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Hibernacula Site Selection of Hatchling Blanding's Turtles (Emydoidea blandingii)

by

Arika Nyhus

A Thesis

Submitted to the Graduate Faculty of

St. Cloud State University

in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

In Biological Sciences: Ecology and Natural Resources

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Thesis Committee: William M. Cook, Chairperson Matthew L. Julius Jennifer Y. Lamb Jeffrey S. Torguson

Abstract

The Blanding's turtle, *Emydoidea blandingii*, is a threatened semi-aquatic freshwater turtle ranging from the upper Midwest to Southeastern Canada, with isolated populations in Eastern states and provinces. Information regarding the spatial ecology and demography of the species is essential to population recovery. Although habitat utilization and spatial ecology of the adult Blanding's turtle has been well studied, little information is known about hatchlings following nest emergence. At Camp Ripley Training Center, hatchlings are relocated from protected nests to wetland complexes following emergence as an attempt to reduce mortality and eliminate a long journey to water. However, the success of this management strategy is still unknown. The objectives of this study are to 1) quantify the distances traveled and survivorship of hatchlings between release strategies, 2) discover whether hatchlings select aquatic or terrestrial habitat for hibernation through a third order habitat selection analysis, 3) identify the selection of habitat characteristics at hatchling locations through a fourth order habitat selection analysis, and 4) determine the most effective hatchling release strategy: either a) release hatchlings into the nearest wetland complex or b) release hatchlings directly at the nest site. In 2017 and 2018, transmitters were attached to hatchlings following nest emergence and escorted to wetland complexes frequently utilized for hatchling release. In 2019, hatchlings were released at the nest-site to compare movement patterns, survivorship, and habitat selections of hatchlings based on release strategy. Spatial distribution and macro-habitat selection were analyzed using ArcGIS and the Euclidean distance method. Micro-habitat selection was quantified through a series of paired t-tests and logistic regressions. The results suggest that hatchlings travel significantly farther when released at the nest site compared to wetland release but there is no significant difference in survival between release strategies. Hatchlings released in wetlands used the edges of uplands and wetlands non-randomly, however, there was no significant difference in habitat use between wetlands and uplands. Hatchlings released at the nest site used uplands nonrandomly and wetlands randomly. Uplands were significantly preferred over wetlands when hatchlings were released at the nest site. Between release strategies, hatchlings selected for greater substrate depths and more moss vegetation. From the findings of this research, it is recommended that wetland release continues, however, hatchlings should be released in wetlands characterized by waterlogged substrates that do not contain large bodies of open water. Additionally, land management practices should be updated to include buffer zones around wetlands, as upland habitat was shown to play an important role during the first hibernation for hatchling Blanding's turtles at Camp Ripley.

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Chapter 1: Introduction

Research Importance

The lineage of Testudines (turtles) began approximately 300 million years ago during the Triassic Era. Since their origin, turtles have survived several catastrophic events, including the Cretaceous extinction that killed the dinosaurs and many other fauna and flora species (Pérez-García, 2020). Today, turtles are the second most at-risk order of vertebrates, with approximately 61 % of turtles considered threatened or extinct (Lovich, Ennen, Agha, & Gibbons, 2018). These declines in turtle populations are mainly due to anthropogenic impacts including habitat loss, pollution, global warming, and over exploitation (Gibbons, Scott, Ryan, Buhlmann, Tuberville, Metts, & Winne, 2000). Turtles are also at greater risk to extinction than most species because of natural history traits such as delayed sexual maturity and low juvenile success (Rhodin, Walde, Horne, van Dijk, Blanck, & Hudson, 2011). Loss of Testudines should be concerning to humans because they play a role in mineral cycling, soil nutrients, seed dispersal, and in some cases, enhanced seed germination (Ernst & Lovich, 2009). Additionally, turtles make great indicators of pollution in an environment and hold cultural importance to humans (Ernst & Lovich, 2009). Despite being at such high risk of extinction, more charismatic species such as large bodied mammals and avian species typically receive more funding for conservation research. Sigouin, Pinedo-Vasquez, Nasi, Poole, Horne, and Lee (2017) reported that 112,290,000 dollars were donated to combat poaching of rhinos and elephants from 2013 – 2016, while only 2,194,271 dollars were allocated towards turtle conservation. It is very important that future research is concentrated on turtles in attempts to reduce the extinction risks these species face.

The Blanding's Turtle

Description and Distribution

Freshwater turtle declines are becoming of increasing concern, with approximately 63% of species requiring conservation action or attention in North America (Ernst & Lovich, 2009). One family of special concern is Emydidae. Emydidae is the largest family of freshwater turtles, with over 40% of the 95 species considered threatened, endangered, or critically endangered (Rhodin, Standford, van Dijk, Eisemberg, Luiselli, Mittermeier, & Walde, 2018). Among these species of concern is the Blanding's turtle (*Emydoidea blandingii*). The Blanding's turtle is a medium sized semi-aquatic turtle commonly known for its bright yellow chin, domed shaped body with yellow flecks, and charismatic smile. The body mass of adults ranges anywhere from 0.69 to 3.1 kg with a carapace length range of 16 to 28 cm. Blanding's turtles are similar in body size across its range apart from the population at Camp Ripley in central-Minnesota. These turtles are larger than all known populations with adult males and females measuring in lengths > 250 mm (Sajwaj, Piepgras, & Lang, 1998). Hatchling body size and carapace length is less documented, though several studies described that hatchlings weigh approximately 10 g with carapace lengths ranging from 29 to 35 mm (Congdon, Tinkle, Breitenbach, & van Loen Sels, 1983; Avery, van Loben Sels & Tinkle, 2000; Pappas, Brecke, & Congdon, 2000).

This species is found in most parts of the upper Midwest and southeastern Canada, with isolated populations existing in Eastern states and provinces. Larger populations in relation to their range occur in Michigan (Gibbons, 1968; Congdon & Gibbons, 1996), Wisconsin (Ross, 1989; Ross & Anderson, 1990), Minnesota (Pappas et al., 2000), and Nebraska (Rowe, 1992). The largest of these populations occur at Valentine NWR area in Nebraska with over 135,000

individuals and at the Weaver Dunes located in southeastern Minnesota with more than 5,000 individuals (Rowe, 1992; Pappas et al., 2000). Aside from these areas, most populations are limited in distribution and size (Congdon & Keinath, 2006). The Blanding's turtle is classified as threatened or endangered across most of its range and has been listed as threatened in Minnesota since 1984 (Department of Natural Resources, n.d.; Rhodin & van Dijk, 2011). This species is not currently federally listed under the Endangered Species Act. Though, in July of 2015, the U.S. Fish and Wildlife Service (USFWS) determined that federal listing of the Blanding's turtle may be warranted. Therefore, a status review has been initiated and a determination will be made whether to propose the Blanding's turtle under the Endangered Species Act following an assessment of the species (U.S. Fish and Wildlife Services, 2015).

Extrinsic Threats

Like most turtles, there are major threats impacting the Blanding's turtle including habitat degradation, road mortality, and collection for trade (Congdon & Keinath, 2006; Compton, 2007; Beaudry, deMaynadier, & Hunter Jr, 2009). Destruction of wetland habitat has been the largest contributor to population loss across the Blanding's turtle range (Dahl, 1990; Ross & Anderson, 1990; Rowe & Moll, 1991; Kinney, 1999; Pappas et al., 2000). This species is also at greater risk of road mortality and loss of habitat than most freshwater turtles because it utilizes both land and water during all life stages (Beaudry, deMaynadier, & Hunter Jr, 2008; current study). Hatchlings emerge from terrestrial nests from August to October (Butler & Graham, 1995; Standing, Herman, Hurlburt, & Morrison, 1997; Pappas et al., 2000). Adults move among several wetland complexes starting in April for foraging and mating purposes and again for overwintering in November (Ross & Anderson, 1990; Rowe & Moll, 1991; Kinney, 1999).

Females also travel up to 4 km from resident wetlands for nesting in late May through early July (Kinney, 1999). As human activity increases in areas with known Blanding's turtle populations, the probability that individuals can make these movements safely decrease dramatically (Congdon & Keinath, 2006).

Removal of individuals due to the pet trade also poses a threat to the stability of populations, especially those that are small and isolated due to the increased risk of loss in genetic diversity (Congdon & Keinath, 2006). It is illegal to possess or collect Blanding's turtles but there seems to be an increasing trend in the pet trade of this species and has been deemed to be one of the most "engaging and interesting" turtle species to collectors (U.S. Fish & Wildlife Service, 2013).

