber constrictor	Blue racer	+	+	+	+	+	+
Heterodon nasicus	Plains hognose snake	+	+	+	+		
Heterodon platirhinos	Eastern hognose snake	+	+		+		+
Lampropeltis triangulum	Milk snake		+		+	+	
Nerodia sipedon	Northern watersnake	+	+	+	+	+	+
Opheodrys vernalis	Smooth greensnake	+		+	+	+	+
Pantherophis vulpinus	Eastern fox snake	+	+	+	+	+	+
Pituophis catenifer	Bullsnake	+	+	+			+
Regina grahamii	Graham's crayfish snake			+	+	+	+
*Regina septemvittata**	Queen snake	+	+			+	+
*Sistrurus catenatus**	Eastern massasauga					+	
Storeria dekayi	Dekay's brownsnake	+	+	+	+	+	+
Storeria occipitomaculata	Red-bellied snake		+			+	
Thamnophis proximus	Western ribbon snake	+					

Scientific Name	Common Name	County					
		Lee	Ogle	Henry	Bureau	DuPage	Grundy
Thamnophis radix	Plains gartersnake	+	+	+	+	+	+
Thamnophis sirtalis	Common gartersnake	+	+	+	+	+	+
Total		32	27	28	28	32	30

Table 1.8. Amphibian and reptile occurrence at grassland-dominated preserves in northern Illinois (● = contemporary record 2002 to present, ☐ = historical record prior to 2002). Cells shaded dark gray represent sites outside of species range. Species in Greatest Need of Conservation are highlighted in light gray. One preserve is left unnamed at the request of stewards and preserve managers. See Table 1.1 for preserve abbreviations.

		Preserve														
Scientific Name	**Common Name**	**FSPSNP**	**Unnamed**	**MPHA**	**MSP**	**MMNP**	**AMNP**	**SPSHA**	**WCPFP**	**GWS**	**RWF**	**GLPSNA**	**GRSWA**	**PWWFP**	**NG**	**FNAL**
Frogs and Toads																
A. americanus	American Toad	●	●			●	●	●	●	●	●	●	●	●	●	●
A. crepitans	Northern Cricket Frog			●		●	●	●		●	●	●	●		●	
H. versicolor/ chrysoscelis	Gray Treefrog complex	●	●		●	●	●	●		●	●	●	●	●	●	●
L. catesbianus	Bullfrog		●			●	●	●	●	●	●	●	●	●	●	●
L. clamitans	Green Frog		●			●	●	●	●	●	●	●	●	●	●	●
L. pipiens	Northern Leopard Frog		●	●		●	●	●	●	●	●	●	●	●	●	●
P. crucifer	Spring Peeper		●								●	●			●	
P. maculata	Chorus Frog	●	●	●		●	●	●	●	●	●	●	●	●	●	●
Salamanders																
A. tigrinum	Tiger Salamander		●			●	●	●	●	●	●	●	●	●	●	●
N. viridescens	Eastern Newt						●									
Turtles																
A. spinifera	Spiny Softshell								●		●			●	●	●
C. serpentina	Common Snapping Turtle		●			●	●	●	●	●	●	●	●	●	●	●
C. picta	Painted Turtle		●			●	●	●	●	●	●	●	●	●	●	●
E. blandingii	Blanding's Turtle		●			☐	●	●	●	●	●	●	●	●	●	●
K. flavescens	Yellow Mud Turtle					●		☐	▨			▨		▨		▨
T. ornata	Ornate Box Turtle	☐	☐		●	☐	☐	☐	▨	●	●	●	●	▨	●	▨
Lizards																
A. sexlineata	Six-lined Racerunner	☐	●		●	●	●	●	▨		●		●	▨		▨
O. attenuatus	Eastern Slender Glass Lizard		●				●		▨			●	●	▨		▨

Scientific Name	Common Name	Preserve														
		FSPSNP	Unnamed	MPHA	MSP	MMNP	AMNP	SPSHA	WCPFP	GWS	RWF	GLPSNA	GRSWA	PWWFP	NG	FNAL
Snakes																
C. constrictor	Blue Racer				●	●		☐				●	☐		●	
H. nasicus	Plains Hog-nosed Snake	☐			●	●	●	☐	▨				●	▨		▨
H. platirhinos	Eastern Hog-nosed Snake	●	●				●	●	▨	●	●		●	▨	●	▨
L. triangulum	Eastern Milk Snake								●						●	
N. sipedon	Northern Watersnake		●				●		●	●		●	☐		●	
O. vernalis	Smooth Greensnake						●		●		●	●	●	●		●
P. vulpinus	Eastern Fox Snake	●		●	●	●	●	●	●		●	●	●	●	●	●
P. catenifer	Bullsnake					☐		☐	▨			☐		▨	●	▨
R. grahamii	Graham's Crayfish Snake								☐			●				
S. dekayi	Dekay's Brown Snake	●	●	●		●	●	●	●	●	●	●	●	●	●	●
S. occipitomaculata	Red-bellied Snake													●		
T. proximus	Western Ribbon Snake		●				●		▨	●				▨		▨
T. radix	Plains Gartersnake								●		●	●	●	●	●	●
T. sirtalis	Common Gartersnake	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Table 1.9. Correlations or t-test results for associations between (A) land cover types within preserves, (B) land cover types within preserve buffers, and (C) preserve history and management factors and residuals of amphibian and reptile species richness (residuals of the semi-log species-area relationship curve).

	Test statistic	**Value**	**P**
A. Preserve Land Cover			
Wetland	r	0.36	0.187
Grassland/Savanna	r	-0.21	0.459
Forest	r	0.15	0.600
Unsuitable	r	-0.15	0.603
B. Buffer Land Cover			
Wetland	r	0.22	0.431
Grassland/Savanna	r	-0.36	0.186
Forest	r	0.50	**0.056**
Unsuitable	r	-0.25	0.368
C. Preserve History and Management			
Decades Managed	r	-0.22	0.425
Grazed	t	-0.34	0.737
Plowed	t	-1.04	0.318
Woody Encroachment	t	-0.09	0.930
Fire	t	-0.92	0.374
Remnant Presence	t	0.65	0.530

Table 1.10. Statistical tests of association between preserve characteristics and species-specific presence or absence. Results of two-sample t-tests are listed for preserve area, years of active management, and grassland/savanna area. The results of Fisher's Exact Test are listed for prescribed fire intensity, presence of remnant prairie, history of plowing, history of grazing, and woody encroachment.

	Area		Wetland Area		Grassland/ Savanna Area		Years of Management		Grazed	Plowed	Woody	Fire	Remnant
	Two-sample t-test								Fisher's Exact Test				
	t	P	t	p	t	P	t	P	P	P	P	P	P
A. crepitans	-2.59	**0.028**	-1.75	0.141	-2.36	**0.041**	0.38	0.715	1.000	1.000	1.000	1.000	0.470
O. vernalis	-2.17	**0.049**	-3.03	**0.023**	-2.00	**0.068**	-2.19	**0.048**	0.132	0.608	1.000	0.619	0.282
T. ornata	-3.11	**0.026**	-0.78	0.454	-2.69	**0.043**	-1.55	0.166	0.242	1.000	1.000	1.000	0.242
T. radix	-3.79	**0.009**	-3.06	**0.022**	-4.90	**0.003**	-3.25	**0.006**	0.132	0.119	1.000	1.000	**0.026**

Table 1.11. Illinois Natural Area Inventory (INAI) status and burn history of 12 artificial cover object (ACO) arrays at Green River SWA (see Appendix 1.1 Figure 1 for array locations).

ACO Array	INAI Status	Prescribed Fire				
		Spring 2015	Fall 2015	Fall 2016	Spring 2018	Spring 2019
A	No		X		X	
B	Yes	X				X
C	No					X
D	No			X		
E	Yes	X				X
F	No	X			X	
G	Yes			X		
H	Yes			X		
I	Yes			X		
J	No			X		
K	No	X				X
L	Yes	X			X	

Table 1.12. Analysis of variance of effect of burn and INAI status on captures per 100 ACO checks for Smooth Greensnakes (A), Dekay's Brownsnake (B), and Common Gartersnake (C). Year and ACO array (nested within INAI status) were included as additional sources of variation. For INAI, F = $MS_{INAI}/MS_{Array(INAI)}$. Significant results are highlighted in bold.

Source	SS	df	MS	F	p
A. Smooth Greensnake					
Year	5.918	2	2.959		
Fire	13.424	1	13.424	28.866	**<0.001**
INAI	3.279	1	3.279	9.315	**0.012**
Array(INAI)	3.522	10	0.352		
Error	9.766	21	0.465		
Total	33.754	35			
B. Dekay's Brownsnake					
Year	2.731	2	1.366		
Fire	7.921	1	7.921	5.502	**0.029**
INAI	28.900	1	28.900	2.732	0.129
Array(INAI)	105.797	10	10.580		
Error	30.236	21	1.440		
Total	177.161	35			
C. Common Gartersnake					
Year	440.356	2	220.178		
Fire	72.335	1	72.335	2.221	0.151
INAI	8.441	1	8.441	0.033	0.859
Array(INAI)	2586.072	10	258.607		
Error	683.998	21	32.571		
Total	3763.408	35			

Table 1.13. Mean (standard deviation) number of captures per 100 ACO checks at array-year combinations differing in burn status (A) and INAI status (B).

	Smooth Greensnake	DeKay's Brownsnake	Common Gartersnake
A. Fire			
no (n = 24)	0.75 (0.67)	1.78 (2.01)	8.35 (10.34)
yes (n = 12)	1.94 (1.05)	2.87 (2.61)	10.71 (10.69)
B. INAI			
no (n = 18)	1.45 (1.08)	3.04 (2.57)	9.62 (11.30)
yes (n = 18)	0.84 (0.79)	1.25 (1.46)	8.65 (9.65)

Table 1.14. Correlations (probability) among vegetation categories. N = 12 for correlations involving plant stature; n = 24 for all other correlations. Significant results are highlighted in bold.

	Forbs	Grasses	Sedges	Thatch	Woody	Stature
Bare Soil	-0.106 (0.621)	0.055 (0.799)	0.048 (0.823)	**-0.865** **(<0.001)**	-0.166 (0.437)	0.265 (0.405)
Forbs		-0.305 (0.147)	0.045 (0.834)	-0.179 (0.403)	-0.275 (0.193)	0.104 (0.749)
Grasses			-0.373 (0.073)	0.017 (0.939)	-0.199 (0.352)	0.169 (0.600)
Sedges				-0.018 (0.932)	-0.124 (0.563)	0.061 (0.850)
Thatch					0.167 (0.434)	-0.268 (0.400)
Woody						-0.314 (0.320)

Table 1.15. Shapiro Wilkes t-tests for differences in vegetation by burn status (A) and INAI status (B). Significant results are highlighted in bold.

	Status	N	Mean	SD	t	df	p
A. Burn status							
Bare Soil	no	17	0.05	0.04	-4.943	6.34	**0.002**
	yes	7	0.37	0.17			
Forbs	no	17	0.23	0.12	-0.901	8.52	0.392
	yes	7	0.29	0.17			
Grasses	no	17	0.26	0.12	-0.150	8.36	0.885
	yes	7	0.27	0.17			
Sedges	no	17	0.07	0.12	-0.334	22.00	0.741
	yes	7	0.09	0.18			
Thatch	no	17	0.92	0.08	-0.279	8.17	0.787
	yes	7	0.08	0.10			
Woody	no	17	0.18	0.16	19.780	9.57	**<0.001**
	yes	7	0.11	0.09			
Stature	no	8	34.88	14.17	1.418	19.33	0.172
	yes	4	46.75	22.21	-0.975	4.27	0.382
B. INAI status							
Bare Soil	no	12	0.15	0.19	0.124	21.184	0.903
	yes	12	0.14	0.16			
Forbs	no	12	0.22	0.13	-0.991	21.481	0.333
	yes	12	0.27	0.15			
Grasses	no	12	0.29	0.16	0.890	18.649	0.385
	yes	12	0.24	0.10			
Sedges	no	12	0.13	0.17	2.367	11.222	**0.037**
	yes	12	0.01	0.02			
Thatch	no	12	0.68	0.42	0.068	21.936	0.947
	yes	12	0.67	0.39			
Woody	no	12	0.13	0.10	-1.287	17.722	0.215
	yes	12	0.20	0.18			
Stature	no	6	43.42	17.88	0.915	9.961	0.382
	yes	6	34.25	16.80			

Table 1.16. Correlations (probability) between Smooth Greensnake, Dekay's Brownsnake, and Common Gartersnake captures and vegetation. N = 12 for correlations involving plant stature; n = 24 for all other correlations. Significant results are highlighted in bold.

	Bare Soil	Forbs	Grasses	Sedges	Thatch	Woody	Stature
Smooth	**0.582**	0.020	0.027	0.040	**-0.684**	-0.339	0.462
Greensnake	**(0.003)**	(0.926)	(0.902)	(0.852)	**(<0.001)**	(0.105)	(0.130)
Dekay's	0.217	-0.170	-0.046	**0.625**	-0.083	-0.312	0.351
Brownsnake	(0.309)	(0.427)	(0.832)	**(0.001)**	(0.701)	(0.137)	(0.263)
Common	0.185	-0.043	-0.171	**0.517**	-0.022	-0.104	0.368
Gartersnake	(0.388)	(0.841)	(0.425)	**(0.010)**	(0.919)	(0.630)	(0.239)

Table 1.17. Fisher's exact tests of independence between species occurrence and fire status and INAI status. Significant results are highlighted in bold. Year-ACO array combinations were treated as independent observations for these analyses.

	Fire Status			INAI Status		
	no	yes	p	no	yes	p
Tiger Salamander						
absent	18	10	0.691	14	14	1.000
present	6	2		4	4	
Six-lined racerunner						
absent	9	7	0.203	13	3	**0.002**
present	15	5		5	15	
Eastern Foxsnake						
absent	14	6	0.729	3	7	0.738
present	10	6		15	11	
Plains Gartersnake						
absent	8	2	0.438	9	11	0.264
present	16	10		9	7	

Table 1.18. Shapiro-Wilkes t-tests comparing vegetation (proportion of ground cover attributable to bare soil, forbs, grasses, sedges, thatch, and woody vegetation and plant stature) at arrays with and without Tiger salamanders (A), Six-lined racerunners (B), Eastern Foxsnakes (C), and Plains Gartersnakes (D). N = the number of arrays, SD = standard deviation, df = degrees of freedom, and p = probability. Significant results are highlighted in bold.

	Occurrence	N	Mean	SD	t	df	p
A. Tiger Salamander							
Bare Soil	Absent	16	0.14	0.16	-0.152	11.731	0.882
	Present	8	0.15	0.20			
Forbs	Absent	16	0.27	0.14	0.923	14.269	0.371
	Present	8	0.21	0.14			
Grasses	Absent	16	0.22	0.10	-2.066	10.106	0.065
	Present	8	0.35	0.15			
Sedges	Absent	16	0.10	0.16	1.819	16.915	0.087
	Present	8	0.02	0.03			
Thatch	Absent	16	0.65	0.42	-0.447	15.507	0.661
	Present	8	0.72	0.37			
Woody	Absent	16	0.19	0.15	1.474	18.205	0.158
	Present	8	0.11	0.12			
Stature	Absent	8	37.56	16.55	-0.319	4.974	0.763
	Present	4	41.38	20.86			
B. Six-lined Racerunner							
Bare Soil	Absent	8	0.20	0.23	0.906	9.892	0.386
	Present	16	0.12	0.14			
Forbs	Absent	8	0.19	0.15	-1.321	12.510	0.210
	Present	16	0.27	0.13			
Grasses	Absent	8	0.32	0.18	1.259	8.766	0.240
	Present	16	0.24	0.09			
Sedges	Absent	8	0.13	0.18	1.179	9.066	0.268
	Present	16	0.04	0.10			
Thatch	Absent	8	0.55	0.46	-1.005	11.387	0.336
	Present	16	0.74	0.36			
Woody	Absent	8	0.09	0.09	-2.146	21.011	**0.044**
	Present	16	0.20	0.16			
Stature	Absent	3	55.50	17.88	1.967	2.838	0.149
	Present	9	33.28	13.75			
C. Eastern Foxsnake							
Bare Soil	Absent	13	0.10	0.12	-1.462	14.947	0.164
	Present	11	0.20	0.22			
Forbs	Absent	13	0.26	0.14	0.462	21.626	0.649
	Present	11	0.23	0.14			

	Occurrence	N	Mean	SD	t	df	p
Grasses	Absent	13	0.27	0.13	0.324	20.995	0.749
	Present	11	0.26	0.14			
Sedges	Absent	13	0.05	0.09	-0.716	14.742	0.485
	Present	11	0.09	0.17			
Thatch	Absent	13	0.70	0.39	0.395	20.878	0.697
	Present	11	0.64	0.42			
Woody	Absent	13	0.19	0.17	0.833	21.086	0.414
	Present	11	0.14	0.11			
Stature	Absent	8	37.19	16.64	-0.418	5.072	0.693
	Present	4	42.13	20.48			
D. Plains Gartersnake							
Bare Soil	Absent	7	0.18	0.20	0.671	9.726	0.518
	Present	17	0.13	0.17			
Forbs	Absent	7	0.17	0.11	-2.036	13.962	0.061
	Present	17	0.28	0.14			
Grasses	Absent	7	0.25	0.14	-0.446	10.475	0.665
	Present	17	0.27	0.13			
Sedges	Absent	7	0.00	0.00	-2.712	16.030	**0.015**
	Present	17	0.10	0.15			
Thatch	Absent	7	0.62	0.41	-0.387	10.981	0.706
	Present	17	0.69	0.40			
Woody	Absent	7	0.24	0.21	1.358	7.334	0.215
	Present	17	0.13	0.11			
Stature	Absent	2	23.25	0.35	-3.424	9.037	**0.008**
	Present	10	41.95	17.25			

Table 1.19. Mean (SD) number of captures per 100 ACO checks at preserves within the Green River COA (see Table 1.1 for preserve abbreviations).

Species	Preserve						Total
	FSPNP	MPHA	SPSHA	GWS	RWF	GRSWA	
Tiger Salamander	0	0	0.56	0	0.39	0.18	0.27
	(-)	(-)	(1.66)	(-)	(0.63)	(0.45)	(0.86)
Six-lined Racerunner	0	0	0.06	0	0	0.84	0.43
	(-)	(-)	(0.22)	(-)	(-)	(1.40)	(1.06)
Plains Hognosed Snake	0	0	0	0	0	0.03	0.01
	(-)	(-)	(-)	(-)	(-)	(0.08)	(0.06)
Eastern Hognosed Snake	0	0	0	0	0.05	0.03	0.02
	(-)	(-)	(-)	(-)	(0.19)	(0.09)	(0.10)
Smooth Greensnake	0	0	0	0	1.01	1.14	0.74
	(-)	(-)	(-)	(-)	(1.66)	(0.98)	(1.10)
Eastern Foxsnake	0.21	0.35	0.31	0	1.48	0.16	0.44
	(0.42)	(0.69)	(0.64)	(-)	(1.87)	(0.25)	(0.98)
Dekay's Brownsnake	1.46	17.29	1.67	9.38	5.60	2.14	3.55
	(2.92)	(10.12)	(2.61)	(-)	(4.36	(2.25)	(5.03)
Plains Gartersnake	0	0	0	0	1.37	0.99	0.73
	(-)	(-)	(-)	(-)	(1.98)	(1.81)	(1.59)
Common Gartersnake	0.63	10.63	9.05	13.02	30.59	9.14	12.61
	(1.25)	(3.92)	(8.23)	(-)	(29.46)	(10.37)	(16.91)

Table 1.20. Mean (SD) proportion of ground cover attributable to bare soil, forbs, grasses, sedges, thatch, and woody vegetation and mean plant stature at preserves within the Green River COA (see Table 1.1 for preserve abbreviations).

Ground Cover	Preserve					Total
	FSPNP	MPHA	SPSHA	RWF	GRSWA	
Bare Soil	0.38 (-)	0.18 (0.37)	0.08 (0.01)	0.20 (0.23)	0.14 (0.17)	0.16 (0.20)
Forbs	0.49 (0.12)	0.52 (0.15)	0.17 (0.08)	0.43 (0.11)	0.25 (0.14)	0.31 (0.17)
Grasses	0.18 (0.05)	0.29 (0.11)	0.46 (0.09)	0.27 (0.13)	0.27 (0.13)	0.30 (0.14)
Sedges	0.14 (0.15)	0.03 (0.05)	0.05 (0.10)	0.08 (0.11)	0.07 (0.13)	0.07 (0.11)
Thatch	0.29 (0.15)	0.81 (0.39)	0.91 (0.11)	0.62 (0.40)	0.67 (0.40)	0.70 (0.37)
Woody	0.17 (0.08)	0.06 (0.06)	0.03 (0.05)	0.04 (0.05)	0.16 (0.15)	0.10 (0.12)
Stature	34.25 (10.25)	41.25 (8.13)	40.10 (12.22)	59.83 (10.03)	38.83 (17.22)	43.57 (15.97)

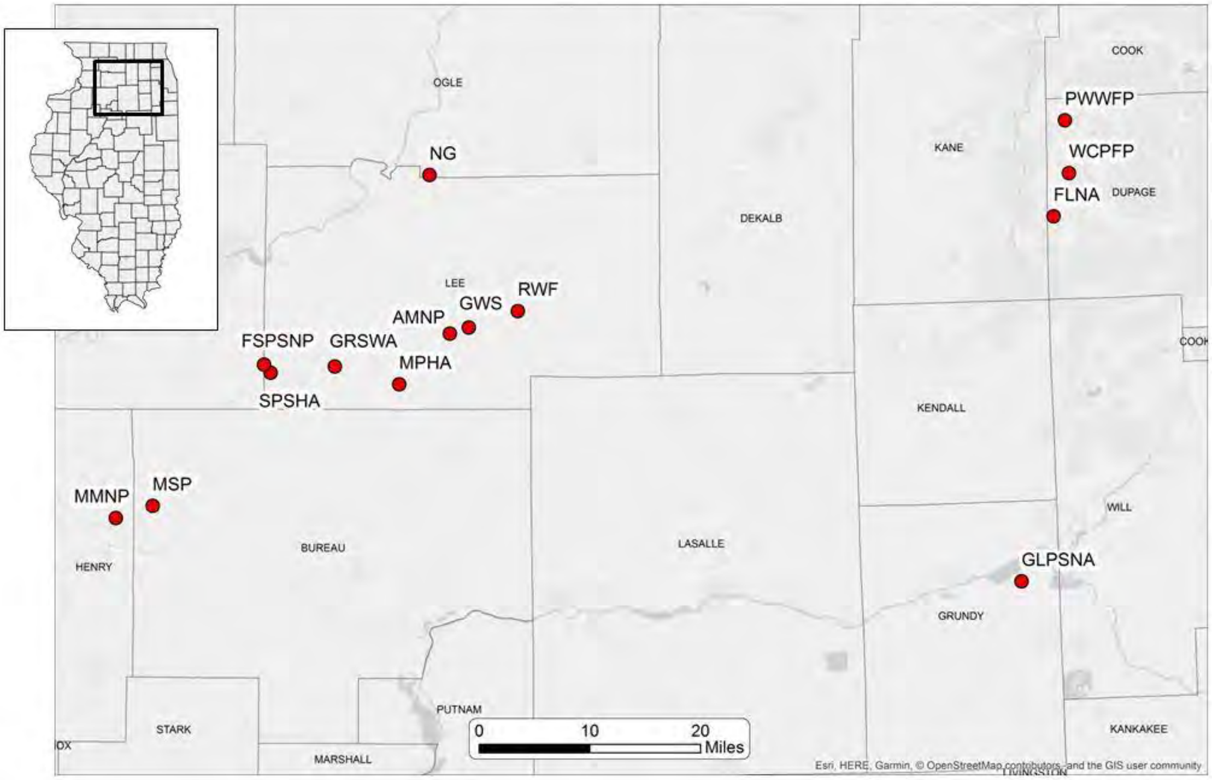


Figure 1.1. Location of preserves included in analyses of land cover, historic land use, and management effects on amphibian and reptile biodiversity. Abbreviations: MMNP – Mineral Marsh Nature Preserve, MSP – McCune Sand Prairie land and Water Reserve, FSPNP – Foley Sand Prairie Nature Preserve, SPSHA – Sand Prairie State Nature Preserve, GRSWA – Green River State Wildlife Area, MPHA – Maytown Pheasant Habitat Area, AMNP – Amboy Marsh Nature Preserve, GWS – Gremel Wildlife Sanctuary, RWF – Richardson Wildlife Foundation, NG – Nachusa Grasslands, GLPSNA – Goose Lake Prairie State Natural Area, FNLA – Fermi National Laboratory, WCPFP – West Chicago Prairie Forest Preserve, PWWFP – Pratt's Wayne Woods Forest Preserve (one preserve is omitted at the request of stewards and preserve managers).

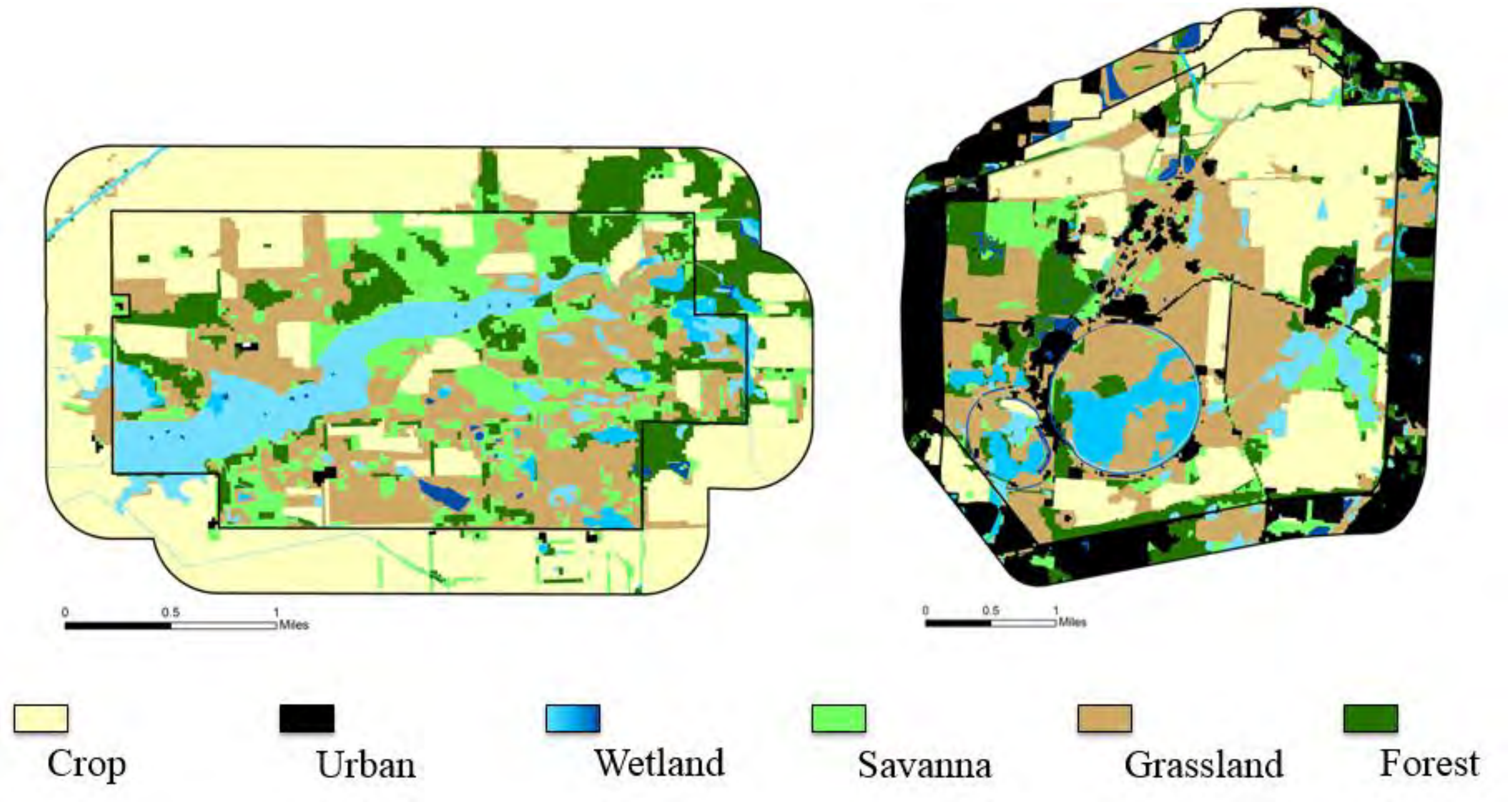


Figure 1.2. Preserve and buffer land cover maps for Green River State Wildlife Area (GRSWA, left) and Fermi National Laboratory (FNAL, right). Preserve boundaries are indicated by the inner polygons. Buffers include the area between preserve boundaries and outer polygons.

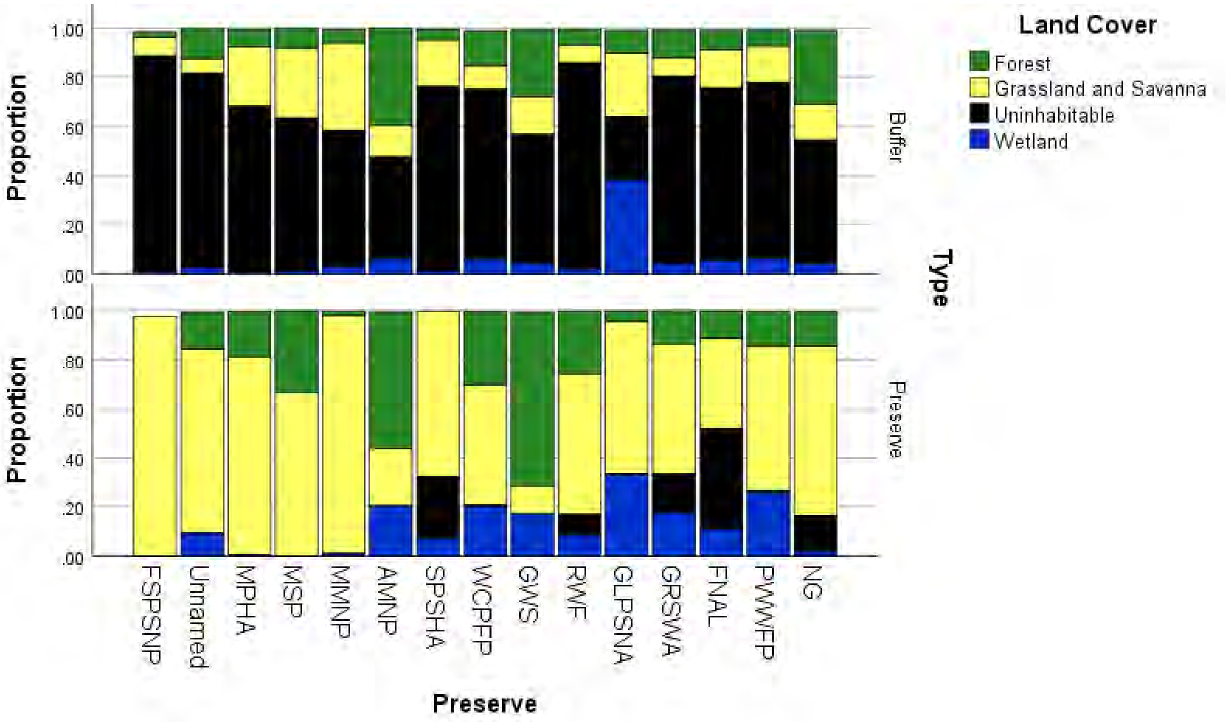


Figure 1.3. Proportion of land cover types within the buffers (top) and preserves (bottom). See Table 1.1 for preserve abbreviations.

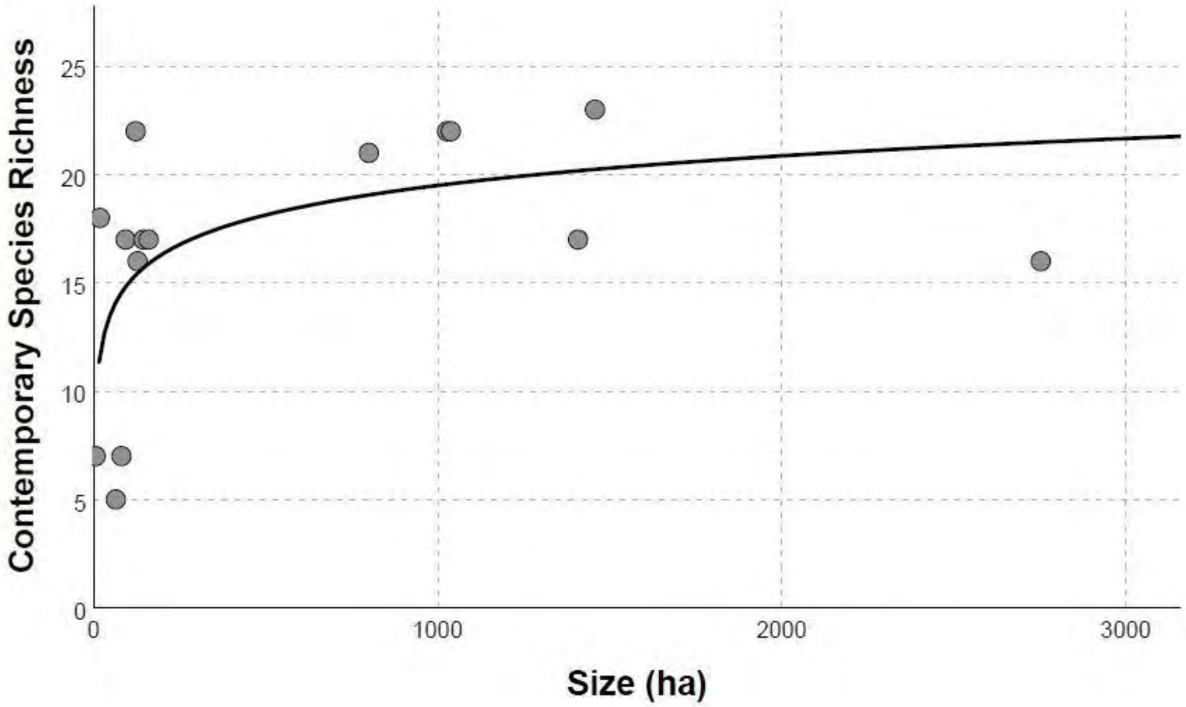


Figure 1.4. Relationship of amphibian and reptile species richness to preserve area (species richness = 5.89 + 1.97*ln(preserve area), R = 0.60, P = 0.019).

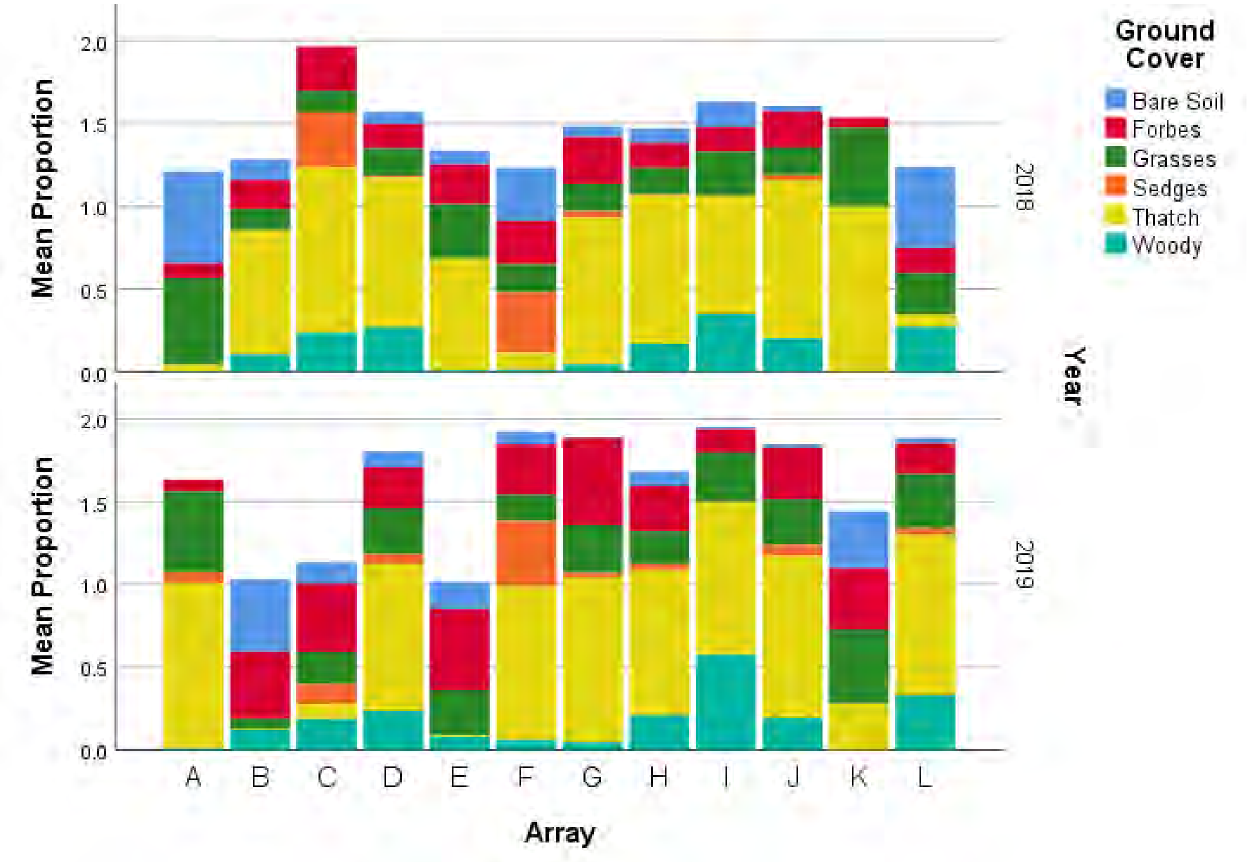


Figure 1.5. Variation in vegetation ground cover among artificial cover object arrays and years at Green River SWA (see Appendix Fig. 1.1 for array locations and Table 1.11 for INAI status and prescribed fire history). Proportions exceed 1 because of overlapping vegetation layers.

Appendix 1.1. Amphibian and reptile encounters during surveys at Green River SWA and at other preserves within the Green River COA, 2017-2019

A comprehensive inventory of amphibians and reptiles at Green River SWA, Foley Sand Prairie Nature Preserve, Maytown Pheasant Habitat Area, McCune Sand Prairie Land and Water Reserve, Mineral Marsh Nature Preserve, Amboy Marsh Nature Preserve, Sand Prairie State Habitat Area, Gremel Wildlife Sanctuary and Richardson Wildlife Foundation was carried out during the 2017, 2018, and 2019 field seasons. Methods employed included artificial cover object arrays, drift fence and funnel trap arrays, aquatic traps, visual and auditory encounter surveys, canine-assisted searches, and radio telemetry (Appendix Fig. 1-8). Day-to-day field operations were carried out by Jay Vecchiet with assistance from Tristan Schramer (2017), Nick Geifer (2018, 2019) and others. These efforts resulted in nearly 6,500 amphibian and reptile records at Green River SWA and more than 8,700 records at all sites combined (Appendix 1.1 Tables 1-6).

Appendix 1.1 Table 1. Numbers of amphibians and reptiles, by species, recovered from under artificial cover object (ACO) arrays at Foley Sane Prairie Nature Preserve (FSPNP), Green River SWA (GRSWA), Gremel Wildlife Sanctuary (GWS), Maytown Pheasant Habitat Area (MPHA), Richardson Wildlife Foundation (RWF) and Sand Prairie State Habitat Area (SPSHA). Also listed is the number of boards comprising each array and the number of times arrays were checked per year. Locations of ACO arrays are shown in Appendix 1.1 Fig. 1-3 and 3-8. Species abbreviations: ATIG –Tiger Salamander (*A. tiginum*), COGA – Common Gartersnake, DEBR – Dekay's Brownsnake, EHOG – Eastern Hog-nosed Snake, FOSN – Eastern Foxsnake, LCLM – Greenfrog (*L. clamitans*), LPIP – Leopard Frog *(L. pipiens*), PLGA – Plains Gartersnake, PTRI – Chorus Frog (*P. triseriata*), SLRR – Six-lined Racerunner, SMGR – Smooth Greensnake, WHOG – Plains (Western) Hog-nosed Snake.

					Species												
Preserve	ACO Array	Year	Boards	Checks	ATIG	COGA	DEBR	EHOG	FOSN	LCLM	LPIP	PLGA	PMAC	PTRI	SLRR	SMGR	WHOG
FSPNP	AB	2018	8	2													
		2019	8	15			7										
	AC	2018	8	2													
		2019	8	15		3			1								
GRSWA	A	2017	32	21		5	19									4	
		2018	32	12	5	24	5		1			3				13	
		2019	32	17	3	37	21					1				7	
	B	2017	32	22											13	3	1
		2018	32	10	1										3	1	
		2019	32	21		3	2								38	16	2
	C	2017	32	20		2	1		3			2				2	
		2018	32	10		1						1					
		2019	32	17		22	6					6				16	
	D	2017	32	21		46	56					17				8	
		2018	32	12		20	10		1			4	1		2	10	
		2019	32	17		29	10					7			28	5	
	E	2017	32	18		2	2					1			2		

Preserve	ACO Array	Year	Boards	Checks	Species												
					ATIG	COGA	DEBR	EHOG	FOSN	LCLM	LPIP	PLGA	PMAC	PTRI	SLRR	SMGR	WHOG
		2018	32	12	4	8	1					4			3	3	
		2019	32	20		25	6					4			23	16	2
	F	2017	32	18		32	39									4	
		2018	32	12		131	25		1			2				12	
		2019	32	16		202	39		3			1			1	3	
	G	2017	32	20		64	14					26			2	11	
		2018	32	12		20	4		2			7			8	3	
		2019	32	18		40	2		1			6			6	3	
	H	2017	32	20		126	34		1			3				4	
		2018	32	10	1	68	7		1							6	
		2019	32	15		131	13					3			11	6	
	I	2017	32	19		1						3			2	1	
		2018	32	12		4									5	3	
		2019	32	17		9	1								9		1
	J	2017	32	20		11	12		1			14				15	
		2018	32	11		47	6		4			15			1	7	
		2019	32	16	1	92	7				1	49			1	3	
	K	2017	32	18		10	6		2			2				1	
		2018	32	8		12	6									3	
		2019	32	19	13	114	21	3	3			1	1			13	
	L	2017	32	19		60	14		1			2				1	
		2018	32	13		94	13	1	1						2	3	
		2019	32	18	4	134	7	1	2			1			8	1	
GWS	GWS	2017	32	6		25	18										
MPHA	AA	2018	8	5		5	4										
		2019	8	9		10	15										

Preserve	ACO Array	Year	Boards	Checks	Species												
					ATIG	COGA	DEBR	EHOG	FOSN	LCLM	LPIP	PLGA	PMAC	PTRI	SLRR	SMGR	WHOG
	Z	2018	8	5		2	12										
		2019	8	9		8	6		1								
RWF	AD	2019	16	7		22	7		2			1					
	S	2018	16	8		21	10		2			7					
		2019	16	14		5	21		3			2					
	T	2018	16	6		2	2										
		2019	16	1													
	U	2018	16	9	1	17	3		10			1				1	
		2019	16	13		67	4		5							1	
	W	2018	16	9	3	48	18	1								1	
		2019	16	12	2	53	15										
	X	2018	16	8	1	79	17		1			3				5	
		2019	16	12	1	80	2		3	2		4		1		10	
	Y	2018	16	8		138	7		3			7				2	
		2019	16	12		79	6		1							1	
SPSHA	M	2017	8	8		3											
		2018	8	4	2	2											
		2019	8	18		25	3		2								
	N	2017	8	8		3											
		2018	8	6	1	4	2										
		2019	8	17		27	5										
	P	2017	8	8		4			1								
		2018	8	4		2	1										
		2019	8	15		33	11		2								
	Q	2017	8	8													
		2018	8	3													

Preserve	ACO Array	Year	Boards	Checks	Species												
					ATIG	COGA	DEBR	EHOG	FOSN	LCLM	LPIP	PLGA	PMAC	PTRI	SLRR	SMGR	WHOG
		2019	8	13		12	3										
	R	2017	8	7		1											
		2018	8	4		6											
		2019	8	15		3									1		

Appendix 1.1 Table 2. Numbers of amphibians and reptiles, by species, recovered from drift fence and funnel trap arrays at Green River SWA (GRSWA) and Sand Prairie State Habitat Area (SPSHA). Also listed is the number of trap nights arrays were monitored per year. Locations of drift fence and funnel trap arrays are shown in Appendix 1.1 Fig. 1 and 6. Species abbreviations: AAMR – American Toad (*A. americanus*), ATIG –Tiger Salamander (*A. tiginum*), COGA – Common Gartersnake, EHOG – Eastern Hog-nosed Snake, FOSN – Eastern Foxsnake, GLIZ – Slender Glass Lizard, LCAT – Bullfrog (*L. catesbiana*), LCLM – Greenfrog (*L. clamitans*), LPIP – Leopard Frog *(L. pipiens*), PLGA – Plains Gartersnake, PTRI – Chorus Frog (*P. triseriata*), SLRR – Six-lined Racerunner, SMGR – Smooth Greensnake, WHOG – Plains (Western) Hog-nosed Snake.

Preserve	Drift Fence Array	Year	Trap Nights	Species															
				AAMR	ATIG	COGA	EHOG	FOSN	GLIZ	HCHV	LCAT	LCLM	LPIP	PLGA	PMAC	PTRI	SLRR	SMGR	WHOG
GRSWA	1	2017	31										8				6		
		2018	7	1			1					2	3						
	10	2018	32		42	1											31		
	11	2018	21		1	1					1		4						1
	17	2019	26			1											7		
	18	2019	26														2	1	
	19	2019	27														3		4
	2	2017	31		2	2							33				42		
	20	2019	21			7											1		
	3	2017	31		1	6							9			1	6	1	
	4	2017	31		1						34	1	35				2	2	
		2018	47		2	4						2	4				3	2	
	5	2017	31			7				3		10	36	1		1		2	
	6	2017	31		3								29				1		
	7	2017	31														3		
		2018	39	4	3	4		1			1	2	5		1		19		

Preserve	Drift Fence Array	Year	Trap Nights	Species															
				AAMR	ATIG	COGA	EHOG	FOSN	GLIZ	HCHV	LCAT	LCLM	LPIP	PLGA	PMAC	PTRI	SLRR	SMGR	WHOG
	8	2018	47		52	2			1			1	2		5		22		1
		2019	21			1													
	9	2018	47	2								9	7	1			1		
SPSHA	12	2018	26		1	7					1								
	13	2018	26			2											1		
	14	2019	20					1									7		
	15	2019	20			6							1				2		
	16	2019	13			4													

Appendix 1.1 Table 3. Numbers of turtles, by species, recovered from aquatic traps at Green River SWA (GRSWA), Sand Prairie State Habitat Area (SPSHA), Mineral Marsh Nature Preserve (MMNP), Gremel Wildlife Sanctuary (GWS), and Amboy Marsh Nature Preserve (AMNP). Also listed is the number of traps deployed and the number of trap nights accrued. Pond locations are shown in Appendix 1.1 Fig. 1 and 4-7. Standard names: *C. picta* – Painted Turtle, *C. serpetina* – Snapping Turtle, *E. blandingii* – Blanding's Turtle, *K. flavescens* – Mud Turtle.