Intrinsic Threats

The natural history of the Blanding's turtle also makes population recovery due to human impacts problematic (Congdon, Dunham, & van Loben Sels, 1993). The Blanding's turtle is a long lived species (\approx 70 years) reaching sexual maturity between the ages of 14 - 20, though the average female does not reach sexual maturity until 17.5 years of age (Brecke & Moriarty, 1989; Congdon & van Loben Sels, 1991; Congdon et al., 1993). The Blanding's turtle experiences high fecundity with a range of 3 to 26 eggs per clutch (Ernst & Lovich, 2009). Despite the species' larger clutch size, this species has low reproductive success with only 10 - 20 % of females producing a maximum of one clutch per year (Congdon et al., 1983; Congdon et al., 1993). Moreover, those nests that are laid each year have a small chance of survival, with nest depredation ranging from 40 to 100 % when nests go unprotected (Avery et al., 2000). However, nest protection has proven to be an effective conservation strategy, as < 1 % of 101 protected

nests were depredated in a Nova Scotia population (Shallow, Standing, Herman, Morrison, & Power, 2000).

The Blanding's turtle also has temperature-dependent sex determination. Temperaturedependent sex determination means that the temperature at which eggs are incubated within the nest cavity determine the sex of the hatchling. For the Blanding's turtle, nesting season generally occurs from early-May to mid-July and eggs are incubated within the nest cavity for 75 – 110 days prior to hatchling emergence (Congdon et al., 1983). Warm incubation conditions will produce females while cooler incubation conditions will produce males. It has been predicted that a mean global temperature rise of 2°C will skew sex ratios while a 4°C increase will end the production of males entirely (Janzen, 1994). Increasing temperatures from climate change will result in biased sex ratios and reduce the probability of Blanding's turtle population persistence (Congdon & Keinath, 2006).

Focus Group

Congdon and associates (1993) state that high annual survivorship of juvenile Blanding's turtles is essential due to the species delayed sexual maturity. Annual survivorship cannot be understood without understanding the movement patterns and habitat use of the species at the micro and macro-level. Movement patterns and habitat use has been extensively researched in adult Blanding's turtles, though hatchlings remain largely understudied and has been considered the least understood period in a turtle's life cycle (Morafka, 1994). Lack of research in hatchlings is largely due to transmitter weight restrictions and battery life limitations in radio-transmitters (Paterson, Steinberg, & Litzgus, 2012; Kingsbury & Robinson, 2016).

The mass of a transmitter being applied to an animal must be restricted because it can affect the behavior, physiology, and survival of the study organism (Goodlett T., Goodlett G., & Hamilton, 1998). The American Society of Ichthyologists and Herpetologists recommend that transmitters weigh ≤ 10 % of an animal's body weight (Beaupre, Jacobson, Lillywhite, & Zamudio, 2004), but more conservative approaches recommend restricting the transmitter weight to ≤ 5 % (Gottwald, Zeidler, Friess, Ludwig, Reudenbach, & Nauss, 2019). Research on hatchling turtles is also time restricted when compared to larger conspecifics. As transmitter size gets smaller, the transmitter battery life also decreases. Due to the mass of hatchling turtles following nest emergence, transmitter weight is often limited to less than one gram. Transmitters that weigh less than one gram only last a few weeks while larger transmitters can last several years (Kingsbury & Robinson, 2016). Despite the constraint transmitters place on hatchling research, examination into the first year of life is critical as it is thought to be the most vulnerable life stage (Frazer, Gibbons, & Greene, 1990; Ernst & Lovich, 2009). Even slight changes in the survivorship of hatchlings can impact a population tremendously due to the already high mortality rate during the first year of life (Frazer et al., 1990; Congdon, Dunham, & Sels, 1994).

Study Purpose

Several conservation management strategies have been implemented in attempts to reduce hatchling mortality rates. These include head starting programs, protection of Blanding's turtle nests, and escorting hatchlings to wetland habitat following nest emergence. Head-starting refers to the process of housing hatchlings in a controlled environment for the winter and subsequently returning them to their original habitat in the spring. Head starting turtles has been reported to increase the survival rate to nearly six times that of hatchlings released at the nest site (Green, 2015). Yet, this management technique is expensive and time consuming (Heppell, Crowder, & Crouse, 1996). Nest protection is a mechanism used in conservation management to reduce predation of turtle nests and has been found to successfully prevent predation with minimal disturbance to turtle clutches (Riley & Litzgus, 2013). Escorting hatchlings is a conservation management strategy that consists of protecting nests and releasing hatchlings into the nearest wetland complex following nest emergence. Escorting hatchlings presumably eliminates a long journey to water, mitigates predation and road mortality, and reduces risks of desiccation. However, this management practice has never been researched to our knowledge.

Therefore, the purpose of this study is to determine the effectiveness of releasing hatchling Blanding's turtles in wetlands compared to releasing hatchlings at the nest site. The effectiveness of each hatchling release strategy will be measured by examining the movement patterns, survivorship, and the macro-micro habitat selections of randomly chosen hatchlings following nest emergence. The subsequent sections will review previous research regarding movements, survival rates, and habitat selections of hatchling Blanding's turtles and closely related species released at the nest site. Following this review, the objectives and hypotheses of this thesis project will be introduced.

Previous Hatchling Literature

Movement Patterns

Female freshwater turtles typically deposit their eggs in sandy soils, cover their nest back up, and retreat to resident wetlands. Eggs incubate for several months and hatchlings typically emerge from nests in autumn. Because hatchling turtles receive no help following nest emergence from parents, hatchlings must exhibit innate behaviors that allow individuals to orient themselves and disperse from the nest site to suitable habitat using environmental cues (Pappas, Congdon, Brecke, & Capps, 2009). Visual, olfaction, and auditory cues have been claimed to be the most important stimulation within the first couple hours of nest emergence (Pappas et al., 2009). These cues simply involve the sensing organs associated with each sense. Dispersal based on visual stimulation occurs when hatchlings use light and dark horizons to orient themselves. Olfaction cues can help hatchlings move towards water when nests are close enough to wetlands, and auditory cues have also been argued as a back-up orientation mechanism for freshwater turtles that must orient towards lotic habitats (Tuttle & Carroll, 2005). In addition to sensory organs, some have suggested that freshwater turtles exhibit internal compasses such as a geomagnetic or a sun compass (Castellano, Behler, & Ultsch, 2008; Pappas et al., 2009; Congdon, Pappas, Krenz, Brecke, & Schlenner, 2015; Pappas, Congdon, & Brecke, 2017). A geomagnetic compass uses the Earth's magnetic field to sense the environment while a sun compass uses the orientation of the sun in conjunction with an internal biological clock that allows an animal to navigate in a single direction (Caldwell & Nams, 2006). For the Blanding's turtle, several studies have concluded that hatchlings first utilize visual stimulation and orient towards dark horizons to navigate towards riparian forests. A sun compass is then developed within two days of nest emergence (Pappas et al., 2009) and hatchlings use this sun compass to continuously navigate towards dark horizons throughout the day. These studies also included methodologies to test for a geomagnetic compass, however, all research led to the conclusion that the presence or absence of a magnet had no effect on hatchling movements. Therefore, there is no evidence that hatchling Blanding's turtles use a geomagnetic compass to orient themselves

while dispersing from nests (Pappas et al., 2009; Congdon et al., 2015; Schlenner, 2015; Krenz, Congdon, Schlenner, Pappas, & Brecke, 2018).

In most cases, orientation becomes increasingly more difficult as distances from nests to water increase (Pappas et al., 2009). The distance that hatchling Blanding's turtles must travel may be substantially farther than most turtles, as females are known to move several km away from wetlands for suitable nesting sites (Pappas, Congdon, & Brecke, 2017). For example, the mean distance to water for 138 Blanding's turtle nests was 622 m while the mean distance to water was 37 m for 87 snapping turtle (Chelydra serpentina) nests at Weaver Dunes (Pappas et al., 2009). Distances traveled by hatchling Blanding's turtles following nest emergence has gained little attention. Though one study was conducted on hatchling Blanding's turtle (n = 48)and hatchling wood turtle (n = 45) movements following nest emergence in Ontario using radio transmitters that weighed 5-8 % of the hatchlings' body mass with a battery capacity of 60 days. They reported that the largest distance traveled by hatchling wood turtles was 195 m while the largest distance traveled by hatchling Blanding's turtles was 449 m (Paterson et al., 2012). Releasing hatchlings in the nearest wetland complex could aid in hatchling orientation and potentially reduce the distances hatchlings need to travel to reach potentially suitable habitat for overwintering.

Survivorship

Because female Blanding's turtles typically nest far away from resident wetlands, hatchlings are at great risk of predation, have longer exposures to fluctuating environmental conditions, and are more susceptible to road mortality (Pappas, Congdon, & Brecke, 2017). In general, the hatchling stage is categorized by low survivorship when compared to adult conspecifics. Annual survivorship in hatchling freshwater turtles has been documented as low as 0.06 while adult survivorship in freshwater turtles has ranged between 0.76 to 0.96 (Heppell, 1998). Low survivorship during this life stage is largely due to small body size which increases their risk of predation and vulnerability to fluctuating environmental conditions (Tamplin, 2009). Hatchlings are also more at risk for desiccation because they have a greater surface area to volume ratio than adults, which in turn, means hatchlings absorb heat faster leading to higher moisture loss (Boyer 1965; Stevenson, Peterson, & Tsuji, 1985; Kolbe & Janzen, 2002).