					Species			
Site	Pond	Year	Traps	Trap Days	*C. picta*	*C. serpentina*	*E. blandingii*	*K. flavescens*
GRSWA	1. Cattail Marsh	2017	16	77				
	2. Atkinson	2018	7	70	4	4		
	3, 4 & 5. West Slough	2017	22	105				
		2018	5	60	1			
	6. Maytown Pond	2017	15	70				
	7. Central Slough	2017	9	45	2	1		
		2018	3	15	2			
	8. Gavin's Pond	2017	32	151	13			
		2018	20	243	57	5		
	9. Savanna Marsh	2017	20	95	1			
		2018	3	30	4			
	10. Farm Pond	2017	4	20				
		2018	4	32	20	1		
	11. Unnamed Pond	2019	8	24	3			
	12. Pothole Pond	2018	3	15				
	13. Peat Marsh	2017	62	272	32	9	1	
		2018	22	210	20	4		
	14. RCG Marsh	2017	5	25				
	15. Little Crow Pond	2017	1	2				
		2018	6	42	10			
	16. Big Crow Pond	2018	8	56	49	18		
		total	275	1659	218	42	1	0

Site	Pond	Year	Traps	Trap Days	*Species* *C. picta*	*C. serpentina*	*E. blandingii*	*K. flavescens*
SPSHA	17. SPSHA North	2017	8	40	33	1		
		2018	10	184	52	7	1	
	18. SPSHA East	2018	5	54	25	1		
		total	23	278	110	9	1	0
MMNP	19. Big Frankenreider	2018	27	216	8	1		2
		2019	10	20	3			
	20. Little Frankenreider	2018	5	40				
	21. Mineral Marsh	2019	11	55				
		total	53	331	11	1	0	2
GWS	22. Iris East Pond	2017	13	65				
	23. Walter's Wetland	2017	2	10				
	24. Iris West Pond	2017	7	35				
	25. Main Pond	2017	28	140	36	5		
	26. Culvert Pond	2017	4	20		1		
	27. Pine Pond	2017	5	25				
		total	59	295	36	6	0	0
AMNP	28. Lily Pond	2019	10	70	17	4		
	29. Main Pond	2019	20	140	7	1		
	30. Willow Pond	2019	14	98	1	1		
		Total	44	308	25	6	0	0

Appendix 1.1 Table 4. Numbers of amphibians and reptiles (excluding turtles), by species, recovered from aquatic traps at Green River SWA (GRSWA), Sand Prairie State Habitat Area (SPSHA), Mineral Marsh Nature Preserve (MMNP), Gremel Wildlife Sanctuary (GWS), and Amboy Marsh Nature Preserve (AMNP). Also listed is the number of traps deployed and the number of trap nights accrued. Pond locations are shown in Fig. 1, 7, 8, 9, and 11. Standard names: *A. tigrinum* – Tiger Salamander, *A. americanus* – American Toad, *H. chrysoscelis/versicolor* – Gray Treefrog complex, *L. catesbiana* – Bullfrog, *L. clamitans* – Greenfrog, *L. pipiens* – leopard frog, *P. maculata* – Boreal Chorus Frog, *N. sipedon* – Northern Watersnake.

					Species								
Site	Pond	Year	Traps	Trap Days	*A. tigrinum*	*A. americanus*	*A. crepitans*	*H. chrysoscelis/versicolor*	*L. catesbiana*	*L. clamitans*	*L. pipiens*	*P. maculata*	*N. sipedon*
GRSWA	1. Cattail Marsh	2017	16	77									
	2. Atkinson	2017					1		2	4	81		
		2018	7	70	5		1				33		
	3, 4, & 5. West Slough	2017	22	105									
		2018	5	60	5				5	1	146		
	6. Maytown Pond	2017	15	70	13	1		1		37	152		
		2018	3	30									
	7. Central Slough	2017	9	45						2	452		
		2018	3	15									
	8. Gavin's Pond	2017	32	151					4	118	130		
		2018	20	243	20		1	2	30	29	84		

	10. Farm Pond	2017	4	20			2		2	5	2		
		2018	4	32									
	11. Unnamed Pond	2019	8	24					9	5			
	12. Pothole Pond	2018	3	15									
	13. Peat Marsh	2017	62	272	10		1		1	29	1239		
		2018	22	210	2				3	2	2		
	14. RCG Marsh	2017	5	25					1	1			
	15. Little Crow Pond	2017	1	2	1								
		2018	6	42	7				3	1	1		
	16. Big Crow Pond	2018	8	56	2				1		1		
		total	275	1659	65	1	6	3	71	241	2435	0	0
SPSHA	17. SPSHA North	2017	8	40	1				19	1			
		2018	10	184	1			1	52	15			
	18. SPSHA East	2018	5	54	1				2	2			
		total	23	278	3	0	0	1	73	18	0	0	0
MMNP	19, 20, & 21	2018	32	256	7	1	1	1	5	11			
		2019	21	75	34		1	13	2	36	71	5	
		total	53	331	41	1	2	14	7	47	71	5	0
GWS	22. Iris East Pond	2017	13	65			1		74	heard	seen		
	23. Walter's Wetland	2017	2	10		1			6	heard			
	24. Iris West Pond	2017	7	35			seen		33	1	seen		
	25. Main Pond	2017	28	140			seen		149	1	seen		
	26. Culvert Pond	2017	4	20				1	62				
	27. Pine Pond	2017	5	25	4		7	6	11	seen			
		total	59	295	4	1	8	7	335	2	0	0	0
AMNP	28. Lily Pond	2019	10	70					1				
	29. Main Pond	2019	20	140	1				10	33			1
	30. Willow Pond	2019	14	98	5				11	31	1		1
		Total	44	308	6	0	0	0	22	64	1	0	2

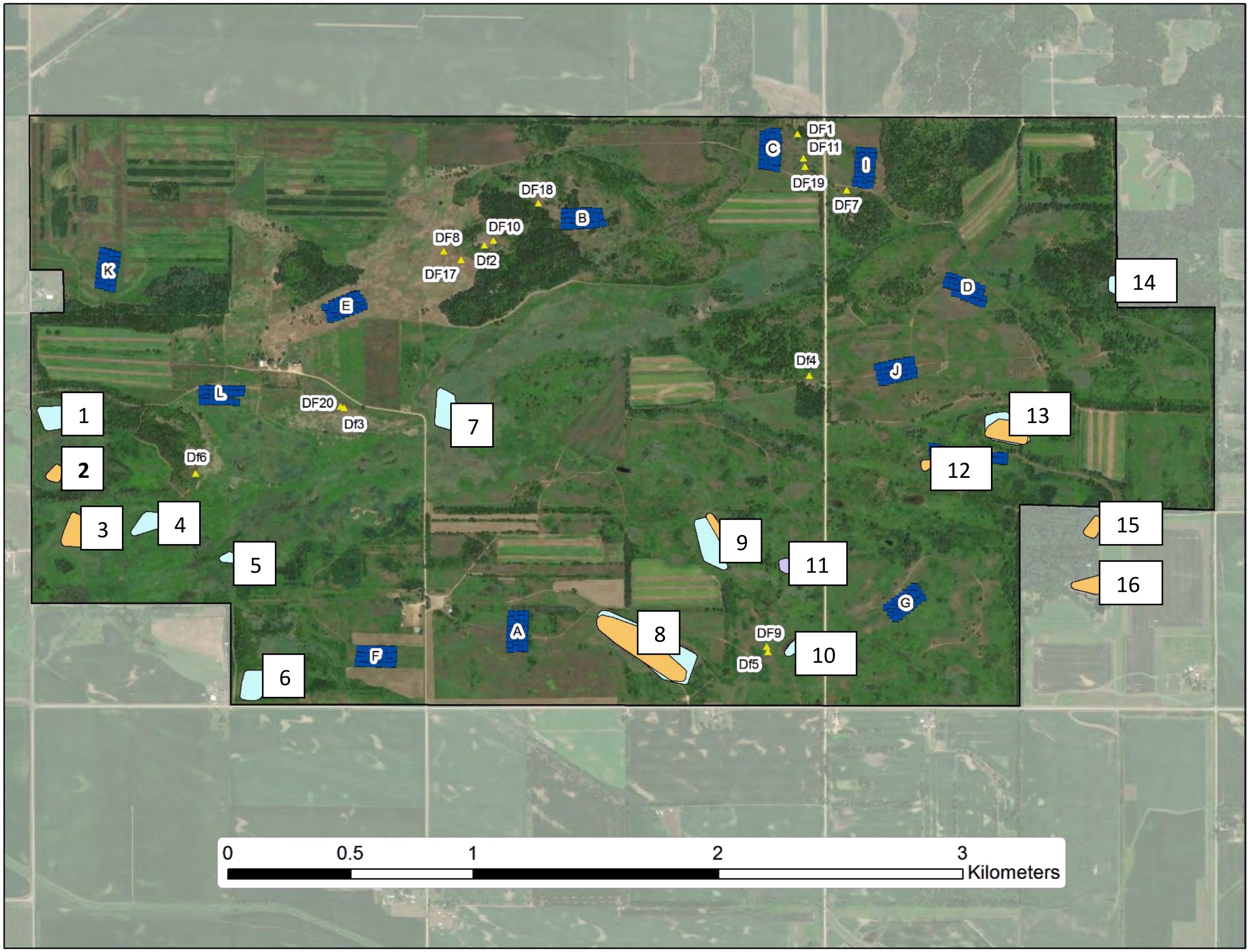
Appendix 1.1 Table 5. Numbers of amphibians and reptiles, by species, recorded via visual encounters at Foley Sand Prairie Nature Preserve (FSPNP), Green River SWA (GRSWA), Gremel Wildlife Sanctuary (GWS), Mineral Marsh Nature Preserve (MMNP), McCune Sand Prairie Land and Water Reserve (MSP), Richardson Wildlife Foundation (RWF), and Sand Prairie State Habitat Area (SPSHA). Species abbreviations: ATIG –Tiger Salamander (*A. tiginum*), COGA – Common Gartersnake, CPICTA – Painted Turtle (C. picta), ACREP – Cricket Frog (A. crepitans), DEBR – Dekay's Brownsnake, EHOG – Eastern Hog-nosed Snake, FOSN – Eastern Foxsnake, GLIZ – Slender Glass Lizard, PLGA – Plains Gartersnake, SLRR – Six-lined Racerunner, SMGR – Smooth Greensnake, WHOG – Plains (Western) Hog-nosed Snake, HVERS – Gray Treefrog (*H. versicolor/chrysoscelis*), TORN – Ornate Box Turtle, AAMR – American Toad (*A. americanus*).

		Species															
Site	Year	ATIG	COGA	CPICTA	EMBL	ACREP	DEBR	EHOG	FOSN	GLIZ	PLGA	SLRR	SMGR	WHOG	HVERS	TORN	AAMR
FSPNP	2019						1										
GRSWA	2017	2	40	1		1	24	3	12	2	5	3	9	1		2	
	2018		8		1		8	3	7	2	3		2	1		3	
	2019		10					5	1	5	1		1	1		2	
GWS	2017		3				1										
MMNP	2018		1			calling	1		1			>20		2	1		calling
	2019		4											2			
MSP	2018		2									>100					
	2019		2									>10			2	1	
RWF	2018							1									
	2019							1									
SPSHA	2017	1	2				5	2									
	2019		2		1		2	1									

Appendix 1.1 Table 6. Dates and locations of canine assisted searches for Ornate Box Turtles. Location abbreviations: GRSWA – Green River SWA, RWS – Richardson Wildlife Foundation, HNP – Hahnaman Nature Preserve, FSPNP – Foley Sand Prairie Nature Preserve, SPSHA – Sand Prairie State Habitat Area.

Year	Date	Preserve	Result	Comment
2015	8 days	GRSWA	8 turtles	Information from R. Blogg; turtles found on last day
2017	23 June	GRSWA		
	24 June	GRSWA		
2018	22 May	RWS	2 turtles	
	23 May	GRSWA	3 turtles	
	24 May	RWS		
	25 May	GRSWA		High temperatures
	26 May	GRSWA		High temperatures
	27 May	HNP	1 turtle	High temperatures
2019	20 June	GRSWA		
	21 June	GRSWA		
	22 June	GRSWA		
	23 June	GRSWA	1 turtle	
	24 June	GRSWA		
	25 June	GRSWA		High temperatures
	26 June	FSPNP, SPSHA		High temperatures

Appendix 1.1 Figure 1 (next page). Locations of artificial cover object arrays (dark blue rectangles labeled A – L), drift fence and funnel trap arrays (yellow triangles labeled DF#), and aquatic traps (colored polygons, pale blue = 2017, gold = 2018, pink = 2019) at Green River SWA. Numbers adjacent to aquatic trap locations correspond to ponds listed in Tables 3 and 4.


DF1
DF11
C
I
DF19
DF7
DF18
B
DF10
DF8
Df2
DF17
K
E
D
14
Df4
J
L
1
DF20
Df3
7
13
Df6
2
12
3
4
9
15
5
11
16
G
A
8
DF9
F
6
Df5
10
0
0.5
1
2
3
Kilometers

Appendix 1.1 Figure 2 (next page). Locations of artificial cover object arrays (dark blue rectangles labeled AB and AC) at Foley Sand Prairie Nature Preserve.


AB
AC
0
0.25
0.5
Kilometers

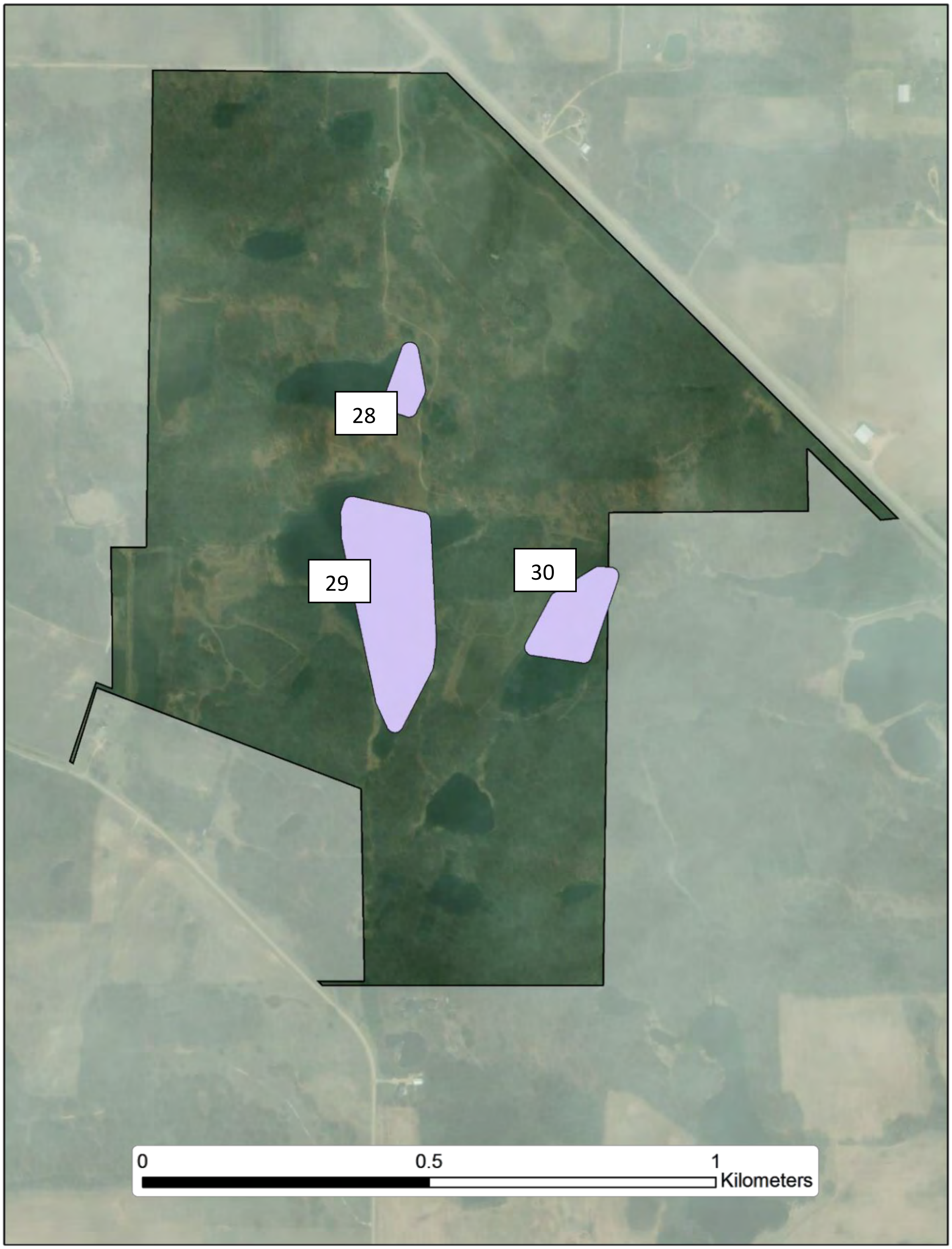
Appendix 1.1 Figure 3 (next page). Locations of artificial cover object arrays (dark blue rectangles labeled Z and AA) at Maytown Pheasant Habitat Area.


Z
AA
0
0.5
1
Kilometers

Appendix 1.1 Figure 4 (next page). Locations of aquatic traps (colored polygons) at Mineral Marsh Nature Preserve in 2018 (gold) and 2019 (lavender). Numbers adjacent to aquatic trap locations correspond to ponds listed in Tables 3 and 4.


18
19
17
0
0.5
1
Kilometers

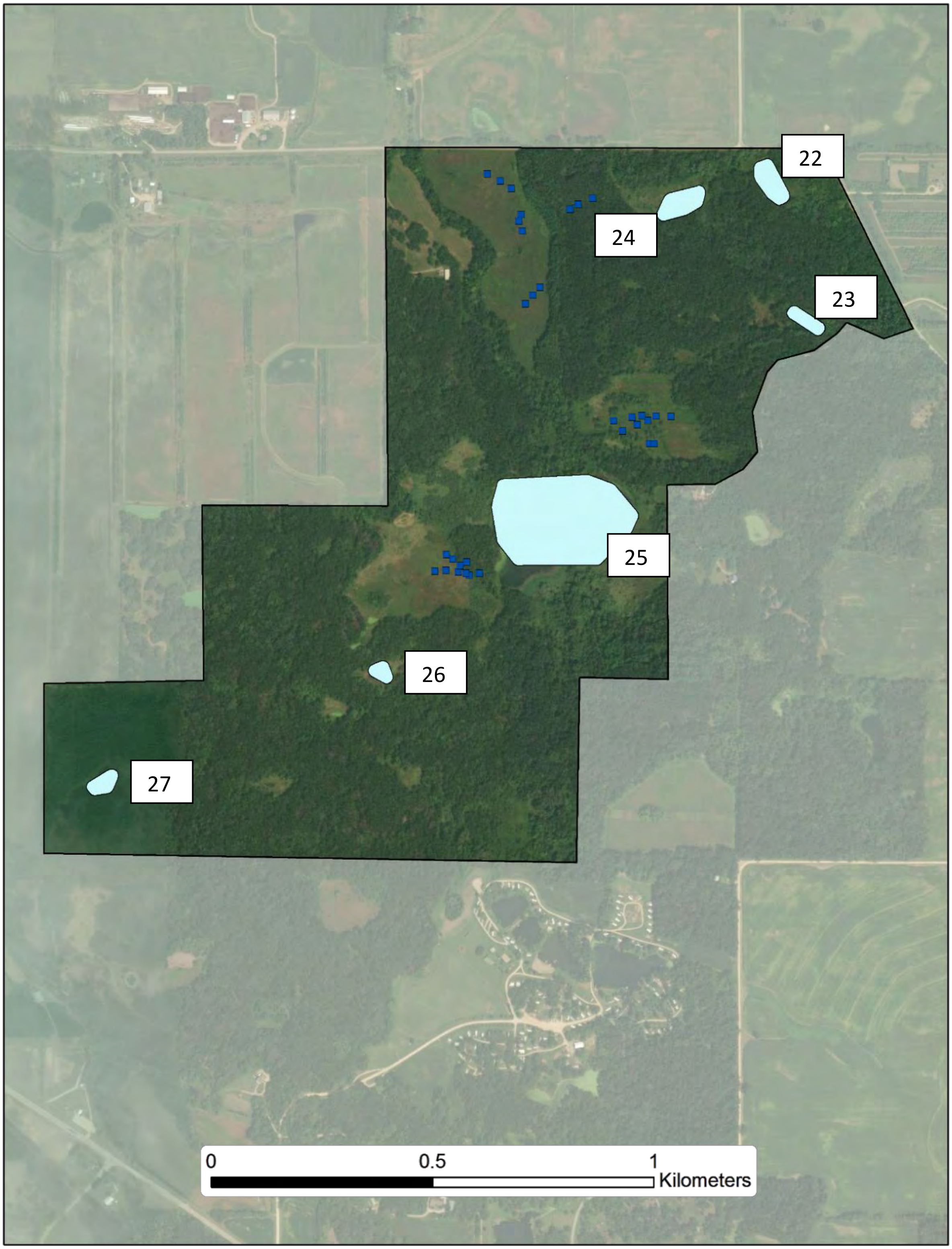
Appendix 1.1 Figure 5 (next page). Locations of aquatic traps (colored polygons) at Amboy Marsh Nature Preserve in 2019. Numbers adjacent to aquatic trap locations correspond to ponds listed in Tables 3 and 4.


28
29
30
0
0.5
1
Kilometers

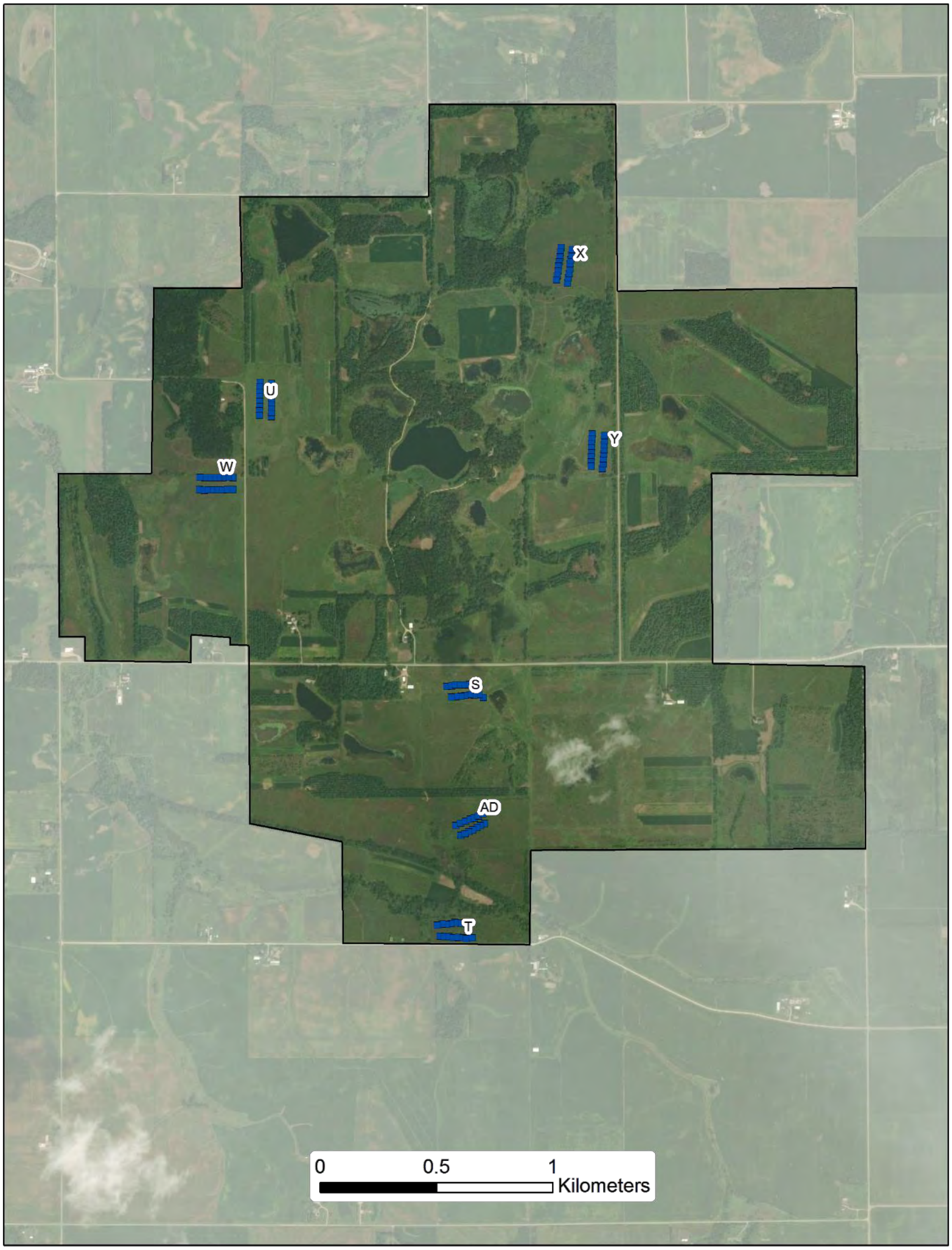
Appendix 1.1 Figure 6 (next page). Locations of artificial cover object arrays (dark blue rectangles labeled M – R), drift fence and funnel trap arrays (yellow triangles labeled DF#), and aquatic traps (colored polygons; gold = 2017, blue = 2018) at Sand Prairie State Habitat Area. Numbers adjacent to aquatic trap locations correspond to ponds listed in Tables 3 and 4.


DF15
DF14
DF12
M
17
N
P
18
DF16
DF13
R
Q
0
0.5
1
Kilometers

Appendix 1.1 Figure 7 (next page). Locations of artificial cover object arrays (dark blue rectangles) and aquatic traps (colored polygons) at Gremel Wildlife Sanctuary in 2017. Numbers adjacent to aquatic trap locations correspond to ponds listed in Tables 3 and 4.


22
24
23
25
26
27
0
0.5
1
Kilometers

Appendix 1.1 Figure 8 (next page). Locations of artificial cover object arrays (dark blue rectangles labeled S – Y and AD) at Richardson Wildlife Foundation.


X
U
Y
W
S
AD
T
0
0.5
1
Kilometers

Part 2. Historic and current status and NatureServe occurrence ranks of SGCN amphibians and reptiles within the Green River COA

Field efforts during 2017-2019 have provided updated locality records for amphibian and reptile species in greatest need of conservation (SGCN), including Eastern Newts, Blanding's Turtles, Ornate Box Turtles, Slender Glass Lizards, Plains Hognose Snakes and Smooth Green Snakes, at 11 preserves within are near the Green River COA (Table 2.1). This information, together with information from other sources has been used to generate provisional occurrence ranks for these species using NatureServe guidelines (Hamerson et al. 2008). These are summarized in Table 2.2 and treated more fully in the species accounts that follow. Although not a focus of work in this study, records from Hahnaman Sand Prairie Nature Preserve are included here because of their significance and the proximity of this site to other preserves in the Green River COA. Incidental observations and the results of radio telemetry conducted during 2020 are included as well.

2.1 Eastern Newt

The eastern newt, *Notophthalmus viridescens*, has been recorded from just one of the eleven preserves within the Green River COA included here, Amboy Marsh Nature Preserve, a site now owned and managed by the Illinois Audubon Society (Table 2.1). The most extensive information on eastern newts at Amoy Marsh Nature Preserve is provided by Phillips (2014) who encountered the species in five ponds on the property during survey efforts in April and June 2014. Site stewards also report regular occurrences (D. Carey and W. Rogers, personal communication).

Given the frequency and time span over which the species has been observed, if current conditions prevail, persistence for the foreseeable future (i.e., at least 20 - 30 years) is reasonably certain, yielding a provisional NatureServe occurrence rank of *AC: Excellent to Fair viability* (Table 2.2). The absence of occurrence records from other preserves in the region suggests that newts at Amboy Marsh are isolated from other breeding populations, placing this population at greater risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes. Altered hydrology and disease are potential risk factors. Information on newt abundance at Amboy Marsh and on the status of newts at nearby sites (e.g., Gremel Wildlife Sanctuary where surveys failed to detect the species in 2017; Vecchiet 2017) might allow assignment of a more specific occurrence rank.

2.2 Blanding's Turtle

The Blanding's turtle, *Emydoidea blandingii*, has been recorded from seven of the eleven preserves in the Green River COA included here (Table 2.1). Recent records are lacking for two of these preserves, Mineral Marsh Nature Preserve (last recorded in 1996) and Gremel Wildlife

Sanctuary (last recorded in 2004) (Table 2.1); trapping efforts at these preserves during this study yielded no captures (Appendix 1.1 Table 3). From 2017-2019, trapping and visual encounter surveys resulted in the capture of two Blanding's turtles at Green River SWA and two Blanding's Turtles at Sand Prairie SHA (Table 2.3, Appendix 1.1 Table 3 and 5). Five additional Blanding's turtles were trapped at Sand Prairie SHA in 2020 (R. Blogg, personal communication, Table 2.3). From 2017-2019, ten live and one dead Blanding's turtles were encountered at RWF through a combination of trapping, visual encounters, and radio telemetry (B. Towey, personal communication). Trapping efforts at Amboy Marsh Nature Preserve in 2019 (this study) and 2020 (Mauger 2020) yielded no captures although preserve stewards observed Blanding's turtles at Amboy Marsh in 2018 and 2019 (one observation each year, D. Carey and W. Rogers, personal communication) and the species was encountered in modest numbers in 1986 (7 records), 1989 (6 records), and 1991 (19 records), prior to its designation as a nature preserve. Five adult and one juvenile Blanding's turtles were encountered through trapping and trail camera surveys between 2002 and 2016 (Nelson et al. 2019) and stewards report additional sightings as recent as 2018 (D. Carey and W. Rogers, personal communication; Table 2.1).

Blanding's turtles at Green River SWA and Sand Prairie SHA were tracked via radio telemetry (Table 2.1), providing information on movement patterns within these preserves and adjacent property (Fig. 2.1, 2.2). At Green River SWA, female L1R2 (tracked from 2018-2020) moved among wetlands spanning the entire east-west extent of the preserve and moved off-site to wetlands southeast of the Green River SWA boundary. This turtle overwintered in these off-site wetlands (winter 2018-2019), in a cattail dominated wetland on the west side of the property (winter 2019-2020), and in a wetland on the east side of the property (winter 2020-2021; Fig. 2.1). Female L1R2 contained eggs in June 2018 (detected via palpation) and apparently nested in sand prairie habitat somewhat west of Pump Factory Road (Fig. 2.1). No eggs were detected in 2019 and her reproductive status was not assessed in 2020. Green River SWA female L1R1, a large juvenile, was tracked for a shorter period of time and exhibited more restricted movements. She too moved off-site to wetlands southeast of the Green River SWA boundary where she overwintered (winter 2017-2018, Fig. 2.1).

At Sand Prairie SHA, two turtles were tracked long term (male L1R1, 2018-2020; female L2R2, 2019-2020); tracking of five other turtles, trapped by environmental consultants (Stantec) began in 2020 (Fig. 2.2). All seven turtles largely restricted their movements to a shallow wetland and associated deeper ponds. Female L2R2 reproduced in both 2019 and 2020, moving to a sand ridge to the east where she presumably nested. Turtle trapping at Sand Prairie SHA has disproportionately focused on the wetland occupied by these turtles (Appendix 1.1 Figure 6; R. Blogg, personal communication). Future trapping efforts might profitably include suitable habitat to the west, south, and southeast (Fig. 2.2). Historically, shallow water habitats suitable for Blanding's turtles were probably more extensive but have been modified by dredging and ditching (Fig. 2.3), potentially reducing available Blanding's turtle habitat, especially during drought conditions.

Provisional NatureServe occurrence ranks for Blanding's turtles at preserves within the Green River COA range from F;X (failed to find, extirpated at Mineral Marsh Nature Preserve), to D (poor viability at Amboy Marsh Nature Preserve, Gremel Nature Preserve, and Green River SWA, to CD (fair to poor viability at an unnamed site, Sand Prairie SHA, and Richardson Wildlife Foundation; Table 2.2). Threats include incompatible land use (e.g., game fields and agricultural leases may pose a risk to females during nesting treks at Green River SWA and Sand Prairie SHA), off-property movements that may place animals in harm's way, non-native vegetation (e.g., invasive reed canary grass), and predators on nests and hatchlings (e.g., raccoons). Given their isolation from each other and from Blanding's turtle populations elsewhere, populations within the Green River COA are also at risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes. Blanding's turtle persistence might also benefit from hydrological monitoring and management.

2.3 Illinois Mud Turtle

The Illinois mud turtle, *Kinosternon flavescens*, has been recorded from two of the eleven preserves in the Green River COA included here. Recent records are lacking for Sand Prairie SHA despite trapping there in 2017 and 2018 (last recorded in 1986; Table 2.1, 2.3; Appendix 1.1 Table 3). Two individuals were encountered at Mineral Marsh Nature Preserve during trapping efforts there (Table 2.1, Fig. 2.4). Trapping focused on wetlands immediately west of the preserve boundary (with land-owner permission negotiated by R. Blogg), a site known to be occupied by Mud turtles in the past (Tuma 1993, 2006). Research at Mineral Marsh in 1992-1993 documented movements via radio telemetry by Mud turtles from these wetlands to Mineral Marsh, where nesting and aestivation. Previous assessments suggest that the status of Mud turtles in Illinois is precarious (Tuma 1993, 2006; Christiansen et al. 2012).

Provisional NatureServe occurrence ranks for Mud turtles at preserves within the Green River COA range from F;X (failed to find, extirpated at Sand Prairie SHA to D (poor viability at Mineral Marsh Nature Preserve; Table 2.2). Given its isolation, the Mud turtle population at Mineral Marsh is at risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes. Private ownership of wetlands utilized by Mud turtles at Mineral Marsh limits options for their protection and management.

2.4 Ornate Box Turtle

The Ornate box turtle, *Terrapene ornata*, has been recorded from ten of the eleven preserves in the Green River COA included here (Table 2.1, 2.3). Recent records are lacking for five of these preserves, Foley Sand Prairie Nature Preserve (last encountered in the 1980s), an unnamed preserve (shell fragments, date not specified, Nelson et al. 2019), Mineral Marsh Nature Preserve (last encountered prior to 1997), Amboy Marsh Nature Preserve (date not specified, Nelson et al. 2015), and Gremel Wildlife Sanctuary (last encountered in 2007). A single individual was encountered at McCune Sand Prairie Land and Water Reserve in 2019 (Fig. 2.5). None were encountered at Sand Prairie SHA in 2017-2019 but five were found by environmental consultants (Stantec) working at the site in 2020, one of which was tracked using radio telemetry (Fig. 2.6). One Ornate box turtle was encountered at Hahnaman Sand Prairie Nature Preserve in May 2018 (Fig. 2.7). Additionally, eight Ornate box turtles were encountered there by environmental consultants in October 2018 (Stantec, G. Wahl, personal communication). Ornate box turtles have been encountered very infrequently at Richardson Wildlife Foundation (B. Towey, personal communication); two that were encountered during this study were tracked using radio telemetry by RWF staff from 2018-2020 (Table 2.3, Fig. 2.8). Eleven ornate box turtles were encountered at Green River SWA during this study (Table 2.3). Among these were five of eight animals captured and marked by R. Blogg in 2015 and six animals captured for the first time during this study. Ten individual ornate box turtles were tracked at Green River SWA using radio telemetry (Table 2.3, Fig. 2.9).

At Green River SWA, Ornate box turtle movements encompass a large portion of the preserve. Individual turtles regularly crossed Game Road and Pump Factory Road within the preserve and sometimes crossed Maytown Road along the southern border of the preserve. Ornate box turtles utilized agricultural lands within the Green River SHA and on private lands north and south of the preserve (Fig. 2.9). On turtle, male L1R1 was found dead apparently of trauma caused by agricultural equipment (Table 2.3).

Provisional NatureServe occurrence ranks for Ornate box turtles at preserves within the Green River COA range from F;X (failed to find, extirpated at Foley Sand Prairie Nature Preserve, an unnamed preserve, Mineral Marsh Nature Preserve, Amboy Marsh Nature Preserve, and Gremel Wildlife Sanctuary) to D (poor viability at McCune Sand Prairie Land and Water Reserve, Sand Prairie SHA, and Richardson Wildlife Foundation) to BC (fair to good viability at Hahnaman Sand Prairie Nature Preserve and Green River SWA; Table 2.2). Threats include incompatible land use (e.g., game fields and agricultural leases may pose a risk at Green River SWA and Sand Prairie SHA), road-crossings and off-property movements that may place animals in harm's way (as observed at Green River SWA), encroachment by woody vegetation (e.g., at Green River SWA, Fig. 2.10, and McCune McCune Sand Prairie Land and Water Reserve, Fig. 2.11), and predators on nests and hatchlings (e.g., raccoons). Given their isolation from each other and from Ornate box turtle populations elsewhere, populations within the Green River COA are also at risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes.

2.5 Slender Glass Lizard

The Slender glass lizard, *Ophisaurus attenuatus*, has been recorded from three of the eleven preserves in the Green River COA included here, an unnamed preserve, Amboy Marsh Nature Preserve, and Green River SWA (Table 2.1). In total, eleven Slender glass lizards were encountered at Green River SWA (Table 2.4, Fig. 2.13). Observations were restricted to the north half of the preserve although the species may occur in suitable sand prairie habitat throughout the preserve.

Provisional NatureServe occurrence ranks for Slender glass lizards at preserves within the Green River COA range from U (unrankable at an unnamed preserve and at Amboy Marsh Nature Preserve to BC (fair to good viability at Green River SWA; Table 2.2). Threats include incompatible land use (e.g., game fields and agricultural leases may pose a risk at Green River SWA), road-crossings that may place animals in harm's way (one specimen was found dead on Pump Factory Road, another was observed basking on Game Road), and encroachment by woody vegetation (e.g., at Green River SWA, Fig. 2.10). Given their isolation from each other and from Slender glass lizard populations elsewhere, populations within the Green River COA are also at risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes.

2.6 Plains Hognose Snake

The Plains hognose snake, *Heterodon nasicus*, has been recorded from seven of the eleven preserves in the Green River COA included here (Tale 2.1). Recent records are lacking for four of these preserves, Foley Sand Prairie Nature Preserve (last encountered in the 1980s), McCune Sand Prairie Land and Water Reserve (last encountered in 2006), Amboy Marsh Nature preserve (last encountered in 1989), and Sand Prairie SHA (last encountered in 1991; Table 2.1). During this study, four individuals were encountered at Mineral Marsh Nature Preserve and 11 were encountered at Green River SWA (Table 2.5). In addition, nine Western hognose snakes were encountered at Hahnaman Sand Prairie Nature Preserve by environmental consultants in October 2018 (Stantec, G. Wahl, personal communication). Encounters at Mineral Marsh Nature Preserve occurred throughout the site (Fig. 2.4); encounters at Green River SWA were restricted to the north half of the preserve (Fig. 2.13) although the species may occur in suitable sand prairie habitat throughout the preserve.

Provisional NatureServe occurrence ranks for Plains hognose snakes at preserves within the Green River COA range from F,X (failed to find, extirpated at Foley Sand Prairie Nature Preserve and Sand Prairie SHA) to F (failed to find at McCune Sand Prairie Land and Water Reserve and Amboy Marsh Nature Preserve) to BC (fair to good viability at Mineral Marsh Nature Preserve, Hahnaman Sand Prairie Nature Preserve and Green River SWA; Table 2.2). Threats include incompatible land use (e.g., game fields and agricultural leases may pose a risk at Green River SWA), road-crossings that may place animals in harm's way, and encroachment by woody vegetation (e.g., at Green River SWA, Fig. 2.10). Possibly, encroachment by woody vegetation is associated with a ack of recent records of Plains hognose snakes at McCune Sand Prairie (Fig. 2.11). Given their isolation from each other and from Plains hognose snake populations elsewhere, populations within the Green River COA are also at risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes.

2.7 Smooth Greensnake

The Smooth Greensnake, *Opheodrys vernalis*, has been recorded from three of the eleven preserves in the Green River COA included here, Amboy Marsh Nature Preserve, Richardson

Wildlife Foundation, and Green River SWA (Table 2.1). Recent records are lacking for Amboy Marsh Nature Preserve. At Green River SWA, 201 individual Smooth Greensnakes were encountered a total of 227 times (26 recaptures) from 2017-2019. At Richardson Wildlife Foundation, 19 individual Smooth Greensnakes were encountered a total of 21 times (2 recaptures) from 2018-2019. All except 18 Smooth Greensnake encounters were of animals found under arrays of artificial cover objects. At Green River SWA, Smooth Greensnakes were encountered at all 12 arrays (Appendix 1.1, Figure 1). At Richardson Wildlife Foundation Smooth Greensnakes were encountered at four arrays located north of Shaw Road (Appendix 1.1, Figure 8). Given the abundance observed in this study, Green River SWA likely encompasses one of largest Smooth Greensnake populations in Illinois.

Provisional NatureServe occurrence ranks for Smooth Greensnakes at preserves within the Green River COA range from F (failed to find at Amboy Marsh Nature Preserve) to BC (fair to good viability at Richardson Wildlife Foundation) to AC (excellent to fair viability at Green River SWA; Table 2.2). Threats include incompatible land use (e.g., game fields and agricultural leases may pose a risk at Green River SWA), road-crossings that may place animals in harm’s way, and encroachment by woody vegetation (e.g., at Green River SWA, Fig. 2.10). Given their isolation from each other and from Plains hognose snake populations elsewhere, populations within the Green River COA are also at risk of decline due to inbreeding depression, demographic and environmental stochasticity, and catastrophes. Despite these threats, Green River SWA may contain the largest Smooth Greensnake population in Illinois.

Hatchling Smooth Greensnakes (animals prior to their first hibernation) were first encountered after Aug 2; gravid females were encountered between May 21 and July 19 (Fig. 2.14). The smallest gravid female encountered measured 246 mm snout-vent length. Growth patterns suggest that this size is achieved during the second full year after hatching (after the second hibernation, Fig. 2.14).

2.8 Literature Cited

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Table 2.1. Most recent records of amphibian and reptile species in greatest conservation need at preserves within the Green River Conservation Opportunity Area through 2020. Observations made during this study are highlighted in green. Preserves are ordered from smallest to largest. Preserve abbreviations: FSPNP – Foley Sand Prairie Nature Preserve; Unnamed – preserve not identified at request of site managers and stewards, MPHA – Maytown Pheasant Habitat Area, MSP – McCune Sand Prairie Land and Water Reserve, MMNP – Mineral Marsh Nature Preserve, AMNP – Amboy Marsh Nature Preserve, SPSHA – Sand Prairie State Habitat Area, HSNA – Hahnaman Sand Prairie Nature Preserve, GWS – Gremel Wildlife Sanctuary, RWS – Richardson Wildlife Foundation, GRSWA – Green River State Wildlife Area.

	Preserve										
	FSPNP	Unnamed	MPHA	MSP	MMNP	AMNP	SPSHA	HSPNA	GWS	RWF	GRSWA
Eastern Newt						2019[1]					
Blanding's Turtle		2018[1]			1996[2]	2019[1]	2017-20		2004[3]	2017-20	2017-20
Illinois Mud Turtle					2018		1986[2]				
Ornate Box Turtle	1980s[2]	-[4]		2019	Pre-1997[2]	–[5]	2020[6]	2018	2007[3]	2018-20	2017-20
Slender Glass Lizard		-[4]				2020[7]					2017-19
Plains Hognose Snake	1980s[2]			2006[8]	2018-19	1989[2]	1991[2]	2018[9]			2017-19
Smooth Greensnake						–[5,10]				2018-19	2017-19

[1] D. Carey and W. Rogers, personal communication; [2] Illinois Department of Natural Resources Biotics5; [3] S. Hager, personal communication; [4] Nelson et al. 2019; [5] Nelson et al. 2015; [6] Two individuals were encountered in 2020, R. Blogg, personal communication; [7] Mauger 2020; [8] Horger 2006; [9] G. Wahl, personal communication; [10] R. Nyboer, personal communication

Table 2.2. Provisional NatureServe occurrence ranks of amphibian and reptile species in greatest conservation need at preserves within the Green River Conservation Opportunity Area. Occurrence rank abbreviations: AB – excellent to good viability (green), AC – excellent to fair viability (green), BC – good to fair viability (pale green), CD – fair to poor viability (yellow), D – poor viability (orange), F – failed to find (red), X – extirpated (red), U – unrankable. See Table 2.1 for preserve abbreviations.

	Preserve										
	FSPNP	Unnamed	MPHA	MSP	MMNP	AMNP	SPSHA	HSNA	GWS	RWF	GRSWA
Eastern Newt						AC					
Blanding's Turtle		CD			F; X	D	CD		D	CD	D
Illinois Mud Turtle					D		F; X				
Ornate Box Turtle	F; X	F; X		D	F; X	F; X	D	BC	F; X	D	BC
Slender Glass Lizard		U				U					BC
Plains Hognose Snake	F; X			F	BC	F	F; X	BC			BC
Smooth Greensnake						F				BC	AC

Table 2.3. Blanding's Turtles (A) Ornate Box Turtles (B) and Illinois Mud Turtles (C) captured at Green River SWA (GRSWA), Sand Prairie State Habitat Area (SPSHA), Richardson Wildlife Foundation (RWF), Hahnaman Sand Prairie Nature Preserve (HSPNP), McCune Sand Prairie Land and Water Reserve (MSP), and Mineral Marsh Nature Preserve (MMNP). Date, latitude, and longitude refers to first capture; carapace length is straight line distance from anterior to posterior carapace margin. Capture locations are shown in Figure 2.1, 2.2, and 2.4.

Preserve	ID	Sex	Capture Method	Date	Latitude, Longitude	Carapace Length (mm)	Mass (g)	Comments
A. Blanding's Turtles								
GRSWA	L1R1	Female	Trap	17-May-17	41.639076, -89.488501	168	620	Large juvenile, telemetry 17-May-17 – 23-Jun18 (transmitter came off)
GRSWA	L1R2	Female	Hand capture	25-May-18	41.571389, -89.516556	231	1785	Telemetry 25-May-18 – present
SPSHA	L1R1	Male	Trap	5-Jun-18	41.633944, -89.624556	199	1122	Telemetry 5-Jun-18 – present
SPSHA	L2R2	Female	Hand capture	13-Jun-18	41.634500, -89.621611	218	1900	Telemetry 13-Jun-18 – present
SPSHA	L1L10	Female	Trap	3-Jun-20	41.635227, -89.626017	211	1453	Telemetry 3-Jun-20 – present; first captured by Stantec crew
SPSHA	L1L3	Female	Trap	5-Jun-20	41.634827, -89.627848	189	1012	Telemetry 5-Jun-20 – present; first captured by Stantec crew
SPSHA	L9R9	Male	Trap	5-Jun-20	41.634143, -89.626653	233	1738	Telemetry 5-Jun-20 – present; first captured by Stantec crew
SPSHA	R1R10	Male	Trap	3-Jun-20	41.635227, -89.626017	216	1392	Telemetry 3-Jun-20 – present; first captured by Stantec crew
SPSHA	R1R3	Male	Trap	5-Jun-20	41.634827, -89.627848	197	1049	Telemetry 5-Jun-20 – present; first captured by Stantec crew

Preserve	ID	Sex	Capture Method	Date	Latitude, Longitude	Carapace Length (mm)	Mass (g)	Comments
B. Ornate Box Turtles								
GRSWA	L1R1	Male	Hand capture	30-May-17	41.647815, -89.495011	106	333	Telemetry 30-May-17 – 26-May-28; found dead of trauma in adjacent ag field
GRSWA	L1R2	Female	Hand capture	19-Jun-17	41.648515, -89.497245	117	398	Telemetry 19-Jun-17 – 25-Sep-19 (transmitter removed)
GRSWA	L12	Female	Dogs	23-May-18	41.631775, -89.511632	119	446	Telemetry 23-May-18 – 21-Oct-18, died during winter 2018-2019; excavated carcass 24Jun-19; previously encountered and marked during dog surveys in May 2015
GRSWA	L10	Female	Dogs	23-May-18	41.632041, -89.512494	115	352	Telemetry 23-May-18 – present; previously encountered and marked during dog surveys in May 2015
GRSWA	L11	Female	Dogs	23-May-18	41.632882, -89.512510	101	248	Telemetry 23-May-18 – 23-Aug-19 (transmitter removed); previously encountered and marked during dog surveys in May 2015
GRSWA	L2	Female	Hand capture	25-May-18	41.635778, -89.527306	119	403	Telemetry 22-May-18 – present; previously encountered and marked during dog surveys in May 2015
GRSWA	L1R3	Female	Hand capture	25-May-18	41.647816, -89.495568	99	189	Telemetry 25-May-18 – present
GRSWA	L9	Female	Hand capture	23-Jun-18	41.631834, -89.516804	110	346	Telemetry 23-Jun-18 – present; previously encountered and marked during dog surveys in May 2015
GRSWA	L1R9	Male	Dogs	23-Jun-19	41.632889, -89.511167	113		Telemetry 23-Jun-19 – present

Preserve	ID	Sex	Capture Method	Date	Latitude, Longitude	Carapace Length (mm)	Mass (g)	Comments
GRSWA	L1R10	Female	Hand capture	31-Jul-19	41.632944, -89.511472	100	186	Telemetry 31-Jul-19 – present
GRSWA	L1R11	Male	Hand capture	25-Sep-19	41.633407, -89.520427	109	286	
RWF	L2	Female	Dogs	22-May-18	41.719515, -89.190898	105	270	Telemetry by RWF staff, 22-May-18 – Aug-20; recapture of previously marked turtle
RWS	R1	Female	Dogs	22-May-18	41.719123, -89.190622	115	372	Telemetry by RWF staff, 22-May-18 – Aug-20; recapture of previously marked turtle
HSPNP	R2	Female	Dogs	26-May-18	41.624075, -89.641730	106	286	
MSP	R2	Female	Hand capture	29-May-19	41.454150, -89.826167			Old adult
SPSHA	R2R11	Female	Hand capture	5-Jun-20	41.634789, -89.622402	115	398	Telemetry 5-Jun-20 – present; first captured by Stantec crew
C. Illinois Mud Turtles								
MMNP	L2R3	Female	Trap	15-May-18	41.440413, -89.897702	114	279	
MMNP	L10R10	Male	Trap	15-May-18	41.440413, -89.897702	130	367	

Table 2.4. Captures of Slender Glass Lizards at Green River SWA. Snout-vent length (SVL) and tail length (Tail) are recorded in mm, mass is recorded in grams. Capture locations are shown in Figure 2.12.