The fate of hatchling Blanding's turtles and closely related species demonstrates the vulnerability of hatchlings following nest emergence. Dragon (2015) found that 52% of hatchling wood turtles (*Glyptemys insculpta*) were predated, 22 % survived the study period (approximately two months), 15% of the fates were unknown, 9 % drowned, 1 % were found desiccated, and 1 % were ran over by a vehicle. Similarly, Paterson and colleagues (2012) found that 56 % of hatchling wood turtles were predated, 24 % were considered lost, 11% survived the study (approximately two months), and the remaining 9 % of the hatchlings drowned. This study also tracked hatchling Blanding's turtles and reported that 42% survived the study period, 38 % of the hatchling were considered lost, 16 % were predated, 2% were found desiccated, and the remaining 2% were found dead on the road. By releasing hatchling in wetland complexes, hatchling survival rates may increase, as desiccation risks, encounters with roads, and predators would presumably decrease.

Habitat Selection

Habitat is the space in which an organism resides and contains biotic and abiotic resources necessary for the survival of a species (Brussard, Ball, Caughley, & Gunn, 1996). Habitat use refers to an extended length of time an animal occupies a habitat, while habitat selection is where animals utilize habitat disproportionately to its availability (Johnson, 1980; Mayor, Schneider, Schaefer, & Mahoney, 2009). Habitat selection can be investigated at four different spatial scales: first order selection, second order selection, third order selection, and fourth order selection. First order selection occurs when climate and geology features are selected for throughout the geographical range of a species. Second order selection occurs when a population spends more time in certain habitats than other habitats within the population range (area that a population is distributed throughout a landscape). Third order selection occurs when individuals spend more time in certain habitats than other habitats within the individual's home range and fourth order selection identifies the characteristics (i.e. vegetation, food, soil) that are being selected for within preferred habitat (Johnson, 1980). First, second, and third order selection are often referred to collectively as macrohabitat selection while fourth order selection refers to microhabitat selection (Mayor et al., 2009).

Adult habitat selection for overwintering has been extensively studied at the macro (second and third order) and micro (fourth order) scale across the range of the Blanding's turtle. Blanding's turtle populations are known to overwinter in permanent water bodies with a preference for shrub swamps at Camp Ripley (Sajwaj et al., 1998). Yet, macro and micro hibernacula site selection in hatchling Blanding's turtles remains largely debated. In cold temperature regions, freshwater hatchlings are faced with the challenge of surviving unpredictable winters. Most hatchlings in northern regions emerge from their nests in the fall (August-October) and it is assumed that hatchlings must retreat to aquatic habitats to reduce predation and desiccation (Ultsch, Draud, & Wicklow, 2007). While some studies support the notion that hatchlings retreat to aquatic habitat for overwintering, others have suggested that hatchlings also overwinter on land.

A study was conducted on hatchling Blanding's turtles (n = 48) in Ontario, Canada and it was concluded that all but two hatchlings entered aquatic habitat for hibernation (Paterson et al., 2012). They reported that hatchlings and adults preferred marsh and swamp habitats over upland forests, and hatchlings selected for more ground cover and woody vegetation when compared to random sites. Butler and Graham (1995) indicated that hatchling Blanding's turtles (n = 63) also sought standing water upon nest emergence in Massachusetts, but they reported that the habitats hatchlings were selecting were different from those of adults. The habitats selected by hatchlings were flooded wetlands and vernal pools characterized by standing water with shallow water depths and largely contained *Sphagnum* and muck. However, they did admit that this population has a lesser distance to travel to get to water (< 200 m) than most respective populations. McMaster and Herman (2000) looked at habitat selection and movement patterns of Blanding's turtles (n = 22) from ages 1 – 20 in Nova Scotia. They found that all life stages selected for sedge meadow aquatic habitat with slow moving, shallow water (1-2 m) containing an abundance of *Sphagnum* vegetation.

In contrast, hatchling Blanding's turtles (n = 78) were tracked for 11 days following nest emergence in Nova Scotia and it was concluded that hatchlings moved random with respect to water (Standing et al., 1997). Furthermore, only 18% (n = 14) of hatchlings from this experiment entered water and many hatchlings seemed to overtly avoid it. Similarly, McNeil, Herman, and Standing (2000) released hatchling Blanding's turtles (n = 36) at various distances to water following nest emergence in Nova Scotia. It was concluded that hatchlings did not seek water upon nest emergence, even when they were released adjacent to the wetland edge. In fact, only 22% of the hatchlings entered water over an average of three days and 66% of the hatchlings released 0.25 m away from wetland edges retreated upland away from the water.

Due to the inconclusive evidence regarding hibernacula site selection in hatchling Blanding's turtles, several studies have attempted to ascertain whether this species is adapted to survive winter on land. Dinkelacker, Castanzo, Iverson, and Lee Jr. (2005) conducted an experiment to compare the hatchling Blanding's turtle's (n = 10) ability to survive in normoxic aquatic habitat to hatchling softshell turtles (*Apalone spinifera*; n = 7) and snapping turtles (n =8), who are known to overwinter in aquatic environments. It was found that all hatchling softshell and snapping turtles survived the duration of the study (77 days) while approximately one-third of the hatchling Blanding's turtles died. These findings concluded that if hatchling Blanding's turtles do overwinter in aquatic environments, they are restricted to highly oxygenated microhabitats.

Dinkelacker, Castanzo, Iverson, and Lee Jr. (2004) also examined the susceptibility to dehydration, supercooling capacity, resistance to inoculative freezing, capacity for freeze tolerance, and physiological responses to somatic freezing in hatchling Blanding's turtles (n = 77). The evaporated water loss mean (4.1 mg·g⁻¹·d⁻¹) was intermediate to those values reported for species known to overwinter on land (range: 0.9 to 3.6 mg·g⁻¹·d⁻¹; n = 5) and species known to overwinter in aquatic environments (range: 6.3 to 11.4 mg·g⁻¹·d⁻¹; n = 3). In this study, the

hatchlings ability to withstand freezing (supercooling capacity) was up to -14.0 °C but they found little evidence that hatchlings could resist freezing (inoculative freezing). They also found that hatchlings were not negatively affected by brief exposure to -8.0 °C or prolonged exposure to -4.0 °C, indicating that hatchling Blanding's turtles can tolerate hypothermic stress. Due to the intermediate evaporated water loss between species that overwinter on land and water, this study suggested hatchlings that may overwinter on land would need to seek moist substrates where they can burrow to avoid severe water loss and minimize freezing.

A similar study done by Packard G., Packard M., Lang, and Tucker (1999) tested the freezing tolerance in hatchling painted turtles (*Chrysemys picta*; n = 7), slider turtles (*Trachemys scripta*; n = 8), snapping turtles (n = 8), and Blanding's turtles(n = 8). They concluded that all species survived freezing at - 2.0 °C for up to 30 hours, however, only hatchling painted turtles and hatchling Blanding's turtles were able to survive past 30 hours and suggested that these species withstand terrestrial freezing better than hatchling slider and snapping turtles.

Statistical Analysis of Habitat Selection

Habitat selection occurs when animals select habitat types more often than would be expected based on the habitat that is available to them (White & Garrott, 1990). To determine which habitats are being used disproportionately to availability, one must determine what is spatially accessible to the study organism. Moreover, the defined accessibility must be assumed to be equally available to all sampling units. Unfortunately, there is no way of directly telling if the habitat deemed available by the investigator is perceived as available to the animal (White & Garrott, 1990). To limit arbitrary available habitat estimates, wildlife studies generally utilize the population range, home range, or average daily distance estimates to quantify the available habitat to the study units. The definition of the amount of habitat available is then based on the spatial scale at which habitat selection is being investigated. In general, second order habitat selection studies use the population range to define habitat availability, third order studies use individual home ranges to select an available habitat boundary, and fourth order studies often use an average daily distance traveled by the study organism (Aebischer, Robertson, & Kenward, 1993; Conner & Plowman, 2001; Edge, Steinberg, Brooks, & Litzgus, 2010; Paterson et al., 2012).

Habitat use by the animals must also be compared to habitat use that is expected based on the amount of area of each habitat type. Habitat selection studies establish the expected habitat use ratios by using the proportion of area made up by each habitat type within the available range and multiply that by the total number of locations (Friedman, 1937; Neu, Byers, & Peek, 1974; Johnson, 1980). In recent decades, habitat selection analyses have used mapping computer programs (i.e. ArcMap) to generate random points within the defined available habitat to compare animal locations to random locations (Conner & Plowman, 2001; Edge et al., 2010; Paterson et al., 2012).

One habitat selection analysis that has been gaining increasing attention is the Euclidean distance approach (Conner & Plowman, 2001). This habitat analysis is a distance-based approach and is desirable because it uses the animal as the sampling unit, analyses can test between groups, and covariates can be included with a MANOVA. Additionally, the Euclidean distance method can be adapted to any spatial scale. This approach evaluates habitat selection by comparing the animal location distances to habitat types to the distances to habitat types from simulated random points. If animals are selecting habitat at random, then the distances between

habitat types should be similar for animal locations and random locations. Moreover, the ratio calculated from animal distances to random distances should equal 1.0. If animals are selecting habitat non-randomly, ratios > 1 indicate the animal used the habitat less than expected and ratios < 1 signify that the animal used the habitat more than expected based on available habitat. These habitats can then be compared to evaluate which habitats significantly differed from the others and a relative rank of habitats can be established.

Study Objectives and Hypotheses

This study compares the movement patterns, survivorship, and macro-micro habitat selections of hatchling Blanding's turtles released into wetlands to hatchlings released at the nest site following nest emergence at Camp Ripley Training Center in Little Falls, Minnesota. The objectives of this study are to 1) quantify the distances traveled and survivorship of hatchlings between release strategies through statistical analyses, 2) discover whether hatchlings select aquatic or terrestrial habitat for hibernation through a third order habitat selection analysis, 3) identify the selection of habitat characteristics at hatchling locations through a fourth order habitat selection analysis, and 4) determine the most effective hatchlings directly at the nest site. It is hypothesized that 1) hatchlings released at the nest site will travel significantly farther and have a higher mortality rate than those released in wetland complexes, and 2) hatchlings will select habitat based on release strategy. Therefore, hatchlings released in wetland complexes will remain in those wetland complexes while hatchlings released at the nest site will overwinter on land. 3) Hatchlings will prefer less open water and more emergent vegetation cover than random

sites, and 4) releasing hatchlings in the nearest wetland complex is the most effective hatchling release strategy.

Chapter 2: Materials and Methods

Spatial locations of state-listed species are considered nonpublic data under the Minnesota Data Practices Act. Due to the status of the Blanding's turtle, specific location information has been withheld from this document. Data pertaining to this thesis project can be requested through the Minnesota Natural Heritage Information System:

https://files.dnr.state.mn.us/eco/nhnrp/natural_heritage_data.pdf

Study Area

The study area for this project is Camp Ripley Training Center, a 53,000-acre military training site located 16 km north of Little Falls, MN (see Figure 2.1). This military training center falls within the transitional zone of northern coniferous and deciduous forests at an elevation between 1122-1535 ft above mean sea level. Human development at Camp Ripley is minimal, with most development impacts occurring in the cantonment area. Down range consists of secondary roads and trails and several designated areas which have man made developments for training purposes. The land at Camp Ripley is surrounded by the Mississippi River to the east and the Crow Wing River to the north (see Figure 2.2). An abundance of continuous and diverse wetlands including shrub swamps, deep and shallow marshes, vernal pools, and inland freshwater habitat surround the training center. Many wetlands across Camp Ripley are protected from military disturbance, including areas with known Blanding's turtle habitat. The uplands of Camp Ripley consist of extensive mixed hardwood and conifer forest regions, and many open fields consisting of short grasses, shrubs, and forbs. Several areas are deemed off limits to military personnel to protect the habitat of threatened and endangered species. Most of the lands at Camp Ripley are maintained through clear cutting and prescribe fires to reduce hazard burns,

limit the spread of invasive species, and to enhance flora and fauna habitats. Additional land is cleared for training and consist of impact areas for military purposes. The soils at Camp Ripley are comprised of dark gray loamy sands with most of the soil being gravelly sand subsoils, making Camp Ripley appropriate Blanding's turtle habitat for nesting purposes.



Figure 2.1 Location of Camp Ripley military training center in Morrison County, MN. Map created by Arika Nyhus (2018).

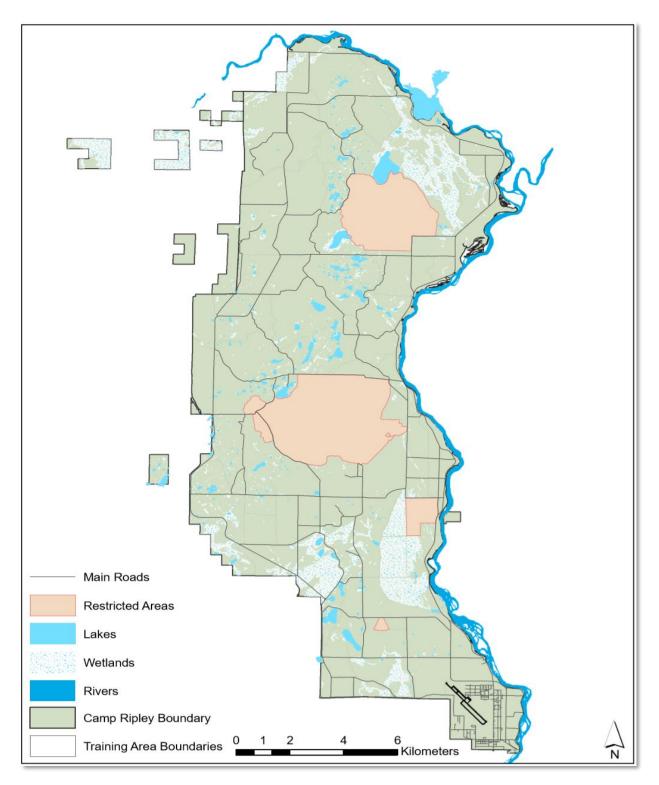


Figure 2.2 Topographical features of Camp Ripley near Little Falls, MN. Map created by Arika Nyhus (2020).

Roadside Surveys

Surveys began prior to the start of nesting season and ended after two to three days of no turtle sightings. Roads were surveyed by conducting vehicle searches through areas of known nesting activity and areas with potential nesting activity. One to three trucks ran circular routes daily starting as early as 1630 and ending as late as 0300, depending on demand. In the southern region, surveys began on Argonne Road and continued through Normandy Road. Survey efforts were primarily focused on Luzon Road, Marne Road, and Manila Road. Secondary routes were focused on Argonne Road, Armor Trail, Cody Road, and Normandy Road. In the northern region, surveys began on East Boundary Road near gate 48A up to the most northern part of camp on Yalu Road. Survey efforts were primarily focused on East Boundary Road, Pusan Road, Wonsan Road, and Chorwan Road. Secondary routes were conducted on Casino Road, Salerno Road, Firebreak Road, Fort Greely Road, and Yalu Road.

During roadside surveys, any turtle tracks were examined in attempts to locate the turtle and areas away from roads with known nesting activity were checked frequently. Once a Blanding's turtle was spotted, the turtle's activity was observed. If the female had her neck close to the ground while walking the gravel road, the activity was deemed as searching behavior. A bright orange flag was dropped near the female and surveying would proceed. After 30-45 minutes, surveyors returned to analyze the spotted female's behavior. If no progress had been made, surveying would continue, and the cycle would repeat. If the female had begun digging, surveyors would remain at the site at least 100 m away from the digging female to further observe her behaviors. Surveyors made the decision if they should continue surveying or to wait for the female to finish based on her progress. Once the female covered her nest, the female was captured for data collection, and four reflective tape squares were pressed onto the carapace to facilitate future sightings and to reduce road mortality.

Nesting Female Data Collection

Females were measured at the midline length of the carapace and plastron to the nearest cm using a large caliper; width was determined at the widest point of the carapace and at the pectoral seam of the plastron. Females were placed into a pre-weighed bag to estimate the mass (g) of the individual by hanging the bag on a Pesola scale. Additionally, the identification code (ID), age, reproductive status, time, location of capture, as well as any morphological anomalies were recorded. First, females were checked for a three-code ID (Cagle, 1939). If an ID code was not present, a unique code was given to the female. No two turtles can have the same ID, so available codes were determined prior to nesting surveys. Unique codes were given to females using a triangular-shaped file that engraved a V indent into the outer scutes, with each scute representing a letter from the alphabet. If the female had previously been marked, the file was used to reinforce the marks already present to ensure future ID. Age of the females were determined by counting the growth rings (annuli) on the plastron. If a female was ≥ 20 years of age, the individual was recorded as 20+ due to the difficulty of accurately counting the growth rings (annuli) past this age. Female reproductive status was established by palpating for the presence of eggs. Turtles were palpated near the rear legs under the plastron. Females were denoted as gravid if lump-like marbles were present. The time of observation was recorded in military hours and females were documented as being found on either the north or south region of camp. Additionally, coordinates were recorded using NAD83/Universal Transverse Mercator

system (UTM) Zone 15 North and a Global Positioning System (GPS) receiver (GPSmap 64st, Garmin) with an accuracy from 0.5-2 m.

Nest Protection Protocol

After data collection, a 1 m² metal cage was placed over the center of where the eggs were laid and was dug into the ground approximately 8-10 cm deep to reduce predation. Two yellow posts with reflective tape were then positioned to face oncoming traffic to prevent vehicle disturbance (see Figure 2.3). Throughout the duration of the summer, nests were checked periodically for human/animal disturbance, standing water, and vegetation coverage. If the nest was disturbed by a human or an animal, the severity of damage was documented, and the cage was re-placed over the center of the nest. If the nest contained standing water from rainfall, a tunnel was created far away from the center of the nest using a shovel to allow the water to drain from the nest area. Lastly, any vegetation that created a shadow over the center of the nest was removed to promote sun exposure for the incubating eggs.