Date	SVL	Tail	Mass	Latitude	Longitude
31-May-17	156	358	19.9	41.638892	-89.516461
1-Aug-17				41.643897	-89.489296
27-Jun-18				41.645243	-89.515498
23-Sep-18	130	285	4.1	41.647924	-89.512003
23-Sep-18				41.647600	-89.511375
24-May-19				41.63853928	-89.4969365
3-Jun-19	80	170	2.6	41.647282	-89.495925
3-Jun-19	85	189	3.2	41.647593	-89.496144
27-Jun-19				41.647644	-89.494784
14-Aug-19	170	382	28.8	41.64788011	-89.4946573
23-Aug-19	62	112	1.3	41.646200	-89.509141

Table 2.5. Captures of Plains Hognose snakes at Green River SWA (GRSWA) and Mineral Marsh Nature Preserve (MMNP). Snout-vent length (SVL) and tail length (Tail) are recorded in mm, mass is recorded in grams. Capture locations are shown in Figure 2.13.

Date	Site	Method	SVL	Tail	Mass	Sex	ID	Latitude	Longitude	Comments
6-Jul-17	GRSWA	CO						41.646382	-89.509295	ACO B22
26-Jul-17	GRSWA	VIS						41.647694	-89.496227	found by bat researchers, not captured
1-Jul-18	GRSWA	DF	467	70	126.4	F		41.645300	-89.515511	Drift Fence 8, dead, gravid, 8 enlarged follicles
16-Jul-18	GRSWA	VIS	179	25	4.3	F	122	41.646946	-89.510682	
12-Sep-18	GRSWA	DF	390	53	45	F	123	41.648639	-89.498038	Drift Fence 11
23-May-19	GRSWA	CO	266	57	24.8	M	124	41.646402	-89.509179	ACO B23
23-May-19	GRSWA	DF	364	85	53.8	M	126	41.648261	-89.498063	Drift Fence 19A
23-May-19	GRSWA	DF	374	79	59.4	M	127	41.648261	-89.498083	Drift Fence 19C
23-May-19	GRSWA	DF	378	95	58.7	M	125	41.648261	-89.498043	Drift Fence 19A
29-May-19	GRSWA	CO				M	124	41.646402	-89.509179	ACO B23, recapture
14-Jun-19	GRSWA	DF	153	28	4.4	F	128	41.648281	-89.498063	Drift Fence 19C
16-Jun-19	GRSWA	CO	175	36	14.6	M	129	41.643350	-89.520244	ACO E31
21-Jun-19	GRSWA	VIS	375	67	48.5	M	132	41.646479	-89.506564	Near ACO Array B
6-Jul-19	GRSWA	CO	380	50	49.5	F	134	41.648624	-89.495006	ACO I15, gravid, 6 enlarged follicles
15-Jul-19	GRSWA	CO	227	36	20.3	F	135	41.643291	-89.520625	ACO E8
11-May-18	MMNP	VIS	376	100	57.3	M		41.438233	-89.895054	
11-May-18	MMNP	VIS	500	76	140	F		41.443197	-89.891451	
25-May-19	MMNP	VIS	249	57	17.3	M		41.443900	-89.884850	
26-May-19	MMNP	VIS	203	35	9.8	M		41.443900	-89.887717	stub tail

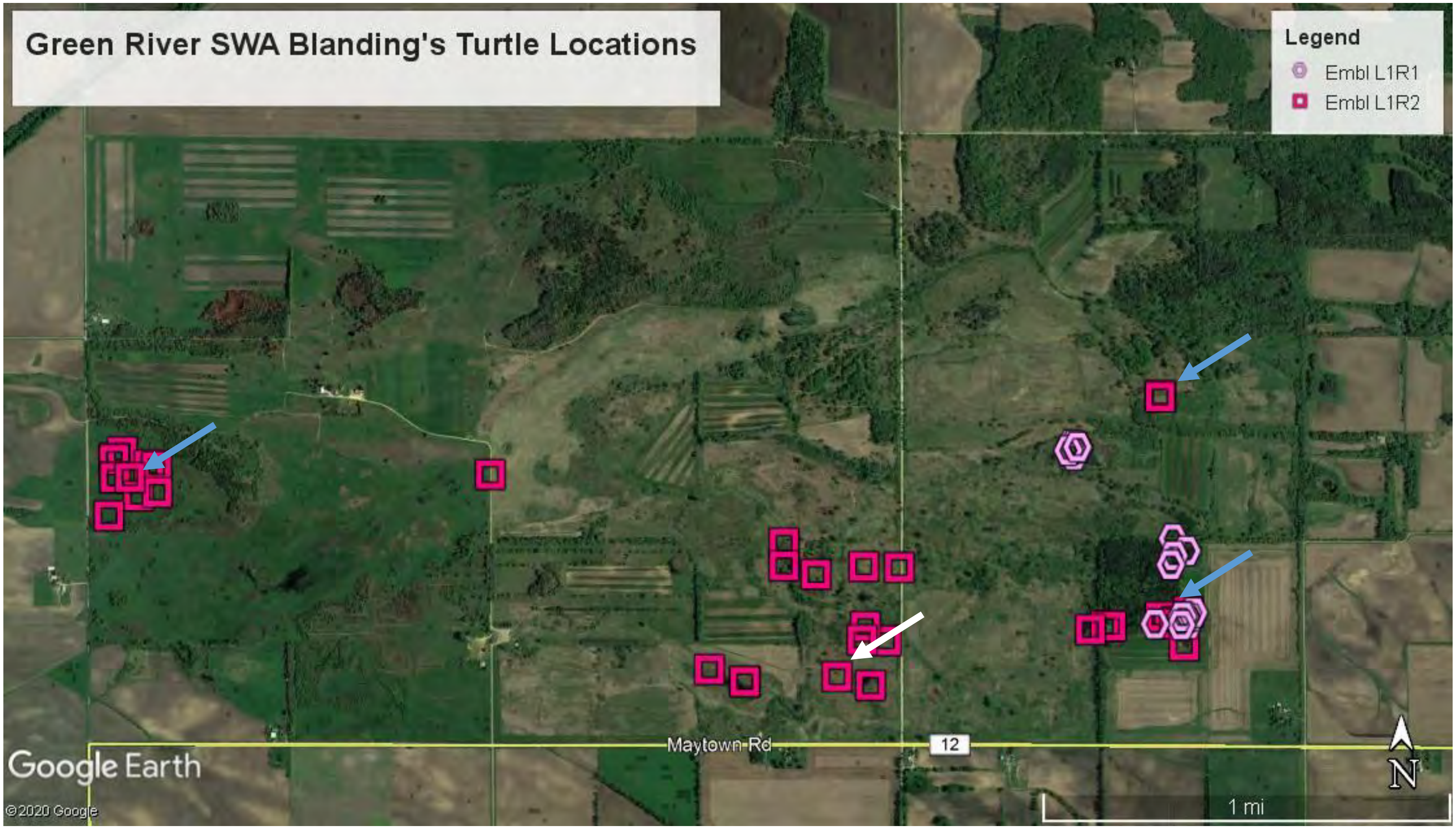

Figure 2.1. Locations of two Blanding's turtles tracked using radio telemetry at Green River SWA. A white arrow denotes sand prairie habitat where nesting activities by female L1R2 were observed in 2018. Blue arrows denote approximate overwintering locations. Turtle locations clustered at the lower right of this figure are on private land adjacent to Green River SWA.

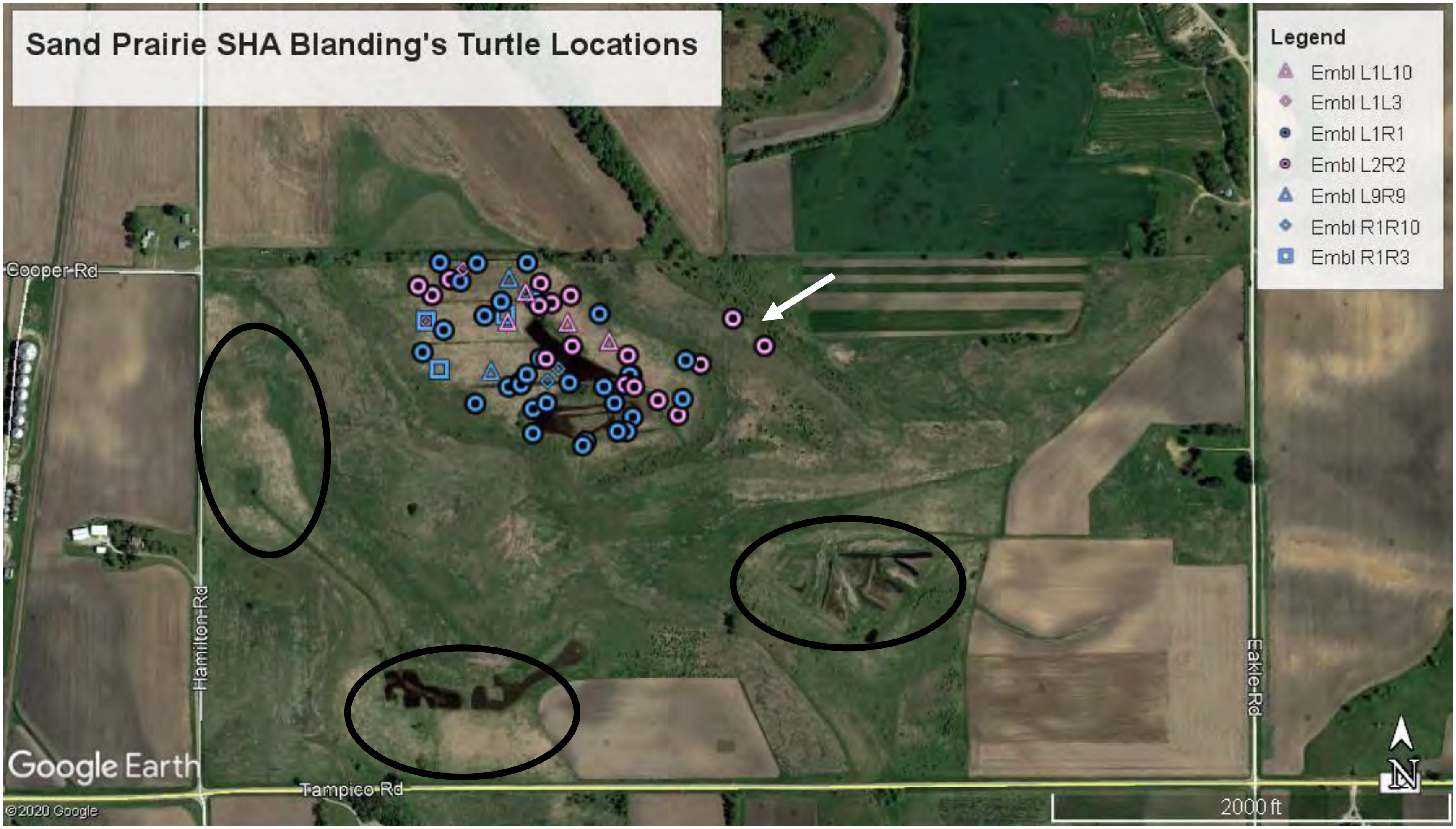

Figure 2.2. Locations of seven Blanding's turtles tracked using radio telemetry at Green River SWA. Wetlands where additional trapping effort is warranted are circled in black. A white arrow denotes the sand prairie ridge where nesting activities by female L2R2 were observed in 2019 and 2020.

Figure 2.3. Historic (1938, upper panel) and current (2011, lower panel) land cover at Sand Prairie SHA showing wetland modification that has occurred at this site. Shallow wetlands dominated by emergent vegetation have been modified into deeper open water habitats (arrows).

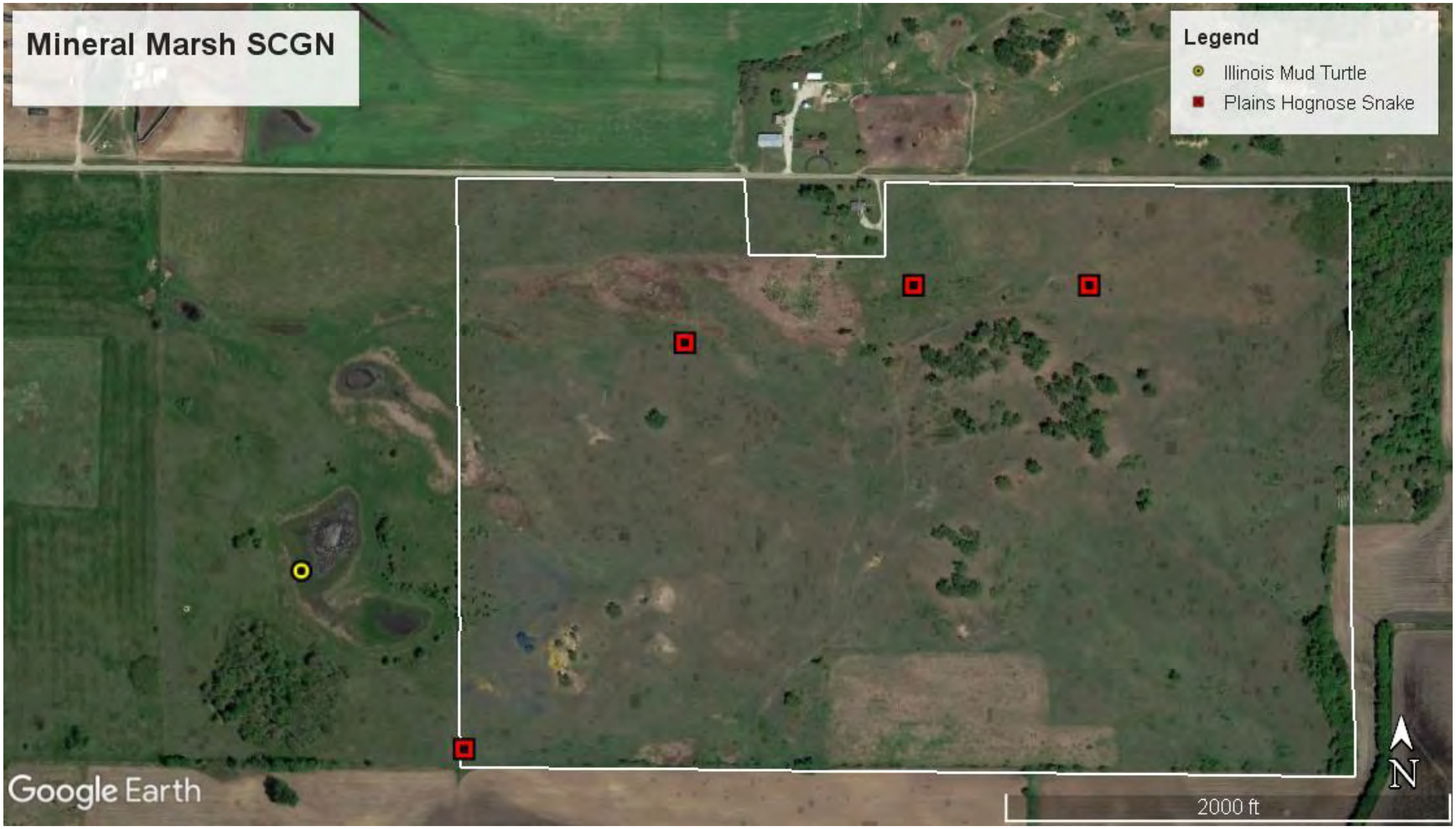

Figure 2.4. Locations of Illinois mud turtles and Plains hognose snakes at Mineral Marsh Nature Preserve. Approximate preserve boundaries are shown in white; Illinois mud turtle locations are on adjacent privately-owned land.

Figure 2.5. Location of Ornate box turtle at McCune Sand Prairie Land and Water Reserve.

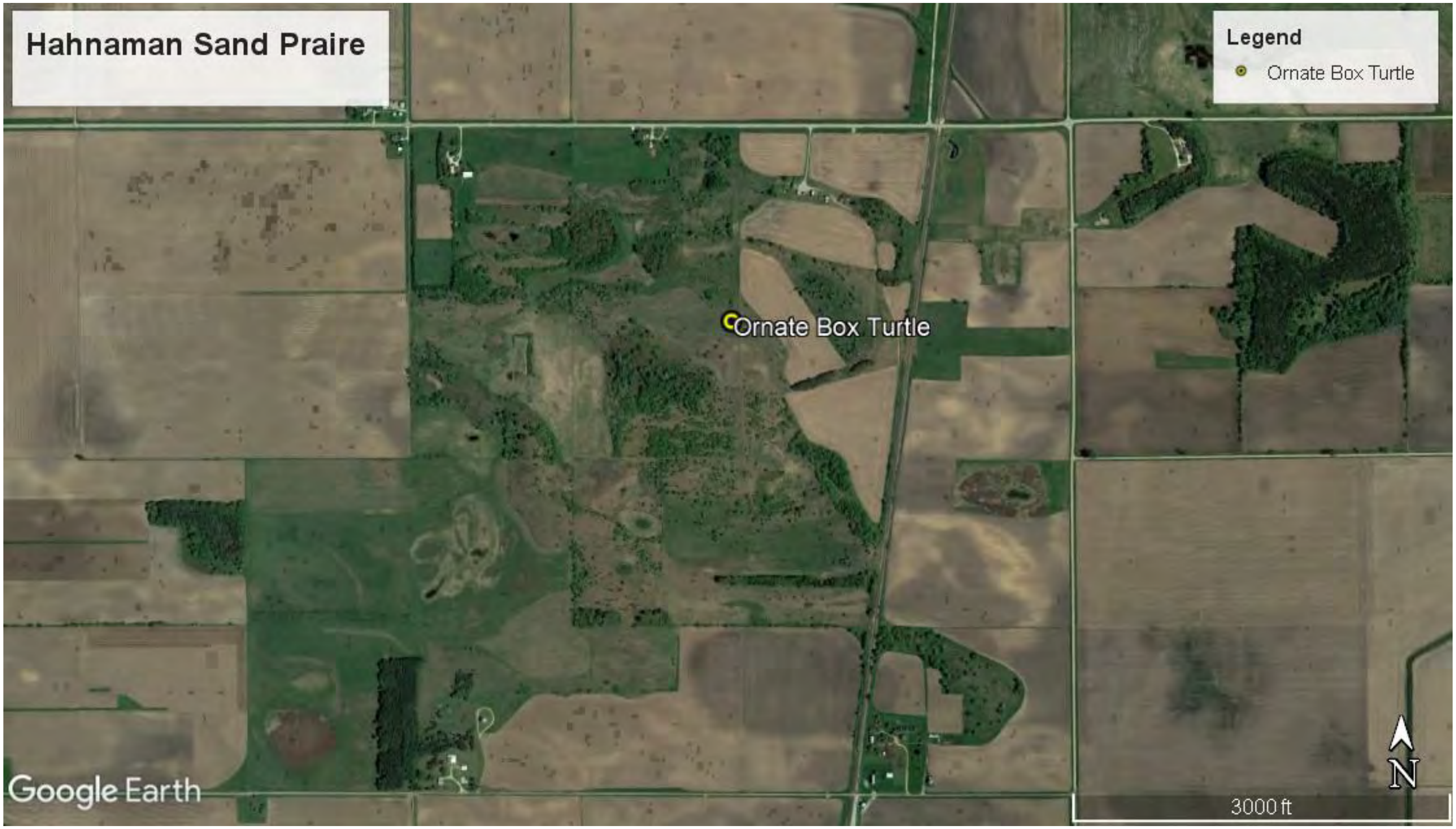

Figure 2.6. Location of Ornate box turtle at Hahnahan Sand Prairie Nature Preserve.

Figure 2.7. Locations of a female Ornate box turtle tracked using radio telemetry at Sand Prairie SHA and of a depredated nest inferred to be hers.

Figure 2.8. Locations of Ornate box turtles at Richardson Wildlife Foundation.

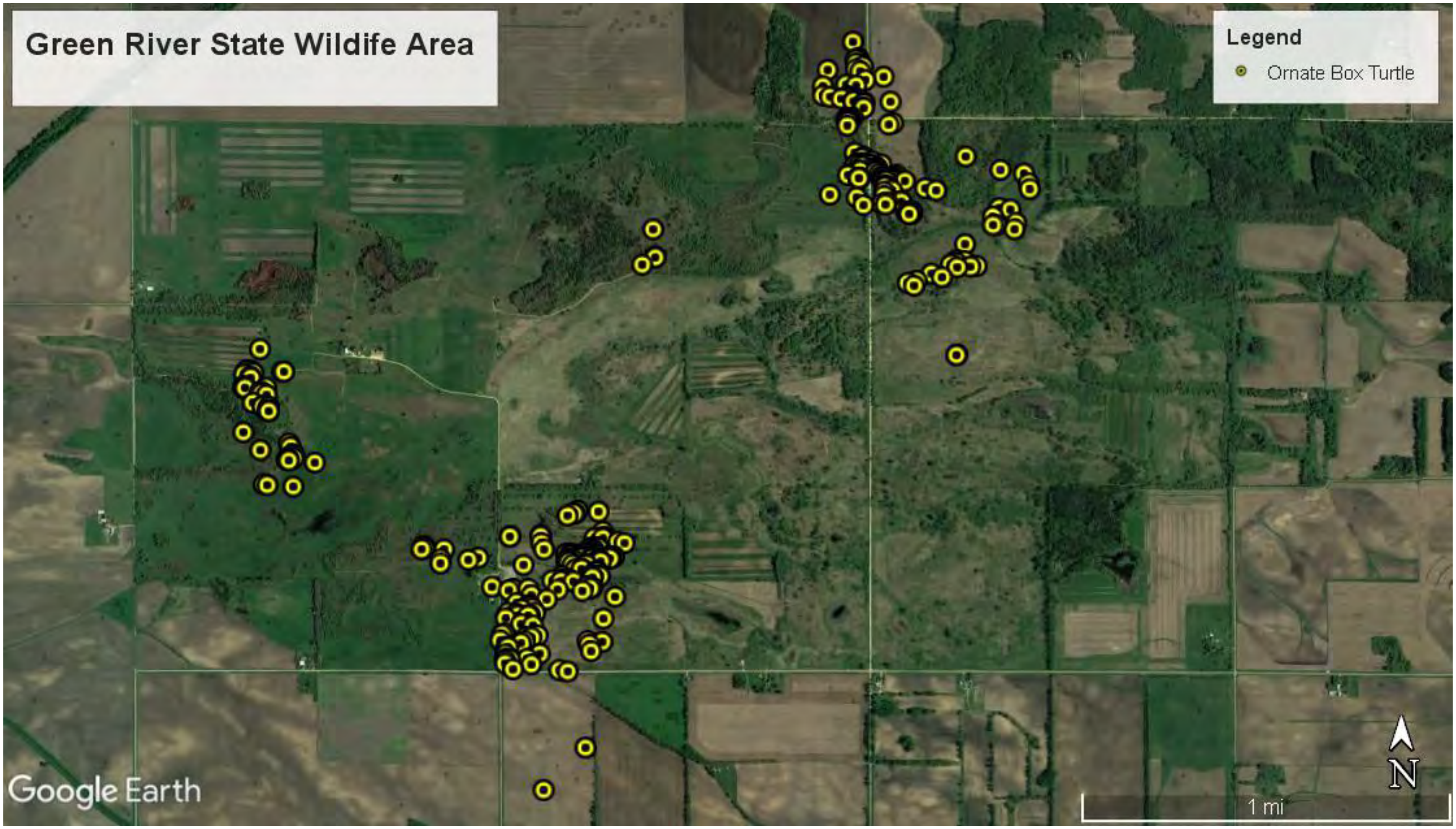

Figure 2.9. Locations of 10 Ornate box turtles at Green River State Wildlife Area tracked using radiotelemetry. Two turtles moved off-property onto agricultural lands north of the preserve; one turtle moved off-property onto agricultural lands south of the preserve.

Figure 2.10. Historic (1938, upper panel) and current (2011, lower panel) land cover at Green River SWA. Areas where woody vegetation now encroaches on Ornate box turtle (cf. Fig. 2.9), Slender glass lizard (cf. Fig. 2.12), and Plains hognose snake habitat (cf. Fig. 2.13) are indicted by arrows in the lower panel. These (and other) areas were largely devoid of woody vegetation in 1938 (upper panel).

Figure 2.11. Historic (1938, upper panel) and current (2011, lower panel) land cover at McCune Sand Prairie Land and Water Reserve showing that woody vegetation now encroaches on Ornate box turtle habitat (cf. Fig. 2.9). This site was mostly devoid of woody vegetation in 1938.

Figure 2.12. Locations of Slender glass lizards at Green River SWA.

Figure 2.13. Locations of Plains hognose snakes at Green River SWA.

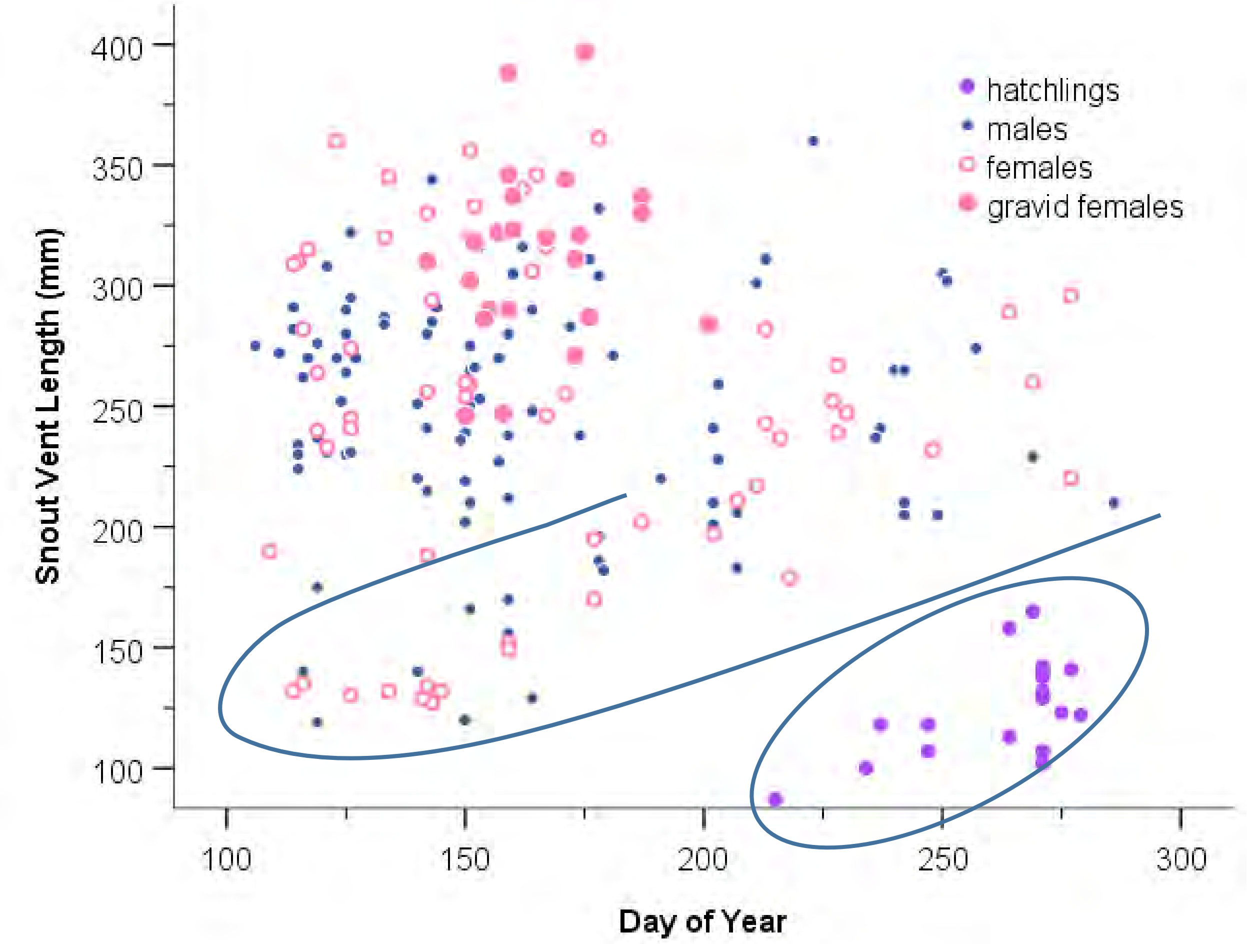

Figure 2.14. Relationship of snout-vent length to day of year in Smooth Greensnakes. Hatchlings (age 0) are enclosed by an oval in the lower right. Animals inferred to be in their first year (age 1) are partially circled. Gravid females (filled pink circles) are inferred to be in their second or later year of life (age 2+).

Part 3. Amphibian and reptile disease status within the Green River COA

3.1 *Batrachochytrium dendrobatidis* occurrence in amphibians at Green River SWA

Batrachochytrium dendrobatidis (*Bd*), the fungus that causes chytridiomycosis, has been implicated in amphibian declines worldwide (Cheng et al. 2011; Collins and Storfer 2003; Wake and Vredenburg 2008), leading to widespread efforts to survey the prevalence and pathogenicity of Bd (e.g., Beyer et al. 2015; Crawford et al. 2017; Lanoo et al. 2011; Julien et al 2019). Commonly, *Bd* is detected in skin swabs using PCR-based laboratory methods.

Skin swabs were collected from Cricket Frogs and Leopard Frogs at four sites in Green River SWA (Fig. 1) in 2017 and 2018 following protocols provided by Pices Molecular (https://www.pisces-molecular.com/, Boulder, CO). These species were selected because of their abundance and because they represent distinct taxonomic lineages (Hylidae, Ranidae). In addition, Leopard Frogs may be a reservoir for *Bd* (Woodhams et al. 2008). Four to eight swabs were combined into 'batches' for analysis by Pices Molecular.

Swabs from a total of 70 Leopard Frogs (47 in 2017, 23 in 2018) and 39 Cricket Frogs (21 in 2017, 18 in 2018) were analyzed (Table 3.1, Fig. 3.1). None of 9 batches of swabs collected in the Central Slough were *Bd* positive; 3 of 4 batches collected in the Cattail Marsh were *Bd* positive, 3 of 4 batches collected in the Peat Marsh were *Bd* positive, and 1 of 1 batch collected at North Pump Factory Road was *Bd* positive (Table 3.1). Six of 12 batches collected from Leopard Frogs where *Bd* positive; 1 of 6 batches collected from Cricket Frogs was *Bd* positive (Table 3.1).

3.2 Snake fungal disease occurrence in snakes at Green River SWA

Snake fungal disease (SFD) is an emerging fungal pathogen caused by *Ophidiomyces ophiodiicola* occurring in a range of snake species primarily in eastern North America (Allender et al. 2015; Lorch et al. 2016). The geographic distribution and range of species affected by SFD is poorly known. Like *Bd*, SFD can be detected in skin swabs using PCR-based laboratory methods.

Skin swabs were collected from Common Gartersnakes and Smooth Greensnakes following protocols provided by Dr. Matt Allender (University of Illinois Veterinary Clinical Medicine) and submitted to the Wildlife Epidemiology Laboratory (https://vetmed.illinois.edu/wel/, Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois Urbana-Champaign) for analysis.

A total of 80 swabs were collected from 73 individual snakes, representing 4 species. The majority of snakes were Smooth Greensnakes *Opheodrys vernalis* (n=38), followed by Common Gartersnakes *Thamnophis sirtalis* (n=30), Plains Hog-nosed Snakes *Heterodon nasicus* (n=4), and Eastern Foxsnakes *Pantherophis vulpinus* (n=1). These species differ in ecology and conservation status. Clinical symptoms or positive PCR results for *O. ophidiicola* were present in all four species, including seven Smooth Greensnakes (18.4%) and seven Common Gartersnakes (23.3%). Detailed methods and results are provided in Appendix 3.1.

3.3 Ranaviruses, herpesviruses, and Mycoplasma in Blanding's Turtles and Ornate Box Turtles within the Green River COA

Ranaviruses, herpesviruses, and Mycoplasma have the potential to cause significant die-offs in turtle populations (Sim et al. 2015, 2016). To test for the occurrence of these pathogens, oral and cloacal swabs were collected opportunistically from Blanding's Turtles (n = 15) and Ornate Box Turtles (n = 14) at several preserves with the Green River COA following protocols provided by Dr. Matt Allender (University of Illinois Veterinary Clinical Medicine) and submitted to the Wildlife Epidemiology Laboratory (https://vetmed.illinois.edu/wel/, Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois Urbana-Champaign) for analysis. Other pathogens were assayed simultaneously.

Three Blanding's turtles tested positive for *Emydoidea* herpesvirus 1 and two Ornate Box Turtles tested positive for *Terrapene* adenovirus. Emydoidea Herpesvirus 1 is the most common pathogen detected in other Illinois Blanding's turtle populations (Lindeman et al. 2019). Detailed methods and results are provided in Appendix 3.2.

3.4 Conclusions

Detection of pathogens including *Batrachochytrium dendrobatidis*, *Ophidiomyces ophiodiicola*, *Emydoidea* herpesvirus 1 and *Terrapene* adenovirus among amphibians, snakes and turtles at preserves in the Green River COA is concerning but not unexpected. The distribution of these pathogens and their impacts on host populations remains poorly known but baseline data like that collected within the Green River COA contributes to a growing knowledge base. Despite the presence of these pathogens, field observations suggest an absence of current disease impacts on amphibian and reptile populations within the Green River COA (e.g., dead or moribund individuals). Future recommendations for continued monitoring are provided in Appendix 3.1 and 3.2.

3.5 Literature Cited

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Table 3.1. Outcome of tests for *Batrachochytrium dendrobatidis* (*Bd*) in Leopard Frogs and Cricket Frogs from four locations at Green River State Wildlife Area, 2017 and 2018. "*Positive*" indicates that *Bd* was detected in all batches of swabs from that site-species-year combination; "*Negative*" indicates that *Bd* was not detected. Each swab corresponds to a unique individual frog.

	Year	Cattail Marsh	Central Slough	Peat Marsh	North Pump Factory Road
Leopard Frog	2017	*Positive* (16 swabs in 2 batches)	*Negative* (20 swabs in 3 batches)	*Positive* (11 swabs in 2 batches)	-
	2018	*Positive* (4 swabs in 1 batche)	*Negative* (14 swabs in 3 batches)	-	*Positive* (5 swabs in 1 batch)
Cricket Frog	2017	*Negative* (8 swabs in 1 batch)	*Negative* (5 swabs in 1 batch)	*Positive* (8 swabs in 1 batch)	-
	2018	-	*Negative* (10 swabs in 2 batches)	*Negative* (8 swabs in 1 batch)	-

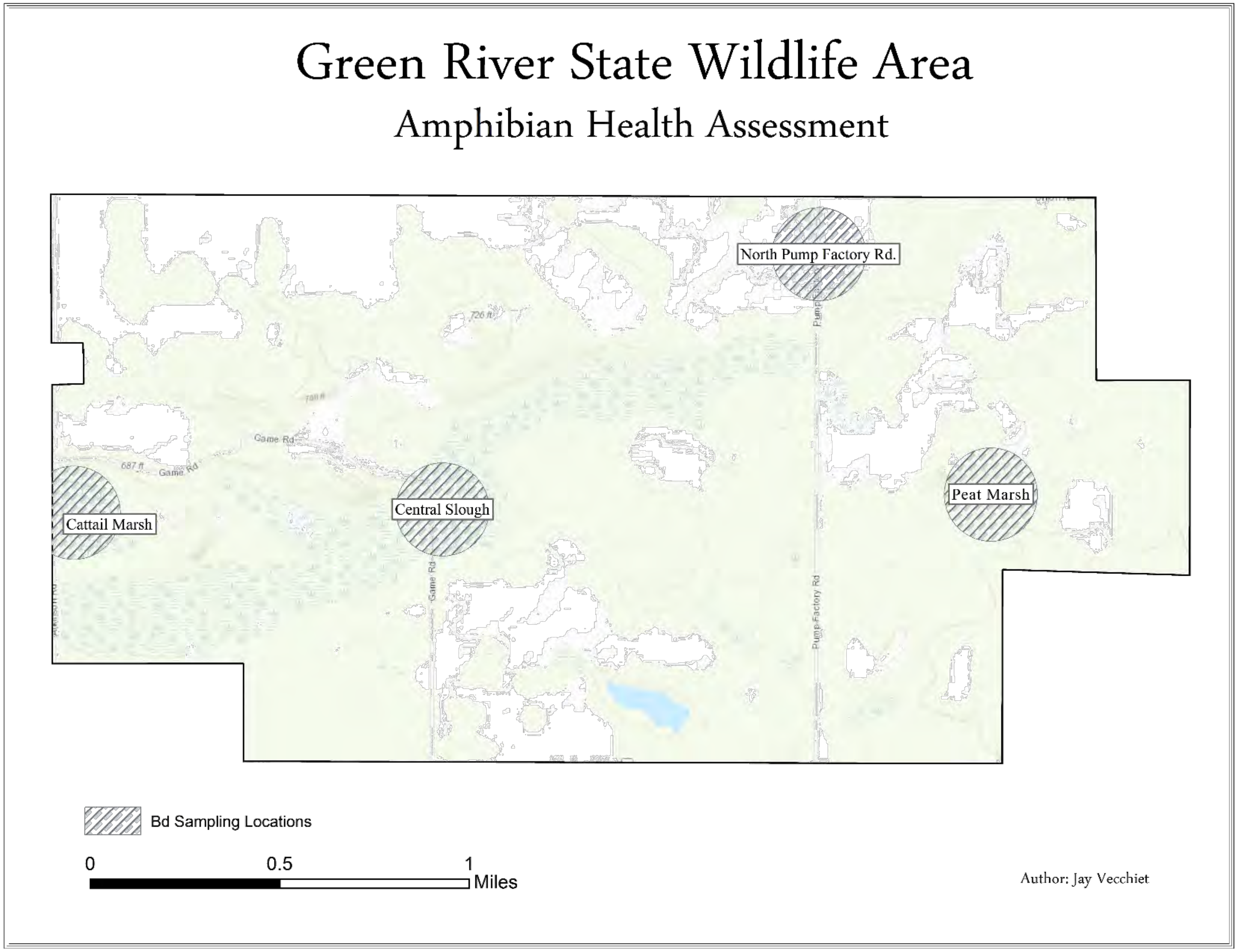

Fig. 3.1. Sampling locations for *Batrachochytrium dendrobatidis* (*Bd*) in Leopard Frogs and Cricket Frogs at Green River State Wildlife Area.

Appendix 3.1. Ophidiomyces Detection and Snake Fungal Disease in Free-Ranging Snakes In Northern Illinois

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OPHIDIOMYCES DETECTION AND SNAKE FUNGAL DISEASE IN FREE-RANGING SNAKES IN NORTHERN ILLINOIS

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26 January 2020

INTRODUCTION

MATERIALS AND METHODS

QUANTITATIVE PCR– Snakes were sampled by swabbing the body and any gross lesions using a cotton-tipped applicator. After collection, swabs were placed in 2 ml Eppendorf tubes and frozen at -20°C until analysis. DNA extraction and quantitative PCR amplification (qPCR) were performed as previously reported (Allender et al., 2015a). DNA extraction followed the manufacturer's recommendations with the addition of an incubation at 37°C with 300U of lyticase prior to the lysis step. Following DNA extraction, each sample was assessed for DNA quantity (measured in ng/ul) and quality (using the ratio of absorbance at 260 nm to 280 nm) using spectrophotometry (Nanodrop, ThermoFisher Scientific). qPCR was performed in triplicate on a QuantStudio3 real time thermocycler. Samples were considered positive if replicates had a lower mean cycle threshold (C_t) value than the lowest detected standard dilution. Copies per reaction were standardized to the total quantity of DNA in the sample by dividing the mean copies/ul for each sample by the DNA concentration, as determined by spectrophotometry.

STATISTICAL ANALYSIS– Descriptive statistics were tabulated including prevalence proportion and 95% confidence intervals. Associations between species and clinical signs, Ophidiomcyes qPCR result, and ophidiomycosis status were performed using a Pearson's chi-squared test. Statistical significance was assigned a $p<0.05$. All analysis was performed using commercial software (SPPS ver. 26, IBM statistics, Chicago, IL 60606).

RESULTS

GENERAL SURVEY RESULTS–A total of 80 swabs were collected from 73 individual snakes, representing 4 species. The majority of snakes were *Opheodrys vernalis* (n=38), followed by *Thamnophis sirtalis* (n=30), *Heterodon nasicus* (n=4), and *Pantherophis vulpinus* (n=1). Individuals were sampled from 1 to 2 times. All 73 individuals had a general body swab, four individuals had an additional swab on the face, and two on the tail taken the same day as the body swab. A single individual (336) was sampled on the body at two different timepoints during the season.

OPHIDIOMYCES DETECTION AND OPHIDIOMYCOSIS CLASSIFICATION–Clinical signs consistent with ophidiomycosis were observed in 13 individuals, with at least a single individual in each species (Appendix 3.1 Table1). *Ophidiomyces ophiodiicola* DNA was detected in samples from 14 snakes for an overall prevalence of 19.2% (95% CI: 10.9 – 30.1%) (Appendix 3.1 Table1). There were significantly more smooth greensnakes (n=4, 10.5%) and western hognose snakes (n=4, 100%) classified with apparent ophidiomycosis than common gartersnakes (n=0, 0%) ($p<0.0001$) (Appendix 3.1 Table1). More common gartersnakes were classified as having

Ophidiomyces present (n=6, 20%) than any other group (p<0.0001). DNA concentration was similar in positive (mean=5.40 ng/ul) than negative samples (mean=5.36 ng/ul) (p=0.977). Thus negative results are unlikely to be a result of low DNA quantity. There was no difference in DNA concentration between body sites (p=0.531). Individual swab samples are listed in Appendix 3.1 Table 2.

DISCUSSION

Ophidiomycosis has potentially serious consequences for the success of snake conservation efforts in Illinois. We detected *Ophidiomyces* DNA in all four species sampled. While ophidiomycosis is associated almost exclusively, to date, with skin lesions, colubrid infections may be associated with more systemic infection. Clinical signs of skin lesions were detected in all species sampled, except fox snakes. qPCR detection has historically been difficult in *Thamnophis* sp. due to low DNA yield from skin swabs. However, DNA yield from fox snakes is usually good and there has been low (anecdotal) false negative occurrence in this species. Thus, it is likely that the skin lesions observed in fox snakes were caused by another pathogen or trauma. For comparison, in indigo snakes, the negative predictive value of skin lesions in identifying apparent ophidiomycosis was 100%, thus giving biologists and veterinarians confidence that animals without skin lesions will test negative for *Ophidiomyces* on qPCR. Conversely, the positive predictive value in this species is poor, making it difficult to use clinical signs to confirm a diagnosis of apparent ophidiomycosis. Previous literature that showed that the rate of false negatives is nearly 10 times higher in animals without lesions than individuals with lesions (Hileman et al., 2017). The current recommendation for sampling to reduce the false negative rate is to repeatedly (8 times) and firmly swab along the entire surface of the skin.

Although the true course of natural disease is unknown in these species, the ability to follow positive snakes over time may allow for a better assessment of mortality rates and impacts on populations. A study in experimentally challenged cottonmouths (*Agkistrodon piscivorus*) demonstrated that, following challenge with *Ophidiomyces,* clinical signs did not present until 1-2 months after exposure (Allender et al., 2015b). Furthermore, clinical signs in most snakes resolved prior to death or euthanasia (Allender et al., 2015b), which further emphasizes the need to test and confirm the outcome of infected snakes.

SFD epidemiologic investigations have required a collaborative effort between biologists, veterinarians, and land managers and have produced a great deal of data about this distribution of this disease. However, it is not the only conservation threat to snakes, and may not even be the only disease facing Arizona snakes. At a time when wildlife diseases are increasingly more important for wildlife populations and public health and wildlife commonly serve as reservoirs for a wide variety of diseases, the need to detect early, or, ideally, to prevent the next disease event, has never been greater.. Future health assessments, pathogen detection, and assessment of contaminant exposure in these indigo snake populations may allow us to identify trends and new threats to both this species and other wildlife species.

Future Recommendations

- Compare prevalence across locations and demographics, i.e. are juveniles more susceptible to disease, specifically the smooth green snake.
- Identify landscape factors, possibly using GIS soil types to determine areas where high ophidiomycosis is likely to occur
- Determine the conservation impact of infection in these species, through things like survival analysis and fecundity.

Appendix 3.1 Table 1. Ophidiomycosis status of snake in Northern Illinois from 2019. Ophidiomycosis is classified as negative (free of clinical signs and qPCR negative), present (free of clinical signs but qPCR positive), possible (clinical signs present but qPCR negative), and apparent (clinical signs present and qPCR positive). Parentheses for prevalence is the 95% confidence interval.

		Ophidiomycosis class			
Species		Negative	Present	Possible	Apparent
Thamnophis sirtalis	n	23	6	1	0
	Prevalence (%)	76.7 (57.7-90.1)	20.0 (7.7-38.6)	3.3 (0.1-17.2)	0 (0-11.6)
Opheodrys vernalis	n	31	0	3	4
	Prevalence (%)	81.6 (65.7-92.3)	0 (0-9.3)	7.9 (1.7-21.4)	10.5 (2.9-24.8)
Pantherophis vulpinus	n	0	0	1	0
	Prevalence (%)	0 (0-97.5)	0 (0-97.5)	100 (2.5-100)	0 (0-97.5)
Heterodon nasicus	n	0	0	0	4
	Prevalence (%)	0 (0-60.2)	0 (0-60.2)	0 (0-60.2)	100 (39.8-100)

Appendix 3.1 Table 2. *Ophidiomyces* qPCR results from individual swab samples from snakes in Ogle and Lee counties, IL in 2019.