Hatchling Data Collection and Transmitter Attachment

Following nest emergence, hatchlings from each clutch were stored in a 10 L bucket for data collection. Turtles were measured for midline length and width on the carapace and plastron to the nearest mm using a digital caliper. Mass of the hatchlings was determined using a 20 g weight limit Pesola scale. Hatchlings were then assigned a number that was attached to the carapace using temporary construction tape. After data was collected from the clutch and numbers were assigned, hatchlings were separated by weight categories. The weight categories included hatchlings from 7.0-8.0 g, 8.5-9.0 g, 9.5-10.0 g, and 10.5-11.0 g. If a hatchling weighed

between the weight categories (e.g., 8.25 g), that hatchling was put in the nearest digit category (e.g., 8.5-9.0 g). Using a random number system (Damon & Harvey, 1987), hatchlings were randomly selected for telemetry attachment from each weight category. Additionally, each hatchling selected for telemetry attachment was given a unique turtle ID. Unlike the adult females, the selected hatchlings were not physically marked with the assigned ID due to a high risk of harming the hatchlings (Plummer, 1979; Davy, Coombes, Whitear, & MacKenzie, 2010). The hatchling IDs were only used in this study to distinguish between turtles. The code assigned to each hatchling was related to the ID code that was provided to the adult maternal female followed by a consecutive number. The letter H was placed in front of each code to differentiate between the mothers and the offspring.

Prior to fitting the transmitter, the carapace of the hatchling was cleaned using water and time was allowed for the carapace to dry to ensure the transmitter set properly. Transmitters were affixed using a fast-drying epoxy compound (Devon Two Part Epoxy). The epoxy was mixed and applied to the carapace approximately midway down the turtle between the dorsal line and the marginal scutes (see Figure 2.4). The turtles were then set in buckets to allow the epoxy to set. Though the recommended wait time to allow the epoxy to set was 5 minutes, turtles were held for approximately one hour prior to release.



Figure 2.3 Representation of nest protection for the Blanding's turtle (*Emydoidea blandingii*) at Camp Ripley using 1 m^2 metal cages and yellow posts with reflective tape to eliminate vehicle disturbance. Photo taken by Arika Nyhus (2017).



Figure 2.4 Position of transmitter on hatchling Blanding's turtles (*Emydoidea blandingii*) at Camp Ripley. Photo taken by Arika Nyhus (2017).

Hatchling Release Sites

In 2017-2018, hatchlings were released in two wetland complexes known as Goose Lake and Range Marsh. Goose Lake and Range Marsh were chosen as release sites for several reasons. During the field work conducted by Sajwaj and associates (1998), they found that Goose Lake and Range Marsh were the most utilized wetland complexes by monitored Blanding's turtles in the southern region of Camp Ripley (~63%; 25 of 40 turtles). If hatchlings prefer the same habitat as adults in this population, we assumed that these habitats would be the best representation of ideal wetland release sites for hatchlings. Additionally, these wetland complexes are concentrated in high nesting activity areas. Therefore, hatchlings selected for this study were not transported significantly far from natal nest sites, with an average distance of 1.9 km from nest site to hatchling release location. Finally, we wanted to pick wetlands that have been frequently utilized for hatchling release in previous years and these two wetland complexes are amongst the most popular in the southern region of camp.

Goose Lake is an 18.46 ha aquatic habitat that contains several wetland types. This waterbody consists mostly of inland open fresh water, with patches of bog and seasonally flooded habitat. This wetland is dominated by hydromorphic-rooted aquatic plants such as Variegated pond-lilies (*Nuphar variegate*) and American White Water-lilies (*Nymphaea odorata*). Woody vegetation and Peat moss (*Sphagnum*) are sparsely present in bog patches, and emergent vegetation covers < 25% of the habitat. This region is permanently flooded with water depths \leq 3 m, and mineral soils lack organic content. Range Marsh is an 14.06 ha habitat that consists mostly of saturated emergent wetlands, with a large region of shrub swamp habitat and smaller non-vegetated aquatic communities. Much of Range Marsh is covered by Sedges (*Carex*)

with patches of woody vegetation containing Alder (*Alnus*), Dogwood (*Cornus*), and Willow (*Salix*) species. This area is semi permanently flooded, and the soils vary from deep organic muck to mineral soils with high organic content.

In 2017-2018, 12 hatchlings were randomly selected to be released in Goose Lake and 11 hatchlings were spread throughout Range Marsh from four different nests. Hatchlings were distributed within the wetland complexes by randomly selecting an area easily accessed by humans and releasing the hatchlings on the edge of the wetland to simulate current management practices. In 2019, 20 hatchlings were randomly selected for tracking from four different nests and released at their specific nest location to compare movement patterns, survivorship, and micro-macrohabitat selections to hatchlings released in wetland complexes. Once released, individuals were located every one to three days using a receiver (model R4100, Advanced Telemetry Systems) and locations were recorded using a hand-held GPS unit (GPSmap 64st, Garmin).

Survivorship and Movement Patterns

The fates of all monitored hatchlings were recorded and characterized from 2017-2019. Categories included survived until end of transmitter battery life, predated, drowned, lost transmitter, or lost hatchling. Hatchlings were considered predated when remains were found that showed evidence of an attack. Predation was recorded if only the hatchling's empty carapace was found or if the hatchling was found deceased with missing limbs and/or organs. Hatchlings categorized as drowned were discovered under water with no indication of external damage. A transmitter loss was documented when only an intact transmitter was found with no signs of predator activity. A lost hatchling could not be assigned to any of these categories and included hatchlings who presumably carried a failed transmitter. Survival rates were compared between Range Marsh and Goose Lake, as well as between release strategies (wetland release vs. nest site release) using a Fisher's exact test. A two-sample t-test was used to test if there was a significant difference in survival rates based on hatchling body mass.

Movement patterns and distances between consecutive points were quantified using ArcMap 10.6 from Environmental Systems Research Institute (ESRI, Redlands, California, USA). Total distance traveled by each hatchling was found by converting the point features (locations) into a line feature based on date using the *points to line* tool within ArcMap. Data points were arranged by date using the sort field within the tool. Therefore, hatchlings had a oneline feature that connected points based on the successive dates the hatchlings were located. Average daily distance for each hatchling was quantified by using the *split line at vertices* tool. This then provided the distances traveled between the successive dates the hatchlings were located. If a hatchling was not tracked daily throughout the study period, the amount of days between observations was divided by the provided distance. Two-sample t-tests were used to assess if total distance traveled among wetland release sites and if release strategies were significantly different.

Third Order Habitat Selection (Macro-Scale)

Because one of the main objectives for this project was to identify if hatchlings were selecting wetlands or uplands following nest emergence, the landscape at Camp Ripley was partitioned into two general categories: wetland and upland habitat. The landscape was digitized using an orthophotograph obtained from the Army National Guard (2016) and ArcMap 10.6. Wetland habitat boundaries were distinguished from uplands through the National Wetland Inventory (NWI) wetland polygon feature class provided by the U.S. Fish and Wildlife Service (2020). Wetland polygons delineating wetland habitat type were combined into one polygon feature class. All other landscapes for this study were considered upland habitat and these areas were digitized as a separate polygon feature class.

Habitat selection by hatchlings at the macro-scale was analyzed using a modification of the Euclidean distance method (Conner and Plowman, 2001; Edge et al., 2010; Paterson et al., 2012). This method essentially compares the average distance to each habitat (upland and wetland) for hatchlings to the average distance to each habitat for random locations within a given distance. The distance used to quantify habitat availability is generally based on a population or home range, however, these ranges are not known for hatchlings and the hatchlings are confined to a fixed starting point (wetland release site or nest site). Therefore, the largest distance traveled by hatchlings was used to define habitat availability (Paterson et al., 2012) and ArcGIS was used to create a boundary that represented the habitat available to each hatchling. The maximum distance traveled by hatchlings released in the wetland complexes was 536 m and the largest distance traveled by hatchlings released at the nest site was 842 m. Therefore, individual boundaries (available habitat) were created around the release point of individual hatchlings and the specific distance used to set this boundary (536 m or 842 m) depended on release strategy (wetland release or nest site release). Individual hatchlings were used as the sampling unit and only hatchlings that had 10 or more locations were included in the analysis. During the duration of the study, some hatchlings were tracked longer than others due to variation in battery life and the quantity of additional transmitters. Therefore, to account for unequal sampling intensity, 10 locations were chosen randomly for hatchlings that were located

more than 10 times. Hatchling locations were randomly selected based on date using the "sample from columns" option in Minitab[®] version 19 (State College, PA, USA).