Cap	Species	ID	Date	Swab Location	**DNA concentration (ng/mL)**	**Result**	**Fungal_copies/ng DNA**
1	2	299	6-May-19	Body	4.2	0	0.00
2	2	284	29-Apr-19	Body	4.28	0	0.00
3	2	286	29-Apr-19	Body	3.96	0	0.00
4	2	131	24-Apr-19	Body	3.53	0	0.00
5	2	325	20-May-19	Body	3.28	0	0.00
6	2	291	1-May-19	Body	3.87	0	0.00
7	2	316	14-May-19	Body	2.9	0	0.00
8	2	323/4	29-May-19	Body	2.69	0	0.00
9	2	339	6-Jul-19	Body	5.6	0	0.00
10	2	336	16-Jun-19	Body	3.03	0	0.00
11	2	336*	22-Jun-19	Body	3.07	0	0.00
12	2	273	24-Apr-19	Face	2	0	0.00
13	2	328	8-Jun-19	Body	3.58	0	0.00
14	2	312	7-May-19	Body	2.74	0	0.00
15	2	292	3-May-19	Body	3.53	0	0.00
16	2	289	1-May-19	Body	3.12	0	0.00
17	2	313	13-May-19	Body	2.51	0	0.00
18	2	275	24-Apr-19	Body	3.51	0	0.00
19	2	277	26-Apr-19	Body	2.47	0	0.00
20	2	315	14-May-19	Body	2.47	0	0.00
21	2	285	29-Apr-19	Body	2.69	0	0.00
22	2	298	6-May-19	Body	2.86	0	0.00
23	2	337	16-Jun-19	Body	3.85	0	0.00
24	2	338	6-Jul-19	Body	1.13	0	0.00
25	2	288	1-May-19	Body	1.95	0	0.00
26	2	274	24-Apr-19	Body	3.2	0	0.00
27	2	282	29-Apr-19	Body	3.93	0	0.00
28	2	279	26-Apr-19	Face	3.28	0	0.00
29	2	296	6-May-19	Body	3.36	0	0.00
30	2	283	29-Apr-19	Body	3.48	0	0.00
31	2	322	23-May-19	Body	2.85	0	0.00
32	2	293	3-May-19	Body	4.56	0	0.00
33	2	297	6-May-19	Body	2.7	0	0.00
34	2	281	29-Apr-19	Body	2.77	0	0.00
35	2	281	29-Apr-19	Face	2.1	0	0.00
36	2	314	13-May-19	Body	2.87	0	0.00
37	2	314	13-May-19	Face	6.09	1	7.13
38	2	327	20-May-19	Body	4.3	1	0.94

39	2	327	20-May-19	Face	3.2	1	14.79
40	2	329	8-Jun-19	Body	2.64	1	1.54
41	2	329	8-Jun-19	Face	4.26	0	0.00
42	2	333	9-Jun-19	Body	3.87	0	0.00
43	2	333	9-Jun-19	Lesion	7.94	1	0.73
44	2	332	9-Jun-19	Body	3.51	1	11.07
45	2	332	9-Jun-19	Lesion	6.18	0	0.00
46	1	462	20-May-19	Body	10.01	0	0.00
47	1		22-Jun-19	Body	6.37	0	0.00
48	1		29-May-19	Body	1.48	1	0.70
49	1		19-Jun-19	Body	4.37	0	0.00
50	1		14-Jun-19	Body	2.79	0	0.00
51	1	445	3-May-19	Body	12.78	0	0.00
52	1	1067	20-May-19	Body	20.27	0	0.00
53	1		22-Jun-19	Body	3.79	0	0.00
54	1		3-May-19	Body	6.83	0	0.00
55	1		31-May-19	Body	21.39	0	0.00
56	1		7-Jun-19	Body	10.1	0	0.00
57	1		7-May-19	Body	4.35	0	0.00
58	1		14-Jun-19	Body	6	0	0.00
59	1		6-Jul-19	Body	7.49	0	0.00
60	1		13-May-19	Body	2.81	0	0.00
61	1		7-May-19	Body	4.58	0	0.00
62	1		29-May-19	Body	3.02	1	2.88
63	1		29-May-19	Body	5.74	0	0.00
64	1		14-May-19	Body	3.5	1	3.22
65	1		22-Jun-19	Body	15.51	0	0.00
66	1		13-May-19	Body	7.49	0	0.00
67	1		8-Jul-19	Body	2.8	0	0.00
68	1		16-May-19	Body	5.36	0	0.00
69	1		20-May-19	Body	5.45	0	0.00
70	1		7-May-19	Body	3.64	0	0.00
71	1		22-Jun-19	Body	6.47	1	1.04
72	1		22-Jun-19	Body	7.23	0	0.00
73	1		26-Sep-18	Body	7.92	0	0.00
74	1		10-Aug-18	Body	22.75	1	0.59
75	1		21-Sep-18	Body	6.17	1	1.32
76	3	182	22-Jun-19	Body	29.79	0	0.00
77	4	127	23-May-19	Body	3.45	1	1.39
78	4	125	23-May-19	Body	3.22	1	4.17
79	4	126	23-May-19	Body	4.56	1	1.12
80	4	124	23-May-19	Body	4.12	1	2.27

Appendix 3.2. Health assessment in Blanding's turtles and Ornate Box Turtles to aid in conservation efforts in Lee and Ogle Counties, IL

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HEALTH ASSESSMENT IN BLANDING'S TURTLES AND ORNATE BOX TURTLES TO AID IN CONSERVATION EFFORTS IN LEE AND OGLE COUNTIES, IL

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26 January 2020

2

INTRODUCTION

Chelonians are one of the most highly endangered vertebrate taxa, with ninety-eight (29%) of the known species considered endangered or critically endangered. Conservation efforts in all chelonians have been multi-faceted and include translocation or captive re-introductions, however, little emphasis has been placed on the wildlife health implications during or following these efforts. Blanding's turtles (*Emydoidea blandingii*), an endangered species in Illinois, have experienced range-wide declines because of habitat loss, degradation, and fragmentation. Since Blanding's turtles are long-lived and do not become reproductively active until 11-20 years of age, adult survival is crucial to population success. However, conservation strategies that support all life stages are critical to a successful outcome. The ornate box turtle (Terrapene ornata) is also state-endangered and efforts to conserve this species has been pursued at numerous sites in Illinois. The presence of pathogens is a threat that will interfere with conservation and jeopardize the health of all individuals in the event of an outbreak. While these ongoing projects have made a positive impact on population sustainability, infectious disease threats have largely been ignored or under-studied. With increased awareness and expertise, the existing conservation program is primed to introduce pathogen surveillance and health assessment into Blanding's turtle management plans.

Techniques that address the void in baseline health require an approach that utilizes specific biomedical diagnostics. Hematologic, plasma biochemical, and pathogen prevalence data have been utilized as a means of determining the wellness of free-ranging reptile populations, but have not been critically evaluated. In mammals and birds, inflammatory responses observed on complete blood counts, elevated concentrations of kidney or liver enzymes, and/or presence of pathogens are fairly straightforward. Unfortunately, assessing health in reptiles is not well-defined and utilizing diagnostic assays designed for mammals often lead to difficulty in interpretation. In addition, the close tie of physiological responses and temperature displayed by ectotherms can complicate interpretations compared to endotherms. Baseline studies that establish the same rigor and criteria for interpretation are lacking in Illinois amphibian and reptile populations.

Infectious diseases, particularly those causing upper respiratory tract infections, have been identified as major sources of morbidity and mortality in chelonians. Ranavirus, *Mycoplasma*, and herpesvirus infections have been reported as emerging causes of upper respiratory tract infections. The clinical signs associated with these three infections overlap and appropriate laboratory testing is necessary to differentiate these pathogens, allowing targeted management decisions. Concurrent infections with more than one etiologic agent have also been identified, complicating interpretation of significance.

Habitat fragmentation and loss of genetic variation over time may increase susceptibility to infectious disease. These factors will play an increasingly important role in the future as results of population fragmentation become more apparent in this long-lived turtle species. Since herpesvirus and Mycoplasma infections may recrudesce in times of stress or concurrent disease, these pathogens provide suitable sentinels for population health. Conservation initiatives such as captive breeding and head-start programs may, therefore, risk introduction of infectious disease into otherwise naïve populations and result in potentially catastrophic morbidity and mortality.

For these reasons, it is imperative to evaluate the health of the free-ranging Blanding's turtle population and of turtles being released or re-introduced to these populations.

The goal of this project was to assess the health of the Blanding's and ornate box turtles through the generation of baseline disease prevalence data and hematologic health. This health monitoring aimed to establish criteria that can be integrated into future conservation assessments of wild and captive collections. Our specific objective was to use quantitative PCR through Fluidigm technology (multi-plex qPCR), test oral-cloacal swabs to determine prevalence of up to 16 pathogens.

MATERIALS AND METHODS

Study sites

Blanding's and Ornate Box turtles were sampled from sites across Lee and Ogle Counties, Illinois during May-September 2017-2019. In addition, Ornate Box turtles were sampled from a site in Sauk County, Wisconsin through collaboration with Dr. J. Kapfer (University of Wisconsin – Whitewater).

Capture Methods

Turtles were captured with the aid of radiotelemetry, in a trap, or incidentally by hand during fieldwork. The turtles captured via telemetry were adults being tracked as a part of the King's Lab Research Program at Northern Illinois University.

Physical examination and Sample collection

Each turtle was assigned a permanent ID and mass, sex, and age status was recorded. Straight carapace length (SCL), straight carapace height (SCH), straight carapace width (SCW), anterior plastron length (APL) and posterior plastron length (PPL) were measured. Physical examinations were performed, noting visual appearance of the eyes, nose, oral cavity, ears, legs, digits, shell, integument, and cloaca. Each Blanding's turtle was characterized as juvenile (<250 grams), sub-adult (250-750 grams), or adult (>750 grams). Sex was classified as male, female or unknown. A combined oral-cloacal swab was collected from each individual.

DNA extraction, pathogen amplification, and amplicon sequencing

DNA was extracted from oral/cloacal swabs using Qiagen DNA Blood mini kit (Qiagen, Valencia, CA), according to the manufacturer's protocol. DNA concentration and purity was assessed spectrophotometrically (NanoDrop 1000, Thermo Fisher Scientific, Waltham, MA, USA) and DNA samples were stored at -20ºC prior to quantitative polymerase chain reaction (qPCR). Quantitative PCR was performed in a multiplex format to evaluate 14 pathogens simultaneously in Blanding's turtles and 12 in ornate box turtles using published or in house primer-probe assays (Appendix 3.2 Table1). Initially, Specific Target Amplification was performed on each sample with pooled pathogen Taqman assays and preamp mastermix (Thermo-Fisher, Waltham, MA 02454 USA). Each reaction was performed under the following cycling program on an MJ Tetrad thermocycler: 95°C (10 min), 14 cycles of 95°C (15 sec) and 60°C (4 min). The qPCR assay was then performed in triplicate using 2.25 µl of amplified DNA from the first reaction on a Fluidigm 96.96 Gene Expression IFC and amplified on the Fluidigm Biomark HD Real Time PCR thermacycler (Fluidigm, South San Francisco, CA 94080 USA) using the following cycling protocol: 70°C (30 min), 25°C (10 min), 95°C (1 min), followed by 35 cycles at 96°C (5 sec) and 60°C (20 sec). Serial dilutions of positive controls for FV3-like ranavirus, *Emydoidea* herpesvirus 1, box turtle *Mycoplasma*, *Mycoplasma agassizii*, and *Mycoplasma testudineum* were prepared from 10^7 to 10^1 copies per reaction. A non-template control was included on each plate. All reactions were then analyzed using Fluidigm Real Time PCR analysis software (Fluidigm, South San Francisco, CA 94080 USA). Positive samples on the Fluidigm were confirmed using a simplex qPCR reaction using the same primers on a QuantStudio 3 (Life technologies) and similar standard curve parameters.

Statistical analysis

Pathogen Surveillance. The binomial qPCR detection status (positive or negative) and 95% confidence intervals (CI) were calculated for each pathogen.

RESULTS

Sampling effort

There were 15 total samples collected from Blanding's turtles and 21 samples from ornate box turtles (Appendix 3.2 Appendix). Of the Blanding's, eight turtles were sampled at Nachusa, three from Richardson Wildlife Foundation, two from Green River, and two from Sand prairie state wildlife habitat. Ten ornate box turtles were sampled from Green River, seven from Spring Green Preserve (Wisconsin), two from Richardson Wildlife Foundation, and one each from Hahnaman Sand Prairie and McCune Sand Prairie Land and Water Reserve.

Pathogen surveillance

Blanding's turtles. Pathogens were detected unequally in turtles at each site. The most common pathogen detected was *Emydoidea* herpesvirus 1 which occurred in 3 of the 15 individuals for an individual prevalence of 15.0% (95% CI: 4.3-48.1%; Appendix 3.2 Table 2). All of the positive individuals occurred at Nachusa for a site prevalence of 42.9% (95% CI: 9.9-81.6%) (Appendix 3.2 Table 2). All of the samples from positive turtles were taken in May. Quantity of pathogens varied, with turtle 1R9R from Nachusa having the highest copy number, and interestingly was sampled in 2018 and observed to be negative. No other pathogens were detected at Nachusa or any other site.

Ornate box turtles. Pathogens were detected unequally in turtles at each site. There were only two detections, both of *Terrapene* adenovirus (Appendix 3.2 Table 3). Interestingly, these samples came from the only individuals caught at Hahnaman Sand Prairie and McCune Sand Prairie Land and Water Reserve.

DISCUSSION

Chelonian Pathogen Survey. We set out to describe the pathogen shedding of populations of Blanding's turtles and ornate box turtles in Lee and Ogle counties. Pathogen studies have been performed in Blanding's in the Chicagoland region for several years and in ornate box turtles at Nachusa for 3 years. The objective of this surveillance was to establish a baseline investigation to provide comparison for the overall health in animals in Illinois. *Emydoidea* herpesvirus 1 was detected in Nachusa turtles only. This is the most common pathogen detected in other populations as well (Lindemann et al., 2019). In other counties, most *Emydoidea* herpesvirus infections occur in May and therefore it seems that across northern Illinois this seasonal pattern holds true. It will be interesting to see if this also occurs in other states or regions. Terrapene adenovirus was the only pathogen detected in OBTs and interestingly found in the only individuals at two sites. This may represent that these populations are under higher environmental and pathogen stress, thus threatening conservation efforts at these sites. Alternatively, little is known about adenovirus in OBT and future efforts should determine the role this pathogen has on disease signs and potential for mortality.

Presence of pathogens in populations of Blanding's and OBTs is an important finding as each of the pathogens surveyed have been associated with morbidity and mortality in species within the order Testudinidae. Herpesvirus has been associated with mortalities in tortoises (Drury et al. 1998; Herva´s et al. 2002; Jungwirth et al. 2014) and recently a northern map turtle who was found dead after exhibiting weakness and nasal discharge (Ossiboff et al. 2015a). *Mycoplasma agassizii* has been identified as a causative agent in URT disease in gopher tortoises and desert tortoises (Brown et al. 1994; Brown et al. 1999) and *Mycoplasma testudineum* has been associated with URT disease in desert tortoises (Brown et al. 2004). URT diseases have been associated with major losses in populations of these species (Rosskopf et al. 1981; Jacobson et al. 1991). These pathogens are proposed to have significant influences on population health in those species. In box turtles monitored during a ranavirus outbreak, individuals that were shedding herpesvirus and *Mycoplasma* had a lower mortality than those with ranavirus alone (Sim et al. 2016) this may reflect that herpesvirus is not associated with disease, but rather associated with a health benefit. Future studies should continue to investigate these factors.

Testing for these pathogens is important for establishing a baseline of prevalence. To determine how environmental factors might affect pathogen load in this species, it would be useful to measure trends over time within this population. Management practices can also be evaluated through pathogen trends as comparing pathogen prevalence in unequally managed sites or over time as management practices change might reflect the ability of these differences to improve population health. Stress from prey and mate competition can increase when home ranges are smaller than ideal (Pough, 1998) which can lead to immunocompromise and increased susceptibility to infection illustrating how management practices can affect these variables.

Future Recommendations

This year identified infrequent pathogens. Future investigations should build on this database to parse out differences in demographic factors and expand the investigation to serially sampling over the season. Thus, the following should be priorities:

- A larger focus on environmental factors (specifically those related to management – aka raccoon abundance, headstarts) and how they correlate with health findings to possibly provide direction to future management
- Multiple samples per individual (up to 4 samples per turtle/year)
- Multiple pathogen surveillance to expand to include RNA pathogens
- Clinical pathological responses (bloodwork)
- Stress response (cortisol) to capture and differences in habitat
- Contamination or toxicologic exposure
- Spatial mapping of pathogens/host response
- Movement of turtles with pathogens
- Impact of pathogens on fitness (number of eggs in females and survival)

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Appendix 3.2 Table 1. Pathogens/co-pathogens tested for in Blanding's turtles and ornate box turtles using PCR or qPCR primers.

Pathogen	Blanding's	Ornate	Source
FV3 - ranavirus	X	X	Allender et al., 2013
Ambystoma tigrinum virus - ranavirus	X	X	Pallister et al., 2007
Bohle iridovirus - ranavirus	X	X	Pallister et al., 2007
Epizootic hemorrhagic necrosis virus - ranavirus	X	X	Pallister et al., 2007
Mycoplasma agassizii	X	X	Braun et al., 2014
Mycoplasma testudineum	X	X	Braun et al., 2014
Emydid *Mycoplasma*	X	X	Internal
Salmonella tymphimurium	X	X	Park et al., 2008
Salmonella enteritidis	X	X	Levin, 2009
Tortoise intranuclear coccidia	X		Alvarez et al., 2013
Emydoidea herpesvirus 1	X		Lindemann et al., 2018
Emydid herpesvirus 1	X		Internal
Emydomyces testovorans	X		Internal
Testudinid herpesvirus 2	X		Braun et al., 2014
Terrapene adenovirus		X	Franzen-Klein et al., in press
Terrapene herpesvirus 1		X	Kane et al., 2017
Terrapene herpesvirus 2		X	Internal

Appendix 3.2 Table 2a. Pathogen surveillance results for Blanding's turtles in Lee and Ogle counties, IL in 2019. Richardson Wildlife Foundation = RWF, Sand prairie state habitat = SPSH

Sample ID	Turtle ID	Location	Ranaviruses				Herpesviruses				Mycoplasmas	
			Ambystoma tigrinum Virus	Bohle Iridovirus	Epizootic Hematopoietic Necrosis Virus	Frog Virus 3	Emydoidea Herpesvirus 1	Emydid Herpesvirus 1	Testudinid Herpesvirus 2	Emydid *Mycoplasma* sp.	*Mycoplasma agassizii*	*Mycoplasma testudineum*
2	L2	RWF	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5	L1R2	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
8	1R9R	Nachusa	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
14	L1R1	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
35	2L3R	Nachusa	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
36	1L8R	Nachusa	Neg	Neg	Neg	Neg	Positive	Neg	Neg	Neg	Neg	Neg
37	1L2R	Nachusa	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
38	1L9R	Nachusa	Neg	Neg	Neg	Neg	Positive	Neg	Neg	Neg	Neg	Neg
39	1R9R	Nachusa	Neg	Neg	Neg	Neg	Positive	Neg	Neg	Neg	Neg	Neg
40	1R8R	Nachusa	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
41	L1R1	SPSH	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
42	L2R2	SPSH	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
50	1L11R	Nachusa	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
51	R2R3	RWF	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
52	L8L9	RWF	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Appendix 3.2 Table 2b. Pathogen surveillance results for Blanding's turtles in Lee and Ogle counties, IL in 2019. Richardson Wildlife Foundation = RWF, Sand prairie state habitat = SPSH

Sample ID	**Turtle ID**	**Location**	Salmonella		**Intranuclear coccidiosis**	***Emydomyces testavorans***
			Salmonella typhimurium	***Salmonella enteritidis***		
2	L2	RWF	Neg	Neg	Neg	Neg
5	L1R2	Green River	Neg	Neg	Neg	Neg
8	1R9R		Neg	Neg	Neg	Neg
14	L1R1	Green River	Neg	Neg	Neg	Neg
35	2L3R	Nachusa	Neg	Neg	Neg	Neg
36	1L8R	Nachusa	Neg	Neg	Neg	Neg
37	1L2R	Nachusa	Neg	Neg	Neg	Neg
38	1L9R	Nachusa	Neg	Neg	Neg	Neg
39	1R9R	Nachusa	Neg	Neg	Neg	Neg
40	1R8R	Nachusa	Neg	Neg	Neg	Neg
41	L1R1	SPSH	Neg	Neg	Neg	Neg
42	L2R2	SPSH	Neg	Neg	Neg	Neg
50	1L11R	Nachusa	Neg	Neg	Neg	Neg
51	R2R3	RWF	Neg	Neg	Neg	Neg
52	L8L9	RWF	Neg	Neg	Neg	Neg

Appendix 3.2 Table 3a. Pathogen surveillance results for ornate box turtles in Lee and Ogle counties, IL in 2019. Richardson Wildlife Foundation = RWF, Spring Green Preserve State Natural Area = SG, Hahnaman sand prairie nature preserve = HSNP, McCune Sand prairie land and water reserve = MSP.

			Ranaviruses				**Mycoplasmas**			**Herpesviruses**	
Sample ID	**Turtle ID**	**Location**	***Ambystoma tigrinum* Virus**	**Bohle Iridovirus**	**Epizootic Hematopoietic Necrosis Virus**	**Frog Virus 3**	**Emydid *Mycoplasma* sp.**	***Mycoplasma agassizii***	***Mycoplasma testudineum***	**Terrapene Herpesvirus 1**	**Terrapene Herpesvirus 2**
15	L1R1	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
20	L12	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
25	R2	HSNP	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
26	2	SG	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
27	6	SG	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
28	410	SG	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
30	1	SG	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
31	231	SG	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
32	431	SG	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
33	211	SG	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
34	R2	MSP	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
43	L1R3	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
44	L11	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
45	L10	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
46	L2	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
47	L1R9	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
48	L9	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
49	L1R2	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
53	L2	RWF	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
54	L1R10	Green River	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
55	R1	RWF	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Appendix 3.2 Table 3b. Pathogen surveillance results for ornate box turtles in Lee and Ogle counties, IL in 2019. Richardson Wildlife Foundation = RWF, Spring green Preserve State Natural Area = SG, Hahnaman sand prairie nature preserve = HSNP, McCune Sand prairie land and water reserve = MSP.

Sample ID	Turtle ID	Location	Terrapene Adenovirus	*Salmonella typhimurium*	*Salmonella enteritidis*	Intranuclear coccidiosis
15	L1R1	Green River	Neg	Neg	Neg	Neg
20	L12	Green River	Neg	Neg	Neg	Neg
25	R2	HSNP	Positive	Neg	Neg	Neg
26	2	SG	Neg	Neg	Neg	Neg
27	6	SG	Neg	Neg	Neg	Neg
28	410	SG	Neg	Neg	Neg	Neg
30	1	SG	Neg	Neg	Neg	Neg
31	231	SG	Neg	Neg	Neg	Neg
32	431	SG	Neg	Neg	Neg	Neg
33	211	SG	Neg	Neg	Neg	Neg
34	R2	MSP	Positive	Neg	Neg	Neg
43	L1R3	Green River	Neg	Neg	Neg	Neg
44	L11	Green River	Neg	Neg	Neg	Neg
45	L10	Green River	Neg	Neg	Neg	Neg
46	L2	Green River	Neg	Neg	Neg	Neg
47	L1R9	Green River	Neg	Neg	Neg	Neg
48	L9	Green River	Neg	Neg	Neg	Neg
49	L1R2	Green River	Neg	Neg	Neg	Neg
53	L2	RWF	Neg	Neg	Neg	Neg
54	L1R10	Green River	Neg	Neg	Neg	Neg
55	R1	RWF	Neg	Neg	Neg	Neg

Appendix 3.2 Appendix. Sample collection dates and locations.

Sample ID	Species	Turtle ID	Date	State	County	Location
2	Emydoidea blandingii	L2	2-Jul-18	IL	Lee	Richardson Wildlife Foundation
5	Emydoidea blandingii	L1R2	25-May-18	IL	Lee	Green River
8	Emydoidea blandingii	1R9R	24-Jul-18	IL	Lee	Nachusa Grasslands
14	Emydoidea blandingii	L1R1	2017	IL	Lee	Green River
35	Emydoidea blandingii	2L3R	9-May-19	IL	Ogle	Nachusa Grasslands
36	Emydoidea blandingii	1L8R	14-May-19	IL	Lee	Nachusa Grasslands
37	Emydoidea blandingii	1L2R	14-May-19	IL	Lee	Nachusa Grasslands
38	Emydoidea blandingii	1L9R	16-May-19	IL	Lee	Nachusa Grasslands
39	Emydoidea blandingii	1R9R	16-May-19	IL	Lee	Nachusa Grasslands
40	Emydoidea blandingii	1R8R	22-May-19	IL	Lee	Nachusa Grasslands
41	Emydoidea blandingii	L1R1	26-Jun-19	IL	Lee	Sand Prairie State Habitat Area
42	Emydoidea blandingii	L2R2	7-Jul-19	IL	Lee	Sand Prairie State Habitat Area
50	Emydoidea blandingii	1L11R	25-Jul-19	IL	Lee	Nachusa Grasslands
51	Emydoidea blandingii	R2R3	22-Jul-19	IL	Lee	Richardson Wildlife Foundation
52	Emydoidea blandingii	L8L9	22-Jul-19	IL	Lee	Richardson Wildlife Foundation
15	Terrapene ornata	L1R1	23-Jun-17	IL	Lee	Green River
20	Terrapene ornata	L12	23-May-18	IL	Lee	Green River
25	Terrapene ornata	R2	26-May-18	IL	Whiteside	Hahnaman Sand Prairie Nature Preserve
26	Terrapene ornata	2	8-Aug-18	WI	Sauk	Spring Green Preserve State Natural Area
27	Terrapene ornata	6	8-Aug-18	WI	Sauk	Spring Green Preserve State Natural Area
28	Terrapene ornata	410	8-Aug-18	WI	Sauk	Spring Green Preserve State Natural Area
30	Terrapene ornata	1	8-Aug-18	WI	Sauk	Spring Green Preserve State Natural Area
31	Terrapene ornata	231	8-Aug-18	WI	Sauk	Spring Green Preserve State Natural Area
32	Terrapene ornata	431	8-Aug-18	WI	Sauk	Spring Green Preserve State Natural Area
33	Terrapene ornata	211	8-Aug-18	WI	Sauk	Spring Green Preserve State Natural Area
34	Terrapene ornata	R2	29-May-19	IL	Bureau	McCune Sand Prairie Land and Water Reserve

43	Terrapene ornata	L1R3	28-Jun-19	IL	Lee	Green River
44	Terrapene ornata	L11	28-Jun-19	IL	Lee	Green River
45	Terrapene ornata	L10	28-Jun-19	IL	Lee	Green River
46	Terrapene ornata	L2	28-Jun-19	IL	Lee	Green River
47	Terrapene ornata	L1R9	23-Jun-19	IL	Lee	Green River
48	Terrapene ornata	L9	28-Jun-19	IL	Lee	Green River
49	Terrapene ornata	L1R2	28-Jun-19	IL	Lee	Green River
53	Terrapene ornata	L2	22-Jul-19	IL	Lee	Richardson Wildlife Foundation
54	Terrapene ornata	L1R10	31-Jul-19	IL	Lee	Green River
55	Terrapene ornata	R1	22-Jul-19	IL	Lee	Richardson Wildlife Foundation

Part 4. Size, condition, and growth of emblematic snake species within the Green River COA

Body size, condition (size-corrected mass), and growth provide indirect measures of animal health. Because snakes continue to grow throughout their lives, body size reflects the combined effects of growth and survival: where resources for growth are abundant and survival is high, body size should be greater than where resources are scarce or survival is low. Thus, variation in body size among habitats or sites may reflect underling differences in demography. Like growth, snake body condition is associated with habitat quality (e.g., fire suppression, land use practice; Beaupre and Douglas 2009; Lomas et al. 2015; Wittenberg and Beaupre 2014), an observation that has led to a new management and restoration plans to improve ecosystem quality for wildlife (Beaupre and Douglas 2009). Growth among the youngest age classes is of particular interest because in many reptiles, the onset of reproductive maturity is determined by size rather than age. Consequently, rapid growth early in life may promote population growth by shortening generation time and increasing the probability of survival to reproduction. By plotting snout-vent length vs. day of capture, growth trajectories of neonatal snakes can be inferred using linear regression analysis. Importantly, such trajectories differ among sites, demonstrating their potential utility in demonstrating effects of habitat type, quality, and management history on animal health.

4.1 Body Size

Effects of INAI status on snake body size at Green River SWA. – Snakes living in higher quality habitats might grow more rapidly or live longer and thus achieve greater adult size than snakes living in lower quality habitats. To test this, the body size distributions of adult snakes captured within INAI boundaries was comparted to that outside INAI boundaries using Kolmogorov–Smirnov tests. Because males and females achieve different body size, sexes were analyzed separately. To exclude neonatal and juvenile snakes, only snakes ≥ 250 mm SVL (Smooth Greensnakes), 200 mm (Dekay's Brownsnakes), and 400 mm SVL (Plains Gartersnakes, Common Gartersnakes) were included. In no cases did size distributions differ between snakes captured within vs. outside INAI boundaries (Table 4.1).

Variation in snake body size among preserves in Northern Illinois. – Long-term capture-mark-recapture studies of snakes at Nachusa Grasslands (Lee County), Goose Lake Prairie (Grundy County), and Potawatomi Woods Forest Preserve (DeKalb County) allows for comparison of snake body size between preserves within the Green River COA and elsewhere (see King and Sacerdote 2015, Virgin and King 2019, and King and Vanek 2020 for further information on data collection at Nachusa Grasslands, Goose Lake Prairie, and Potawatomi Woods). As above, recaptures were excluded and comparisons were restricted to snakes ≥ 200 mm (Dekay's Brownsnakes), 400 mm (Plains Gartersnakes, Common Gartersnakes) and 500 mm SVL (Eastern Foxsnakes). Due to sexual dimorphism in size, sexes were analyzed separately for all species except Eastern Foxsnakes. Size distributions were compared using Kruskal-Wallace tests. Body size of Dekay's Brownsnakes (males only) and Common Gartersnakes (both sexes) differed significantly among sites (Table 4.2, Fig. 4.1). Dekay's Brownsnakes were significantly longer at Nachusa Grasslands than at Green River SWA; Common Gartersnakes were significantly longer at Goose Lake than at Green River, Nachusa, or Richardson.

4.2 Body Condition

Effects of fire and INAI status on snake body condition at Green River SWA. – Body condition can be an indicator of overall health and so may vary with habitat quality and management activities. At Green River SWA, prescribed fire is used widely with burns conducted on a ca. 3 year interval. Additionally, portions of Green River SWA are recognized as Category I or II INAI sites based on vegetation characteristics and may represent higher quality habitat than areas outside INAI boundaries.

To assess potential effects of fire and INAI status on snake body condition within the Green River SWA, residuals from the regression of mass on length were computed, thus yielding a size-independent measure of mass (snakes that are heavier than expected given their length have positive residuals (better condition) than snakes that are lighter than expected given their length (negative residuals, poorer condition).

Recaptures were omitted from analysis. Because the relationship between length and mass is curvilinear, data were transformed using natural logarithms (lnSVL = ln(SVL+1) and lnMass = ln(Mass + 1)). lnMass was regressed on lnSVL separately for males and females because length-mass relationships differ between the sexes. Analysis of variance was used to test the main effects of fire status, INAI status, and year on condition separately for Smooth Greensnakes, Dekay's Brownsnakes, Plains Gartersnakes, and Common Gartersnakes (sample sizes in Table 4.3).

Results were consistently non-significant except for a difference in Common Gartersnake condition between INAI and non-INAI areas (P = 0.050; Table 4.4). However, the magnitude of this difference was small; mean condition = -0.016 (CI = -0.036-0.004) for non-INAI and 0.011 (=0.007-0.029) for INAI captures.

Variation in snake body condition among preserves in Northern Illinois. – Sample sizes were sufficient to compare condition of Eastern Foxsnakes, Dekay's Brownsnakes, Plans Gartersnakes, and Common Gartersnakes among two or more preserves (Table 4.5). Significant differences were seen in all four species (Table 4.5, Fig. 4.2). Body condition of snakes at Green River tended to be higher than at least some other preserves (Fig. 4.2).

4.3 Growth

Effects of fire and INAI status on neonatal growth at Green River SWA. – Neonatal temperate-zone snakes are born (live-bearing species) or hatch (egg-laying species) in a pulse in late summer and can be identified as a distinct age class from plots of SVL vs. day of year (DOY; Figure 4.3). Many species exhibit rapid growth prior to entering hibernation for the first time and growth can be characterized by regression analysis (Fig. 4.4). Year-to-year variation in weather potentially influences growth functions through effects on date of birth and on growth rate (Fig. 4.4). Consequently, for two species with sufficient sample sizes (Dekay's Brownsnakes, Common Gartersnakes), SVL was regressed on DOY separately for each of two years. Residuals from these regressions were then analyzed using Anova to test for effects of fire and INAI status and their interaction.

Analysis of variance revealed that there was a significant INAI-by-fire interaction and main effect of fire on residual SVL of neonatal Common Gartersnakes (Table 4.6, Fig. 4.5). There were no significant main effects or INAI-by-fire interaction on residual SVL of neonatal Dekay's Brownsnakes (Table 4.6) but the directionality matched that observed in Common Gartersnakes (Fig. 4.5).

4.4 Conclusions

Effects of fire and INAI status on snake body size and condition tended to be small and generally non-significant. However, both body size and condition varied among Northern Illinois preserves. Possibly, this variation reflects differences in resource availability or survival. There was an interactive effect of fire and INAI status on neonate growth (as reflected in the residual of SVL following regression on day-of-year) in Common Gartersnakes such that snakes grew more rapidly in burned sites outside INAI boundaries and more slowly within INAI boundaries (regardless of fire). Follow-up studies on the mechanisms underlying these patterns would be of interest.

4.5 Literature Cited

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Table 4.1. Results of Kolmogorov-Smirnov tests comparing body size distributions for snakes captured within vs. outside INAI boundaries at Green River SWA.

		Sample Size		
Species	Sex	Non-INAI	INAI	P
Smooth Greensnake	Female	32	14	0.366
	Male	19	22	0.970
Dekay's Brownsnake	Female	78	33	0.231
	Male	25	9	0.100
Plains Gartersnake	Female	45	8	0.683
	Male	10	7	0.480
Common Gartersnake	Female	80	121	0.816
	Male	51	29	0.656

Table 4.2. Results of Kruskal-Wallace tests comparing body size distributions of snakes among northern Illinois preserves. Shown is the median SVL (sample size) and associated test statistic, degrees of freedom, and P value for Eastern Foxsnakes (A), Dekay's Brownsnake (B), Plains Gartersnake (C), and Common Gartersnake (D). Significant P values are highlighted in bold.

	Preserve							
	Goose Lake	Green River	Nachusa	Potawatomi	RWF	Test Statisitic	df	P
A. Eastern Foxsnake								
	770.0	188.0	163.3		226.7			
	(16)	(25)	(59)		(18)	6.256	3	0.100
B. Dekay's Brownsnake								
male		228.5	246.0	240.0				
		(34)	(119)	(180)		11.484	2	**0.003**
female		275.0	273.3	282.0				
		(111)	(213)	(237)		1.340	2	0.512
C. Plains Gartersnake								
male		435.0	445.0					
		(17)	(25)			2.210	1	0.137
female		467.0	503.0					
		(53)	(31)			3.336	1	0.068
D. Common Gartersnake								
male	484.0	439.0	453.5	446.0	462.0			
	(71)	(80)	(108)	(133)	(55)	37.808	4	**<0.001**
female	567.5	490.0	510.0	483.5	522.0			
	(176)	(209)	(120)	(208)	(92)	125.716	4	**<0.001**

Table 4.3. Sample sizes included in analyses of year, fire status, and INAI status on snake body condition. Fire status = ‘no’ for areas not burned and ‘yes’ for captures in areas that were burned in fall or spring prior to field work. INAI status = ‘no’ for captures outside INAI boundaries and ‘yes’ for captures within INAI boundaries. Dekay’s Brownsnakes and Common Gartersnakes were not measured in 2019.

		Smooth Greensnake	Dekay’s Brownsnake	Plains Gartersnake	Common Gartersnake
Year	2017	48	151	58	259
	2018	57	68	31	322
	2019	61		57	
Fire	no	63	104	81	229
	yes	103	115	65	352
INAI	no	104	152	98	268
	yes	62	67	48	313
Total		166	219	148	581

Table 4.4. P-values associated with main effects of year, fire status, and INAI status on snake body condition. Significant results are shown in bold.

Source of Variation	Smooth Greensnake	Dekay's Brownsnake	Plains Gartersnake	Common Gartersnake
Year	0.636	0.065	0.624	0.186
Fire	0.765	0.185	0.435	0.867
INAI	0.185	0.922	0.708	**0.050**

Table 4.5. Results of analyses of variance comparing condition (residuals from regression of ln(mass) on ln(SVL) among Northern Illinois preserves. Shown is the mean residual (sample size) and associated Fstatistic, degrees of freedom, and P value for Eastern Foxsnakes (A), Dekay's Brownsnake (B), Plains Gartersnake (C), and Common Gartersnake (D). Significant P values are highlighted in bold.

	Preserve							
	Goose Lake	Green River	Nachusa	Potawatomi	RWF	F	df	P
A. Eastern Foxsnake								
	0.062	0.031	0.021		0.009			
	(26)	(44)	(149)		(25)	3.375	3,240	**0.019**
B. Dekay's Brownsnake								
male		0.077	-0.058	0.016				
		(88)	(265)	(547)		36.841	2,897	**<0.001**
female		0.082	-0.100	0.038				
		(198)	(461)	(802)		79.857	2,1458	**<0.001**
C. Plains Gartersnake								
male		0.005	-0.005					
		(71)	(54)			0.119	1,123	0.963
female		0.053	-0.079					
		(104)	(70)			20.077	1,173	**<0.001**
D. Common Gartersnake								
male	-0.065	0.058	-0.052	0.009	0.071			
	(135)	(283)	(509)	(1183)	(110)	38.188	4,2215	**<0.001**
female	0.021	0.074	-0.094	-0.001	0.063			
	(291)	(476)	(536)	(1280)	(141)	56.487	4,2719	**<0.001**

Table 4.6. P-values from analysis of variance of residual neonatal SVL following regression on day-of-year. Significant results are shown in bold.

Source of Variation	Dekay's Brownsnake	Common Gartersnake
Fire	0.111	**<0.001**
INAI	0.603	0.125
Fire-by- INAI	0.503	0.035

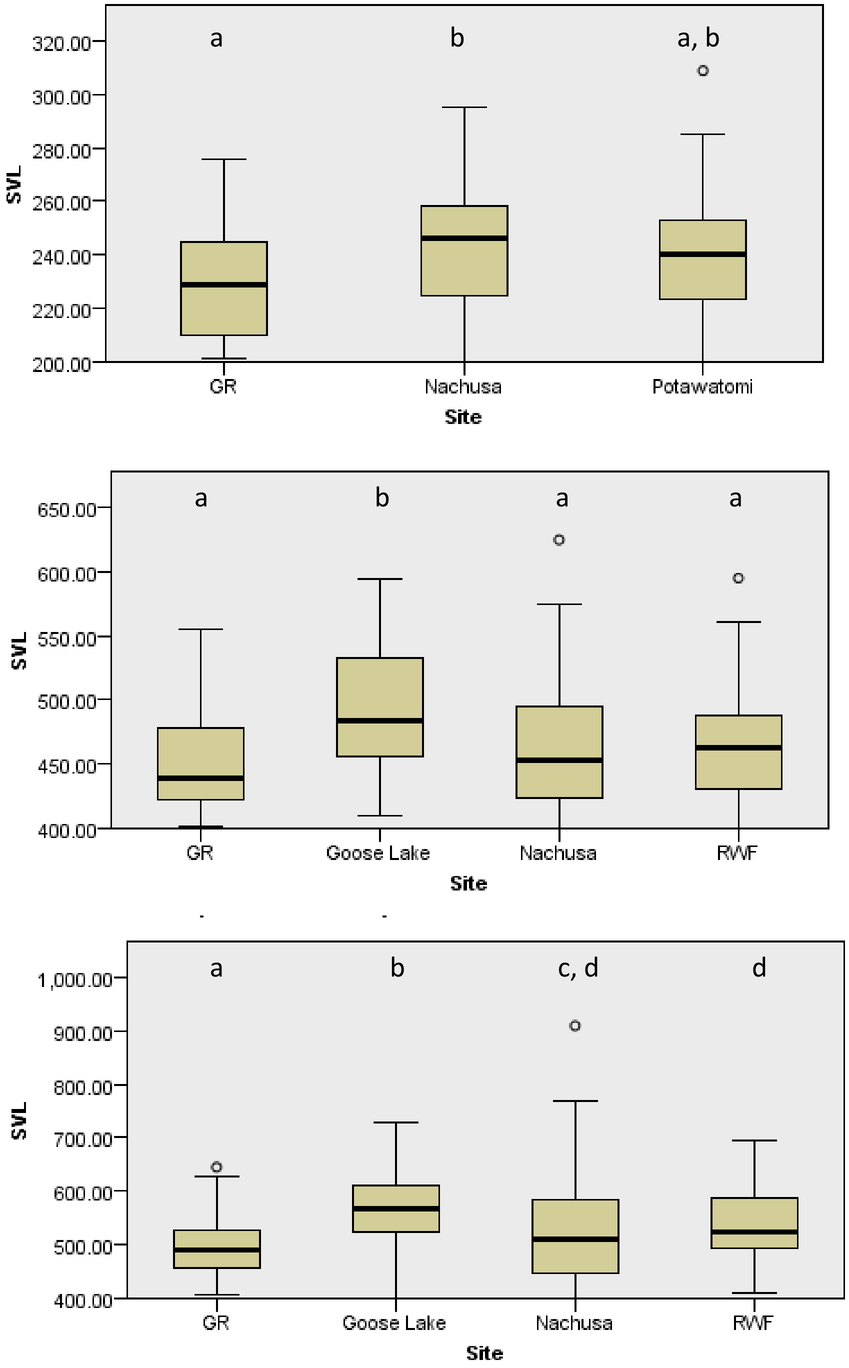

Figure 4.1. Box plots showing body size (SVL in mm) distributions for male Dekay's Brownsnakes (top panel), male Common Gartersnakes (middle panel) and female Common Gartersnakes (bottom panel). Lower case letters in each panel identify sites that do not differ significantly. Other comparisons were non-significant (Table 4.2). Dark bars represents median, boxes represent quartiles, whiskers represent range, open circles represent outliers.

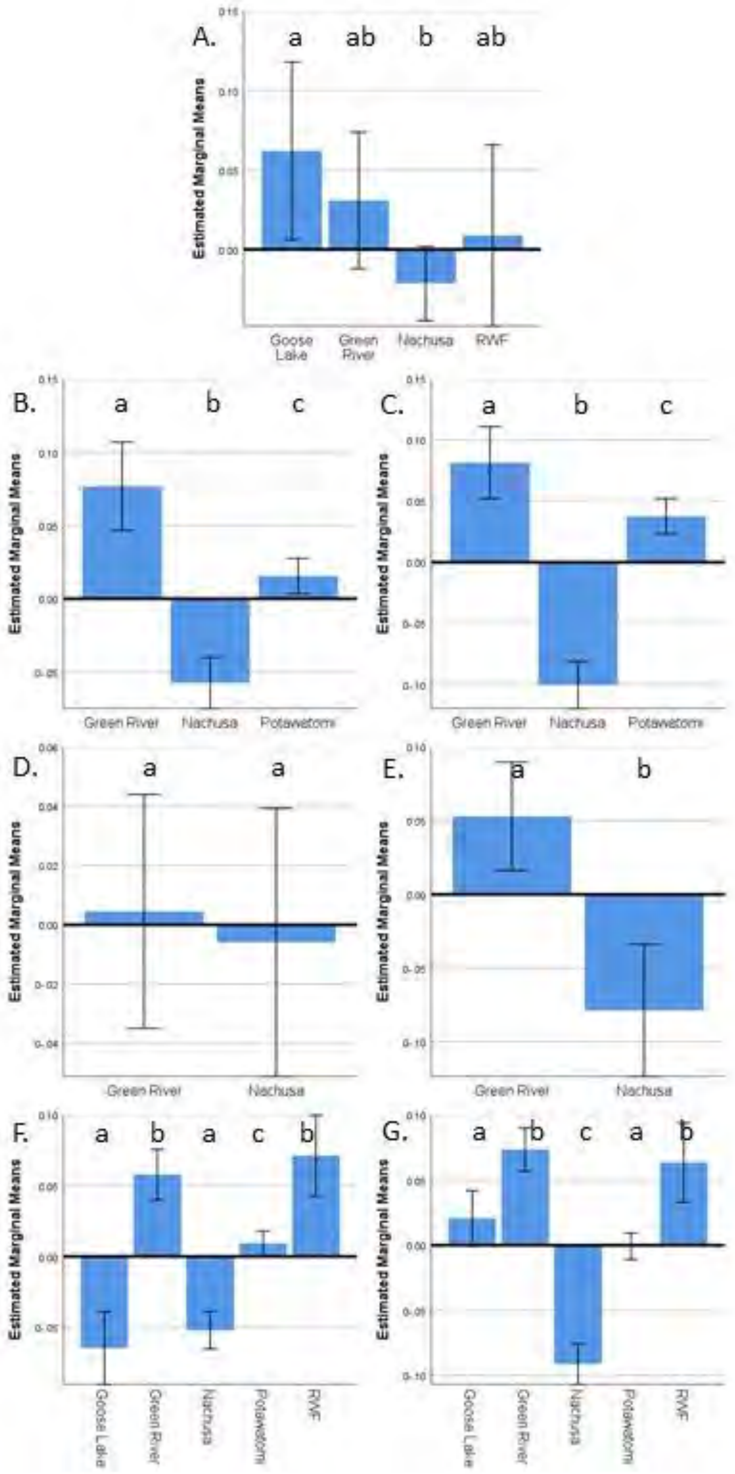

Figure 4.2. Variation in condition of Eastern Foxsnakes (A), Dekay's Brownsnakes (males – B, females – C), Plains Gartersnakes (males – D, females – E, and Common Gartersnakes (males – F, females – G) among Northern Illinois preserves. Bars represent residuals from regression of ln(Mass) on ln(SVL); whiskers represent 95% confidence intervals; lower case letters identify preserves that do not differ significantly.

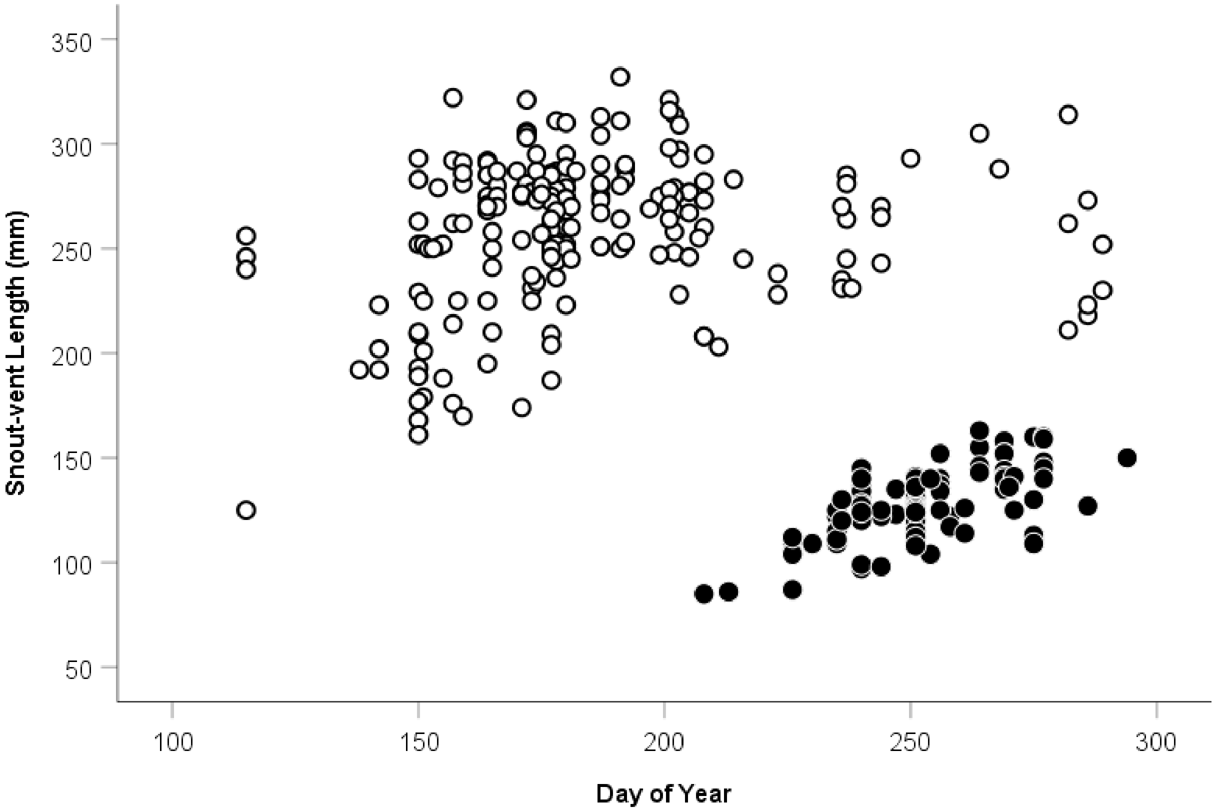

Figure 4.3. Relationship between day of year and snout-vent length of Dekay's Brownsnakes at Green River SWA. Neonates appear as a distinct age class (fill circles) starting around day 200.

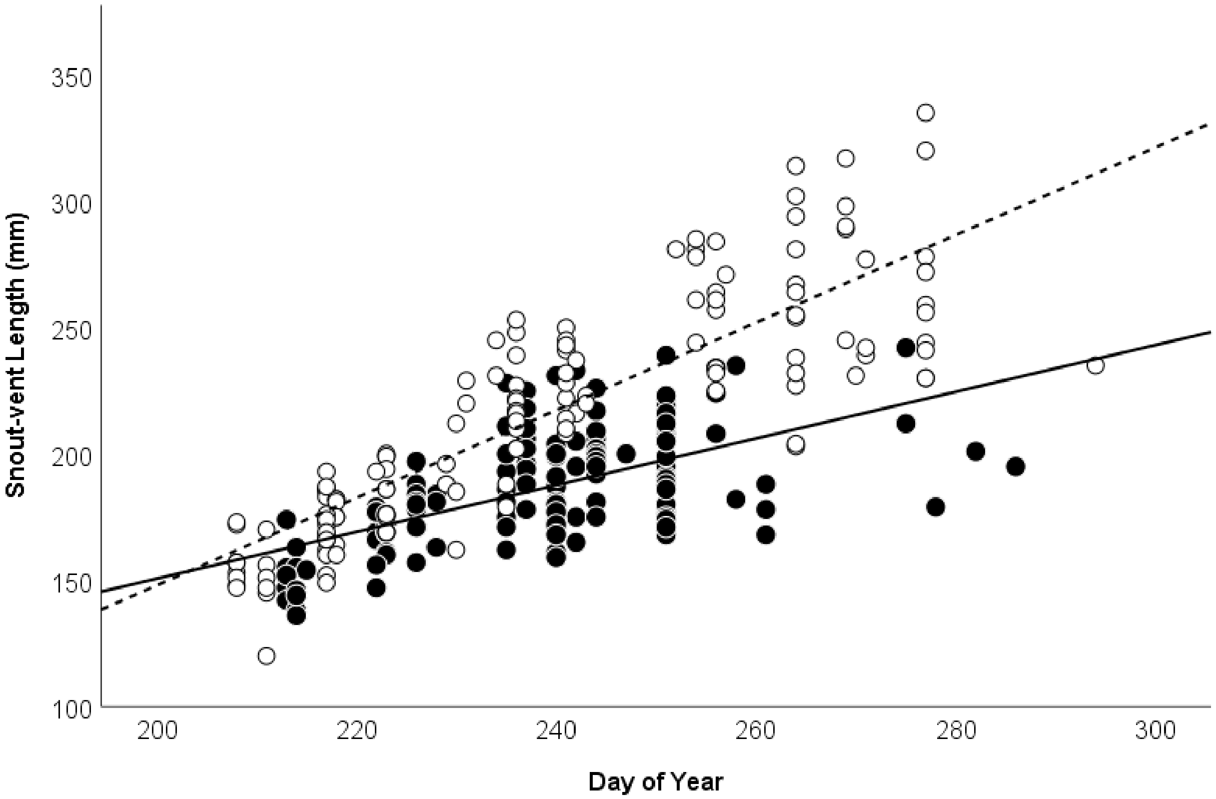

Figure 4.4. Relationship between day of year and snout-vent length of neonatal Common Gartersnakes at Green River SWA in 2017 (filled circles, solid line) and 2018 (open circles, dashed line).

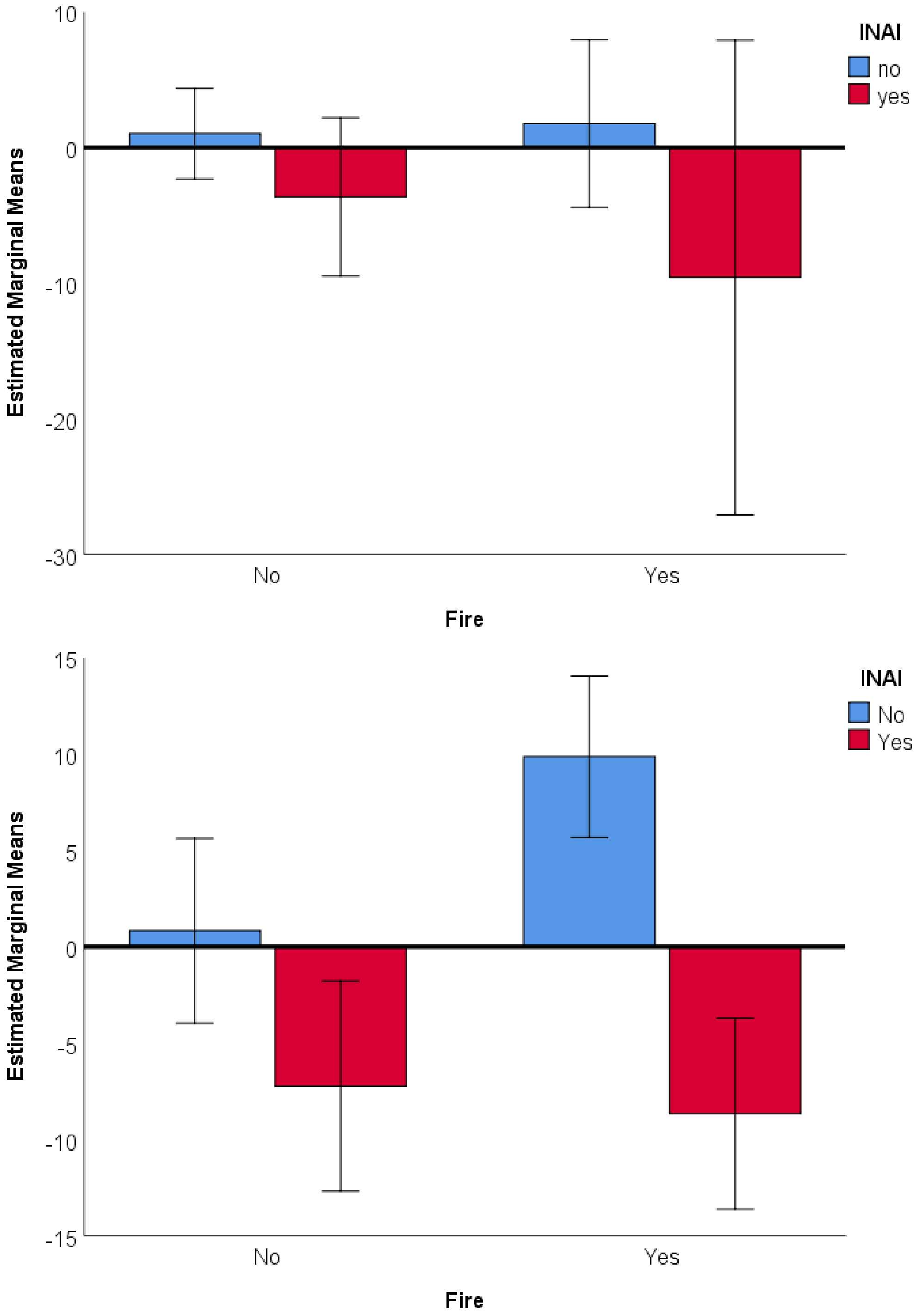

Figure 4.5. Profile plots showing the effects of Fire and INAI status on residual SVL following regression on day-of-year of neonatal Dekay’s Brownsnakes (upper panel) and Common Gartersnakes (lower panel). Error bars represent 95% confidence intervals. Scale of Y axis differs between panels.

Part 5. Blanding’s turtle population viability and sensitivity

Objective 6 of this project is to develop a baseline population viability analysis for Blanding’s turtle using new and existing data. Specific outcomes are to

a. Provide updated estimates of Blanding’s Turtle vital rates based on the Spring-Bluff – Chiwaukee Prairie population
b. Compile estimates of Blanding’s Turtle vital rates range-wide
c. Generate a baseline Illinois Blanding’s Turtle PVA
d. Explore PVA outcomes over a range of initial population sizes and degrees of connectivity
e. Conduct sensitivity analysis of model parameters

A manuscript addressing objective 6 was submitted to the Journal of Fish and Wildlife Management on 21 August 2020. Reviewers were generally positive about the manuscript but requested significant revisions. A revised version, submitted on 12 February 2021 and currently in review, provides information relating to outcomes a-e and is included in this report (Appendix 5.1). An abstract is provided below followed by additional information on PVA outcomes over a range of degrees of connectivity (outcome d).

5.1. Blanding’s Turtle Demography and Population Viability – Manuscript Abstract

In anticipation of US federal status classification (warranted, warranted but precluded, not warranted), scheduled for 2023, we provide population viability analysis of the Blanding’s turtle *Emydoidea blandingii*, a long-lived, late-maturing, semi-aquatic species of conservation concern throughout its range. We present demographic data from long-term study of a population in northeastern Illinois and use these data as the basis for viability and sensitivity analyses focused on parameter uncertainty and geographic parameter variation. We use population viability analysis to identify population sizes necessary to provide population resiliency to stochastic disturbance events and catastrophes and demonstrate how alternative definitions of ‘foreseeable future’ might affect status decisions. Demographic parameters within our focal population resulted in optimistic population projections (probability of extinction = 0% over 100 years) but results were less optimistic when catastrophes or uncertainty in parameter estimates were incorporated (probability of extinction = 3% and 16%, respectively). Uncertainty in estimates of age-specific mortality had the biggest impact on population viability analysis outcomes but uncertainty in other parameters (age of first reproduction, environmental variation in age-specific mortality, % females reproducing, clutch size) also contributed. Blanding’s turtle demography varies geographically and incorporating this variation resulted in both mortality- and fecundity-related parameters affecting population viability analysis outcomes. Possibly, compensatory variation among demographic parameters allows for persistence across a wide range of parameter values. We found that extinction risk decreased and retention of genetic diversity increased rapidly with increasing initial population size. In the absence of catastrophes, demographic conservation goals could be met with a smaller initial population size than could genetic conservation goals; ≥20-50 adults were necessary for

extinction risk <5% whereas ≥50-110 adults were necessary to retain >95% of existing genetic diversity over 100 yrs. These thresholds shifted upward when catastrophes were included; ≥50-200 adults were necessary for extinction risk <5% and ≥110 to more than 200 adults were necessary to retain >95% of existing genetic diversity over 100 yrs. Impediments to Blanding's turtle conservation include an incomplete understanding of geographic covariation among demographic parameters, the large amount of effort necessary to estimate and monitor abundance, and uncertainty regarding the impacts of increasingly frequent extreme weather events.

5.2. PVA Outcomes over a Range of Degrees of Connectivity

The zero growth population viability model descried in Section 5.1 and Appendix 5.1 was modified to include one immigrant female every five years for initial adult population sizes ranging from 2-200 adults. Initial adult population size had a large effect on extinction probability and retention of genetic diversity (Table 5.1, Fig. 5.1). These relationships were strongly curvilinear; extinction risk was highest and genetic diversity was lowest at small initial adult population sizes but leveled off at about 50 adults and remained relatively constant as initial adult population size increased further (Table 5.1, Fig. 5.1). A more complete treatment of the effect of initial adult population size on PVA outcomes, including results with and without catastrophes, is provided in Appendix 5.1.

A modest rate of immigration (1 adult female every 5 yr) reduced extinction risk and increased genetic diversity (Table 5.1, Fig. 5.1). These relationships of extinction risk and of retention of genetic diversity to initial adult population size remained strongly curvilinear but leveled off at an initial population size of ca. 20 adults vs. 50 adults in the absence of immigration (Table 5.1, Fig. 5.1).

Population viability analyses must be interpreted carefully. Outcomes are dependent on the accuracy with which parameters are estimated and the assumption that these parameters remain unchanged over the period modeled. For this reason, comparisons among scenarios can be more meaningful than the results of any give scenario. Such comparisons demonstrate that larger populations fare better than smaller populations and at small population sizes, extinction risk rises and genetic diversity decreases rapidly. Immigration has beneficial effects, reducing extinction risk and increasing the genetic diversity of small populations, thus improving demographic and genetic characteristics of Blanding's turtle populations.

Table 5.1. Summary statistics of Illinois Blanding's turtle population viability analyses for initial adult population sizes ranging from 2-200 adults with (A) no immigration and with (B) one adult female immigrant every five years (0.2/yr). N_i and N_a refer to the initial population size and initial adult population size, respectively. Stochastic r refers to the mean growth rate of extant populations prior to carrying capacity truncation averaged across years. Probability of extinction is the proportion of populations that went extinct in 100 yr. N-extant is the mean size of extant populations after 100 yr. Genetic diversity is the expected heterozygosity as a proportion of initial heterozygosity after 100 yr. A more complete treatment of the effect of initial adult population size on PVA outcomes, including results with and without catastrophes, is provided in Appendix 5.1.

N_i	N_a	Stochastic r	Probability of Extinction (95% Confidence Interval)		N-extant (95% Confidence Interval)		Genetic Diversity (95% Confidence Interval)	
A. No Immigration								
6	2	-0.023	0.95	(0.93, 0.97)	38	(7, 67)	0.59	(0.54, 0.64)
12	4	-0.018	0.81	(0.77, 0.84)	42	(30, 53)	0.65	(0.62, 0.68)
19	6	-0.015	0.65	(0.61, 0.69)	50	(38, 61)	0.71	(0.68, 0.73)
25	8	-0.014	0.55	(0.50, 0.59)	46	(37, 55)	0.72	(0.70, 0.74)
31	10	-0.013	0.47	(0.42, 0.51)	53	(45, 61)	0.75	(0.73, 0.77)
62	20	-0.013	0.29	(0.25, 0.33)	82	(69, 95)	0.82	(0.81, 0.83)
93	30	-0.012	0.18	(0.15, 0.22)	97	(84, 108)	0.85	(0.84, 0.86)
124	40	-0.012	0.14	(0.11, 0.17)	124	(108, 139)	0.88	(0.87, 0.89)
154	50	-0.010	0.09	(0.07, 0.12)	154	(135, 173)	0.90	(0.89, 0.91)
185	60	-0.011	0.11	(0.08, 0.13)	158	(140, 175)	0.92	(0.91, 0.92)
216	70	-0.010	0.07	(0.04, 0.09)	166	(148, 184)	0.92	(0.91, 0.93)
247	80	-0.011	0.05	(0.03, 0.07)	171	(153, 188)	0.92	(0.92, 0.93)
278	90	-0.011	0.06	(0.04, 0.08)	201	(178, 224)	0.93	(0.93, 0.94)
309	100	-0.010	0.04	(0.02, 0.05)	213	(191, 234)	0.94	(0.93, 0.94)
370	120	-0.009	0.03	(0.02, 0.05)	231	(209, 253)	0.95	(0.94, 0.95)
432	140	-0.008	0.02	(0.01, 0.03)	240	(218, 260)	0.95	(0.95, 0.96)
493	160	-0.010	0.03	(0.01, 0.04)	218	(198, 237)	0.95	(0.95, 0.95)
555	180	-0.008	0.03	(0.02, 0.04)	258	(236, 279)	0.96	(0.95, 0.96)
617	200	-0.009	0.02	(0.01, 0.03)	244	(221, 265)	0.96	(0.95, 0.96)
B. One Adult Female Immigrant Every Five Years								
6	2	0.013	0.53	(0.49, 0.58)	71	(60, 81)	0.84	(0.83, 0.85)
12	4	0.006	0.39	(0.35, 0.44)	73	(62, 82)	0.86	(0.85, 0.86)
19	6	0.005	0.23	(0.19, 0.26)	79	(70, 87)	0.86	(0.86, 0.87)
25	8	0.003	0.20	(0.16, 0.23)	94	(80, 107)	0.88	(0.87, 0.88)
31	10	0.002	0.15	(0.12, 0.18)	92	(80, 102)	0.88	(0.87, 0.88)

N_i	N_a	Stochastic r	Probability of Extinction (95% Confidence Interval)		N-extant (95% Confidence Interval)		Genetic Diversity (95% Confidence Interval)	
62	20	-0.002	0.10	(0.07, 0.12)	124	(108, 140)	0.90	(0.90, 0.91)
93	30	-0.002	0.05	(0.03, 0.07)	150	(133, 167)	0.92	(0.91, 0.92)
124	40	-0.004	0.05	(0.03, 0.07)	158	(139, 176)	0.93	(0.92, 0.93)
154	50	-0.005	0.04	(0.02, 0.06)	188	(167, 208)	0.93	(0.93, 0.94)
185	60	-0.005	0.02	(0.01, 0.04)	188	(169, 206)	0.94	(0.94, 0.94)
216	70	-0.005	0.01	(0.00, 0.02)	207	(187, 226)	0.94	(0.94, 0.95)
247	80	-0.006	0.01	(0.00, 0.02)	210	(189, 229)	0.95	(0.95, 0.95)
278	90	-0.007	0.01	(0.00, 0.02)	209	(190, 226)	0.95	(0.95, 0.96)
309	100	-0.007	0.01	(0.00, 0.02)	219	(200, 237)	0.95	(0.95, 0.96)
370	120	-0.007	0.02	(0.01, 0.03)	239	(217, 260)	0.96	(0.95, 0.96)
432	140	-0.006	0.01	(0.00, 0.01)	251	(230, 272)	0.96	(0.96, 0.96)
493	160	-0.008	0.00	(0.00, 0.01)	232	(211, 253)	0.96	(0.96, 0.96)
555	180	-0.006	0.01	(0.00, 0.01)	264	(242, 284)	0.97	(0.96, 0.97)
617	200	-0.007	0.01	(0.00, 0.02)	264	(243, 284)	0.97	(0.96, 0.97)

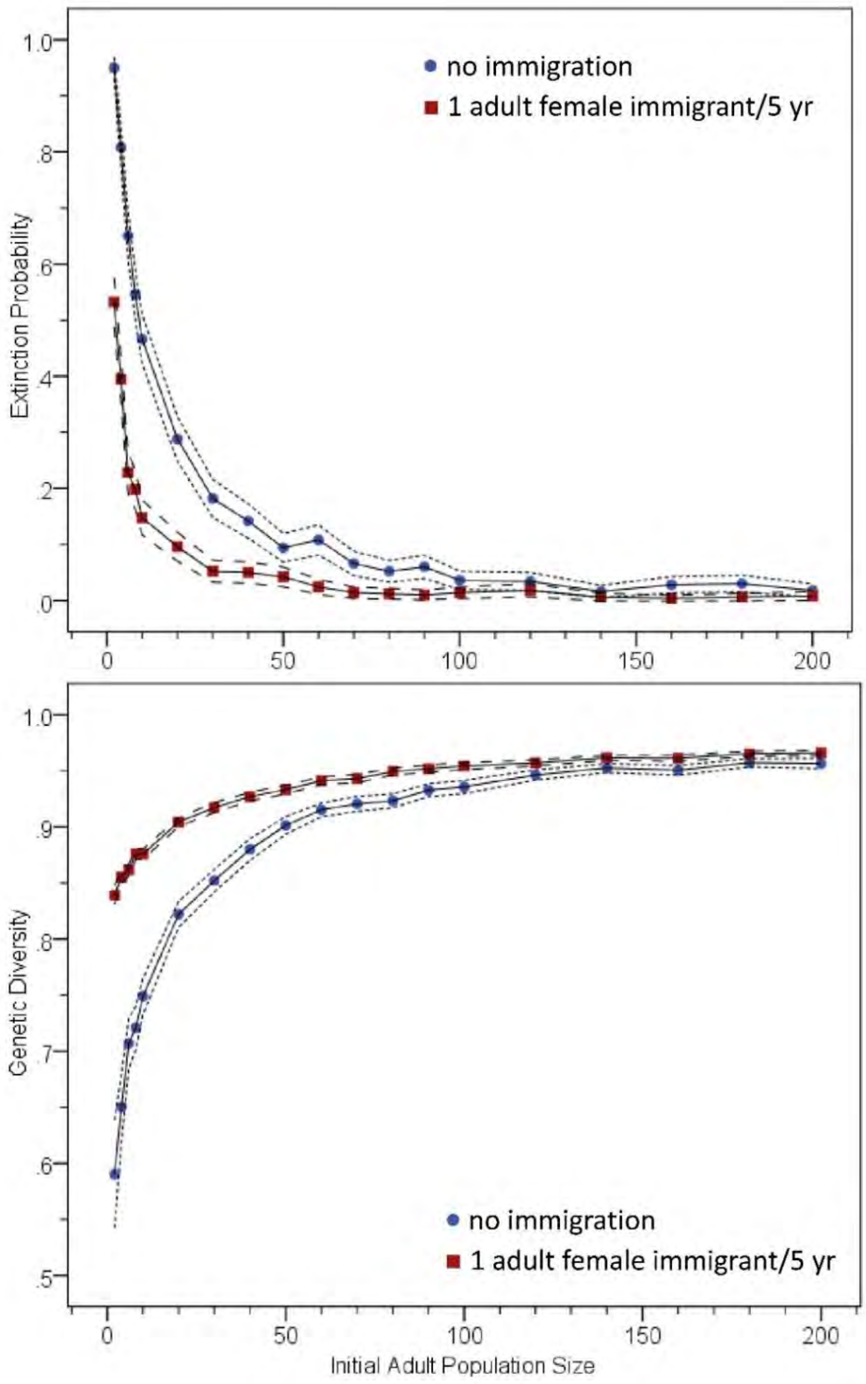

Figure 5.1. Effect of initial adult Blanding's Turtle population size on extinction probability (upper panel) and genetic diversity (lower panel) with (red squares) and without (blue circles) immigration (one adult female every five years). Shown are means (symbols, solid lines) and 95% confidence intervals (dotted, dashed lines) for 500 iterations of each initial adult population size and immigration combination. A more complete treatment of the effect of initial adult population size on PVA outcomes, including results with and without catastrophes, is provided in Appendix 5.1.

Appendix 5.1. Blanding's Turtle Demography and Population Viability

A manuscript addressing objective 6 and currently in review for publication in the Journal of Fish and Wildlife Management is provided (with the exception Supplemental Materials, References S1 – S6 and Supplemental Materials, File S1). Supplemental Materials, Tables S2-10 in Excel files format (.xlsx) and the Vortex .xml file (Supplemental Materials, File S1) are available upon request.

Blanding's Turtle Demography and Population Viability

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Abstract

In anticipation of US federal status classification (warranted, warranted but precluded, not warranted), scheduled for 2023, we provide population viability analysis of the Blanding's turtle *Emydoidea blandingii*, a long-lived, late-maturing, semi-aquatic species of conservation concern throughout its range. We present demographic data from long-term study of a population in northeastern Illinois and use these data as the basis for viability and sensitivity analyses focused on parameter uncertainty and geographic parameter variation. We use population viability analysis to identify population sizes necessary to provide population resiliency to stochastic disturbance events and catastrophes and demonstrate how alternative definitions of 'foreseeable future' might affect status decisions. Demographic parameters within our focal population resulted in optimistic population projections (probability of extinction = 0% over 100 years) but results were less optimistic when catastrophes or uncertainty in parameter estimates were incorporated (probability of extinction = 3% and 16%, respectively). Uncertainty in estimates of age-specific mortality had the biggest impact on population viability analysis outcomes but uncertainty in other parameters (age of first reproduction, environmental variation in age-specific mortality, % females reproducing, clutch size) also contributed. Blanding's turtle demography varies geographically and incorporating this variation resulted in both mortality- and fecundity-related parameters affecting population viability analysis outcomes. Possibly, compensatory variation among demographic parameters allows for persistence across a wide range of parameter values. We found that extinction risk decreased and retention of genetic diversity increased rapidly with increasing initial population size. In the absence of catastrophes, demographic conservation goals could be met with a smaller initial population size than could genetic conservation goals; ≥20-50 adults were necessary for extinction risk <5% whereas ≥50-110 adults were necessary to retain >95% of existing genetic diversity over 100 yrs. These thresholds shifted upward when catastrophes were included; ≥50-200 adults were necessary for extinction risk <5% and ≥110 to more than 200 adults were necessary to retain >95% of existing genetic diversity over 100 yrs. Impediments to Blanding's turtle conservation include an incomplete understanding of geographic covariation among demographic parameters, the large amount of effort necessary to estimate and monitor abundance, and uncertainty regarding the impacts of increasingly frequent extreme weather events.

Introduction

Assessments of population viability have become increasingly important in identifying and making management decisions for species of conservation concern (Akçakaya et al. 1999; Morris and Doak 2002; Lacy 2019; IUCN 2012; USFWS 2016). Unfortunately, as the number of species under threat increases worldwide (Gibbons et al. 2000; Houlahan et al. 2000; Schipper et al. 2008; Butchart et al. 2010), practitioners are often faced with an unsolvable dilemma of making decisions based on projections from incomplete data over uncertain time periods and under changing environmental conditions (Reed et al. 1998; Morrison et al. 2016). As a consequence, it is important to have a clear understanding of the uncertainties associated with population viability assessments and the decisions they engender. This is especially true for species that are long-lived and rare because these traits increase extinction risk and make precise estimates of key demographic parameters difficult to obtain (Mace and Kershaw 1997; Burnham and Anderson 2002).

The Blanding's turtle *Emydoidea blandingii* (Figure 1) is a long-lived, late-maturing, semi-aquatic species that often occurs at low density across large expanses of wetland and adjacent upland habitat (Congdon et al. 2008, 2011; Reid et al. 2016). Its distribution is centered on the North American Great Lakes, extends westward to the Sandhills of Nebraska, and includes disjunct populations in northeastern North America. Blanding's turtles are threatened by habitat loss, elevated rates of nest predation due to subsidized predators, and road mortality (Congdon et al. 2008). As a consequence of these threats, populations are frequently small and isolated (Congdon et al. 2008). The Blanding's turtle is ranked as endangered by the International Union for Conservation of Nature and is recognized as being in need of conservation or listed as threatened or endangered in each state and province in which it occurs (IDNR 2009; IUCN 2012; MNDNR 2013; MDIFW 2015; PWAP 2015; COSEWIC 2016; NEWP 2017; NGPC 2018; NYDEC 2019; IDNR 2020; IESPB 2020; MNHP 2020; NHESP 2020; ODNR 2020; MSU 2021; NHI 2021; SDGFP 2020). A petition to list the Blanding's turtle under the US Endangered Species Act (ESA 1973, as amended) was found to present "substantial scientific or commercial information indicating that the petitioned actions may be warranted," thus triggering status review with a 12-month finding that listing is warranted, warranted but precluded, or not warranted anticipated in 2023 (USFWS 2015, 2021).

Much of our knowledge of Blanding's turtle demography comes from long-term study at the E. S. George Reserve (University of Michigan) in southern Michigan that demonstrates low nest survival, delayed reproductive maturity, and high adult survival (Congdon et al. 1993, 2000). Other long term studies corroborate these characteristics of Blanding's turtle demography (e.g., Standing et al. 1999; Reid et al. 2016). Associated life-table analyses indicate that population stability is most sensitive to juvenile and adult survival and less sensitive to nest survival, age at first reproduction, and fecundity (Congdon et al. 1993, 2000). But even for the Michigan study, where field research spans nearly four decades (Congdon et al. 2000), precise estimates for some demographic parameters are lacking. For example, age 0 mortality is equated to nest failure or nest failure combined with hatch failure and juvenile mortality is inferred from other demographic parameters by assuming a constant population size (Congdon et al. 1993, 2000).

Available information on Blanding's turtle demography has been used in population viability analyses (PVA) to assess impacts of road mortality (Beaudry et al. 2008), alternative management and urban development scenarios (Dillon Consulting Limited 2013), and head-starting (in which eggs are collected and hatchlings are reared in captivity to minimize mortality during vulnerable early life stages) as a management strategy (Buhlmann et al. 2015; Thompson 2020). In general, PVA makes use of mathematical models to generate future projections of population dynamics (Morris and Doak 2002). Often, PVA incorporates one or more sources of stochasticity in vital rates and may include sensitivity analyses to assess how variation in parameter values affects PVA outcomes (McCarthy et al. 1995; Cross and Beissinger 2001; Prowse et al. 2016; Manlik et al. 2017). This variation might be the result of parameter uncertainty (e.g., the 95% confidence limits of a parameter estimate), parameter variability (e.g., from population to population), or parameter manipulation (e.g., observed or hypothetical changes in a parameter as the result of management or other human activities). Ideally, PVA is based on knowledge of demographic parameters estimated from long-term study of a single population (Morris and Doak 2002). Instead, existing Blanding's turtle PVAs have used a combination of estimates obtained from short-term studies and 'borrowed' from the long-term Michigan study (Baudry et al. 2008; Dillon Consulting Limited 2013; Buhlmann et al. 2015). Blanding's turtle sensitivity analyses have included only a subset of demographic parameters using life-table or matrix methods without stochasticity (Heppell 1998; Congdon et al. 1993, 2000). A comprehensive Blanding's turtle PVA based on locally-derived parameter estimates and incorporating stochasticity and global sensitivity analysis is lacking.

To build on our understanding of Blanding's turtle demography and population viability, we (1) characterize Blanding's turtle demography from long-term study of a centrally located population, (2) model population viability and assess sensitivity, and (3) use population viability analysis to explore the possibility of setting population size thresholds for conservation. Importantly, we are able to estimate nearly all parameters used in PVA from a single Blanding's turtle population. This includes estimates of environmental (= process) variance, the component of the total variance in a demographic parameter attributable to year-to-year environmental variation separate from the error variance attributable to sampling (Franklin et al. 2002). These estimates of demographic parameters and environmental variances allow us to conduct sensitivity analyses designed specifically to address uncertainty in parameter estimates in our focal population, thus guiding future demographic study. We also conduct sensitivity analyses that address geographic variation in demography among Blanding's populations, thus potentially identifying key variables affecting persistence range-wide.

Population size criteria are often included among the goals of policy makers and managers tasked with endangered species protection and recovery despite active debate regarding their utility and generality (Jamieson and Allendorf 2012; Frankham et al. 2013; Frankham et al. 2014; Franklin et al. 2014). Blanding's turtles exhibit remarkable variation in population size. At the upper extreme is the Valentine National Wildlife Refuge population in the Sandhills of north-central Nebraska which is thought to exceed 100,000 animals (Lang 2004). Perhaps next largest is the Weaver Dunes population in southeastern Minnesota numbering ca. 5,000 animals (Pappas et al. 2000; Lang 2003). More typically, Blanding's turtle populations number from 10's to 100's of individuals (Graham and Doyle 1977; Herman et al.

1995; Joyal et al. 2000; McNeil 2002; Kiviat et al. 2004; Rubin et al. 2004; Compton 2007; Congdon et al. 2008; Ruane et al. 2008; MWPARC 2010; COSEWIC 2016), a size where demographic and environmental stochasticity are likely to accelerate loss of genetic variability and magnify extinction risk (Ovaskainen and Meerson 2010). To address this variation in population size, we use PVA to identify population sizes for which extinction risk is projected to remain below and genetic diversity is projected to remain above threshold values, repeating our analyses with varying degrees of environmental stochasticity with and without catastrophes and running simulations for differing durations to demonstrate how alternative definitions of 'foreseeable future' might affect conclusions (USDOI 2009; Almy 2017; Lake and Petersen 2017; USFWS 2019b).

We use our results to identify demographic characteristics associated with Blanding's turtle population resiliency, the ability of a population to withstand stochastic disturbance events and local catastrophes (Shaffer and Stein 2000; Wolf et al. 2015). Resiliency, redundancy (ability to withstand regional catastrophic events), and representation (ability to adapt to environmental change) constitute the three Rs used by the USFWS to inform Endangered Species Act decisions (Shaffer and Stein 2000; Wolf et al. 2015; USFWS 2016), which, for Blanding's turtles, are scheduled to occur in 2023 (USFWS 2019a). Blanding's turtles share life history traits and conservation threats with a number of other North American freshwater turtles, including snapping turtles, wood turtles, bog turtles, and spotted turtles (Congdon et al. 1994; Enneson and Litzgus 2008; Shoemaker et al. 2013; Feng et al. 2019), making our work relevant to turtle conservation more generally.

Study Site

Our data on Blanding's turtle demography mostly comes from long-term (2004-2018) monitoring at the Spring Bluff-Chiwaukee Prairie (SBCP) complex in Lake County, Illinois and Kenosha County, Wisconsin. This site consists of 215 ha of high-quality coastal wetland habitat along Lake Michigan and is part of the Chiwaukee Illinois Beach Lake Plain, recognized among Wetlands of International Significance (https://rsis.ramsar.org/, January 2021). It is managed by the Lake County Forest Preserve District (LCFPD), Wisconsin Department of Natural Resources, and The Nature Conservancy and has been the focus of efforts aimed at promoting Blanding's turtle recruitment, survival and habitat quality, including prescribed fire, mechanical and chemical treatment of invasive plants, turtle head-starting, and meso-predator removal (Thompson et al. 2020; Urbanek et al. 2016). Supplemental information comes from shorter-term (2017-2019) Blanding's turtle monitoring at Illinois Beach State Park (IBSP), an adjacent 1,680 ha coastal wetland complex immediately south of SBCP, and from the work of researchers elsewhere.

Materials and Methods

Field methods

From 2004 to 2018, capture-mark-recapture data were collected by capturing turtles in baited collapsible minnow traps (Promar, 30 X 30 X 60 cm, 0.6-cm mesh), nylon hoop traps (Memphis Net and Twine, 76 cm diameter with 2.5 cm mesh), and by hand during the active season (April-August). Turtles were marked with PIT tags and notching of marginal scutes and were photographed to aid in future recognition (Cagle 1939; Buhlmann and Tuberville 1998). Turtles weighing less than 750g were classified as juveniles and assigned ages by counting annuli from plastron photos (Germano and Bury 1999; Wilson et al. 2003). Photos that could not be scored consistently by two independent observers were excluded (n=40 older juveniles with indistinct annuli). Sex of adults was determined by observing the concavity of the plastron (Graham and Doyle 1979). A subset of adult Blanding's turtles, mostly females, were equipped with radio-transmitters, facilitating collection of reproductive data (Thompson et al. 2020).

Demography

We palpated inguinal pockets of known-age females during the weeks prior to nesting to determine reproductive status (non-gravid, gravid), providing us with information on age at first reproduction. We used logistic regression to characterize the relationship between age and reproductive status of these females, providing us with an estimate of reproductive frequency early in life. We also palpated transmitter-equipped females of unknown age to obtain reproductive frequency among older females. We computed environmental variance in the proportion of females reproducing by subtracting mean annual binomial variance from the among-year variance following Akçakaya (2002). We obtained data on number of eggs per clutch from 120 clutches included in the Lake County head-starting program from 2008-2018 (Thompson et al. 2020). We estimated environmental variance in clutch size (= among-year variance) from variance components analysis computed using the restricted maximum likelihood method in IBM SPSS 25 with year and female ID as random factors.

To facilitate PVA, we defined age 0 to encompass the period from oviposition (late June – early July) through the resumption of activity following a turtle's first winter (typically in April or May). Mortality during this period includes (1) clutch failure mostly due to nest predation, (2) hatch failure of eggs within intact clutches, (3) post-hatch mortality, including mortality prior to the cessation of activity in fall and over winter. Rates of clutch failure were obtained by using telemetry to observe nesting at SBCP and IBSP coupled with follow-up monitoring to determine nest outcome (depredated, destruction by other causes, undisturbed; Urbanek et al. 2016). Hatch failure was estimated from the LCFPD head-starting program (Thompson et al. 2020) based on eggs incubated from 2008-2018. Rates of post-hatch mortality were obtained from Kastle et al. (in press) who used telemetry to monitor 82 hatchlings for up to 88 days at sites in northern Illinois and southern Wisconsin, including SBCP, and then extrapolated mortality from hatching through resumption of spring activity. We combined these components to estimate Age 0 mortality as 1 – (clutch survival*hatch success*post-hatch and over-winter survival).

We have data from too few years to estimate environmental variance of clutch failure at SBCP. Instead, we used data from a Michigan study where the fates of 238 nests were monitored from 1976-1998 (Table 1 in Congdon et al. 2000) and estimated environmental variance following Akçakaya (2002). Environmental variance in hatch failure was estimated from the LCFPD head-starting program following Akçakaya (2002). Environmental variance in post-hatch and overwinter mortality is unknown but was assumed to be no greater than the environmental variance of clutch failure, allowing us to estimate lower and upper limits to environmental variance in age 0 mortality by combining these components following Goodman (1960).

Age-specific juvenile survival was estimated from 265 captures of 127 wild-born turtles (recaptures = 52% of total captures) first captured as juveniles using Cormack-Jolly-Seber model selection in program MARK (Golba 2019). Adult mortality was estimated from 531 captures of 148 adults (recaptures = 72% if total captures) using Cormack-Jolly-Seber model selection. Environmental variance in adult mortality was estimated using the variance component option in program MARK (Golba 2019). We estimated adult population size from 531 captures of 148 adults using the Jolly–Seber model (Jolly 1965; Seber 1965) as implemented in program JOLLY (http://www.mbr-pwr.usgs.gov/software.html). Confidence intervals computed using Manly's method (Krebs 1998; Manly 1984).

Population viability and sensitivity

Spring Bluff Chiwaukee Prairie model. We used the PVA software program Vortex because of its flexibility and repeatability (Lacy and Pollak 2018; Lacy et al. 2018). We modeled a single Blanding's turtle population for 1,000 iterations over a time-frame of 100 years using the default order of simulation events. We defined extinction as occurring when only one sex remained. We assumed no inbreeding depression; evidence of inbreeding is equivocal for Blanding's turtles (Anthonysamy et al. 2017; Sethuraman et al. 2014) and for turtles generally (e.g., Alacs et al. 2007; Buchanan et al. 2019; Davy and Murphy 2014; Gallego-Garcia et al. 2018) and evidence of inbreeding depression is scarce (Velo-Antón et al. 2011). We assumed no correlation between environmental variance in reproduction and survival based on the absence of a correlation between year-to-year variation in residual clutch size and survival at SBCP ($r = 0.20$, $n = 8$, $P = 0.628$). We specified a population state variable to track adult population size along with default tracking of total population size.

Demographic parameters were set to values observed at SBCP except as described here and in the Results. Environmental variances were used to compute environmental standard deviations (SD) by which Vortex specifies binomial distributions to simulate environmental variation in reproductive and mortality rates (Lacy et al. 2018). We assumed a polygynous mating system from marker-based analyses elsewhere (Refsnider 2009; Anthonysamy 2012; McGuire et al. 2015). Maximum lifespan and maximum age of reproduction are unknown at SBCP but were set to 83 years based on a Michigan population (https://news.umich.edu/oldest-well-documented-blanding-s-turtle-recaptured-at-u-m-reserve-at-age-83/, January 2021). Females at SBCP and other study sites produce at most one clutch per year (personal observation, Congdon et al. 1983; Standing et al. 1999). We set the sex ratio at birth to parity. Many turtles, including Blanding's turtles (Gutzke and Packard 1987), have temperature-dependent sex determination but lack secondary sex characteristics until maturity (Graham and Doyle 1979) so field data on hatchling sex ratio are mostly lacking (for an exception, see Schwanz et al. 2010). We allowed reproduction to be density-independent because information regarding density dependence in Blanding's turtles is lacking and because outcomes such as extinction vs. persistence are determined at densities below which density-dependence is expected to occur. We assumed mortality schedules were the same for males and females; adult mortality does not differ between sexes at SBCP but tests for differences in mortality for other age classes are lacking (Golba 2019). The frequency and severity of catastrophes affecting Blanding's turtle populations are poorly known. Catastrophes have not been observed over 15 years of study at SBCP nor over nearly four decades at a Michigan study site (Congdon et al. 2000) but otter predation resulted in 53 deaths among 100 Blanding's turtles at an Ontario site (Gassbarini 2016) and 49 deaths of unknown cause were documented at another site (Sheppard 2014). Mass mortality events attributable to predation, disease, and severe weather have been documented in other freshwater and terrestrial turtles (Supplemental Material, Table S1; see Fey et al. 2015 for a review of mass mortality events in animals more generally). To assess how catastrophes might affect PVA outcomes, we contrasted no-catastrophe and catastrophe scenarios with catastrophes occurring at an average frequency of 4% (ca. once per generation) and resulting in survival 75% that of the no catastrophe scenario, plausible values based on mass mortality events in freshwater and terrestrial turtles generally (Supplemental Material, Table S1). We assumed that all adult males were potential breeders. Because mortality in turtles typically decreases with size (e.g., Feng et al. 2019), we restricted juvenile mortality to be greater than or equal to adult mortality by setting mortality to 5.3% for age 4 and older (confidence intervals for mortality of age 4 and older juveniles broadly overlapped those of adults). We used the stable age distribution option in Vortex to generate the number of individuals in each year class for a very large population (100,000) and then treated these as proportional values for a population in which the adult year classes summed to our observed adult population (Lacey et al. 2018, p. 53). The carrying capacity of SBCP for Blanding's turtles is unknown. To allow for realistic amounts of population growth, we set carrying capacity equal to twice our initial adult population size. We specified that carrying capacity truncation be based on adult (rather than total) population size to reduce the impact that years with high reproductive output had on population limitation. Neither harvest nor supplementation were included. Default settings, in which each individual in the initial population is assigned a unique heterozygous genotype at a single neutral locus and descendent genotypes are determined according to Mendel's principles, were used to track the loss of genetic variability over time (Lacy et al. 2018). Additional details are provided in Supplemental Material, Text S1, File S1.

Head-started Blanding's turtles have been released annually since 2007 and predators (primarily raccoons) have been removed annually since 2013 at SBCP (Urbanek et al. 2016; Thompson et al.2020). However, head-starts had not yet reached reproductive maturity as of the completion of data collection in 2018 (Thompson et al. 2020) and thus do not affect estimates of adult mortality and population size. In addition, for PVA, head-starts were excluded from estimates of juvenile mortality (Golba 2019) and nest survival rates observed prior to predator removal were used in estimating age 0 mortality. Thus, while parameter estimates in our SBCP model may reflect the effects of habitat management (prescribed fire, chemical and mechanical methods), we sought to minimize effects of head-starting and predator removal.

Zero-growth model. Our SBCP model resulted in positive population growth and low extinction risk (Results), making extinction risk of limited use in assessing sensitivity and contrasting with separate analyses indicating that adult SBCP Blanding's turtle population size is stable or only slightly increasing (personal observation). For these reasons we created a zero-growth model by increasing age-specific mortality rates and reducing clutch size to achieve a deterministic population growth rate close to zero. Catastrophes were implemented as in the Spring Bluff Chiwaukee Prairie model.

Sensitivity to parameter uncertainty. We assessed the effect of parameter uncertainty on PVA outcomes using our zero-growth model and a combination of single-variable and multi-variable tests in Vortex. Single-variable tests consisted of 1,000 iterations at each of 10 uniformly distributed parameter values while holding other parameters constant. Seventeen parameters were included in single parameter tests (Table 1) and were evaluated for the magnitude of their effects on population growth rate and probability of extinction. Ranges of parameter values used in sensitivity tests were based on 95% confidence limits for parameter estimates observed at SBCP to the extent possible (clutch size and mortality confidence intervals were shifted to match shifts in parameter estimates necessary to achieve zero growth). For parameters for which confidence limits were lacking, plausible values were derived from knowledge of Blanding's turtle biology. Specifically, inbreeding depression was modeled by assuming 0-7 lethal equivalents with 50% due to lethal recessive alleles, thus somewhat exceeding the mean number of lethal equivalents (6.29) affecting fecundity and first-year survival in a meta-analysis of wild birds and mammals (O'Grady et al. 2006). Age of first reproduction was allowed to vary between 12 and 14, allowing for 1 year uncertainty in our assessment of the age of primiparous females (values selected for age of first reproduction were rounded to integer values within Vortex). Maximum lifespan and maximum age of reproduction were allowed to vary jointly from 55 to 85 years. Mean offspring sex ratio was allowed to vary from 40-60% males. We allowed carrying capacity to vary from 104-312 and the environmental SD in carrying capacity to vary from 0 to 50 adults. We allowed the environmental SD in juvenile and adult mortality to vary jointly from 0-4%.

Multi-variable tests included a subset of nine parameters identified as having moderate to large effects in single-variable tests or of intrinsic interest. We used Latin hypercube sampling (LHS) to select 30,000 unique uniformly distributed combinations of parameter values and ran a single iteration of each (Prowse et al. 2016). We evaluated the sensitivity of PVA outcomes to variation in these nine parameters using logistic regression (McCarthy et al. 1995; Cross and Beissinger 2001). Analyses focused on four response variables, stochastic population growth rate (observed per capita growth rate averaged across years and iterations = stochastic-r), probability of extinction, gene diversity (proportion of initial expected heterozygosity remaining), and adult population size. To facilitate analysis via logistic regression, stochastic-r, gene diversity, and adult population size were transformed into binomial variables by equating values below and above the median equal to 0 and 1, respectively (with single iterations, probability of extinction is necessarily binomial). We opted to use logistic regression (vs. e.g., linear regression) to analyze stochastic-r, gene diversity, and adult population size because of non-normality (gene diversity and adult population size equal 0 for simulations resulting in extinction, giving strongly bimodal distributions) and because relationships to independent variables are potentially non-linear. Multiple logistic regression analyses were conducted using IBM SPSS 25 with forced entry of the nine parameters as independent variables and stochastic-r, probability of extinction, gene diversity, or adult population size (analyzed separately) as dependent variables. Sufficiency of sampling was assessed by selecting three sets of 10,000 random samples each and repeating analyses on each subsample (Prowse et al. 2016). Partial logistic regression coefficients were standardized by dividing each by its standard error, providing a relative measure of the influence of each parameter (Cross and Beissinger 2001). To facilitate comparisons among dependent variables and among data sub-samples, standardized partial logistic regression coefficients were scaled so that absolute values summed to 100.

Sensitivity to geographic parameter variation. We reviewed published and unpublished sources for information on geographic variation in demographic parameters across the Blanding's turtle distribution. We excluded estimates of juvenile survival based on studies of head-started turtles (Arsenault 2011; d'Entremont 2014; Ritchie 2017; Starking-Szymanski et al. 2018; Golba 2019) or inferred from survival of other age classes by assuming constant population size (Congdon et. al. 1993; Hawkins 2016). For sites where long-term study has produced multiple estimates, we used the estimate derived from the longest time interval. We assessed the effect of geographic parameter variation on PVA outcomes by repeating our multi-variable tests in Vortex using an expanded range of parameter values. For parameters for which only our SBCP estimate was available (SD of age 0 mortality, juvenile mortality, SD of juvenile and adult mortality), we used parameter uncertainty to set the range of parameter values.

Sensitivity to population size. We used our zero-growth model to investigate the impact of population size on PVA outcomes with and without catastrophes. We included a low, intermediate, and high environmental stochasticity scenarios by setting the environmental SD in % females reproducing, clutch size, and % mortality to the lower limits used in sensitivity testing, the base values used in the zero-growth model, and the upper limits used in sensitivity testing (Table 1). Catastrophes were implemented as in the Spring Bluff Chiwaukee Prairie and zero-growth models. For each scenario, we ran 1,000 iterations with initial population sizes ranging from 5 to 200 adults (total population = 15 to 585 with a stable age distribution) and durations of 50 and 100 years.

Results

Demography

Nine females, first captured as juveniles, initiated reproduction during our study. The youngest of these primiparous females was 13 years old when she first reproduced. Estimates of reproductive frequency among females from 13-18 years of age ranged from 30-89% (Supplemental Material, Table S2, Figure S1). Among adult females of unknown age (n = 11-48 per year from 2012-2018), we detected that females were gravid in 185 of 200 cases (92.5%, 95% confidence interval = 87.9-95.7%). Environmental variance in reproductive frequency (and the environmental SD as used in Vortex) was estimated to equal 0.00 (Supplemental Material, Table S3). From 2008-2018, we obtained 120 clutches from 41 females (1-7 per female; Supplemental Material, Table S4). Number of eggs per clutch averaged 13.0 (range = 7-24; Supplemental Material, Table S4). The estimated variance component attributable to year was 0.74 (environmental SD = 0.86).

We monitored 13 nests at SBCP prior to implementation of mesopredator control and 15 nests at IBSP, a site without mesopredator control. At SBCP, 12 nests were depredated; at IBSP, 6 nests were depredated and 3 others failed due to shoreline erosion, resulting in a clutch failure rate of 75%. We monitored 60 nests at SBCP during years with mesopredator control (n=6-14 nests/year from 2013-2018 and observed a clutch failure rate of 33.3% (0-64.2% per year). Hatch failure among 1,380 eggs incubated for the LCFPD head-starting program (n = 117-222 eggs per year from 2010-2018) averaged 19% (Supplemental Material, Table S5). Post-hatching survival of 82 hatchlings tracked for up to 88 days using telemetry was 79.6% (standard error = 0.06; Kastle et al. in press). Extrapolating to the entire post-hatch and over-winter period (ca. 240 days) using a Weibull survival function gave an anticipated mortality of 35% (Kastle et al. in press). Combining these components, age 0 mortality = 1 – ((1-0.750)*(1-0.187)*(1-0.350)) = 0.868 (86.8%) with uncertainty (sampling variance) = 0.0020 and 95% confidence interval = 78.0-95.6% (Supplemental Material, Table S6.A).

Based on 238 nests monitored at a Michigan study site (n = 4-16 nests per year from 1976-1998; Congdon et al. 2000), environmental variance in clutch failure = 0.034 (Supplemental Material, Table S7). At SBCP, environmental variance in hatch failure was 0.0043 (Supplemental Material, Table S5). An estimate of environmental variance in post-hatch mortality is lacking but assuming it falls between 0.000 and 0.034 (the variance in clutch failure) results in a combined estimate of the environmental variance in age 0 mortality of 0.0096-0.0110 (environmental SD = 0.0980-0.1050 or 9.8-10.5%; Supplemental Material, Table S6.B).