In ArcGIS, hatchling habitat use was quantified by finding the average nearest distance to each habitat type (u_{ij}) for each habitat (j) for individual hatchlings (i). Nearest distance to each habitat (*j*) from each hatchling location within the specified boundary (available habitat) was found using the nearest distance table in ArcGIS. Boundaries were centered on individual (i) release points (wetland release location or nest site location) and all location points for that individual (i) were within the radius of the boundary. Ten random locations were generated within each hatchling's (i) boundary using the random points tool to compare hatchling habitat use to the random locations. Hatchling habitat use was compared to the random locations by finding the average nearest distance to each habitat type (r_{ii}) for each habitat (j) for the hatchling's corresponding random locations (i). A vector of ratios was calculated for individuals (*i*) by dividing the average nearest distance to each habitat type for the hatchling locations by the average nearest distance to each habitat for the corresponding random locations ($d_{ij} = u_{ij} / r_{ij}$). If hatchlings were using habitat randomly, the expected ratio for each habitat type should be close to 1. Hatchlings that produced ratios > 1 were farther from the habitat than expected based on the random points and hatchlings that produced ratios < 1 were closer to the habitat than expected relative to the random points (Conner & Plowman, 2001).

For each hatchling release strategy (wetland release and nest site release), a two-sample ttest was used to assess if the mean distances significantly differed between hatchling locations (u_{ij}) and random locations (r_{ij}) . If the hatchling mean distances were significantly different from the mean distances of random locations, there was evidence of non-random habitat use occurring. A one-sample t-test was then used to test if the mean ratio (\overline{d}) for each habitat type (j) was significantly different from 1. If habitat ratios were significantly different from 1, the value of each habitat ratio was used to determine whether hatchlings were using habitats more or less than expected based on habitat availability. Paired t-tests were then used to determine if the mean ratio (\overline{d}) for each habitat type (j) were significantly different from one another. If there was a significant difference between habitats, then the habitat type with the lowest habitat ratio was considered more preferred.

Fourth Order Habitat Selection (Micro-Scale)

Twelve microhabitat characteristics were quantified within a 1 m² PVC quadrat frame (Daubenmire, 1959) for each hatchling location to investigate selection of locations at the microlevel (see Table 2.1). When a hatchling was located, the 1 m² PVC quadrat frame was positioned over the hatchling and the hatchling was situated in the center of the quadrat. These microhabitat characteristics included: open water percent, vegetation percent (emergent, herbaceous, floating, woody, detritus, and moss), water and soil depth (cm), and water, soil, and air temperature (°C). Open water was categorized as the percentage of standing water within the quadrat frame. Vegetation was categorized as emergent if the plant was rooted to the bottom of the wetland and extended out of the water. Herbaceous vegetation consisted of plants that did not have woody stems including annuals, biennials, forbs, and graminoids. Vegetation was considered emergent if the hatchling was in a wetland and vegetation was deemed herbaceous if the hatchling was located on land. Floating vegetation was categorized as plants that float on the surface of the waterbody and woody vegetation was documented if the plants present had a woody stem. Detritus was recorded if there was organic matter produced by the decomposition of organisms and moss vegetation included plants in the Bryophyta division.

Additionally, the water and soil depth were documented at the exact location of the hatchling within the quadrat frame using a ruler or meter stick. Water depth was found by sticking the ruler or meter stick down into the water until the top of the substrate was reached. Water depth was only documented if there was surface water present. Substrate depth was measured by sticking the ruler or meter stick down into the ground as far as possible. The water depth was then subtracted from this number. Water temperature was recorded at the exact location of the hatchling using a water thermometer (-5 to 50 °C). The water thermometer was placed approximately halfway down between the top of the surface and the soil. Soil temperature was recorded at the exact location of the hatchling using a Rapitest® soil thermometer (-10 to 50 °C). The soil thermometer was inserted into the substrate approximately 2.5 cm. Air temperature was taken approximately 30 cm above the hatchling using a traceable mini-thermistor thermometer (-50 to 150 °C; $\pm 1 °C$ accuracy).

The same 12 variables were also collected at a random plot at the same time as measurements were taken for the corresponding hatchling location to account for habitat availability (see Table 2.1). All measurements were evaluated in the center of the quadrat frame for random plots and followed the same protocols as described above. A random plot was selected by producing a number between 0-360 and then associating that number with a compass bearing to walk 10 m away from the hatchling location. The distance of 10 m was selected because this distance was the average daily distance traveled by hatchlings in 2017. The random plot was always bounded within the same macrohabitat as the corresponding hatchling location.

If the distance to the random plot required entering a different macrohabitat, then microhabitat characteristics were collected on the borderline of the distinct habitats to remain in the same macrohabitat.

Hatchling microhabitat selection was quantified by comparing the microhabitat variables at hatchling locations to the corresponding random plots using matched pair t-tests and logistic regression models. Because an average daily distance was unknown in 2017, there was no data collected at random plots. Therefore, only data from 2018 and 2019 were analyzed. These data were analyzed separately based on release strategy. Additionally, separate logistic regression models were developed for hatchlings found in terrestrial habitats compared to aquatic habitats due to differences in microhabitat variables. Hatchlings and random plots were considered terrestrial if there was no standing water present and considered aquatic if there was measurable standing water. Matched pair t-tests were used to determine which variables were significantly different ($p \le 0.05$) between hatchling locations and the corresponding random locations. Variables found to be significantly different were then used to develop logistic regression models using the stepwise backwards method. Analyses began with all variables found to be significantly predict a turtle location over a random location. Description of variables measured in the assessment of microhabitat selection by Blanding's turtle (Emydoidea blandingii) hatchlings at Camp Ripley in Little Falls, MN.

Microhabitat Variable	Description
Open Water %	Percentage of quadrat with standing water above the surface.
	Percentage of quadrat covered with water plants that are rooted to
Emergent Vegetation %	the bottom of the water body and extend out of the water.
	Percentage of quadrat covered with water plants that float on the
Floating Vegetation %	surface.
	Percentage of quadrat covered with plants that produce wood as its
Woody Vegetation %	structural tissue.
	Percentage of quadrat covered with organic matter produced by
Detritus Vegetation %	the decomposition of organisms.
	Percentage of quadrat covered with plants in the Bryophyta
Moss Vegetation %	division.
	Percentage of quadrat covered with plants that do not have woody
Herbaceous Vegetation %	stems including annuals, biennials, forbs, and graminoids.
	The water depth (cm) at the exact location of the hatchling within
	the quadrat using a meter stick or ruler. In the center of the plot for
Water Depth	random points.
	The substrate depth (cm) at the exact location of the hatchling within the guadrat using a mater stick or ruler. In the contex of the
Soil Depth	within the quadrat using a meter stick or ruler. In the center of the plot for random points.
Son Depui	The water temperature (°C) at the exact location of the hatchling
	within the quadrat using a water thermometer. In the center of the
Water Temperature	plot for random points.
1	The soil temperature (°C) at the exact location of the hatchling
	within the quadrat using a soil thermometer. In the center of the
Soil Temperature	plot for random points.
	The air temperature (°C) at the exact location of the hatchling
	within the quadrat using an air thermometer. In the center of the
Air Temperature	plot for random points.

Chapter 3: Results Hatchling Data Collection and Release Sites

In 2017, there were a total of eight hatchlings affixed with transmitters from two different nests and hatchlings were monitored September 18 - November 7. Hatchlings that were affixed with transmitters (n = 8) ranged from 7.5 – 11.0 g with an average mass of 9.28 g (see Table 3.1). Three hatchlings were randomly selected from 7.5 - 8.0 g, two hatchlings were chosen to be attached with transmitters from 9.5 - 10.0 g, and three hatchlings were in the weight category 10.5 - 11.0 g. Transmitters used on selected hatchlings were model R1614 (Advanced Telemetry Systems, Isanti, Minnesota, USA; 0.3 g). Transmitter weight ratios ranged 2.73 – 5 % of the hatchlings' body mass with an average percent body weight of 3.31 % and had a maximum battery capacity of 24 days (30 ppm). Six hatchlings were randomly selected to be distributed in Goose Lake and three hatchlings were dispersed in Range Marsh (see Figures 3.1 & 3.2). Additionally, two hatchlings from nest AKY were randomly selected for tracking and were released at the nest site as a pilot study (see Figure 3.3). Hatchlings that were not selected for tracking were released in wetland complexes nearest to their nest site.