Age-specific juvenile mortality decreased from 28.9% at age 1 to 14.6%, 6.9%, 3.6% 2.5%, and 2.2% at age 2, 3, 4, 5, and 6 and older (converted from survival estimates in Golba 2019). Confidence intervals were broadest at age 1 (10.9-48.4%) and generally decreased with age (to 0.8-5.7% for age 6 and older; Golba 2019). Adult mortality was estimated to be 5.3% (CI = 3.8-7.3%) with environmental variance = 0.0011 (environmental SD = 0.0332 or 3.3%; Golba 2019). Adult population size estimates ranged from 62 to 111 among years (Supplemental Material, Table S8). For population viability analysis, we used the maximum three-year running average ($\bar{N}$ = 104 over the years 2015-2017, CI = 87-138) as our initial adult population size and assumed a stable age distribution to determine the total population size.

Population viability and sensitivity

Spring Bluff Chiwaukee Prairie model. In the absence of catastrophes, SBCP model settings and parameter values (Table 1; Supplemental Material, Text S1, File S1) resulted in a generation time of 25 years, deterministic population growth rate of 5.8% per year, and stochastic population growth rate of 5.5% per year. No extinctions occurred among 1,000 iterations. Population size grew to carrying capacity within about 20 years (Supplemental Material, Figure S2). Extant populations averaged 1,031 individuals (253 adults) and retained 99% of initial genetic heterozygosity after 100 years. Individual iterations showed wide variation in population size over time (Figure 2). This variation was at least partly due to the high environmental SD of age 0 mortality (10.5%) which, when coupled with high age 0 mortality (86.8%), resulted in runs of years with no surviving offspring and declining population size interspersed with occasional boom years of higher offspring survival and rapid increases in population size (Figure 2, Supplemental Material, Figure S3). Including catastrophes had only small effects compared to the no-catastrophe scenario; deterministic population growth rate was 4.8%, stochastic population growth was 4.5%, no extinctions occurred among 1,000 iterations, and extant population size averaged 993 individuals (240 adults) and retained 99% of initial genetic heterogeneity after 100 years.

Zero-growth model. Zero growth was achieved by increasing age-specific mortality by 4.5% for ages 0-4, 3.5% for ages 5-9, and 2.5% for ages 10 and above and reducing clutch size from 13.0 to 10.5 (Table 1), yielding a generation time of 25 year, deterministic population growth rate of 0.00% per year and stochastic population growth rate of -0.40% per year (Supplemental Material, Figure S2). Three extinctions occurred among 1,000 iterations (probability of extinction = 0.003). Extant populations averaged 248 individuals (88 adults) and retained 96.3% of initial genetic heterozygosity after 100 years. Including catastrophes had mostly modest effects compared to the no-catastrophe scenario; deterministic population growth rate was -1.0%, stochastic population growth was -1.6%, 29 extinctions occurred among 1,000 iterations (probability of extinction = 0.029) and 93% of initial genetic heterogeneity was retained after 100 years. Catastrophes had a larger effect on extant population size which averaged 110 individuals (38 adults), about 44% that of the no-catastrophe scenario.

Sensitivity to parameter uncertainty. In single-variable tests, uncertainty in age 0 mortality, juvenile mortality, and adult mortality had moderate to large effects on stochastic-r and probability of extinction (Supplemental Material, Table S9). Uncertainty in offspring sex ratio, age at first reproduction, % females reproducing, clutch size, the environmental SD in age 0 mortality, and the environmental SD in juvenile and adult mortality had more modest effects (Supplemental Material, Table S9). Uncertainty in other parameters had generally negligible effects (Supplemental Material, Table S9).

We selected nine variables for inclusion in multi-variable sensitivity tests, age at first reproduction, % females reproducing, clutch size, age 0 mortality, the environmental SD in age 0 mortality, juvenile mortality, adult mortality, environmental SD in juvenile and adult mortality (combined), and initial population size. Although uncertainty in initial population size had a negligible effect in single-variable tests, its demographic significance led us to include it regardless. We did not include uncertainty in offspring sex ratio because, while it had a modest effect in single-variable tests, there is little information on offspring sex ratios in nature to guide selection of meaningful values for sensitivity testing.

Across 30,000 samples, stochastic-r ranged from -0.150 to 0.070 (median = -0.011, stochastic-r equaled or exceeded zero in 14,696 samples), gene diversity ranged from 0.000 to 0.991 (median = 0.961), and adult population size ranged from 0 to 363 (median = 43). Probability of extinction was 0.159 with median time to extinction of 78 years (range = 28-100; 5.5% of extinctions occurred within the first 50 years). Logistic regression revealed that uncertainty in age 0 mortality, followed by juvenile mortality and then adult mortality, had the largest effects on stochastic-r, probability of extinction, gene diversity, and adult population size with standardized relative influence of 19-33% (Table 2; Figure 3; Supplemental Material, Table S10, Figure S4, S5, S6). Uncertainty in age of first reproduction, environmental variation in age 0 mortality, % females reproducing, clutch size, and environmental variation in juvenile and adult mortality had smaller effects. In general, parameters related to mortality rates, together with initial population size, more strongly influenced extinction probability whereas parameters related to reproduction more strongly influenced stochastic-r, genetic diversity, and adult population size (Table 2; Figure 3; Supplemental Material, Table S10, Figure S4, S5, S6).

Analysis of random subsamples of n = 10,000 gave highly concordant results (standardized relative influence varied by less than 1.8 from subsample to subsample; Supplemental Material, Table S10), confirming sufficiency of sampling.

Sensitivity to geographic parameter variation. We documented considerable geographic variation in Blanding's turtle demography, including variation in age at first reproduction (10-22 years), % females reproducing (40.5-94.0%), clutch size (7.4-17.7 eggs), and adult mortality (3.5-31.0%; Supplemental Material, Table S11). Components of age-0 mortality were also variable (Supplemental Material, Table S11), including nest failure (33-94%), hatch failure (13-53%), and hatch-to-hibernation mortality (20-80%). Combining variation in nest failure and hatch failure with interpolated hatch-to-spring emergence mortality gave lower and upper bounds on age 0 mortality of 72-99% (Supplemental Material, Table S11; Kastle et al. in press).

We repeated our multi-variable sensitivity tests using observed geographic variation in age at first reproduction, % females reproducing, clutch size, age 0 mortality and adult mortality. Ranges for juvenile mortality, environmental SD in age 0 mortality, and environmental SD in juvenile and adult mortality were identical to those used in sensitivity tests of parameter uncertainty. We varied initial population size from 15 to 403 (equivalent to a stable age distribution with ca. 5 to 138 adults), thus encompassing small to moderately sized Blanding's turtle populations. Larger Blanding's turtle populations occur at some sites but given the results of our parameter uncertainty sensitivity tests, increasing initial population size above ca. 400 is unnecessary in evaluating population viability.

Across 30,000 samples, stochastic-r ranged from -0.558 to 0.131 (median = -0.077, stochastic-r equaled or exceeded zero in 4,705 samples), gene diversity ranged from 0.000 – 0.991 (median = 0.000 because gene diversity following extinction = 0), and adult population size ranged from 0 to 670 (median = 0). Probability of extinction was 0.691 with median time to extinction of 36 years (range = 4-100; 71.2% of extinctions occurred within the first 50 years). Because the median was zero for both gene diversity and adult population size, logistic regression analysis was restricted to stochastic-r (transformed to a binomial variable as before) and probability of extinction. As in sensitivity tests of parameter uncertainty, geographic variation in age-specific mortality consistently had strong effects on both stochastic-r and probability of extinction (Table 2; Figure 4; Supplemental Material, Table S10, Figure S7). In addition, geographic variation in age of first reproduction, clutch size, and % females reproducing had greater effects on stochastic-r and probability of extinction than was the case for sensitivity tests of parameter uncertainty (Table 2; Figure 4; Supplemental Material, Table S10, Figure S7). Variation in initial population size had a noticeable impact on probability of extinction but little impact on stochastic-r (Table 2; Figure 4; Supplemental Material, Table S10, Figure S7). Effects of environmental variation in age 0 mortality and in juvenile and adult mortality were small (Table 2; Figure 4; Supplemental Material, Table S10, Figure S7).

Parameter values resulting in persistence (vs. extinction) and having stochastic-r ≥ 0 included the full range of values sampled except for age 0 mortality (only values less than 97.7% resulted in persistence with stochastic-r ≥ 0). However, combinations of parameter values resulting in persistence with stochastic-r ≥ 0 were more restrictive. For example, high adult mortality (e.g., ≥ 15%) resulted in persistence with stochastic-r ≥ 0 only if age 0 mortality was low (e.g., < 89%) and reproduction commenced at a young age (e.g., ≤ 16 years; Figure 5). Partial correlation analysis revealed that among samples resulting in persistence and stochastic-r ≥ 0, age of first reproduction was positively correlated with % females reproducing and with clutch size and negatively correlated with mortality rates (Table 3). That is, as age at first reproduction increased, % females reproducing and clutch size increased and mortality rates, especially adult mortality rate, decreased (Table 3). Similarly, as % females reproducing and clutch size increased, mortality rates also increased (Table 3). As % females reproducing increased, clutch size decreased and as mortality in one age class increased, mortality in other age classes decreased (Table 3).

Sensitivity to population size. – In the absence of catastrophes, extinction risk dropped rapidly with increasing initial population size for all three environmental stochasticity scenarios (Figure 6; Supplemental Material, Figure S8). The drop was most rapid when environmental variation was low and somewhat less rapid as environmental variation increased. At initial population sizes above ca. 100 adults, differences among environmental stochasticity scenarios were negligible. A projected extinction rate less than 5% over 100 years required an initial population size ≥ 20-50 adults, depending on scenario (Figure 6); a projected extinction rate less than 5% over 50 years required an initial population size ≥ 10-20 adults, depending on scenario (Supplemental Material, Figure S8). The proportion of genetic diversity retained increased rapidly with increasing initial population size for all three environmental stochasticity scenarios (Figure 6). The increase was most rapid when environmental variation was zero and was somewhat less rapid as environmental variation increased. At initial population sizes above 200 adults, differences among scenarios were negligible. To retain ≥ 95% of genetic diversity over 100 years required an initial population size ≥ 50-110 adults, depending on scenario (Figure 6.); to retain ≥ 95% of genetic diversity over 50 years required an initial population size ≥ 25-40 adults, depending on scenario (Supplemental Material, Figure S8).

Including catastrophes resulted in a more gradual drop in extinction risk with increasing initial population size and greater population size thresholds to achieve conservation criteria (Figure 6; Supplemental Material, Figure S8). A projected extinction rate less than 5% over 100 years required an initial population size ≥ 50-200 adults depending on environmental stochasticity scenario (Figure 6). A projected extinction rate less than 5% over 50 years required an initial population size ≥ 20-30 adults depending on scenario (Supplemental Material, Figure S8). To retain ≥ 95% of genetic diversity over 100 years required an initial population ≥ 110 to more than 200 adults depending on scenario (Figure 6.). To retain ≥ 95% of genetic diversity over 50 years required an initial population size ≥ 25-65 adults depending on scenario (Supplemental Material, Figure S8).

Discussion

Demography

Long-term study at SBCP provides an unusually complete assessment of Blanding's turtle demography. Such studies are necessary to understand temporal heterogeneity in demographic processes and develop predictive models (Reinke et al. 2019). As is the case elsewhere (Congdon et al. 1993, 2000; Reid 2016), Blanding's turtles at SBCP experience low nest survival (25%), delayed reproductive maturity (13 years), and high adult survival (94.7%). Our study goes further to fill knowledge gaps regarding Blanding's turtle demography. In particular, we confirm the existence of a positive relationship between the % of females reproducing and age among young adults, as hypothesized by Congdon et al. (1993). The SBCP study also provides more comprehensive estimates of juvenile survival than previously available. For example, prior studies have equated age 0 survival to nest success or nest success + hatch success (Congdon et al. 1993, 2000) whereas we combine these components with post-hatch survival to estimate survival from egg deposition to resumption of activity in spring (Kastle et al. in press). Similarly, juvenile survival beyond age 0 has sometimes been represented by a single value inferred by computing the mean annual survival necessary to maintain a constant population size given observed rates of nest predation and adult survival (Congdon et al. 1993, 2000; Hawkins 2016). Capture-mark-recapture analysis of data from SBCP provides age-specific estimates of survival for many juvenile age classes (1, 2, 3, 4, 5, and 6+ juveniles; Golba 2019). These refinements provide a more complete understanding of Blanding's turtle survival and reproduction and facilitate population viability analysis.

We also provide the first estimates of environmental variation in Blanding's turtle demographic parameters, including environmental variation in % females reproducing, clutch size, age 0 mortality, and (from Golba 2019) adult mortality. We show that environmental variation in % females reproducing is surprisingly low (environmental SD = 0.00%) and while this estimate is based on just 7 years of data from SBCP, analysis of 18 years of data from the DuPage County, Illinois head-starting program (Thompson et al. 2020) gives identical results (% reproducing = 93%, environmental SD = 0.00%). Among other freshwater turtles, total variation in % females reproducing is greater in mud turtles (SD = 10.1%) and pond sliders (13.9%; Frazer et al. 1991) than we observed in Blanding's turtles, but the amount attributable to environmental vs. error variance is not known. We found that environmental variation in clutch size and adult survival were somewhat greater than for % females reproducing (environmental SD = 0.9 offspring and 3.3%, respectively), but what is most striking is the high environmental variation in age 0 mortality (environmental SD = 10.5%). This is primarily due to high year-to-year variance in nest predation rates (Congdon et al. 2000), a feature that may be common to fresh-water, estuarine, and marine turtle demographics (Engeman et al. 2016; Feinberg and Burke 2003; Munscher et al. 2012; Schwanz et al. 2010). Population viability analysis indicates that high environmental variation in age 0 mortality has the potential to generate dramatic changes in population size and age structure over time as a result of runs of years with 100% age 0 mortality (Figure 3; Supplemental Material, Figure S3). Because predator removal began at SBCP in 2013 (Urbanek et al. 2016), information on year-to-year variance in natural predation rates on Blanding's turtle nests is limited to Congdon's long-term study in Michigan (Congdon et al. 2000). Neither the generality of Congdon's result, nor its actual impact on population size and age structure are known. However, in other vertebrates, fluctuations in age structure affect population dynamics (Hoy et al. 2019). Comparable data on nest failure in painted turtles (Table 3 in Schwanz et al. 2010) yield an estimated environmental variance of 0.064 as a result of nest predation and 0.054 if failure due to other causes (mostly parasite or fungus infestation) are included. These values are roughly similar to that computed for Michigan Blanding's turtles (0.034), suggesting that the environmental variance used in our analyses is not atypical. Hatch failure rates of intact nests of Michigan Blanding's turtles (19.5%, Congdon et al. 2000) are also similar to what we observed for SBCP (19%), despite our estimate being based on the results of artificial incubation.

Meaningful estimates of environmental variation in demographic parameters require long-term study, particularly if temporal trends or other variance sources are to be identified (Gould and Nichols 1998; Burnham & Anderson 2002; Anderson 2008; Reinke et al. 2019). As a consequence, the number of species for which estimates of environmental variation are available is limited (Franklin et al. 2002; King et al. 2018; Milligan et al. 2018; Cayuela et al. 2019). Among turtles, we are aware of just one other such estimate. In bog turtles, the environmental SD in survival is 7.2% for young juveniles and 1.9% for adults (computed from coefficients of variation in Shoemaker et al. 2013), values similar to what we report for age 0 and adult Blanding's turtles (10.5% and 3.3% respectively). In the absence of such estimates, values used when generating population projections are sometimes arbitrary and potentially inaccurate. For example, one might be tempted to use default Vortex settings for the environmental SD of % females reproducing (10%), age 0 mortality (10%), juvenile mortality (3%) and adult mortality (3%). And in the absence of long-term study with repeated observations of the same females, one might use the observed SD in clutch size (= 3.3 offspring at SBCP, a value that includes both environmental and sampling variation) in lieu of a variance component estimate (environmental SD = 0.9 offspring at SBCP). Population projections are likely to be biased as a result, potentially causing unwarranted optimism or pessimism about conservation status. Despite spanning 15 years, data from SBCP still represent less than one Blanding's turtle generation. Consequently, temporal trends in environmental variation and the magnitude of environmental variation over longer time frames remains unknown.

In turtle populations, an absence of juveniles has sometimes been interpreted to indicate unsustainably low recruitment (Browne and Hecnar 2007; Howell et al. 2019), leading managers to implement corrective measures aimed at increasing juvenile survival (nest caging, artificial hibernation, head-starting, predator control). Our results suggest that high variance in age 0 survival can also result in an absence of some juvenile classes (Supplemental Material, Figure S1). This observation, when coupled with possible differences in detection probability between juvenile and adult Blanding's turtles, suggests caution when interpreting observed population age structure. Possibly, years with high recruitment may occur frequently enough to ensure population persistence. This is not to say that measures to increase juvenile survival are ineffective; our sensitivity analyses suggest that such efforts should fuel population growth and reduce extinction risk. Alternatively, surplus animals could be used in augmentation or reintroduction programs (Spencer et al. 2017).

Our review of Blanding's turtle studies demonstrates that as in other turtles (Miller and Dinkelacker 2007), demographic parameters vary geographically. For example, we report two-fold or greater differences in age at first reproduction, % females reproducing, clutch size, components of age 0 mortality, and adult mortality. Some of this variation may reflect geographic clines; for example age at first reproduction = 10-11 years in Nebraska, 12-14 years in the Midwest, and 17-22 years in Nova Scotia suggesting a latitudinal gradient as seen in Wood turtles (Greaves and Litzgus 2009). Other parameters vary greatly even at a fine spatial scale. For example, mean clutch size varies from 8-14.5 among northern Illinois Blanding's turtle populations, presumably as a consequence of corresponding variation in mean female size (Ruane et al. 2008). Unfortunately, data are too few to characterize geographic clines and parameter covariation statistically as has been done for some reptiles (Moll 1973; Iverson et al. 1993; Ashton et al. 2003; Litzgus and Mousseau 2006; Greaves and Litzgus 2009; Jones et al. 2012; Hileman et al. 2017), leaving open the question of whether variation in one parameter (e.g., age at first reproduction) is offset by compensatory variation in other parameters (% females reproducing, clutch size, survival) as predicted from partial correlations among parameters resulting in population persistence and stochastic-r ≥ 0 in our sensitivity analysis of geographic parameter variation.

Population viability and sensitivity

Population viability analysis of the SBCP Blanding's turtle population suggests a low risk of extinction, even after mortality rates were increased and clutch size was reduced to achieve zero population growth. This result is consistent with the realized adult population growth parameter, λ, which indicates that population size at SBCP is stable or only slightly increasing (personal observation). Including catastrophes (at an average rate of one per generation and with a reduction in survival to 75% base-line levels) had only small to modest effects on PVA outcomes except for population size, which was markedly smaller (43%) in zero-growth models. Sensitivity tests designed to encompass uncertainty in parameter estimates at SBCP also result in a somewhat more cautionary interpretation; with parameter uncertainty, probability of extinction over 100 years is 16% compared to 0% when uncertainty is ignored. Uncertainty in age 0, juvenile, and adult mortality rates were the largest contributors to population growth rate and probability of extinction. The environmental SD in age 0 mortality had a modest effect on extinction risk and age at first reproduction had a modest effect on population growth rate. Because uncertainty varies among parameters (e.g., uncertainty is greater for age 0 and juvenile mortality than for adult mortality), our sensitivity tests of parameter uncertainty differ from what might be generated e.g., using matrix methods (Mills et al. 1999; Caswell 2019). Thus, while life-table and matrix methods suggest that adult mortality may have the largest impact on Blanding's turtle population viability (Congdon et al. 1993, 2000; Heppell 1998), our results indicate that precise estimates of other demographic parameters (age 0 mortality, juvenile mortality) may be equally important in generating meaningful population projections. Our results also serve to identify demographic parameters that might effectively be targeted for research and management (Klein et al. 2017; Manlik et al. 2017). For example, viability analyses could be improved with more precise estimates of age-specific mortality. Given that adult mortality is already relatively low, management efforts might most effectively be focused on reducing age 0 and juvenile mortality and their environmental variation. Put simply, it may be easier to reduce age 0 mortality by 10% than to reduce adult morality by 1%. Similarly, while adult mortality was the most influential contributor to population growth in diamondback terrapins, reducing age 0 mortality is also necessary to ensure population growth (Crawford et al. 2014). This and other studies (e.g., Reed et al. 2009; Klein et al. 2017; Spencer et al. 2017; Mullin et al. 2020) argue for pluralistic approaches to turtle conservation management.

Sensitivity tests designed to encompass geographic variation in demography demonstrate that in addition to mortality rates, age at first reproduction, % females reproducing, clutch size, and initial population size all have the potential to influence Blanding's turtle population growth, extinction risk, or both. Furthermore, population growth and persistence is possible for nearly the full range of values sampled for any given parameter, but only when other parameters fall within certain bounds; of 30,000 parameter value combinations, just 16% resulted in population persistence with stochastic-r ≥ 0. Thus, while seven of eight estimates of annual adult mortality were less than 6.5%, the exception, a Nebraska population where adult mortality = 31% (Ruane et al. 2008), could represent a viable population if the values of other parameters compensated for high adult mortality. First reproduction is estimated to occur at 10 years at this site (Germano et al. 2000), the youngest age of first reproduction of any Blanding's turtle population. High and female biased (41% in adult females vs. 10% in adult males) mortality at this site has been attributed transportation infrastructure; a state highway lies immediately south and a two-track rail line lies immediately north of wetlands inhabited by Blanding's turtles (Ruane et al. 2008). However, given the early age of reproductive maturity at the site, Blanding's turtles may persist even with adult mortality rates that exceed those reported elsewhere. In contrast, in Nova Scotia, where age of reproductive maturity is ≥ 17 years (Standing 1997; McNeil 2002; The Blanding's Turtle Recovery Team 2003), low mortality rates, perhaps coupled with high reproductive rates (% female reproducing, clutch size), are likely necessary for persistence. Similar conclusions regarding expected patterns of parameter covariation emerge from life-table analyses but in the absence of demographic and environmental stochasticity (Congdon et al. 2000). Demographic data are too incomplete for most Blanding's turtle populations to meaningfully test for such patterns or to assess the degree to which observed variation is the result of sampling error. However, the direction and relative magnitude of parameter covariation can be predicted from partial correlations among parameters in PVA iterations resulting in population persistence and stochastic-r ≥ 0 in our sensitivity analysis (e.g., that there is strong negative covariation between age at first reproduction and adult survival across populations).

Sensitivity to Population Size

For populations with demographic characteristics similar to the SBCP population, our models demonstrate that when initial adult population size is small, risk of extinction is high and loss of genetic variation is rapid. In the absence of catastrophes, extinction risk drops and retention of genetic variation increases rapidly as initial adult population size increases such that even in our high environmental stochasticity scenario, extinction risk is low (<5% over 100 years) at initial population sizes >50 adults and retention of genetic variation is high (>95% over 100 years) at initial population sizes >120 adults. Initial population size thresholds to avoid extinction and retain genetic variation are even lower in 50 year scenarios (>20 and >50 adults in our high environmental stochasticity scenario, respectively). Including catastrophes shifts these population size thresholds upward (e.g., to >200 adults in 100 year scenarios and >65 adults in 50 year scenarios if catastrophes occur at an average rate of once per generation and reduce survival to 75% base-line levels). Given the geographic variation seen in Blanding's turtle demography, these results are likely to be most relevant for populations in the southern Great Lakes region and less so for populations to the southwest and northeast where age at first reproduction is lower and higher, respectively.

Our results also demonstrate the need for clear goals when setting population size criteria. As noted above, the initial adult population size necessary to retain some threshold proportion of genetic variation may differ from that necessary to exceed an extinction risk threshold. Furthermore, small populations necessarily retain less absolute genetic variation (number of alleles) than large populations even if the proportion of variation retained is high, potentially limiting adaptive responses within small isolated populations. Consequently, small Blanding's turtle populations may require genetic management (e.g., via facilitated gene flow, Frankham et al. 2017, 2019; Jordan et al. 2019). Likewise, the initial population size necessary to meet genetic and extinction risk thresholds differs depending on the time period over which goals are to be met (e.g., 50 vs. 100 year in our analyses). Population projections generated using PVA assume that demographic parameters remain unchanged for the duration of simulations, an unlikely assumption over long time periods. But population dynamics play out on a time scale of generations which for Blanding's turtles can be 25 years or more; consequently, simulations of 50 or 100 years represent just 2-4 generations. Although our thresholds for extinction risk and retention of genetic variability (5% extinction risk or 95% retention of genetic variation over 50 or 100 years) are widely used (IUCN 2012), more stringent thresholds and longer time frames are not uncommon (e.g., 150 years, Howell and Seigel 2019; 1% extinction risk in 40 generations, Reed et al. 2003, Trail et al. 2007; 1% extinction risk in 100 years, Wang et al. 2019). This problem has practical implications for listing decisions for long-lived species as reflected in recent controversy surrounding the meaning of "foreseeable future" under the U.S. Endangered Species Act (USDOI 2009; Almy 2017; Lake and Petersen 2017; USFWS 2019b).

Ongoing management at SBCP and the protected status of Blanding's turtles in Illinois provides some assurance that the demographic parameter estimates we generated are unlikely to change dramatically in the near term. We sought to exclude the effects of predator removal on hatching success in our PVA but beneficial effects of management on other parameters (e.g., juvenile survival) are unknown. Even at this site, the possibility exists that disease, climate change, invasive species, increased human population density, or catastrophic events will impact demographic parameters in the future. Climate change is expected to result in an increasing frequency of extreme weather, possibly resulting in mass mortality events that may increase extinction risk (IPCC 2014). This is of particular concern for turtles because of the magnitude of anticipated impacts and their limited ability to respond demographically (Ihlow et al. 2012; Keevil et al. 2018; Mullin et al. 2020). As linkages among extreme weather, mortality, and extinction risk are resolved for Blanding's turtles and other species, more sophisticated population projection models incorporating changing future conditions will be possible (e.g., Chan et al. 2005; Cardoso et al. 2008; Frederiksen et al. 2008) but even our fairly basic catastrophe scenario suggests that increasing extreme weather events and other catastrophes will increase extinction risk and the adult population size needed to withstand such events.

Regardless of conservation goals, our results demonstrate that small Blanding's turtle populations (fewer than ca. 50 adults) are unlikely to persist in the absence of active management. In theory, this population size cut-off could provide a criterion for prioritizing populations for management or selecting among management tactics. In practice, accurate estimates of population size, even for small populations, are challenging to generate. Effective Blanding's turtle population monitoring is a multi-year labor-intensive low-yield endeavor

(Congdon et al. 2008, 2011; Reid et al. 2016). At SBCP, effort averaged more than 1,665 trap nights per year and capture rate averaged just 0.02 adults per trap night from 2004-2018. For many Blanding's turtle populations, systematic monitoring is lacking and information on abundance is limited to element occurrences of unmarked animals in state natural heritage databases without corresponding information on effort. Consequently, our ability to apply population size criteria for conservation and management decisions is currently limited.

Accurate estimates of population size are just one of several data gaps hindering effective Blanding's turtle conservation. Age-specific survival, particularly for younger age classes, has been estimated infrequently and with wide confidence intervals, limiting our understanding of variation among populations and precision of population projections. Environmental drivers of demographic parameters are also poorly known. Our review suggests that age of first reproduction may vary clinally, possibly driven by climate. Habitat quality (e.g., resource availability) might also influence reproductive parameters (age at first reproduction, reproductive frequency, clutch size). If so, reproductive rates might be increased by targeting habitat characteristics for management (Tracy et al. 2006). Similarly, patterns of offspring sex ratio variation are unknown, limiting our ability to meaningfully model sex ratio effects on population projections or predict responses to climate change (Janzen 1994; Valenzuela et al. 2019). Models incorporating offspring sex ratio variation might also include differential male vs. female mortality, particularly that resulting from roads and railways that are thought to inflate female mortality in Blanding's and other freshwater turtles (Gibbs and Steen 2005; Steen et al. 2006; Ruane et al. 2008; Vanek and Glowacki 2019).

As with Blanding's turtles, detailed demographic data and associated population viability analyses are accumulating for other freshwater turtles, providing insights into threat impacts and alternative management strategies (Fordham et al. 2008; Famelli et al. 2012; Folt et al. 2016; Rachmansah et al. 2020). Some turtle PVAs result in quite pessimistic prognoses such that even small changes in demographic parameters (e.g., slightly increased adult mortality) result in a high probability of extinction (Bulté et al. 2009; Midwood et al. 2015; Howell and Seigel 2019; Piczak et al. 2019; but see Shoemaker et al. 2013). More pessimistic results may arise from the use of a longer time frame (150-500 years), use of parameters borrowed from other locations or species, or inappropriate specification of initial population size (e.g., setting initial population size equal to estimated adult population size but then applying a stable age distribution which, in Vortex, results in a smaller number of adults than intended; Bulté et al. 2009; Midwood et al. 2015; Howell and Seigel 2019). Regardless, freshwater turtle PVAs highlight the potential importance of metapopulation structure, inbreeding depression, catastrophes, road mortality, fisheries bycatch, invasive predators, and subsistence harvest (Fordham et al. 2008; Bulté et al. 2009; Enneson and Litzgus 2009 Famelli et al. 2012; Midwood et al. 2015; Howell and Seigel 2019; Piczak et al. 2019). Population viability analyses also demonstrates opportunities for management interventions, including head-starting, predator reduction, and roadside barriers (Spencer et al. 2017; Crawford et al. 2018; Mullin et al. 2020), suggesting future directions for Blanding's turtle PVA.

Population viability and sensitivity analyses indicate that Blanding's turtle populations with demographic characteristics similar to the SBCP population possess resiliency to withstand annual environmental stochasticity but are less resilient to catastrophes (Shaffer and Stein 2000;

Wolf et al. 2015). Demographic characteristics contributing to resiliency include a population size sufficient to ensure persistence over a time frame of 50-100 years (20-30 adults for 50 year persistence and 50-150 adults for 100 year persistence with catastrophes as modeled here), annual adult survival that exceeds 90%, high reproductive rates (more than 90% of older adult females reproduce each year), and age 0 and juvenile survival sufficient to maintain a stable or growing population. Demographic characteristics of resilient populations may vary geographically, depending for example, on age of first reproduction. Resiliency also arises from habitat characteristics. Our SBCP study site encompasses 215 ha of high-quality coastal wetland that provides active-season, nesting, and over-winter habitat and is actively managed to promote Blanding's turtle recruitment and survival. Given the density of adult Blanding's turtles at SBCP (ca. 0.5/ha), sites less than 40-100 ha may be too small to support resilient populations. However, Blanding's turtle population density and home-range area can be quite variable (Lang 2004; Piepgras and Lang 2000; Schuler and Thiel 2008; Edge et al. 2010; Millar 2010; Congdon et al. 2011), making area a poor proxy for quantitative estimates of population size.

Future modeling efforts might profitably focus on regional patterns of Blanding's turtle persistence by including multiple populations and catastrophes of varying spatial extent, thus providing guidance on the level of redundancy necessary to meet conservation goals (Shaffer and Stein 2000; Wolf et al. 2015). Such models might also incorporate realistic rates of natural or facilitated gene flow, as well as the effects of inbreeding depression, to better guide genetic management (Frankham et al. 2017; 2019). Simultaneously, an expanded understanding of patterns of neutral and adaptive genetic variation, potentially coupled with species distribution modeling, would aid in determining the extent of range-wide representation necessary to ensure the potential for adaptation to environmental change (Shaffer and Stein 2000; Wolf et al. 2015; Hamilton et al. 2018; Jordan et al. 2019).

Supplemental Material

Table S1. Examples of mass mortality events among freshwater and terrestrial turtles, including information on the number of deaths ("Deaths"), % population decline ("Decline"), and cause.

Table S2. Reproductive status (0 = nongravid, 1 = gravid) of known age female Blanding's turtles Emydoidea blandingii that reached reproductive maturity during the course of our study at Spring Bluff Chiwaukee Prairie 2004-2018. Proportion reproducing for turtles ages 13-18 is based on the results of logistic regression (Supplemental Material, Figure S1). Proportion reproducing for turtles ages 19 and older is based on the observed proportion among adult females of unknown age (Results).

Table S3.Annual proportion of adult female Blanding's turtles Emydoidea blandingii reproducing at Spring Bluff Chiwaukee Prairie 2012-2018 as determined by palpation. Total variance is the variance across years; error variance is the mean of year-specific binomial variances (= p(1-p)/(n-1); environmental variance = total variance - error variance (following Akçakaya 2002).

Table S4. (A) Size (number of eggs laid) of 120 Blanding's turtle Emydoidea blandingii clutches produced by 40 females from Spring Bluff Chiwaukee Prairie 2008-2018 and (B) associated variance components computed using the restricted maximum likelihood method in SPSS (13 females for which we have data for only a single clutch were excluded).

Table S5. Annual proportion of Blanding's turtle Emydoidea blandingii eggs that hatched from Spring Bluff Chiwaukee Prairie females 2010-2018 as part of the Lake County Forest Preserve District head-starting program. Total variance is the variance across years; error variance is the mean of year-specific binomial variances (= p(1-p)/(n-1); environmental variance = total variance - error variance (following Akçakaya 2002).

Table S6. Worksheet for the calculation of Blanding's turtle Emydoidea blandingii age 0 survival uncertainty (A) and age 0 survival environmental variance (B). Nest success and hatch success are estimated from data collected at Spring Bluff Chiwaukee Prairie 2004-2018; post-hatch survival is from Kastle et al. (in press). Calculaton of the variance of a product (nest success*hatch success*post-hatch survival) follows Goodman (1960) and assumes covariances among survival components are zero. Because environmental variance in post-hatch survival is unknown, we estimate lower and upper bounds by setting this variance to 0.0000 and 0.0340 (to equal environmental variance in nest success), respectively.

Table S7. Annual variation in the proportion of Blanding's turtle Emydoidea blandingii nests surviving at a Michigan study site 1976-1998 (from Table 1 in Congdon et al. 2000) and its associated environmental variance. Total variance is the variance across years; error variance is the mean of year-specific binomial variances (= p(1-p)/(n-1); environmental variance = total variance - error variance (following Akçakaya 2002).

Table S8. Estimated Spring Bluff Chiwaukee Prairie adult Blanding's turtle Emydoidea blandingii population size 2004-2018. Estimates (Ni) and confidence limits are highlighted in yellow, three year running average estimates and confidence limits are highlighted in blue. Symbolism and table organization follows Manly (1984).

Table S9. Results of single-variable sensitivity tests of parameter uncertainty from the Blanding's turtle Emydoidea blandingii PVA. Parameter values and parameter uncertainty eere mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1). Shown are the ranges of Stochacstic-r and Probability of Extinction across parameter values and correaltions between Stochastic-r and Probablity of Extinction and parameter values.

Table S10. Results of logistic regression analysis of sensitivity test results and associated calculations of relative influence from the Blanding's turtle Emydoidea blandingii PVA. Parts A-D represent analyses of sensitivity to parameter uncertainty; parts E-F represent analyses of sensitivity to geographic parameter varition. Included in A-D are analyses based on all 30,000 LHS samples and 3 random subsamples of n = 10,000 each. Dependent variables include the binomial transformation of stochastic-r(A and E), probability of extinction (B and F), binomal transformation of gene diversity,(C) and binomial transformation of adult poulation size (D). Parameter values in sensitivity tests of parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1). Parameter values used in sensitivity tests of range-wade parameter uncertainty are from throughout the species' range in North America (Table 1; Supplemental Material, Table S11). Shown are standardized partial logistic regression coefficients (B), their standard errors (S.E.), Wald statistic (Wald) and associated degrees fo freedom (df) and significance (Sig.), and odds ratio (Exp(B)). Relative influence was acheived by calculated by computing B/S.E. and its absolute value (Abse(B/S.E.)), dividing the latter by the sum of Abs(B/S.E.) across parmaters and multiplying by 100.

Table S11. Geographic variation in Blanding's turtle *Emydoidea blandingii* demographic parameters from throughout the species' range in North America. Parameters are listed in the order that they are used in Vortex PVA software. Locations are ordered from northeast to southwest.

Figure S1. Logistic regression of reproductive status (gravid = 1, not gravid = 0) on age for 9 Blanding's turtle *Emydoidea blandingii* females achieving reproductive maturity during our study at Spring Bluff Chiwaukee Prairie 2004-2018 (Supplemental Material, Table S1). Vertical dashed line at 13 years represents the youngest age of first reproduction in our study. The likelihood of reproducing increases with age as $1/(1+(e^{(-1*(-7.839+(0.551*Age)))}))$ ($\chi^2 = 34.26$, $P < 0.001$), yielding probabilities of 0.34, 0.47, 0.73, 0.82, and 0.88 for 13, 14, 15, 16, 17, and 18 year old turtles.

Figure S2. Blanding's turtle *Emydoidea blandingii* population viability analysis results showing mean (standard deviation) population size over 1,000 iterations of our SBCP (blue) and zero-growth (red) models. Parameter values for the SBCP model and in sensitivity tests of parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018. Clutch size and mortality rates were adjusted to achieve deterministic r = 0 for the zero-growth model.

Figure S3. Example of annual variation in Blanding's turtle *Emydoidea blandingii* age structure in the zero-growth population model that result from years in which age 0 mortality = 100% (A) and the corresponding deterministic stable age distribution (B). Parameter values were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). Shown in A from top to bottom is a sequence of 20 years from one Vortex iteration. Histograms represent the number of individuals surviving to the end of a given age class. Adult age classes (≥13) are pooled.

Figure S4. Sensitivity of probability of extinction to parameter uncertainty in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values and parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). For each relationship, other parameters were held constant at their mean values. P(Extinction) refers to the probability of extinction. EV refers to environmental variation (standard deviation).

Figure S5. Sensitivity of gene diversity to parameter uncertainty in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values and parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). For each relationship, other parameters were held constant at their mean values. P(Gene Diversity > median) refers to the probability that the final genetic diversity is above the median (0.961). EV refers to environmental variation (standard deviation).

Figure S6. Sensitivity of adult population size to parameter uncertainty in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values and parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). For each relationship, other parameters were held constant at their mean values. P(Adults > median) refers to the probability that the final adult population size is above the median (43). EV refers to environmental variation (standard deviation).

Figure S7. Sensitivity of probability of extinction to geographic parameter variation in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values are from throughout the species' range in North America (Table 1; Supplemental Material, Table S11). For each relationship, other parameters were held constant at their mean values. (Extinction) refers to the probability of extinction. EV refers to environmental variation (standard deviation).

Figure. S8. Relationship of probability of extinction (upper panel) and genetic diversity (lower panel) to initial adult Blanding's turtle *Emydoidea blandingii* population size with (filled diamonds) and without (open circles) catastrophes in scenarios lasting 50 yr. Parameter values were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). Solid lines represent a scenario with intermediate environmental stochasticity and are bracketed by dashed (with catastrophes) and dotted (without catastrophes) lines that represent scenarios with low and high environmental stochasticity. Solid horizontal solid lines correspond to 5% probability of extinction (upper panel) and 95% retention of genetic diversity (lower panel).

Text S1. Comments on Blanding's turtle *Emydoidea blandingii* PVA implementation in Vortex.

File S1. Vortex xml file.

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Table 1. Demographic parameter values used in Blanding's turtle *Emydoidea blandingii* population viability analyses and sensitivity tests. Parameter values for the SBCP model and in sensitivity tests of parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018. Clutch size and mortality rates were adjusted to achieve deterministic r = 0 for the zero-growth model. Parameter values used in sensitivity tests of range-wade parameter uncertainty are from throughout the species' range in North America (Supplemental Material, Table S11). Parameters are ordered to match their order in Vortex. SD refers to environmental standard deviation.

Parameter		**SBCP model**	**Zero-growth model**	**Sensitivity tests**	
				Parameter uncertainty	**Range-wide parameter variation**
Inbreeding depression[a]		0[b]	0	0-7[c]	0
Age of first reproduction		13	13	12-14	10-22
Maximum age[a]		83[b]	83	55-85	83
Sex ratio (% male)		50[b]	50	40-60	50
% Females reproducing	1st yr	33	33	29-37[d]	33[d]
	2nd yr	47	47	43-51[d]	47[d]
	3rd yr	60	60	56-64[d]	60[d]
	4th yr	73	73	69-77[d]	73[d]
	5th yr	83	83	79-87[d]	83[d]
	6th yr	89	89	85-93[d]	89[d]
	7th+ yr	92.5	92.5	88.5-96.5[d]	40.5-96.5[d]
SD in % females reproducing[a]		0	0	0-10	0
Clutch size		13.0	10.50	9.9-11.1	7.4-17.7
SD in clutch size[a]		0.9	0.9	0-3.75	0.9
Age 0 mortality (%)		86.8	91.3	82.5-98.0	72-99
SD in age 0 mortality (%)		10.5	10.5	5.3-15.8	5.3-15.8

Juvenile mortality (%)	Age 1	28.9	33.4	28.3-46.2[e]	28.3-46.2[e]
	Age 2	14.6	19.1	13.0-34.3[e]	13.0-34.3[e]
	Age 3	6.9	11.4	6.4-23.9[e]	6.4-23.9[e]
	Age 4	5.3[f]	9.8	6.8-17.3[e]	6.8-17.3[e]
	Age 5	5.3[f]	8.8	7.2-12.9[e]	7.2-12.9[e]
	Age 6	5.3[f]	8.8	7.4-12.3[e]	7.4-12.3[e]
	Age 7	5.3[f]	8.8	7.4-12.3[e]	7.4-12.3[e]
	Age 8	5.3[f]	8.8	7.4-12.8[e]	7.4-12.8[e]
	Age 9	5.3[f]	8.8	7.4-12.8[e]	7.4-12.8[e]
	Age 10+	5.3	7.8	6.4-11.3[e]	6.4-11.3[e]
SD in juvenile mortality (%)[g]		3.3[f]	3.3	0-4	0-4
Adult mortality (%)		5.3	7.8	6.3-9.8	3.5-31
SD in adult mortality (%)[g]		3.3	3.3	0-4	0-4
Initial N		430[h]	304[h]	254-403[i]	15-403[j]
Carrying capacity (adults)[a]		208[b]	208	104-312	208
SD in carrying capacity[a]		0[b]	0	0-50	0

[a] Excluded from LHS analysis

[b] Unknown for SBCP, see text

[c] Lethal equivalents with 50% due to recessive lethal alleles

[d] For parameter uncertainty sensitivity analysis, % females reproducing was varied jointly across age classes by ±4%; for geographic parameter variation sensitivity analysis, % females reproducing for females in their 1st to 6th year of reproduction was set to the minimum of the estimated age-specific value and the sensitivity test value selected for females in their 7th or later year of reproduction

[e] Juvenile mortality was varied jointly across age classes in proportion to the age-specific confidence interval

[f] Adjusted to equal adults

[g] Combined for LHS analysis

[h] Stable age distribution with 104 adults

[i] Stable age distribution with 87-138 adults

[j] Stable age distribution with 5-138 adults

Table 2. Relative influence of parameter uncertainty and geographic parameter variation on Blanding's turtle *Emydoidea blandingii* population growth (Stochastic-r), probability of extinction, gene diversity, and adult population size. Darker shading indicates greater relative influence; sign indicates direction. SD refers to environmental standard deviation. Parameter values in sensitivity tests of parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1). Parameter values used in sensitivity tests of range-wade parameter uncertainty are from throughout the species' range in North America (Table 1; Supplemental Material, Table S11).

	Parameter uncertainty				Geographic Parameter variation	
Parameter	Stochastic-r	Probability of extinction	Gene diversity	Adult population size	Stochastic-r	Probability of extinction
Age of first reproduction	-8.8	3.2	-7.6	-7.9	-17.5	15.0
% Females reproducing	3.5	-0.6	2.1	2.7	7.2	-8.0
Clutch size	4.9	-2.7	3.6	3.7	9.2	-9.9
Age 0 mortality	-29.8	33.4	-28.0	-28.7	-21.2	18.6
SD in age 0 mortality	-2.6	6.7	-4.0	-2.8	-1.6	1.4
Juvenile mortality	-27.4	25.2	-25.9	-26.8	-13.2	13.7
Adult mortality	-19.0	19.9	-18.7	-19.1	-27.7	22.4
SD in juvenile & adult mortality	-2.8	3.8	-2.8	-2.6	-0.7	1.1
Initial population size	1.2	-4.4	7.3	5.7	1.8	-9.9

Table 3. Bivariate correlations (above diagonal) and partial correlations (holding all other parameters constant, below diagonal) among Blanding's turtle *Emydoidea blandingii* demographic parameters resulting in population persistence and stochastic-r ≥ 0. Results are based on n = 4,704 iterations (out of a total of 30,000) generated in sensitivity analyses of geographic parameter variation using parameter values from throughout the species' range in North America (Table 1; Supplemental Material, Table S11).

	Age of first reproduction	% Females reproducing	Clutch size	Age 0 mortality	SD in age 0 mortality	Juvenile mortality	Adult mortality	SD in juvenile & adult mortality	Initial population size
Age of first reproduction		0.01	0.02	-0.01	-0.02	0.01	-0.38	0.01	-0.01
% Females reproducing	0.09		-0.03	0.06	0.00	0.05	0.11	-0.00	-0.01
Clutch size	0.14	-0.08		0.06	0.01	0.04	0.18	-0.00	0.01
Age 0 mortality	-0.18	0.13	0.16		-0.02	-0.12	-0.26	-0.02	-0.01
SD in age 0 mortality	0.01	0.00	0.01	-0.02		0.02	0.02	0.00	-0.01
Juvenile mortality	-0.16	0.13	0.14	-0.24	0.02		-0.26	0.02	0.00
Adult mortality	-0.44	0.19	0.27	-0.38	0.01	-0.36		-0.02	0.00
SD in juvenile & adult mortality	-0.01	0.00	0.00	-0.03	0.00	0.01	-0.02		-0.01
Initial population size	-0.01	-0.01	0.01	-0.01	-0.01	0.00	-0.00	-0.01	

Figure 1. Adult Blanding's turtle *Emydoidea blandingii*. Photo by Dee Hudson.

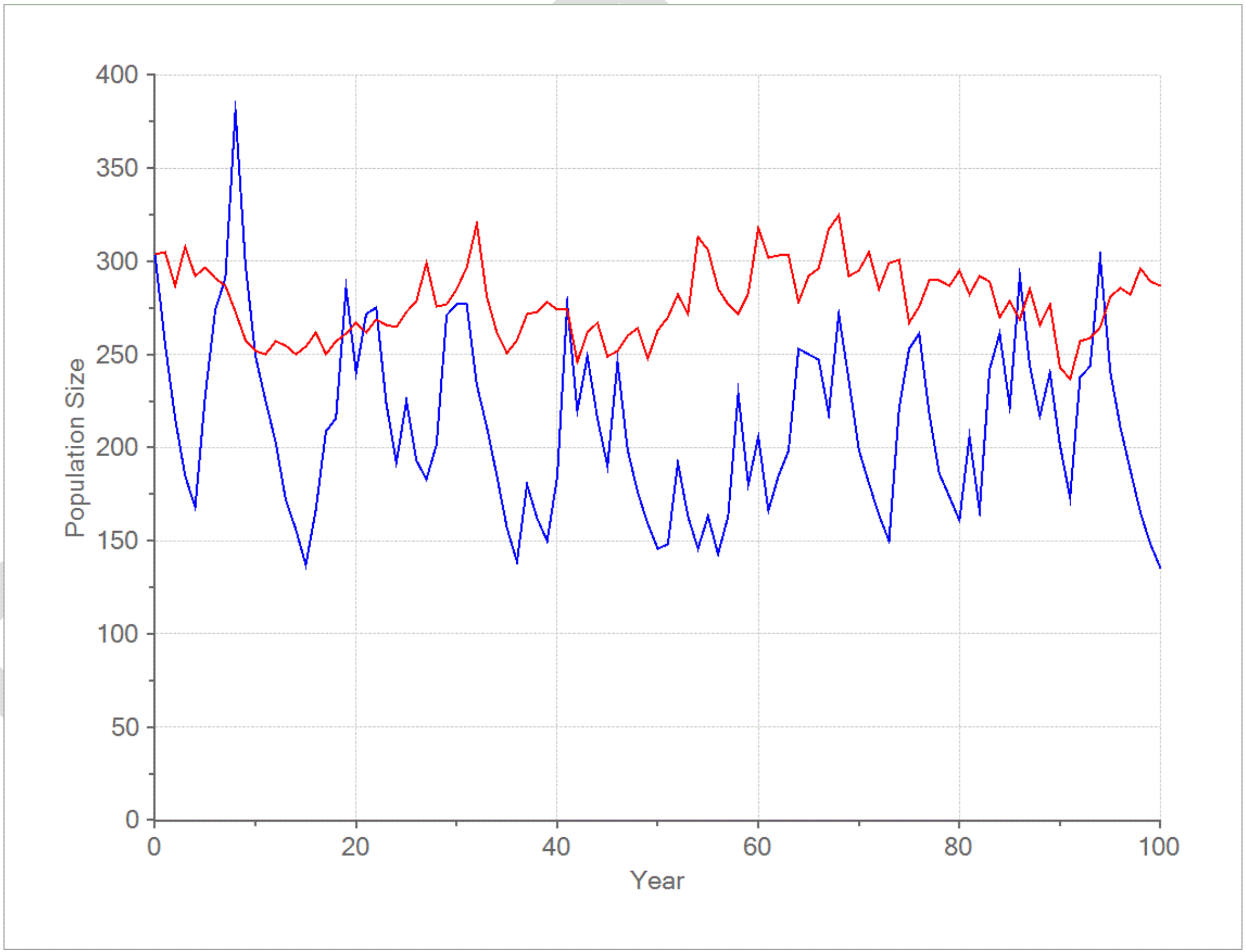

Figure 2. Single iterations of the zero-growth Blanding's turtle *Emydoidea blandingii* population model showing the impact of the presence (blue) or absence (red) of environmental variation in age 0 survival on variation in population size. Parameter values were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1).

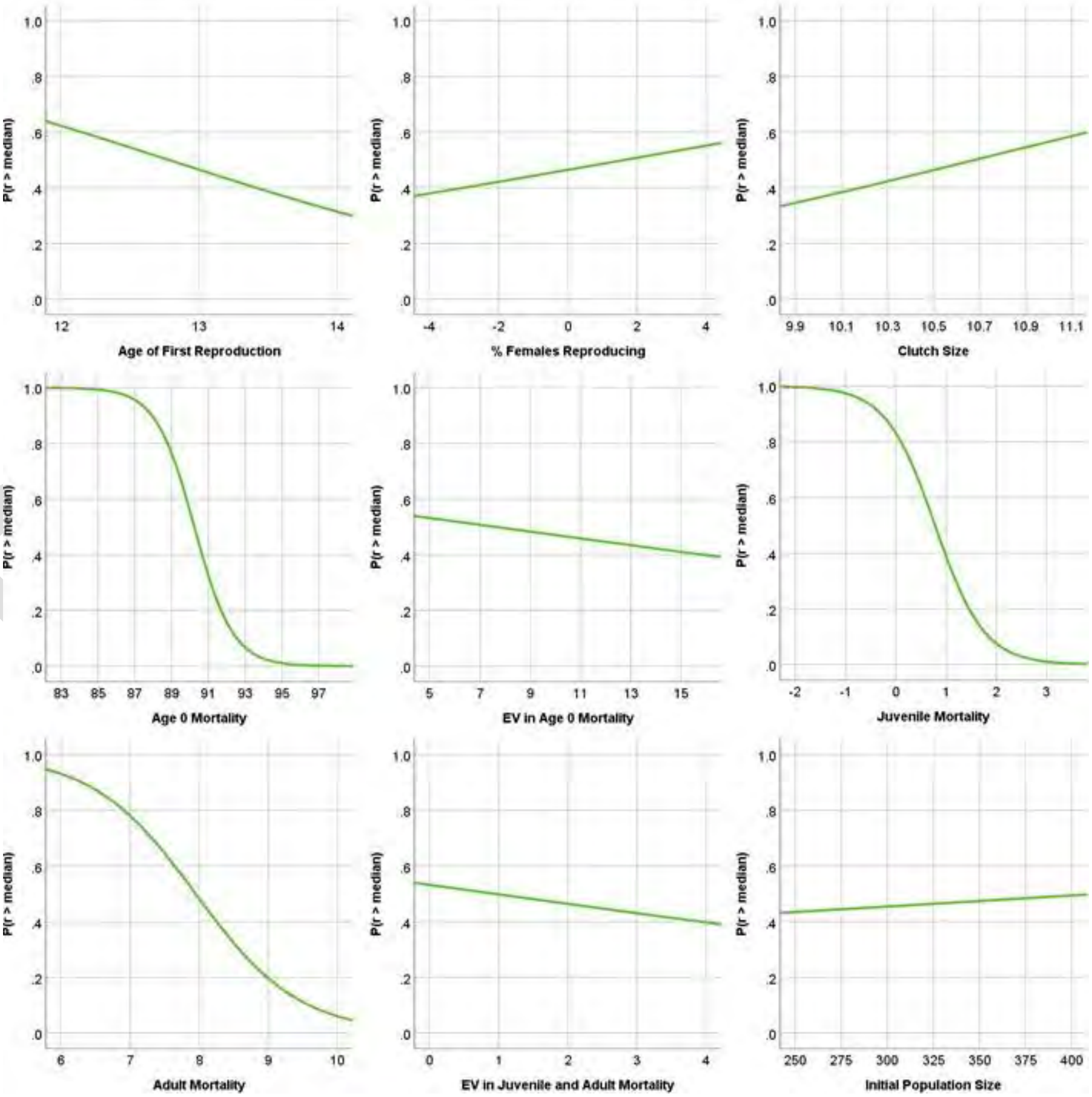

Figure 3. Sensitivity of stochastic-r to parameter uncertainty in the zero-growth Blanding's turtle *Emydoidea blandingii* model. Parameter values were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1). For each relationship, other parameters were held constant at their mean values. P(r > median) refers to the probability that the average population growth rate is above the median (-0.011). EV refers to environmental variation (standard deviation).

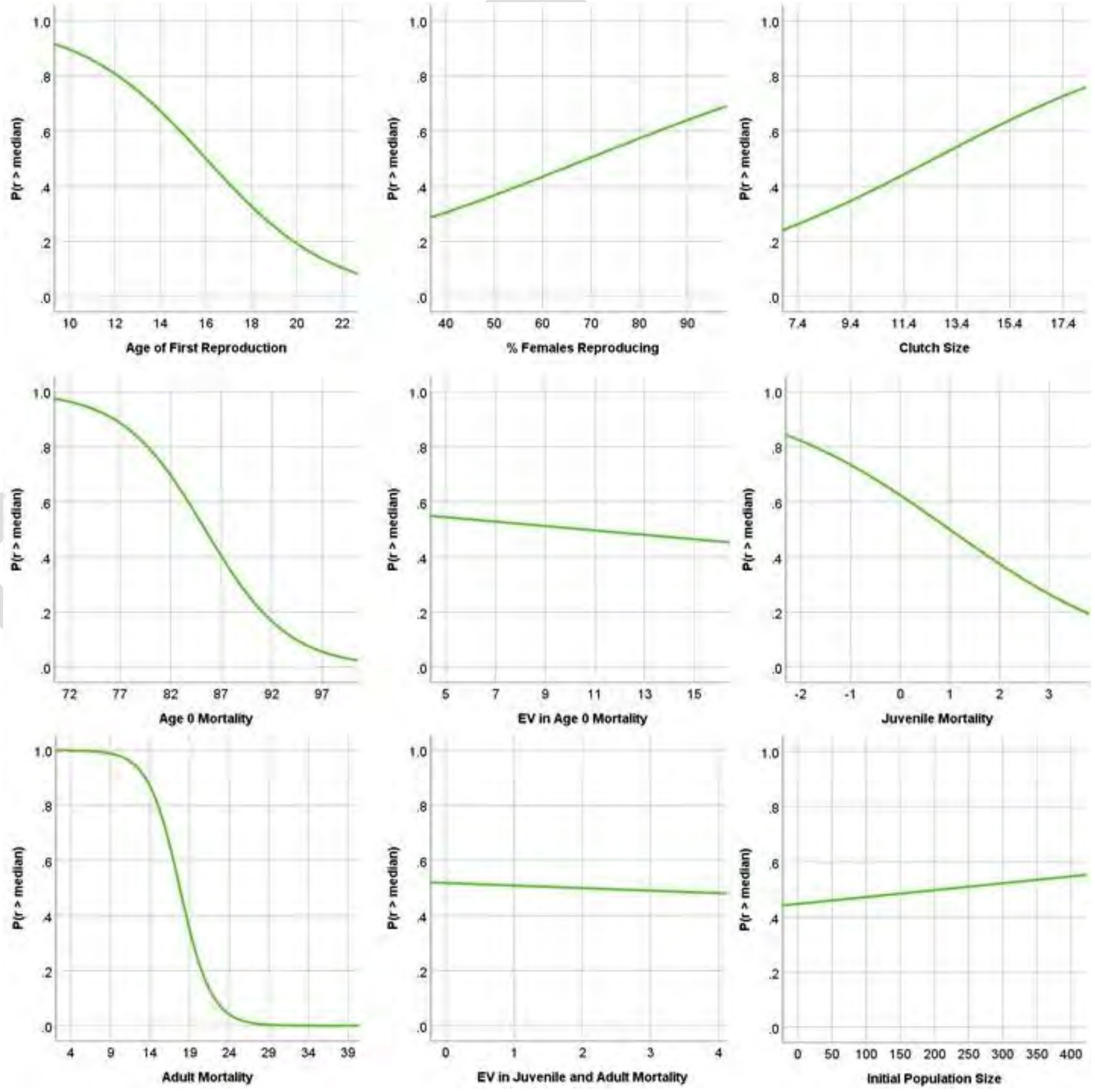

Figure 4. Sensitivity of stochastic-r to geographic parameter variation in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values are from throughout the species' range in North America (Table 1; Supplemental Material, Table S11). For each relationship, other parameters were held constant at their mean values. P(r > median) refers to the probability that the average population growth rate is above the median (-0.077). EV refers to environmental variation (standard deviation).

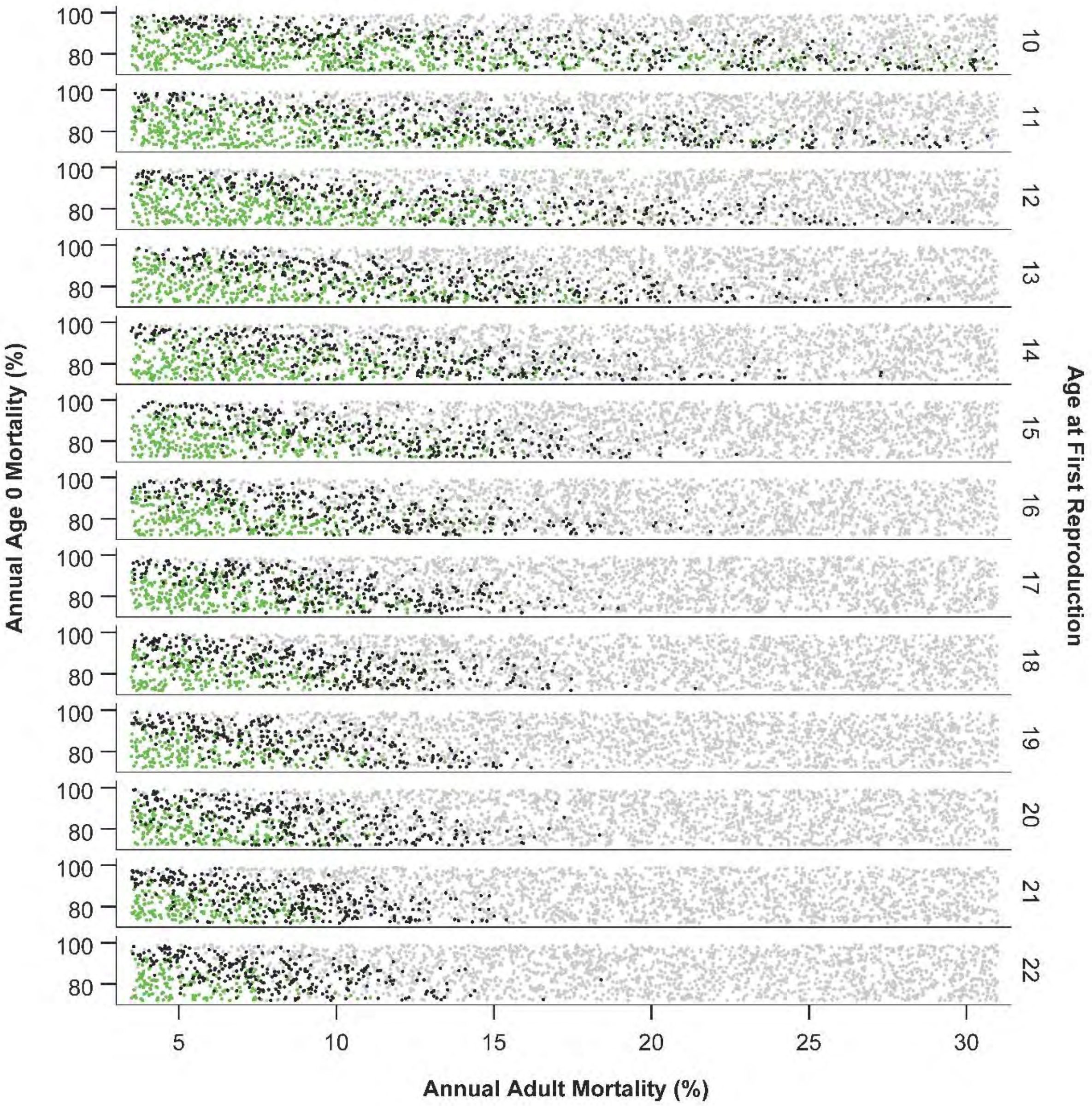

Figure 5. Combinations of parameter values for annual adult mortality, annual age 0 mortality, and age at first reproduction resulting in persistence with stochastic-r ≥ 0 (green points), persistence but with stochastic-r < 0 (black points) or extinction (gray points) in sensitivity analyses of Blanding's turtle *Emydoidea blandingii* geographic parameter variation. Parameter values are from throughout the species' range in North America (Table 1; Supplemental Material, Table S11).

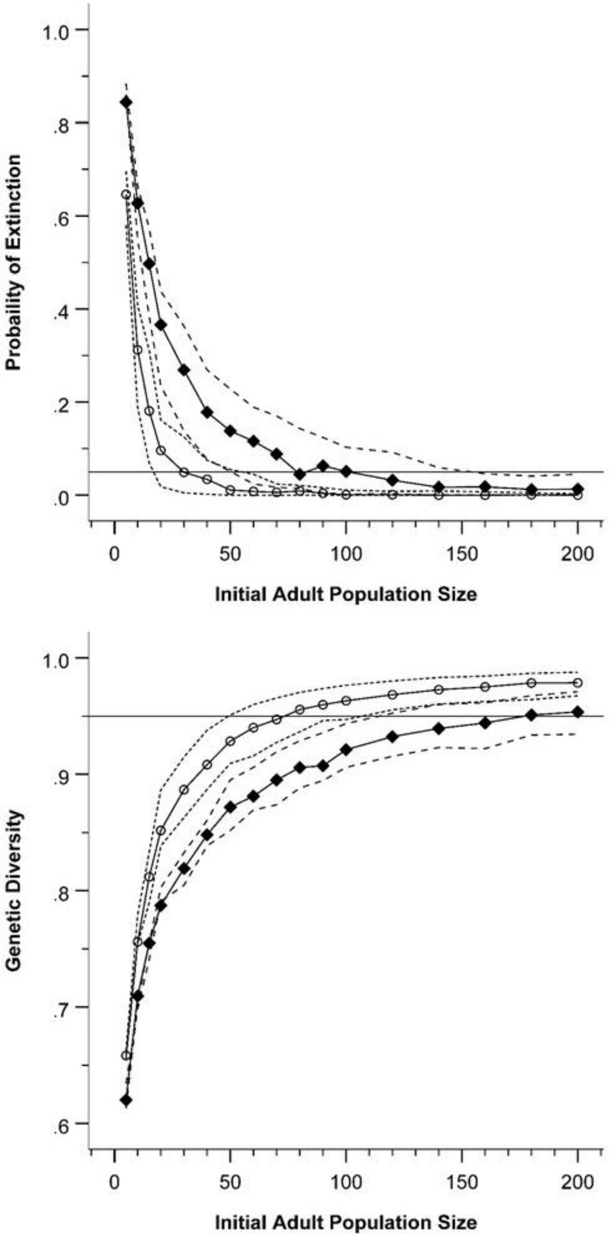

Figure 6. Relationship of probability of extinction (upper panel) and genetic diversity (lower panel) to initial adult Blanding's turtle *Emydoidea blandingii* population size with (filled diamonds) and without (open circles) catastrophes in scenarios lasting 100 yr. Parameter values were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1). Solid lines represent a scenario with intermediate environmental stochasticity and are bracketed by dashed (with catastrophes) and dotted (without catastrophes) lines that represent scenarios with low and high environmental stochasticity. Solid horizontal solid lines correspond to 5% probability of extinction (upper panel) and 95% retention of genetic diversity (lower panel).

Table S1. Examples of mass mortality events among freshwater and terrestrial turtles, including information on the number of deaths ("Deaths"), % population decline ("Decline"), and cause.

Common name	Scientific name	Habitat	Deaths	Decline	Cause	Comments	References
Softshell turtle	*Apalone ferox*	Freshwater	76		Predation	North American river otter predation during drought	Stacy et al 2015
Snapping turtle	*Chelydra serpentina*	Freshwater	34		Predation	North American River otter predation during winters of 1986-1989 (population monitored from 1980-2013, Keevil et al. 2018)	Brooks et al. 1990
Snapping turtle	*Chelydra serpentina*	Freshwater	3	43	Predation	North American river otter predation	Gassbarini 2016
Snapping turtle	*Chelydra serpentina*	Freshwater	6		Winterkill	Winterkill	Seburn 2015
Snapping turtle	*Chelydra serpentina*	Freshwater	12		Winterkill	Winterkill in 1 of eight years	Christiansen and Bickham 1989
Painted turtle	*Chrysemys picta*	Freshwater	31		Roads	Hatchling road mortality	Baxter-Gilbert et al. 2013
Painted turtle	*Chrysemys picta*	Freshwater	132		Winterkill	Winterkill in 1 of eight years	Christiansen and Bickham 1989
Painted turtle	*Chrysemys picta*	Freshwater	8		Predation	Predation on nesting females possibly by foxes	Cochran 1987
Painted turtle	*Chrysemys picta*	Freshwater			Hurricane	Inferred from change in age distribution; attributed to mortality associated with Hurricane Agnes	Ernst 1974
Painted turtle	*Chrysemys picta*	Freshwater	13	45	Predation	North American river otter predation	Gassbarini 2016

Common name	Scientific name	Habitat	Deaths	Decline	Cause	Comments	References
Painted turtle	*Chrysemys picta*	Freshwater	44		Predation	Predation by raccoons	Ross 1988
Wood turtle	*Clemmys insculpta*	Freshwater	35		Enigmatic	Adult female carcasses recovered from nesting area; predation and boat strikes were excluded as cause	Catrysse et al. 2015
Wood turtle	*Clemmys insculpta*	Freshwater		100	Recreation	Recreational activities	Garber and Burger 1995
River turtle	*Dermatemys mawii*	Freshwater	36		Predation	South American otter predation in 1 of 7 years	Platt and Rainwater 2011
Johnstone River snapping turtle	*Elseya irwini*	Freshwater	10		Disease	Disease?	Areial et al. 2017
Blanding's turtle	*Emydoidea blandingii*	Freshwater	53	53	Predation	North American river otter predation	Gassbarini 2016
Blanding's turtle	*Emydoidea blandingii*	Freshwater	49		Enigmatic	Uncertain cause	Sheppard 2014
Blanding's turtle	*Emydoidea blandingii*	Freshwater	25		Wetland draw-down	Predation, road morality, winterkill	Hall and Cuthbert 2000
European pond turtle	*Emys orbicularis*	Freshwater	182		Predation	European otter predation in winters of 2002-2003; turtles absent from otter diets in 1991-1998; Lanszki et al. 2001 J. Zool. 255:97-103	Lanszki et al. 2006

Common name	Scientific name	Habitat	Deaths	Decline	Cause	Comments	References
Desert tortoise	*Gopherus agassizii*	Terrestrial		50	Multiple	Collecting, vandalism, vehicle kills, habitat degradation, infectious disease, hyperpredation by ravens.	Berry et al. 2020
Desert tortoise	*Gopherus agassizii*	Terrestrial		30	Drought	Drought in 1 of 10 yr at 1 of 2 sites	Longshore et al. 2003
Map turtle	*Graptemys geographica*	Freshwater	13		Predation	Predation on nesting females possibly by foxes	Cochran 1987
Map turtle	*Graptemys geographica*	Freshwater		53	Harvest	Inferred from population estimates over time; attributed to harvest	Nickerson and Pitt 2012
Map turtle	*Graptemys geographica*	Freshwater	8		Winterkill	Winterkill in 1 of eight years	Christiansen and Bickham 1989
Yellow mud turtle	*Kinosternon flavescens*	Freshwater		8	Winterkill	Winterkill in 1 of eight years	Christiansen and Bickham 1989
Sonoran mud turtle	*Kinosternon sonoriense*	Freshwater	14	10	Winterkill	Wnter mortality (flood-frost event)	Massengill et al. 2014
Eastern mud turtle	*Kinosternon subrubrum*	Freshwater	300		Roads	Road mortality	Crawford and Doyle 2010
Bellinger River snapping turtle	*Myuchelys georgesi*	Freshwater	400		Disease & Drought	Disease coupled with drought?	Spencer et al. 2018
Toadhead turtle	*Phrynops hilarii*	Freshwater	23		Roads	Road mortality	Attademo et al. 2011
Florida cooter	*Pseudemys floridana*	Freshwater	>48		Predation	North American river otter predation during drought	Stacy et al 2014

Common name	Scientific name	Habitat	Deaths	Decline	Cause	Comments	References
Eastern box turtle	*Terrapene carolina*	Terrestrial	17	22	Disease & Winterkill	Upper respiratory disease and cold weather	Agha et al. 2017
Eastern box turtle	*Terrapene carolina*	Terrestrial	15	23	Disease	Ranavirus affecting relocated animals with a fenced 200 ha area	Johnson et al. 2008
Eastern box turtle	*Terrapene carolina*	Terrestrial	30		Disease	Ranavirus	Johnson et al. 2008
Eastern box turtle	*Terrapene carolina*	Terrestrial	7	21	Disease	Disease	Rossell 2009
Eastern box turtle	*Terrapene carolina*	Terrestrial	13	14	Winterkill	Winterkill in 1 of 23 yr	Seibert and Belzer 2016
Hermann's tortoise	*Testudo hermanni*	Terrestrial		50	Fire & Mechanical	Fire, mechanical (see also Stubs et al, 1985, Biological Conservation 31:125-152)	Hailey 2000
Spiny softshell turtle	*Trionyx spinifera*	Freshwater	26		Winterkill	Winterkill in 1 of eight years, hatchlings only	Christiansen and Bickham 1989
various turtle species		Freshwater	8842		Roads	Road mortality	Aresco 2005

Table S2. Reproductive status (0 = nongravid, 1 = gravid) of known age female Blanding's turtles *Emydoidea blandingii* that reached reproductive maturity during the course of our study at Spring Bluff Chiwaukee Prairie 2004-2018. Proportion reproducing for turtles ages 13-18 is based on the results of logistic regression (Supplemental Material, Figure S1). Proportion reproducing for turtles ages 19 and older is based on the observed proportion among adult females of unknown age (Results).

Turtle	Age (years)																						
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Davey									0	0						1	1	1	1	1			
Drew			0	0	0							0	0		1								
Gene										0								1	1	1	1	1	1
Geri														0				1	0	1			
Leslie						0			0								1	1					
Nico															1	1	1	1	1	1			
Shelley	0	0							0		1	1	1										
Starla					0							1	1										
Stefanie						0									1								
Proportion Reproducing											0.34	0.47	0.6	0.73	0.82	0.88	0.94	0.94	0.94	0.94	0.94	0.94	0.94

Table S3. Annual proportion of adult female Blanding's turtles Emydoidea blandingii reproducing at Spring Bluff Chiwaukee Prairie 2012-2018 as determined by palpation. Total variance is the variance across years; error variance is the mean of year-specific binomial variances (= p(1-p)/(n-1); environmental variance = total variance - error variance (following Akçakaya 2002).

Year	# of Females	# Gravid	Proportion Gravid	Binomial Variance
2012	11	10	0.9091	0.0083
2013	19	19	1.0000	0.0000
2014	25	24	0.9600	0.0016
2015	27	26	0.9630	0.0014
2016	26	25	0.9615	0.0015
2017	44	39	0.8864	0.0023
2018	48	42	0.8750	0.0023
Total	200	185	0.9250	
	Total Variance			0.0022
	Error Variance			0.0025
	Environmental Variance			-0.0003

Table S4. (A) Size (number of eggs laid) of 120 Blanding's turtle *Emydoidea blandingii* clutches produced by 40 females from Spring Bluff Chiwaukee Prairie 2008-2018 and **(B)** associated variance components computed using the restricted maximum likelihood method in SPSS (13 females for which we have data for only a single clutch were excluded).

A.

	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**# of Clutches**	**Mean Clutch Size**
Abigail	10						13					2	11.5
Beatrice						13	14		14	16	9	5	13.2
Betsy			9						8		9	3	8.7
Betty Ann									10		11	2	10.5
Beullah	11	10		15	13							4	12.3
Carter							11	10	9	11	9	5	10.0
Char								10		9	10	3	9.7
Davey									14			1	14.0
Dee Dee					11	13	12			10	14	5	12.0
Drew											10	1	10.0
Delia	16											1	16.0
Doreen			12	15								2	13.5
Elle Mae	14		16	17	19							4	16.5
Elma										14		1	14.0
Ester	10	8	13	16		15	15					6	12.8
Gene						7						1	7.0
Geri								15				1	15.0
Gillian	13	18	13	18	14				14			6	15.0
Josephine								9				1	9.0
Leslie										14	15	2	14.5
Lucinda				16	19		15		15			4	16.3
Lyda Jane	14		13	10	17	12				12		6	13.0
Mabel			10	12		7	11	11				5	10.2
Marcie						13	11	12				3	12.0
Mary			11	18	15	8		14				5	13.2
Miranda								9	10			2	9.5
Misty									13			1	13.0
Myrna				17	18		17					3	17.3
Nancy	8		10	14	15			12		12	12	7	11.9
Nico						12			11			2	11.5
Paulie							7				11	2	9.0
Rose										11	12	2	11.5
Sadie											9	1	9.0
Sara			16	17								2	16.5
Shelley									13			1	13.0
Shirley	8											1	8.0
Stefanie											10	1	10.0
Taylor											10	1	10.0
Venus								12		14	15	3	13.7
Viola	19			22	16	17	18	24		17		7	19.0
Zelda		17	13	15				13	15			5	14.6
# of Clutches	10	4	11	14	10	10	11	12	12	11	16	120	12.4
Mean Clutch Size	12.3	13.3	12.4	15.9	15.7	11.7	13.1	12.6	12.2	12.7	11.1		13.0

B.

Component	**Estimate**
Var(Year)	0.742
Var(TurtleID)	5.093
Var(Error)	4.170

Table S5. Annual proportion of Blanding's turtle Emydoidea blandingii eggs that hatched from Spring Bluff Chiwaukee Prairie females 2010-2018 as part of the Lake County Forest Preserve District head-starting program. Total variance is the variance across years; error variance is the mean of year-specific binomial variances (= p(1-p)/(n-1); environmental variance = total variance - error variance (following Akçakaya 2002).

Year	**Eggs**	**Hatched**	**Proportion Hatched**	**Binomial Variance**
2010	136	123	0.9044	0.0006
2011	222	176	0.7928	0.0007
2012	157	129	0.8217	0.0009
2013	117	87	0.7436	0.0016
2014	144	123	0.8542	0.0009
2015	151	104	0.6887	0.0014
2016	146	132	0.9041	0.0006
2017	140	112	0.8000	0.0012
2018	167	145	0.8683	0.0007
Total	1213	986	0.8197	
	Total Variance			0.0052
	Error Variance			0.0010
	Environmental Variance			0.0043

Table S6. Worksheet for the calculation of Blanding's turtle *Emydoidea blandingii* age 0 survival uncertainty (A) and age 0 survival environmental variance (B). Nest success and hatch success are estimated from data collected at Spring Bluff Chiwaukee Prairie 2004-2018; post-hatch survival is from Kastle et al. (in press). Calculaton of the variance of a product (nest success*hatch success*post-hatch survival) follows Goodman (1960) and assumes covariances among survival components are zero. Because environmental variance in post-hatch survival is unknown, we estimate lower and upper bounds by setting this variance to 0.0000 and 0.0340 (to equal environmental variance in nest success), respectively.

A. Age 0 survival uncertainty

	φ	**var(φ)**		
Nest Success	0.2500	0.0067	$\varphi_2^2\varphi_3^2(var(\varphi_1))$ =	0.001869
Hatch Success	0.8129	0.0001	$\varphi_1^2\varphi_3^2(var(\varphi_2))$ =	0.000003
Post-hatch Survival	0.6500	0.0037	$\varphi_1^2\varphi_2^2(var(\varphi_3))$ =	0.000154
$\varphi_1\varphi_2\varphi_3$ =	0.1321		$var(\varphi_1\varphi_2\varphi_3)$ =	0.002026
			$sd(\varphi_1\varphi_2\varphi_3)$ =	0.045015
lower 95% CL for age 0 mortality:			0.780	
upper 95% CL for age 0 mortality:			0.956	

B. Age 0 survival environmental variance

Lower Bound: environmental variance in post-hatch survival = 0.0000

	φ	**var(φ)**		
Nest Success	0.2500	0.0340	$\varphi_2^2\varphi_3^2(var(\varphi_1))$ =	0.009492
Hatch Success	0.8129	0.0043	$\varphi_1^2\varphi_3^2(var(\varphi_2))$ =	0.000114
Post-hatch Survival	0.6500	0.0000	$\varphi_1^2\varphi_2^2(var(\varphi_3))$ =	0.000000
$\varphi_1\varphi_2\varphi_3$ =	0.1321		$var(\varphi_1\varphi_2\varphi_3)$ =	0.009605
			$sd(\varphi_1\varphi_2\varphi_3)$ =	0.098006

Upper Bound: environmental variance in post-hatch survival = 0.0340

	φ	**var(φ)**		
Nest Success	0.2500	0.0340	$\varphi_2^2\varphi_3^2(var(\varphi_1))$ =	0.009492
Hatch Success	0.8129	0.0043	$\varphi_1^2\varphi_3^2(var(\varphi_2))$ =	0.000114
Post-hatch Survival	0.6500	0.0340	$\varphi_1^2\varphi_2^2(var(\varphi_3))$ =	0.001404
$\varphi_1\varphi_2\varphi_3$ =	0.1321		$var(\varphi_1\varphi_2\varphi_3)$ =	0.011009
			$sd(\varphi_1\varphi_2\varphi_3)$ =	0.104925

Table S7. Annual variation in the proportion of Blanding's turtle Emydoidea blandingii nests surviving at a Michigan study site 1976-1998 (from Table 1 in Congdon et al. 2000) and its associated environmental variance. Total variance is the variance across years; error variance is the mean of year-specific binomial variances (= p(1-p)/(n-1); environmental variance = total variance - error variance (following Akçakaya 2002).

Year	Nests Monitored	Proportion of Nests Surviving	Binomial Variance
1976	8	0.375	0.033
1977	14	0.500	0.019
1978	13	0.538	0.021
1979	13	0.308	0.018
1980	13	0.231	0.015
1981	13	0.077	0.006
1982	14	0.500	0.019
1983	14	0.500	0.019
1984	5	0.600	0.060
1985	15	0.000	0.000
1986	13	0.000	0.000
1987	9	0.000	0.000
1988	7	0.000	0.000
1989	11	0.000	0.000
1990	6	0.333	0.044
1991	6	0.000	0.000
1992	12	0.000	0.000
1993	12	0.000	0.000
1994	4	0.000	0.000
1995	10	0.400	0.027
1996	6	0.333	0.044
1997	16	0.313	0.014
1998	4	0.000	0.000
Total	238	0.049	0.015
	Total Variance		0.049
	Error Variance		0.015
	Environmental Variance		0.034

Table S8. Estimated Spring Bluff Chiwaukee Prairie adult Blanding's turtle Emydoidea blandingii population size 2004-2018. Estimates (Ni) and confidence limits are highlighted in yellow, three year running average estimates and confidence limits are highlighted in blue. Symbolism and table organization follows Manly (1984).

			Year of last previous capture																										3-year Running Average		
Year	ni	Ri	2004	2005	2006	2007	2008	2009	2010	2013	2014	2015	2016	2017	2018	ri	mi	Zi	Mi	Ni	var	sd	Ti(Ni)	var(Ti(Ni))	Tlower	Tupper	lower CL	upper CL	Ni	lower CL	upper CL
2004	9	9														9															
2005	61	61	8													57	8	1	9.1	62.5	9.40	3.1	3.03	0.10	2.52	3.80	61.62	80.43	62.5	61.6	80.4
2006	69	69	1	47												59	48	10	59.7	85.2	12.91	3.6	3.78	0.01	3.63	4.02	80.07	95.31	73.9	70.8	87.9
2007	56	56	0	5	43											48	48	21	72.4	84.3	14.38	3.8	3.96	0.01	3.84	4.15	78.61	94.39	77.3	73.4	90.0
2008	37	37	0	1	3	32										35	36	33	70.8	72.7	5.70	2.4	3.96	0.00	3.89	4.08	69.08	78.84	80.7	75.9	89.5
2009	38	38	0	2	5	8	21									31	36	32	75.0	79.1	23.18	4.8	4.07	0.01	3.93	4.27	71.88	91.93	78.7	73.2	88.4
2010	24	24	0	0	5	3	3	12								16	23	40	81.8	85.2	108.13	10.4	4.29	0.02	4.06	4.63	70.40	115.10	79.0	70.5	95.3
2013	34	34	0	1	0	0	4	7	8							26	20	36	66.7	111.1	250.74	15.8	4.54	0.03	4.26	4.95	89.01	158.24	91.8	77.1	121.8
2014	26	26	0	1	1	1	2	3	4	10						22	22	40	69.0	80.9	58.94	7.7	4.21	0.01	4.03	4.49	69.75	102.22	92.4	76.4	125.2
2015	44	44	0	0	1	2	0	4	3	9	9					34	28	34	71.7	111.3	149.79	12.2	4.48	0.02	4.25	4.82	93.83	146.42	101.1	84.2	135.6
2016	41	41	0	0	0	1	1	4	0	3	7	18				25	34	34	88.9	106.7	144.12	12.0	4.44	0.02	4.21	4.79	89.61	141.25	99.6	84.4	130.0
2017	43	43	0	0	1	1	1	1	1	3	3	10	17			20	38	21	82.0	92.5	134.23	11.6	4.24	0.03	3.97	4.65	76.42	127.19	103.5	86.6	138.3
2018	48	48	0	0	0	0	3	0	0	1	3	6	8	20		0	41														
	530	530	9	57	59	48	35	31	16	26	22	34	25	20	0																
			r1	r2	r3	r4	r5	r6	r7	r8	r9	r10	r11	r12	r13																
		mi	**8**																												
			1	**48**																											
			0	5	**48**																										
			0	1	4	**36**																									
			0	2	7	15	**36**																								
			0	0	5	8	11	**23**																							
			0	1	1	1	5	12	**20**																						
			0	1	2	3	5	8	12	**22**																					
			0	0	1	3	3	7	10	19	**28**																				
			0	0	0	1	2	6	6	9	16	**34**																			
			0	0	1	2	3	4	5	8	11	21	**38**																		
			0	0	0	0	3	3	3	4	7	13	21	**41**																	
			1	10	21	33	32	40	36	40	34	34	21																		
			Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10	Z11	Z12																		

Table S9. Results of single-variable sensitivity tests of parameter uncertainty from the Blanding's turtle *Emydoidea blandingii* PVA. Parameter values and parameter uncertainty eere mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1). Shown are the ranges of Stochacstic-r and Probability of Extinction across parameter values and correaltions between Stochastic-r and Probablity of Extinction and parameter values.

Parameter	Ranges		Correlations	
	Stochastic-r	Probability of Extinction	Stochastic-r	Probability of Extinction
Inbreeding_Depression	0.002	0.004	-0.866	0.051
Age at First Reproduction	0.006	0.003	-0.990	-0.012
Maximum Age	0.002	0.003	0.727	-0.528
Sex Ratio	0.011	0.005	-0.996	0.663
% Females Reproducing	0.004	0.004	0.981	-0.337
SD in % Females Reproducing	0.001	0.003	-0.502	0.036
Mean Offspring per Brood	0.005	0.002	0.978	-0.359
SED in Offspring per Brood	0.000	0.004	0.097	-0.135
Age 0 Mortality	0.085	0.802	-0.974	0.708
SD in Age 0 Mortality	0.004	0.005	-0.982	0.817
Juvenile Mortality	0.052	0.321	-1.000	0.814
SD in Juvenile Mortality	0.002	0.003	0.870	0.283
Adult Mortality	0.022	0.027	-0.998	0.899
SD in Adult Mortality	0.001	0.003	-0.790	0.745
SD in Juvenila and Adult Mortality	0.003	0.002	-0.977	0.649
Initial Population Size	0.001	0.005	-0.214	-0.825
Carrying Capacity	0.001	0.003	-0.255	-0.055
SD in Carrying Capacity	0.001	0.003	-0.453	-0.194

Table S10. Results of logistic regression analysis of sensitivity test results and associated calculations of relative influence from the Blanding's turtle *Emydoidea blandingii* PVA. Parts A-D represent analyses of sensitivity to parameter uncertainty; parts E-F represent analyses of sensitivity to geographic parameter varition. Included in A-D are analyses based on all 30,000 LHS samples and 3 random subsamples of n = 10,000 each. Dependent variables include the binomial transformation of stochastic-r(A and E), probability of extinction (B and F), binomal transformation of gene diversity,(C) and binomial transformation of adult poulation size (D). Parameter values in sensitivity tests of parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and morality adjusted to achieve deterministic r = 0 (Table 1). Parameter values used in sensitivity tests of range-wade parameter uncertainty are from throughout the species' range in North America (Table 1; Supplemental Material, Table S11). Shown are standardized partial logistic regression coefficients (B), their standard errors (S.E.), Wald statistic (Wald) and associated degrees fo freedom (df) and significance (Sig.), and odds ratio (Exp(B)). Relative influence was acheived by calculated by computing B/S.E. and its absolute value (Abse(B/S.E.)), dividing the latter by the sum of Abs(B/S.E.) across parmaters and multiplying by 100.

A.1. Dependent variable = binomial transformation of stochastic-r (r < median = 0, r > median = 1), n = 30,000

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)		B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.641	0.029	483.459	1	0.000	0.527		-22.0	22.0	8.8
% Females Reproducing	0.087	0.010	77.006	1	0.000	1.091		8.8	8.8	3.5
Clutch Size	0.818	0.067	150.002	1	0.000	2.267		12.2	12.2	4.9
Age 0 Mortality	-0.954	0.013	5527.144	1	0.000	0.385		-74.3	74.3	29.8
Juvenile Mortality	-2.045	0.030	4666.298	1	0.000	0.129		-68.3	68.3	27.4
Adult Mortality	-1.336	0.028	2254.935	1	0.000	0.263		-47.5	47.5	19.0
Initial Population Size	0.002	0.001	8.910	1	0.003	1.002		3.0	3.0	1.2
SD in Age 0 Mortality	-0.049	0.008	42.122	1	0.000	0.952		-6.5	6.5	2.6
SD in Juvenile and Adult Mortality	-0.136	0.020	47.389	1	0.000	0.873		-6.9	6.9	2.8
Constant	98.582	1.551	4037.421	1	0.000	#######				
									249.5	

A.2. Dependent variable = binomial transformation of stochastic-r (r < median = 0, r > median = 1), n =10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.583	0.051	131.966	1	0.000	0.558	-11.5	11.5	8.0
% Females Reproducing	0.097	0.017	31.981	1	0.000	1.102	5.7	5.7	4.0
Clutch Size	0.817	0.116	49.253	1	0.000	2.264	7.0	7.0	4.9
Age 0 Mortality	-0.964	0.022	1850.733	1	0.000	0.381	-43.0	43.0	30.1
Juvenile Mortality	-2.051	0.052	1564.838	1	0.000	0.129	-39.6	39.6	27.7
Adult Mortality	-1.330	0.049	747.462	1	0.000	0.265	-27.3	27.3	19.1
Initial Population Size	0.002	0.001	4.528	1	0.033	1.002	2.1	2.1	1.5
SD in Age 0 Mortality	-0.035	0.013	6.997	1	0.008	0.966	-2.6	2.6	1.9
SD in Juvenile and Adult Mortality	-0.137	0.034	15.864	1	0.000	0.872	-4.0	4.0	2.8
Constant	98.468	2.690	1340.250	1	0.000	#######			
								142.8	

A.3. Dependent variable = binomial transformation of stochastic-r (r < median = 0, r > median = 1), n =10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.630	0.050	159.010	1	0.000	0.533	-12.6	12.6	8.8
% Females Reproducing	0.078	0.017	21.234	1	0.000	1.081	4.6	4.6	3.2
Clutch Size	0.776	0.116	44.818	1	0.000	2.173	6.7	6.7	4.7
Age 0 Mortality	-0.952	0.022	1849.819	1	0.000	0.386	-43.0	43.0	29.9
Juvenile Mortality	-2.010	0.051	1562.391	1	0.000	0.134	-39.5	39.5	27.5
Adult Mortality	-1.298	0.048	733.287	1	0.000	0.273	-27.1	27.1	18.8
Initial Population Size	0.001	0.001	2.321	1	0.128	1.001	1.5	1.5	1.1
SD in Age 0 Mortality	-0.060	0.013	21.359	1	0.000	0.942	-4.6	4.6	3.2
SD in Juvenile and Adult Mortality	-0.140	0.034	17.030	1	0.000	0.869	-4.1	4.1	2.9
Constant	98.544	2.666	1366.633	1	0.000	#######			
								143.8	

A.4. Dependent variable = binomial transformation of stochastic-r (r < median = 0, r > median = 1), n =10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.597	0.050	143.380	1	0.000	0.551	-12.0	12.0	8.4
% Females Reproducing	0.080	0.017	22.128	1	0.000	1.083	4.7	4.7	3.3
Clutch Size	0.898	0.116	60.304	1	0.000	2.456	7.8	7.8	5.5
Age 0 Mortality	-0.941	0.022	1854.000	1	0.000	0.390	-43.1	43.1	30.3
Juvenile Mortality	-2.015	0.051	1555.911	1	0.000	0.133	-39.4	39.4	27.8
Adult Mortality	-1.354	0.049	773.736	1	0.000	0.258	-27.8	27.8	19.6
Initial Population Size	0.001	0.001	0.581	1	0.446	1.001	0.8	0.8	0.5
SD in Age 0 Mortality	-0.052	0.013	16.262	1	0.000	0.949	-4.0	4.0	2.8
SD in Juvenile and Adult Mortality	-0.086	0.034	6.524	1	0.011	0.917	-2.6	2.6	1.8
Constant	96.306	2.638	1332.406	1	0.000	#######			
								142.1	

B.1. Dependent variable = probability of extinction, n = 30,000

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	0.179	0.030	35.728	1	0.000	1.197	6.0	6.0	3.2
% Females Reproducing	-0.013	0.010	1.464	1	0.226	0.987	-1.2	1.2	0.6
Clutch Size	-0.360	0.070	26.077	1	0.000	0.698	-5.1	5.1	2.7
Age 0 Mortality	0.844	0.014	3883.271	1	0.000	2.326	62.3	62.3	33.4
Juvenile Mortality	0.949	0.020	2219.896	1	0.000	2.583	47.1	47.1	25.2
Adult Mortality	1.020	0.027	1383.378	1	0.000	2.774	37.2	37.2	19.9
Initial Population Size	-0.005	0.001	66.185	1	0.000	0.995	-8.1	8.1	4.4
SD in Age 0 Mortality	0.102	0.008	158.859	1	0.000	1.107	12.6	12.6	6.7
SD in Juvenile and Adult Mortality	0.151	0.021	50.505	1	0.000	1.163	7.1	7.1	3.8
Constant	-88.473	1.635	2928.785	1	0.000	0.000			
								186.8	

B.2. Dependent variable = probability of extinction, n = 10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	0.183	0.052	12.481	1	0.000	1.201	3.5	3.5	3.3
% Females Reproducing	-0.012	0.018	0.426	1	0.514	0.988	-0.7	0.7	0.6
Clutch Size	-0.261	0.123	4.521	1	0.033	0.771	-2.1	2.1	2.0
Age 0 Mortality	0.817	0.023	1284.506	1	0.000	2.263	35.8	35.8	33.8
Juvenile Mortality	0.900	0.034	693.645	1	0.000	2.459	26.3	26.3	24.9
Adult Mortality	0.972	0.046	441.456	1	0.000	2.642	21.0	21.0	19.8
Initial Population Size	-0.005	0.001	23.340	1	0.000	0.995	-4.8	4.8	4.6
SD in Age 0 Mortality	0.099	0.014	51.798	1	0.000	1.104	7.2	7.2	6.8
SD in Juvenile and Adult Mortality	0.161	0.036	19.709	1	0.000	1.175	4.4	4.4	4.2
Constant	-86.472	2.811	946.008	1	0.000	0.000			
								106.0	

B.3. Dependent variable = probability of extinction, n = 10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	0.202	0.052	15.266	1	0.000	1.224	3.9	3.9	3.6
% Females Reproducing	-0.010	0.018	0.288	1	0.591	0.990	-0.5	0.5	0.5
Clutch Size	-0.342	0.123	7.743	1	0.005	0.710	-2.8	2.8	2.5
Age 0 Mortality	0.839	0.023	1286.372	1	0.000	2.314	35.9	35.9	32.8
Juvenile Mortality	0.926	0.034	727.082	1	0.000	2.524	27.0	27.0	24.7
Adult Mortality	1.081	0.048	508.981	1	0.000	2.948	22.6	22.6	20.6
Initial Population Size	-0.005	0.001	29.196	1	0.000	0.995	-5.4	5.4	4.9
SD in Age 0 Mortality	0.100	0.014	50.960	1	0.000	1.105	7.1	7.1	6.5
SD in Juvenile and Adult Mortality	0.150	0.036	17.006	1	0.000	1.161	4.1	4.1	3.8
Constant	-88.698	2.846	971.478	1	0.000	0.000			
								109.3	

B.3. Dependent variable = probability of extinction, n = 10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	0.181	0.052	12.242	1	0.000	1.198	3.5	3.5	3.2
% Females Reproducing	0.008	0.018	0.204	1	0.651	1.008	0.5	0.5	0.4
Clutch Size	-0.340	0.122	7.804	1	0.005	0.712	-2.8	2.8	2.6
Age 0 Mortality	0.835	0.023	1309.278	1	0.000	2.304	36.2	36.2	33.6
Juvenile Mortality	0.938	0.035	730.112	1	0.000	2.556	27.0	27.0	25.1
Adult Mortality	0.981	0.047	429.313	1	0.000	2.666	20.7	20.7	19.2
Initial Population Size	-0.005	0.001	27.612	1	0.000	0.995	-5.3	5.3	4.9
SD in Age 0 Mortality	0.107	0.014	58.762	1	0.000	1.113	7.7	7.7	7.1
SD in Juvenile and Adult Mortality	0.155	0.037	17.905	1	0.000	1.168	4.2	4.2	3.9
Constant	-87.384	2.814	964.502	1	0.000	0.000			
								107.8	

C.1. Dependent variable = binomial transformation of gene diversity (gene diversity < median = 0, gene diversity > median = 1), n = 30,000

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.571	0.028	428.254	1	0.000	0.565	-20.7	20.7	7.6
% Females Reproducing	0.053	0.009	31.530	1	0.000	1.055	5.6	5.6	2.1
Clutch Size	0.614	0.063	93.499	1	0.000	1.848	9.7	9.7	3.6
Age 0 Mortality	-0.839	0.011	5819.596	1	0.000	0.432	-76.3	76.3	28.0
Juvenile Mortality	-1.906	0.027	4981.407	1	0.000	0.149	-70.6	70.6	25.9
Adult Mortality	-1.386	0.027	2602.630	1	0.000	0.250	-51.0	51.0	18.7
Initial Population Size	0.010	0.001	391.551	1	0.000	1.010	19.8	19.8	7.3
SD in Age 0 Mortality	-0.079	0.007	119.053	1	0.000	0.924	-10.9	10.9	4.0
SD in Juvenile and Adult Mortality	-0.143	0.019	57.651	1	0.000	0.867	-7.6	7.6	2.8
Constant	87.230	1.374	4029.106	1	0.000	#######			
								272.2	

C.2. Dependent variable = binomial transformation of gene diversity (gene diversity < median = 0, gene diversity > median = 1), n = 10,000 sampled at random