In 2018, 17 hatchlings were affixed with transmitters from three different nests and hatchlings were tracked September 9 - November 14. Hatchlings that were affixed with transmitters (n = 17) ranged from 4.5 - 9.5 g with an average mass of 7.97 g (see Table 3.1). Ten hatchlings were affixed with transmitters from 7.0 - 8.0 g, six hatchlings were randomly chosen from 8.5 -9.0 g and one hatchling was chosen from the weight category 9.5 - 10.0 g. Due to the discontinuation of model R1614 and shipping issues, selected hatchlings were outfitted with

several radio transmitter series in 2018. These transmitters included models BD-2X (Holohil Systems Ltd., Ontario, Canada; 0.38 g) and A1025 (Advanced Telemetry Systems; 0.65 g). Transmitter weight ratios ranged 4.47 - 8.4 % of the hatchlings' body mass with an average percent body weight of 5.77 % and had a maximum battery capacity of 28 days (30 ppm). Two hatchlings from nest ACY were randomly chosen to be affixed with radio transmitters, with one hatchling transported to Range Marsh and one to Goose Lake (see Figures 3.2 & 3.4). There were also two hatchlings picked from nest ADU, with each hatchling being released in either Range Marsh or Goose Lake (see Figures 3.2 & 3.4). Finally, there were 13 hatchlings chosen to be tracked from nest ACW. Six hatchlings were released in Range Marsh and seven hatchlings were released in Goose Lake (see Figures 3.2, 3.4, & 3.5). Hatchlings that were not selected for tracking were released in wetland complexes nearest to their nest site.

In 2019, 18 hatchlings were randomly selected for tracking from four different nests and released at their nest site. These hatchlings were monitored from August 26 to November 4 and hatchlings that were selected for tracking ranged from 8.0 - 10.0 g with an average mass of 9.57 g (see Table 3.1). One hatchling was tracked from 7.0 - 8.0 g, two hatchlings were chosen from 8.5 - 9.0 g, fourteen hatchlings were affixed with transmitters from 9.5 - 10.0 g and one hatchling was selected from 10.5 - 11.0 g. Hatchlings were affixed with transmitter model BD-2X (Holohil Systems Ltd., Ontario, Canada; 0.38 g). Transmitter weight ratios ranged 3.6 - 4.75 % of the hatchlings' body mass with an average percent body weight of 3.98 % and had a maximum battery capacity of 21 days (30 ppm). Four hatchlings were randomly selected for transmitter attachment from nest ADW, four hatchlings were selected from nest AJK, four hatchlings from nest ACQ, and six hatchlings were chosen from nest BH (see Figures 3.3 & 3.6). Hatchlings that

were not released at the nest site with transmitters were released in wetland complexes nearest to their nest site.

Table 3.1

Selected hatchling Blanding's turtles (Emydoidea blandingii) mass and release locations.

				2017 Hat	chlings			
Hatchling #	Hatch Date	Mass (g)	CL (mm)	CW (mm)	PL (mm)	PW (mm)	Hatchling ID	Hatchling Release
2	17-Sep-17	10.5	39.96	29.97	31.85	22.79	H_PW01	Range Marsh
6	17-Sep-17	10.5	34.8	28.58	31.48	20.66	H_PW02	Goose Lake
8	17-Sep-17	9.5	34.62	27.75	30.46	20.48	H_PW03	Goose Lake
1	17-Sep-17	8	33.9	28.57	29.83	23.48	H_PW04	Goose Lake
7	17-Sep-17	11	35.44	28.67	31.93	19.83	H_PW05	Range Marsh
9	17-Sep-17	9.75	35.39	29.05	29.67	20.9	H_PW06	Range Marsh
5	5-Oct-17	7.5	33.04	27.1	27.71	20.32	H_AKY01	Nest Site
6	5-Oct-17	7.5	32.55	25.71	22.52	18.83	H_AKY02	Nest Site
Ave	rage	9.28	34.96	28.18	29.43	20.91	_	
				2018 Hat				
Hatchling #	Hatch Date	Mass (g)	CL (mm)	CW (mm)	_	PW (mm)	Hatchling ID	Hatchling Release
7	9-Sep-18	9	33	28.5	28.5	21	H_ACY01	Goose Lake
8	9-Sep-18	9	36	30	31	22	H_ACY02	Range Marsh
1	12-Sep-18	7.5	31.5	26	28	23	H_ACW01	Goose Lake
2	12-Sep-18	8.5	33	28	29.5	22	H_ACW02	Range Marsh
3	12-Sep-18	8	33.5	28	30	22.5	H_ACW03	Range Marsh
4	12-Sep-18	7	31	25	27	22	H ACW04	Goose Lake
5	14-Sep-18	7.5	32	28	29	20	H_ACW05	Goose Lake
6	16-Sep-18	8	30	24	28	19	H_ACW06	Goose Lake
7	16-Sep-18	9	32	27	29	21	H_ACW07	Range Marsh
8	17-Sep-18	4.5	29	20	22	17	H_ACW08	Goose Lake
9	17-Sep-18	8	33	26	28	23	H_ACW09	Goose Lake
10	17-Sep-18	7.5	34	25	26	21	H_ACW10	Range Marsh
11	21-Sep-18	8.5	35	28	30	22	H_ACW11	Range Marsh
12	21-Sep-18	9.5	34	27	29	23	H_ACW12	Range Marsh
13	21-Sep-18	9	33	29	28	23	H_ACW13	Goose Lake
1	27-Sep-18	7	33	28	28	23	H_ADU01	Goose Lake
2	6-Oct-18	8	31.5	26	20 29	21	H_ADU02	Range Marsh
	rage	7.97	32.62	26.68	28.24	21.50	11_110 001	Tunge Muibh
1170	Tuge		02:02	2019 Hat		21.00		
Hatchling #	Hatch Date	Mass (g)	CL (mm)	CW (mm)	PL (mm)	PW (mm)	Hatchling ID	Hatchling Release
1	26-Aug-19	8.00	34.14	29.22	29.58	23.89	H_BH01	Nest Site
3	26-Aug-19	9.00	34.31	30.05	30.84	22.38	H_BH02	Nest Site
5	26-Aug-19	9.50	34.81	29.54	32.12	23.87	H_BH03	Nest Site
8	26-Aug-19	9.50	35.47	29.07	31.57	21.9	H_BH04	Nest Site
10	26-Aug-19	10.00	35.14	28.42	32.55	22.63	H_BH05	Nest Site
11	26-Aug-19	10.00	35.74	27.57	32.19	22.95	H_BH06	Nest Site
5	10-Sep-19	9.75	34.53	32.45	31.4	21	H_ACQ01	Nest Site
11	10-Sep-19	10.00	35.56	32.45	32.44	21	H_ACQ02	Nest Site
12	10-Sep-19	9.50	35.58	33.47	33.47	20.98	H_ACQ03	Nest Site
14	10-Sep-19	9.50	35.57	33.5	32.46	21.1	H_ACQ04	Nest Site
1	10-Sep-19	10.00	35.57	31.65	32.26	22.39	H_AJK01	Nest Site
6	10-Sep-19	10.00	33.47	28.63	31.31	20.97	H_AJK02	Nest Site
9	10-Sep-19	10.50	35.31	31.06	32.47	22.47	H_AJK03	Nest Site
10	10-Sep-19	9.75	34.79	28.65	30.82	21.54	H_AJK04	Nest Site
1	10-Sep-19	9.50	33.67	30.3	30.46	20.42	H_ADW01	Nest Site
	10-Sep-19	9.25	34.52	29.33	30.34	21.01	H_ADW02	Nest Site
2								
2 3	-		33.49	27.23	30.35	20.96	H_ADW03	Nest Site
	10-Sep-19 11-Sep-19	9.00 9.50	33.49 34.16	27.23 29.25	30.35 30.26	20.96 19.68	H_ADW03 H_ADW04	Nest Site Nest Site

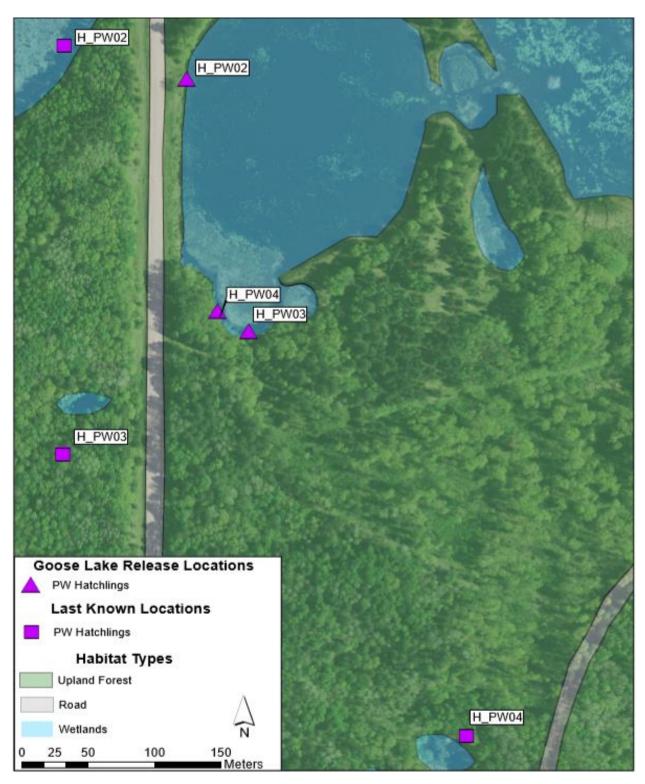


Figure 3.1 Hatchling Blanding's turtle (*Emydoidea blandingii*) release locations in Goose Lake and last known locations during the study at Camp Ripley. Map created by Arika Nyhus (2020).