	Parameter	B	S.E.	Wald	df	Sig.	Exp(B)		B/S.E.	Abs(B/S.E.)	Relative Influence
	Age of First Reproduction	-0.554	0.048	134.002	1	0.000	0.575		-11.6	11.6	7.3
	% Females Reproducing	0.047	0.016	8.326	1	0.004	1.048		2.9	2.9	1.8
	Clutch Size	0.617	0.110	31.439	1	0.000	1.853		5.6	5.6	3.5
	Age 0 Mortality	-0.837	0.019	1961.644	1	0.000	0.433		-44.3	44.3	27.9
	Juvenile Mortality	-1.888	0.046	1683.362	1	0.000	0.151		-41.0	41.0	25.8
	Adult Mortality	-1.384	0.047	875.727	1	0.000	0.250		-29.6	29.6	18.6
	Initial Population Size	0.012	0.001	178.596	1	0.000	1.012		13.4	13.4	8.4
	SD in Age 0 Mortality	-0.067	0.012	29.160	1	0.000	0.935		-5.4	5.4	3.4
	SD in Juvenile and Adult Mortality	-0.172	0.033	27.796	1	0.000	0.842		-5.3	5.3	3.3
	Constant	86.088	2.350	1341.631	1	0.000	#######				
										159.0	

C.3. Dependent variable = binomial transformation of gene diversity (gene diversity < median = 0, gene diversity > median = 1), n = 10,000 sampled at random

	Parameter	B	S.E.	Wald	df	Sig.	Exp(B)		B/S.E.	Abs(B/S.E.)	Relative Influence
	Age of First Reproduction	-0.646	0.049	175.821	1	0.000	0.524		-13.3	13.3	8.3
	% Females Reproducing	0.072	0.017	19.020	1	0.000	1.075		4.4	4.4	2.7
	Clutch Size	0.635	0.113	31.758	1	0.000	1.887		5.6	5.6	3.5
	Age 0 Mortality	-0.873	0.020	1903.479	1	0.000	0.418		-43.6	43.6	27.4
	Juvenile Mortality	-1.955	0.048	1635.759	1	0.000	0.142		-40.4	40.4	25.4
	Adult Mortality	-1.415	0.048	861.537	1	0.000	0.243		-29.4	29.4	18.4
	Initial Population Size	0.010	0.001	119.682	1	0.000	1.010		10.9	10.9	6.9
	SD in Age 0 Mortality	-0.098	0.013	59.459	1	0.000	0.907		-7.7	7.7	4.8
	SD in Juvenile and Adult Mortality	-0.133	0.033	16.126	1	0.000	0.876		-4.0	4.0	2.5
	Constant	91.564	2.478	1364.813	1	0.000	#######				
										159.3	

C.4. Dependent variable = binomial transformation of gene diversity (gene diversity < median = 0, gene diversity > median = 1), n = 10,000 sampled at random

	Parameter	B	S.E.	Wald	df	Sig.	Exp(B)		B/S.E.	Abs(B/S.E.)	Relative Influence
	Age of First Reproduction	-0.569	0.048	142.248	1	0.000	0.566		-11.9	11.9	7.7
	% Females Reproducing	0.046	0.016	8.027	1	0.005	1.047		2.8	2.8	1.8
	Clutch Size	0.537	0.110	23.692	1	0.000	1.712		4.9	4.9	3.2
	Age 0 Mortality	-0.842	0.019	1931.155	1	0.000	0.431		-43.9	43.9	28.5
	Juvenile Mortality	-1.905	0.047	1640.535	1	0.000	0.149		-40.5	40.5	26.2
	Adult Mortality	-1.419	0.048	891.219	1	0.000	0.242		-29.9	29.9	19.3
	Initial Population Size	0.010	0.001	113.617	1	0.000	1.010		10.7	10.7	6.9
	SD in Age 0 Mortality	-0.086	0.012	47.340	1	0.000	0.918		-6.9	6.9	4.5
	SD in Juvenile and Adult Mortality	-0.095	0.033	8.510	1	0.004	0.909		-2.9	2.9	1.9
	Constant	88.752	2.413	1352.912	1	0.000	#######				
										154.4	

D.1. Dependent variable = binomial transformation of adult population size (adult population size < median = 0, adult population size > median = 1), n = 30,000

	Parameter	B	S.E.	Wald	df	Sig.	Exp(B)		B/S.E.	Abs(B/S.E.)	Relative Influence
	Age of First Reproduction	-0.590	0.029	426.945	1	0.000	0.554		-20.7	20.7	7.9
	% Females Reproducing	0.068	0.010	48.409	1	0.000	1.070		7.0	7.0	2.7
	Clutch Size	0.638	0.066	94.761	1	0.000	1.893		9.7	9.7	3.7
	Age 0 Mortality	-0.903	0.012	5571.992	1	0.000	0.406		-74.6	74.6	28.7
	Juvenile Mortality	-2.080	0.030	4869.731	1	0.000	0.125		-69.8	69.8	26.8
	Adult Mortality	-1.395	0.028	2464.276	1	0.000	0.248		-49.6	49.6	19.1
	Initial Population Size	0.008	0.001	221.992	1	0.000	1.008		14.9	14.9	5.7
	SD in Age 0 Mortality	-0.053	0.007	51.923	1	0.000	0.948		-7.2	7.2	2.8
	SD in Juvenile and Adult Mortality	-0.132	0.019	46.155	1	0.000	0.876		-6.8	6.8	2.6
	Constant	93.631	1.481	3996.093	1	0.000	#######				
										260.3	

D.2. Dependent variable = binomial transformation of adult population size (adult population size < median = 0, adult population size > median = 1), n = 10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.583	0.050	137.346	1	0.000	0.558	-11.7	11.7	7.7
% Females Reproducing	0.073	0.017	18.570	1	0.000	1.075	4.3	4.3	2.8
Clutch Size	0.669	0.114	34.434	1	0.000	1.952	5.9	5.9	3.9
Age 0 Mortality	-0.908	0.021	1873.522	1	0.000	0.403	-43.3	43.3	28.5
Juvenile Mortality	-2.073	0.051	1637.565	1	0.000	0.126	-40.5	40.5	26.6
Adult Mortality	-1.389	0.048	821.000	1	0.000	0.249	-28.7	28.7	18.9
Initial Population Size	0.009	0.001	93.250	1	0.000	1.009	9.7	9.7	6.4
SD in Age 0 Mortality	-0.042	0.013	10.516	1	0.001	0.959	-3.2	3.2	2.1
SD in Juvenile and Adult Mortality	-0.161	0.034	22.592	1	0.000	0.852	-4.8	4.8	3.1
Constant	93.222	2.555	1330.775	1	0.000	#######			
								152.0	

D.3. Dependent variable = binomial transformation of adult population size (adult population size < median = 0, adult population size > median = 1), n = 10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.621	0.049	160.435	1	0.000	0.537	-12.7	12.7	8.3
% Females Reproducing	0.090	0.017	28.649	1	0.000	1.094	5.4	5.4	3.5
Clutch Size	0.655	0.114	33.142	1	0.000	1.926	5.8	5.8	3.8
Age 0 Mortality	-0.895	0.021	1872.362	1	0.000	0.409	-43.3	43.3	28.2
Juvenile Mortality	-2.043	0.051	1634.718	1	0.000	0.130	-40.4	40.4	26.4
Adult Mortality	-1.358	0.048	802.081	1	0.000	0.257	-28.3	28.3	18.5
Initial Population Size	0.007	0.001	59.331	1	0.000	1.007	7.7	7.7	5.0
SD in Age 0 Mortality	-0.076	0.013	35.946	1	0.000	0.926	-6.0	6.0	3.9
SD in Juvenile and Adult Mortality	-0.125	0.033	14.124	1	0.000	0.882	-3.8	3.8	2.5
Constant	93.291	2.537	1352.165	1	0.000	#######			
								153.3	

D.4. Dependent variable = binomial transformation of adult population size (adult population size < median = 0, adult population size > median = 1), n = 10,000 sampled at random

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.535	0.049	120.451	1	0.000	0.586	-11.0	11.0	7.4
% Females Reproducing	0.061	0.017	13.223	1	0.000	1.063	3.6	3.6	2.5
Clutch Size	0.614	0.113	29.356	1	0.000	1.848	5.4	5.4	3.7
Age 0 Mortality	-0.895	0.021	1867.965	1	0.000	0.408	-43.2	43.2	29.3
Juvenile Mortality	-2.025	0.051	1604.011	1	0.000	0.132	-40.1	40.1	27.2
Adult Mortality	-1.451	0.049	869.339	1	0.000	0.234	-29.5	29.5	20.0
Initial Population Size	0.007	0.001	67.034	1	0.000	1.007	8.2	8.2	5.6
SD in Age 0 Mortality	-0.054	0.013	18.191	1	0.000	0.947	-4.3	4.3	2.9
SD in Juvenile and Adult Mortality	-0.073	0.033	4.751	1	0.029	0.930	-2.2	2.2	1.5
Constant	92.944	2.547	1331.769	1	0.000	#######			
								147.4	

E. Dependent variable = binomial transformation of stochastic-r (r < median = 0, r > median = 1), n = 30,000

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	-0.360	0.007	2472.159	1	0.000	0.698	-49.7	49.7	17.5
% Females Reproducing	0.028	0.001	413.331	1	0.000	1.029	20.3	20.3	7.2
Clutch Size	0.201	0.008	683.582	1	0.000	1.222	26.1	26.1	9.2
Age 0 Mortality	-0.244	0.004	3628.986	1	0.000	0.784	-60.2	60.2	21.2
Juvenile Mortality	-0.599	0.016	1406.464	1	0.000	0.550	-37.5	37.5	13.2
Adult Mortality	-0.504	0.006	6189.357	1	0.000	0.604	-78.7	78.7	27.7
Initial Population Size	0.001	0.000	25.893	1	0.000	1.001	5.1	5.1	1.8
SD in Age 0 Mortality	-0.032	0.007	19.958	1	0.000	0.968	-4.5	4.5	1.6
SD in Juvenile and Adult Mortality	-0.037	0.019	3.892	1	0.049	0.963	-2.0	2.0	0.7
Constant	31.543	0.488	4172.652	1	0.000	#######			
								284.1	

F. Dependent variable = probability of extinction, n = 30,000

Parameter	B	S.E.	Wald	df	Sig.	Exp(B)	B/S.E.	Abs(B/S.E.)	Relative Influence
Age of First Reproduction	0.399	0.009	2110.672	1	0.000	1.490	45.9	45.9	15.0
% Females Reproducing	-0.040	0.002	601.130	1	0.000	0.960	-24.5	24.5	8.0
Clutch Size	-0.281	0.009	921.067	1	0.000	0.755	-30.3	30.3	9.9
Age 0 Mortality	0.293	0.005	3232.554	1	0.000	1.340	56.9	56.9	18.6
Juvenile Mortality	0.844	0.020	1746.566	1	0.000	2.327	41.8	41.8	13.7
Adult Mortality	0.625	0.009	4711.706	1	0.000	1.868	68.6	68.6	22.4
Initial Population Size	-0.007	0.000	911.848	1	0.000	0.993	-30.2	30.2	9.9
SD in Age 0 Mortality	0.035	0.008	18.039	1	0.000	1.036	4.2	4.2	1.4
SD in Juvenile and Adult Mortality	0.073	0.022	11.258	1	0.001	1.076	3.4	3.4	1.1
Constant	-32.154	0.568	3206.640	1	0.000	0.000			
								305.9	

Table S11. Geographic variation in Blanding's turtle *Emydoidea blandingii* demographic parameters from throughout the species' range in North America. Parameters are listed in the order that they are used in Vortex PVA software. Locations are ordered from northeast to southwest.

Parameter	Value	Location	Reference
Age of first reproduction (minimum)	17	Nova Scotia	The Blanding's Turtle Recovery Team 2003
	19	Nova Scotia	Standing 1997
	19	Nova Scotia	McNeil 2002
	22	Nova Scotia	McNeil 2002
	11	Ontario	Petokas 1986
	12	Ontario	Petokas 1986
	14	Michigan	Congdon et al. 1993
	14	Minnesota	Pappas et al. 2000
	13	Illinois	this study
	11	Nebraska	Ruane et al. 2008
	10	Nebraska	Germano et al. 2000
% Females reproducing	65.8	Nova Scotia	Table 3 in Standing 1997
	82.6	Maine	Beaudry et al. 2008
	55	Ontario	Hawkins 2016
	80	Michigan	Congdon and Keinath 2006
	79	Minnesota	Piepgras et al. 1998
	40.5	Illinois	Banning 2007
	92	Illinois	D. Thompson, Forest Preserve District of DuPage County, personal communication
	77	Illinois	D. Thompson, Forest Preserve District of DuPage County, personal communication
	94	Illinois	this study
	72	Nebraska	Ruane et al. 2008
	93	Nebraska	Lang 2004
Clutch size (mean)	10.6	Nova Scotia	Power 1989, Standing et al. 1999, Standing et al. 2000

		8.0	Nova Scotia	McNeil 2002
		10.4	Nova Scotia	McNeil 2002
		11.7	Maine	Beaudry et al. 2008
		9.8	Maine	Joyal et al. 2000
		13.0	Massachusetts	DePari et al. 1987
		10.6	Massachusetts	Butler and Graham 1995
		12.6	Ontario	Petokas 1986
		7.4	Ontario	Petokas 1986
		10.0	Michigan	Congdon and Keinath 2006
		14.0	Ohio	Spetz 2008
		17.7	Minnesota	Sajwaj et al. 1988
		15.8	Minnesota	Piepgras et al. 1998
		9.8	Minnesota	Pappas et al. 2000
		10.8	Illinois	Banning 2007
		13.3	Illinois	Dreslik et al. 2011
		13.4	Illinois	D. Thompson, Forest Preserve District of DuPage County, personal communication
		14.1	Illinois	D. Thompson, Forest Preserve District of DuPage County, personal communication
		12.4	Illinois	C. Jablonksi, McHenry County Conservation District personal communication
		14.1	Illinois	C. Jablonksi, McHenry County Conservation District personal communication
		14.5	Illinois	W. Graser, Forest Preserve District of Kane County, personal communication
		8.0	Illinois	R. King, personal observation
		13.0	Illinois	this study
		14.9	Nebraska	Rowe 1992
		15.0	Nebraska	Ruane et al. 2008
		12.0	Nebraska	Lang 2004
Age 0 mortality (%)	Nest failure	65	Nova Scotia	Standing et al. 2000

		94	Massachusetts	Butler and Graham 1995
		78.2	Michigan	Congdon et al. 2000
		59	Wisconsin	Reid et al. 2016
		75	Illinois	this study (pre-mesopredator removal)
		33.3	Illinois	this study (post-mesopredator removal)
	Hatch failure	30	Nova Scotia	Standing et al. 2000
		53	Maine	Joyal et al. 2000
		13	Massachusetts	Butler and Graham 1995
		40	New York	Emrich 1991
		19.5	Michigan	Congdon et al. 2000
		21	Illinois	Anthonysamy 2012
		19	Illinois	Dreslik et al. 2011
		22	Illinois	D. Thompson, Forest Preserve District of DuPage County, personal communication
		18	Illinois	D. Thompson, Forest Preserve District of DuPage County, personal communication
		19	Illinois	this study
	Hatch to hibernation	40-80	Nova Scotia	Table 2.4 in Arsenault 2011
		20-58	Ontario	Paterson et al. 2012
	Hatch to hibernation	20.4	Illinois and Wisconsin	Kastle et al. in press
	Hatch to spring Emergence	72-99	Range-wide	Kastle et al. in press
	Egg deposition to spring emergence	86.8	Illinois and Wisconsin	this study
Adult mortality (%)		3.5	Massachusetts	Windmiller et al. 2016
		6.5	Michigan	Congdon et al. 1993
		6.1	Wisconsin	Reid et al. 2016
		3.9	Illinois	Rubin et al. 2004

	5.7	Illinois	Ross and Dreslik 2018
	5.3	Illinois	Golba 2019, Golba et al. in preparation
	31	Nebraska	Ruane et al. 2008

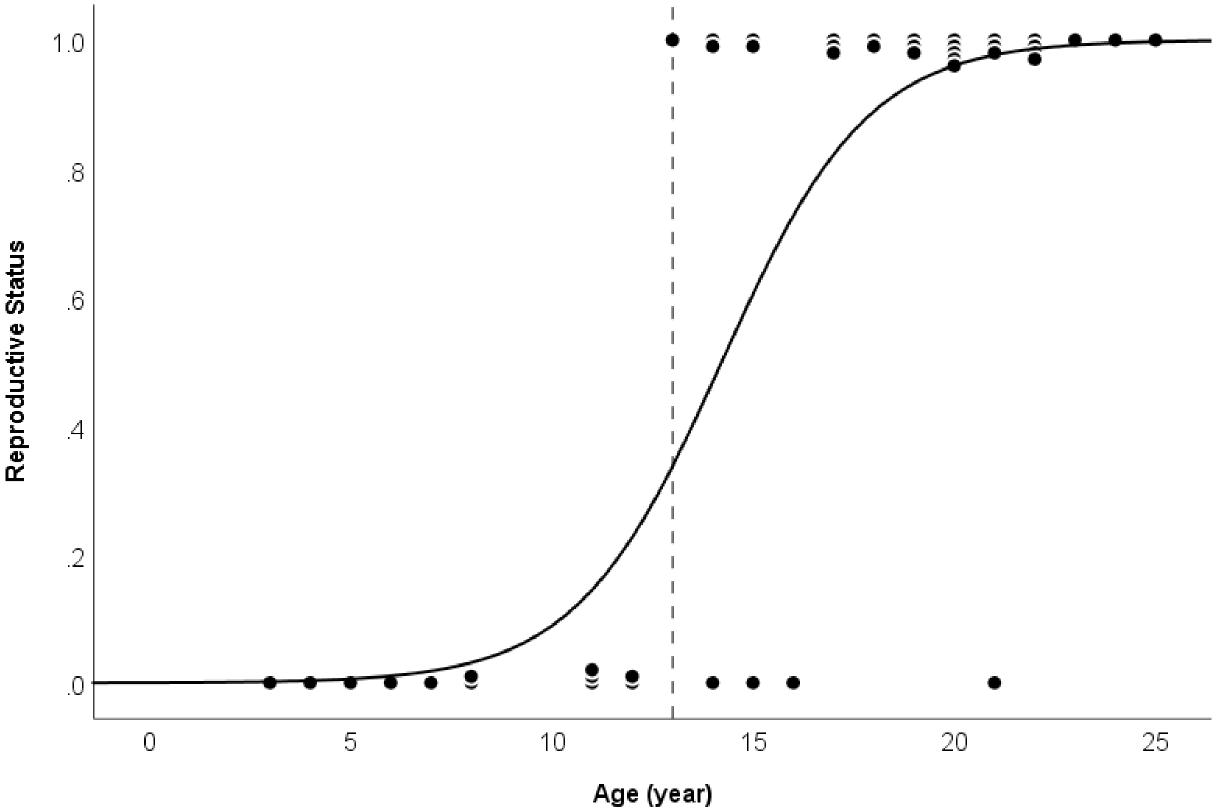

Figure S1. Logistic regression of reproductive status (gravid = 1, not gravid = 0) on age for 9 Blanding's turtle *Emydoidea blandingii* females achieving reproductive maturity during our study at Spring Bluff Chiwaukee Prairie 2004-2018 (Supplemental Material, Table S2). Vertical dashed line at 13 years represents the youngest age of first reproduction in our study. The likelihood of reproducing increases with age as $1/(1+(e^{(-1*(-7.839+(0.551*Age)))}))$ ($\chi^2 = 34.26$, $P < 0.001$), yielding probabilities of 0.34, 0.47, 0.73, 0.82, and 0.88 for 13, 14, 15, 16, 17, and 18 year old turtles.

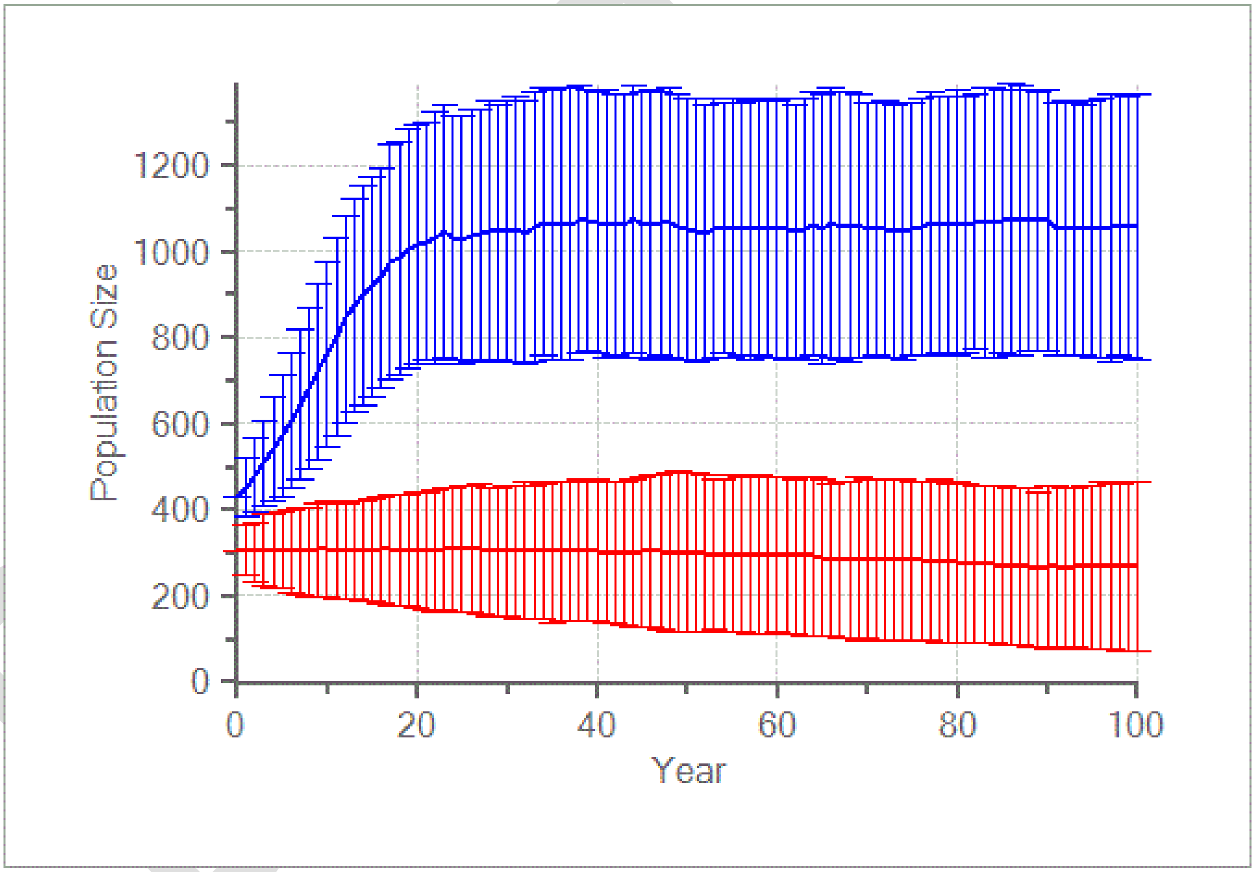

Figure S2. Blanding's turtle *Emydoidea blandingii* population viability analysis results showing mean (standard deviation) population size over 1,000 iterations of our SBCP (blue) and zero-growth (red) models. Parameter values for the SBCP model and in sensitivity tests of parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018. Clutch size and mortality rates were adjusted to achieve deterministic r = 0 for the zero-growth model.

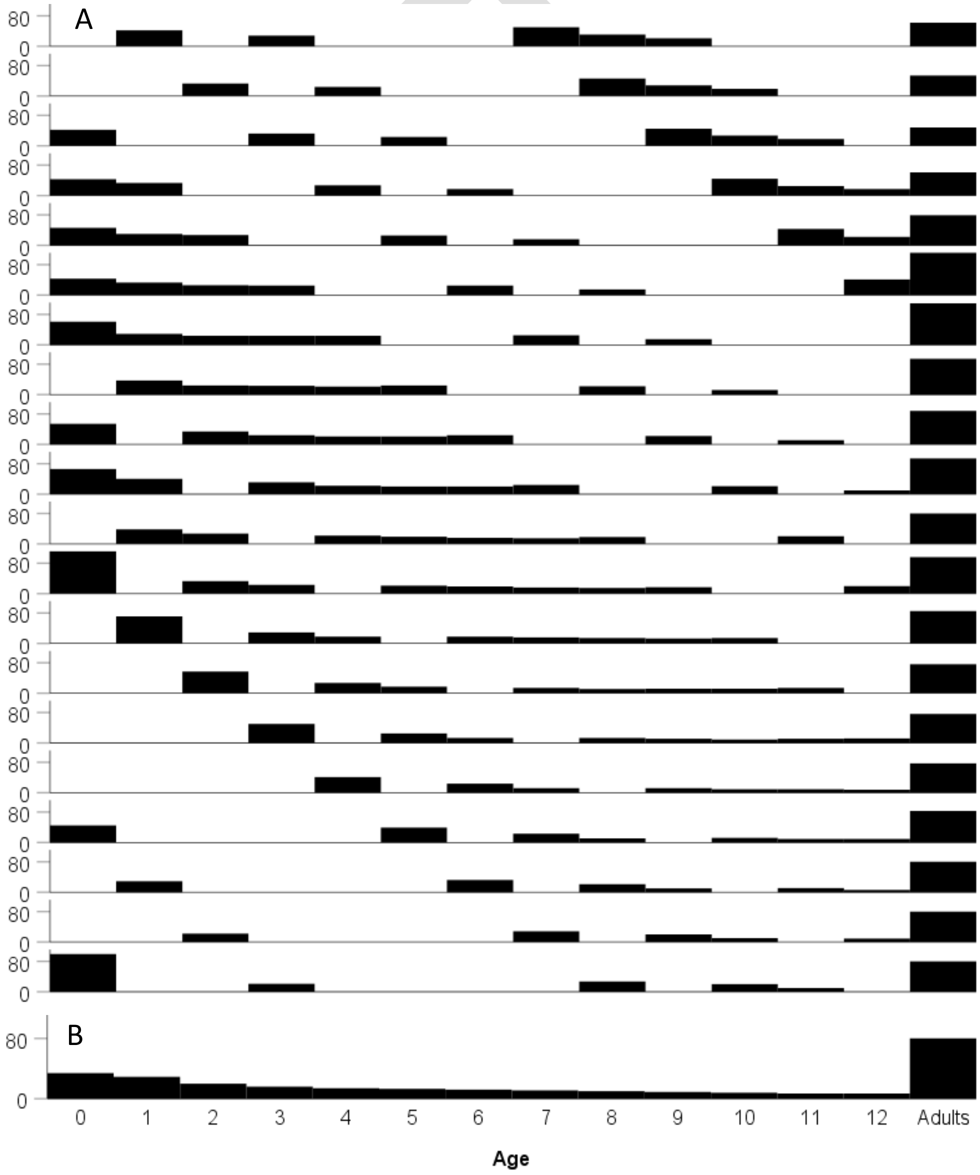

Figure S3. Example of annual variation in Blanding's turtle *Emydoidea blandingii* age structure in the zero-growth population model that result from years in which age 0 mortality = 100% (A) and the corresponding deterministic stable age distribution (B). Parameter values were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). Shown in A from top to bottom is a sequence of 20 years from one Vortex iteration. Histograms represent the number of individuals surviving to the end of a given age class. Adult age classes (≥13) are pooled.

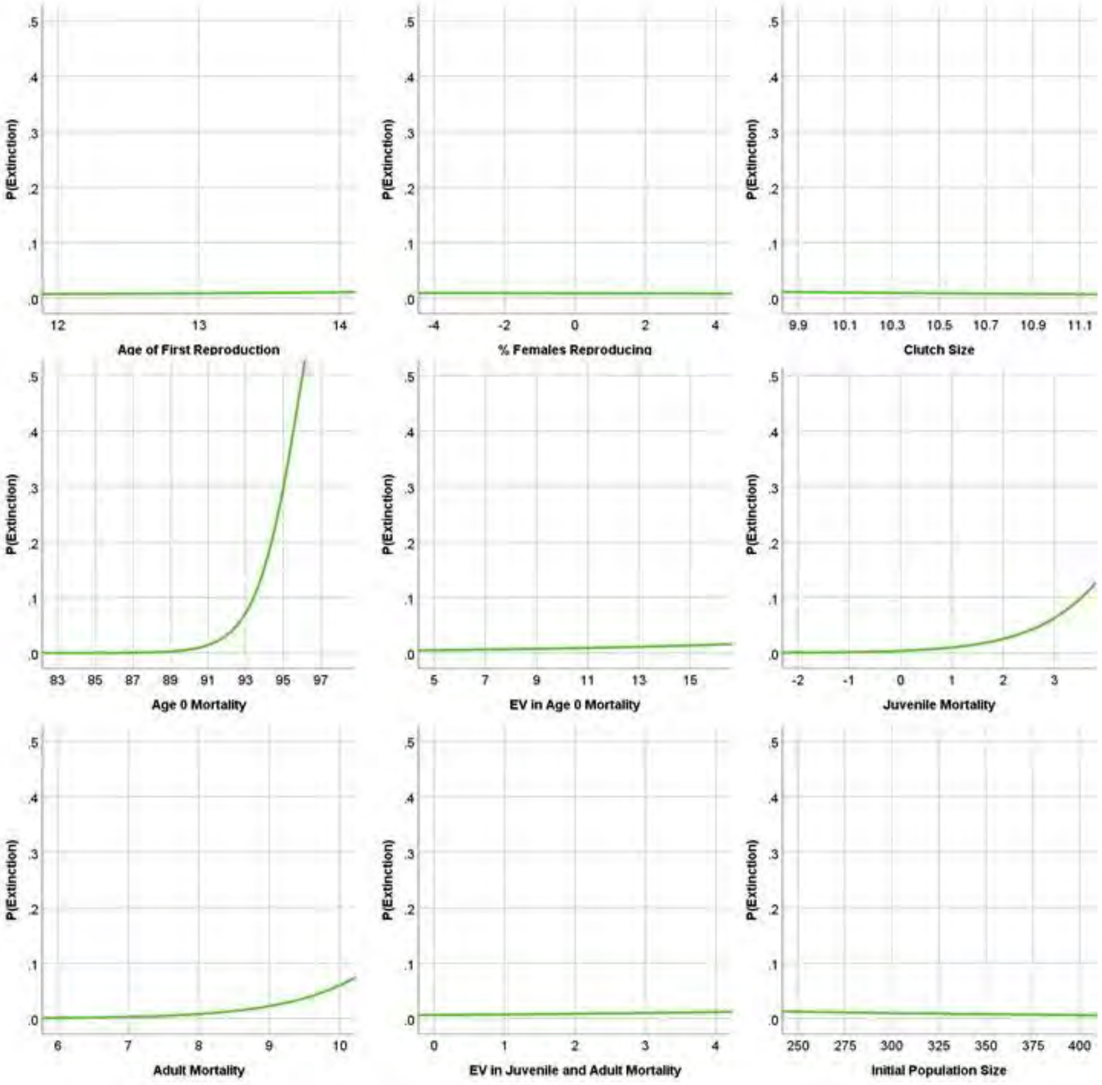

Figure S4. Sensitivity of probability of extinction to parameter uncertainty in the zero-growth Blanding’s turtle *Emydoidea blandingii* population model. Parameter values and parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). For each relationship, other parameters were held constant at their mean values. P(Extinction) refers to the probability of extinction. EV refers to environmental variation (standard deviation).

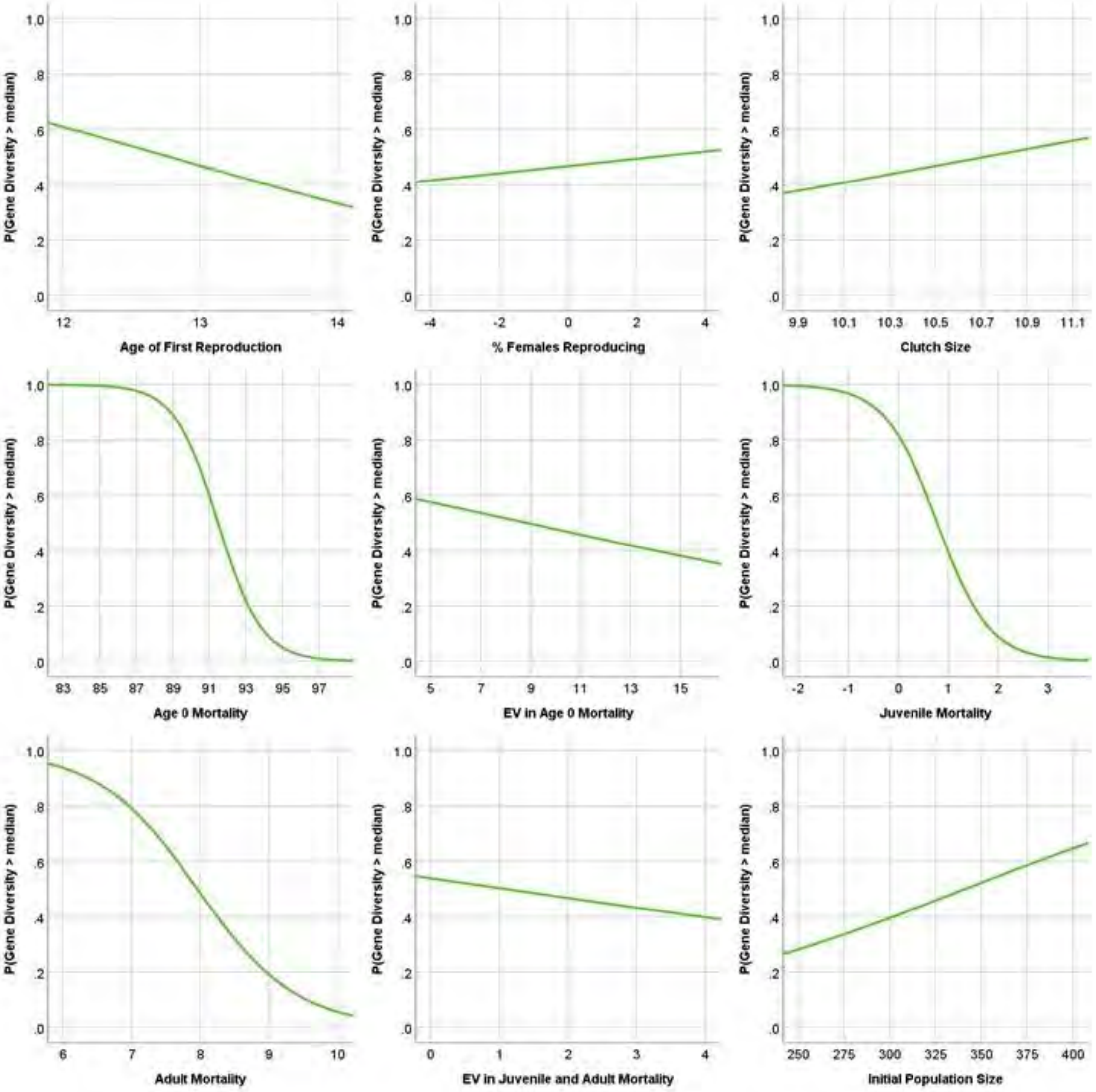

Figure S5. Sensitivity of gene diversity to parameter uncertainty in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values and parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). For each relationship, other parameters were held constant at their mean values. P(Gene Diversity > median) refers to the probability that the final genetic diversity is above the median (0.961). EV refers to environmental variation (standard deviation).

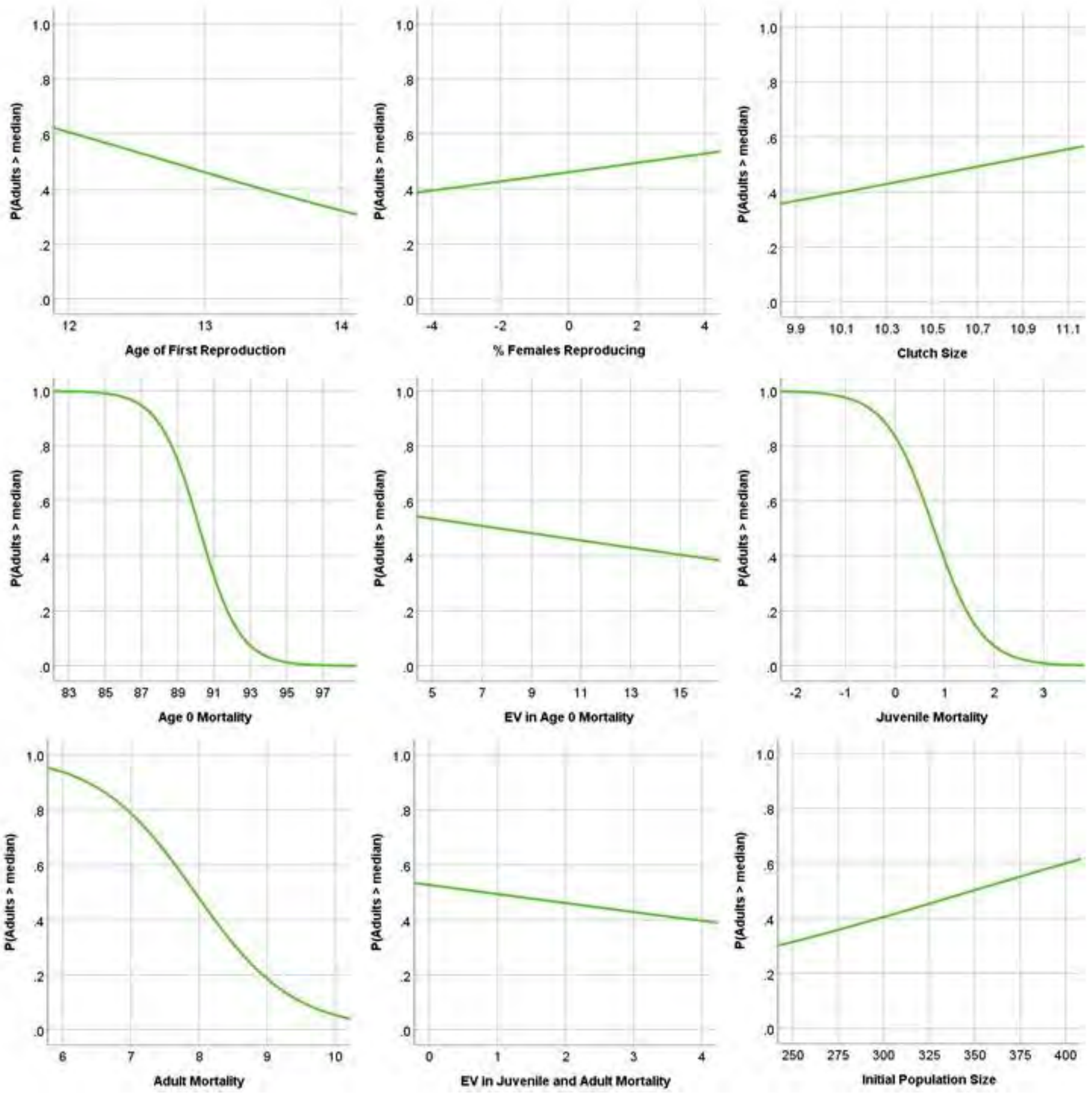

Figure S6. Sensitivity of adult population size to parameter uncertainty in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values and parameter uncertainty were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). For each relationship, other parameters were held constant at their mean values. P(Adults > median) refers to the probability that the final adult population size is above the median (43). EV refers to environmental variation (standard deviation).

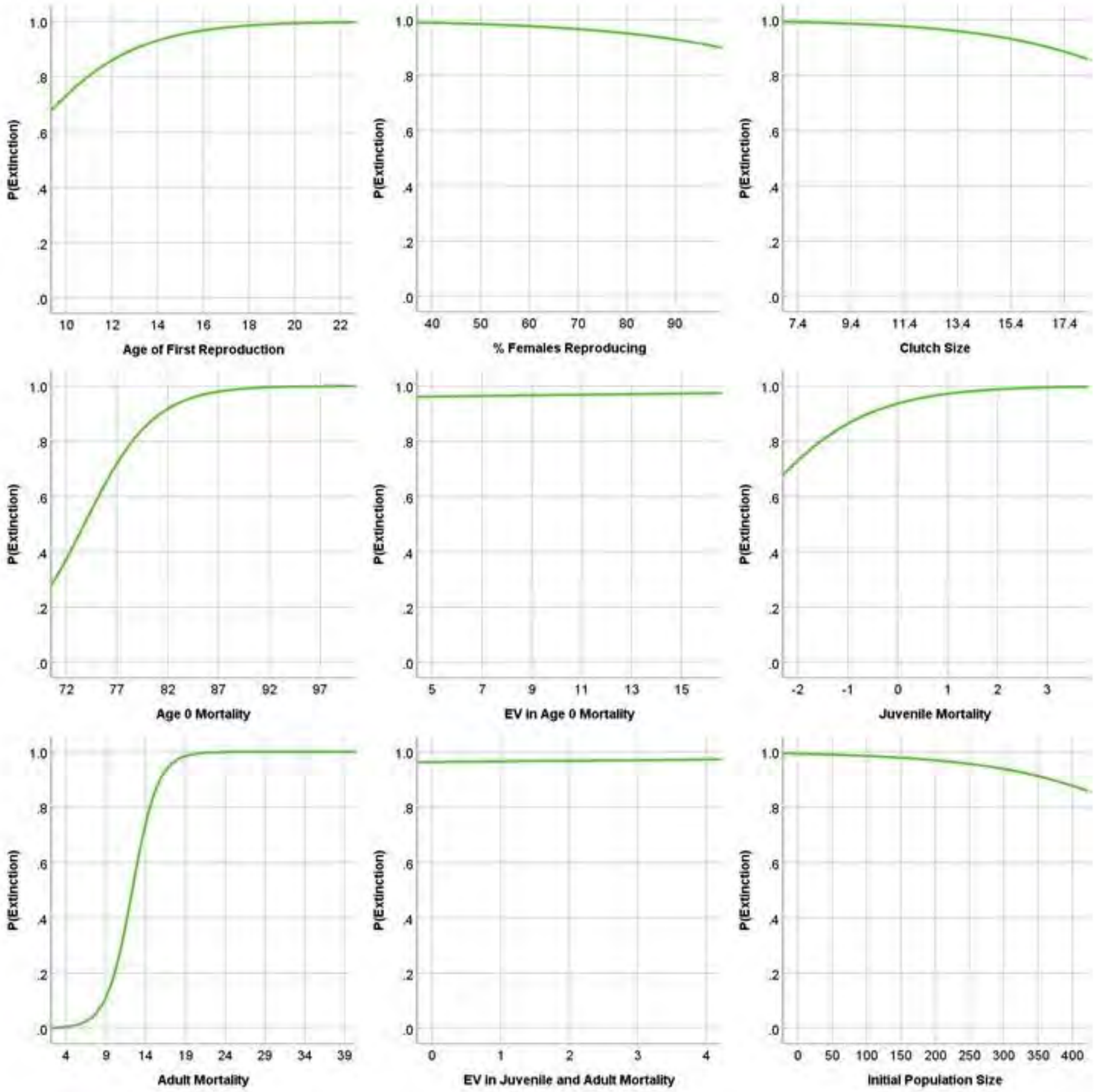

Figure S7. Sensitivity of probability of extinction to geographic parameter variation in the zero-growth Blanding's turtle *Emydoidea blandingii* population model. Parameter values are from throughout the species' range in North America (Table 1; Supplemental Material, Table S11). For each relationship, other parameters were held constant at their mean values. (Extinction) refers to the probability of extinction. EV refers to environmental variation (standard deviation).

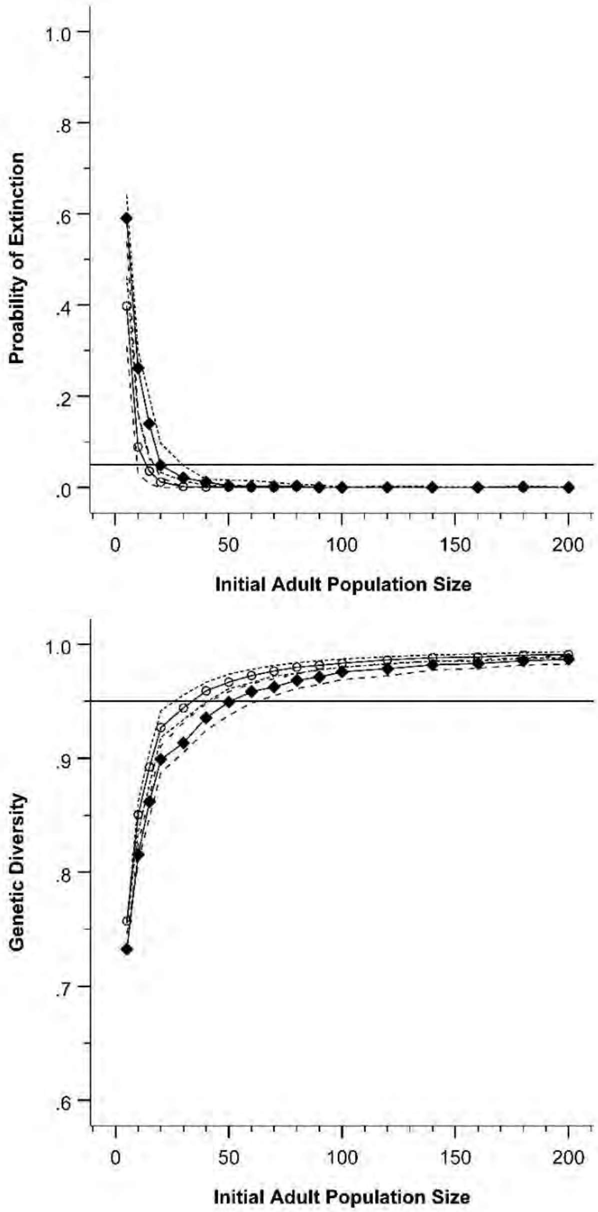

Figure. S8. Relationship of probability of extinction (upper panel) and genetic diversity (lower panel) to initial adult Blanding's turtle *Emydoidea blandingii* population size with (filled diamonds) and without (open circles) catastrophes in scenarios lasting 50 yr. Parameter values were mostly estimated from data collected at the Spring Bluff Chiwaukee Prairie study site from 2004-2018 with clutch size and mortality rates adjusted to achieve deterministic r = 0 (Table 1). Solid lines represent a scenario with intermediate environmental stochasticity and are bracketed by dashed (with catastrophes) and dotted (without catastrophes) lines that represent scenarios with low and high environmental stochasticity. Solid horizontal solid lines correspond to 5% probability of extinction (upper panel) and 95% retention of genetic diversity (lower panel).

Text S1. Comments on Blanding's turtle *Emydoidea blandingii* PVA implementation in Vortex.

Here we provide formulas used in Vortex to allow for variation in % females reproducing and age at first reproduction.

To allow the % females reproducing to vary with age, we used the following formula in Vortex:

```
(A=13)*(33)+(A=14)*(47)+(A=15)*(60)+(A=16)*(73)+(A=17)*(82)+(A=18)*(89)+(A>=19)*(94).
```

To allow age at first reproduction to vary in sensitivity tests for parameter uncertainty, we set age at first reproduction to the minimum desired age and then specified the annual % reproducing using the following formula in Vortex:

```
(A=(ROUND(SV1)))*(33+SV2)+(A=((ROUND(SV1))+1))*(47+SV2)+(A=((ROUND(SV1))+2))*(60+SV2)+(A=((ROUND(SV1))+3))*(73+SV2)+(A=((ROUND(SV1))+4))*(83+SV2)+(A=((ROUND(SV1))+5))*(89+SV2)+(A>=((ROUND(SV1))+6))*(93+SV2).
```

For example, to allow age at first reproduction to vary from 12 to 14, age at first reproduction is set to 12 and SV1 is allowed to vary from 11.5000 to 14.4999. This function also allows for the % females reproducing at a given age to vary according to SV2.

To allow age at first reproduction to vary in sensitivity tests for geographic parameter variation, a modified version of the Vortex code above was used:

```
(A=(ROUND(SV1)))*(MIN(33;SV2))+(A=((ROUND(SV1))+1))*(MIN(47;SV2))+(A=((ROUND(SV1))+2))*(MIN(60;SV2))+(A=((ROUND(SV1))+3))*(MIN(73;SV2))+(A=((ROUND(SV1))+4))*(MIN(83;SV2))+(A=((ROUND(SV1))+5))*(MIN(89;SV2))+(A>=((ROUND(SV1))+6))*(SV2))
```