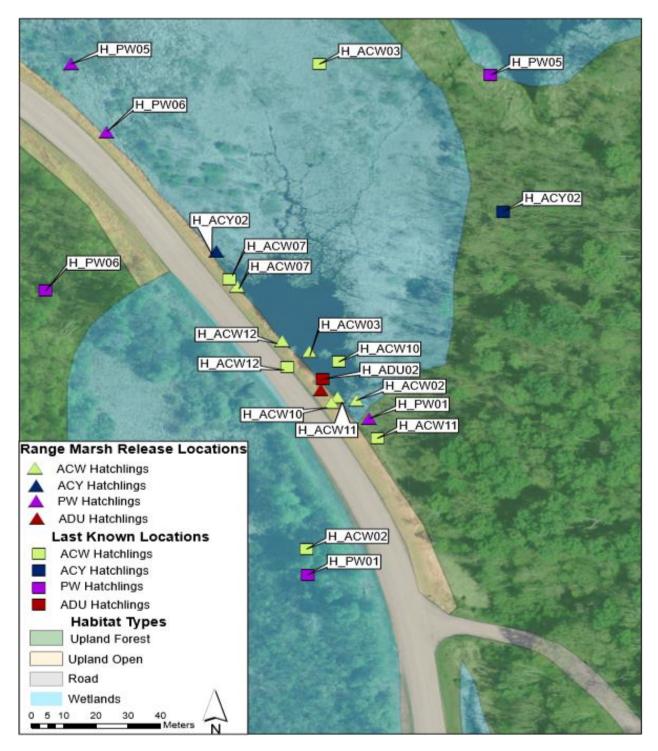


Figure 3.2 Hatchling Blanding's turtle (*Emydoidea blandingii*) release locations in Range Marsh and last known locations during the study at Camp Ripley.

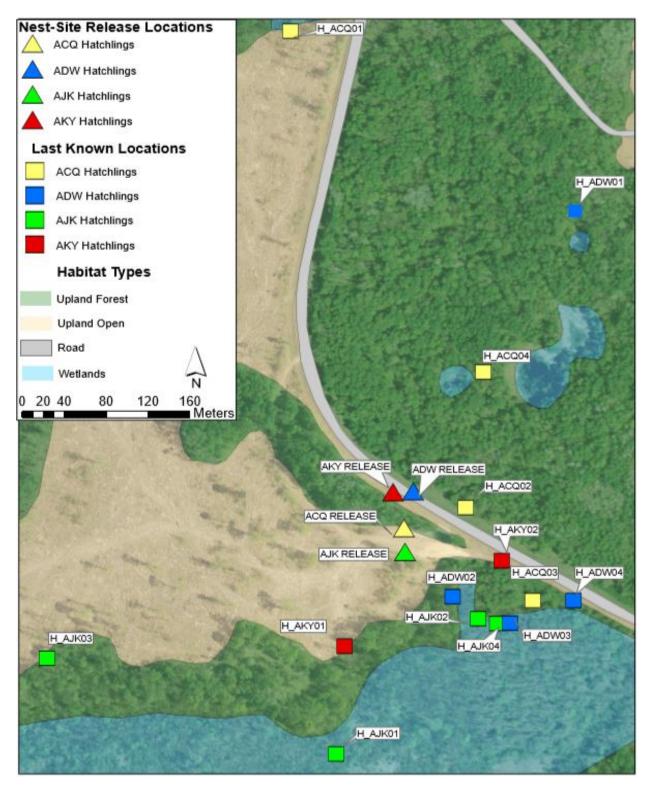


Figure 3.3 Hatchling Blanding's turtle (*Emydoidea blandingii*) nest site release locations and last known locations during the study at Camp Ripley. Map created by Arika Nyhus (2020).

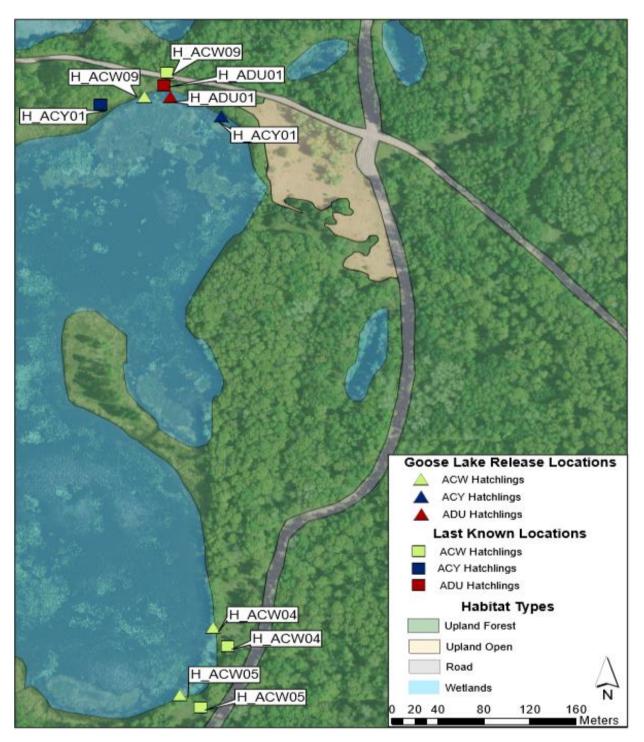


Figure 3.4 Hatchling Blanding's turtle (*Emydoidea blandingii*) release locations in Goose Lake and last known locations during the study at Camp Ripley. Map created by Arika Nyhus (2020).

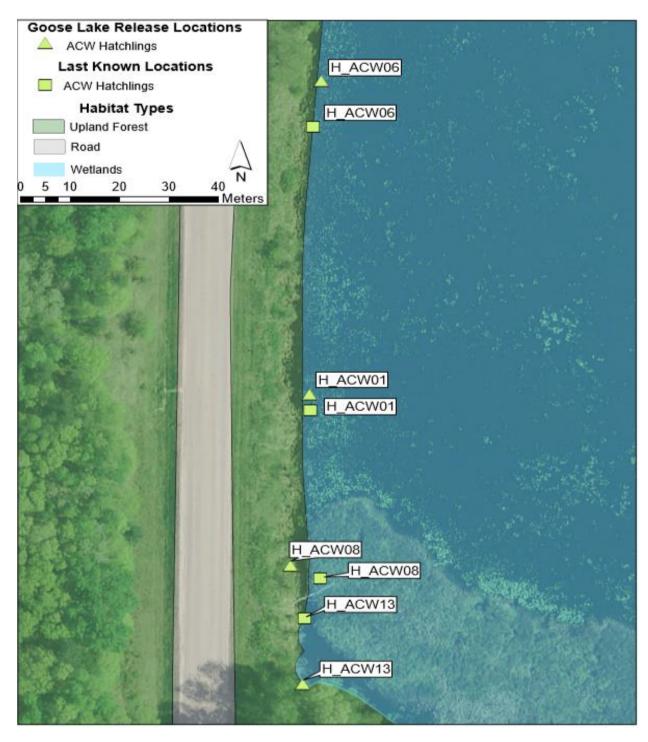


Figure 3.5 Hatchling Blanding's turtle (*Emydoidea blandingii*) release locations in Goose Lake and last known locations during the study at Camp Ripley. Map created by Arika Nyhus (2020).

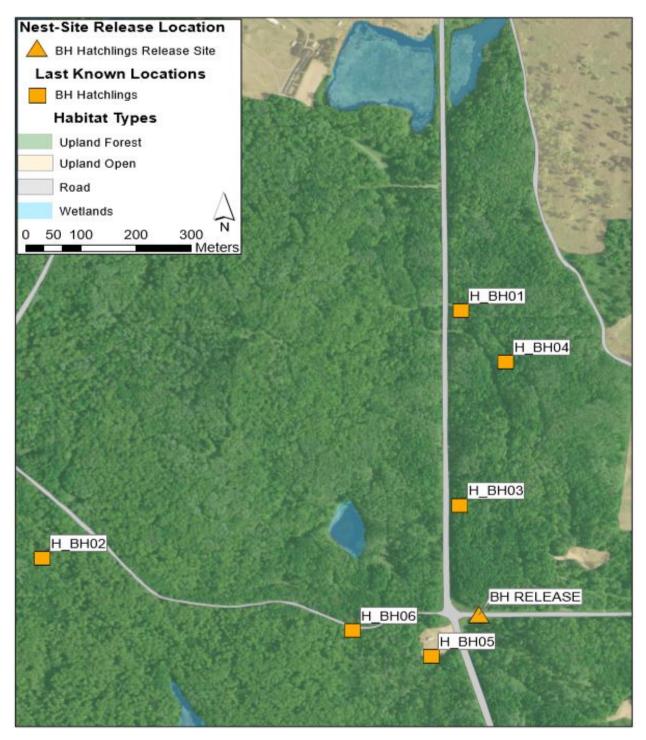


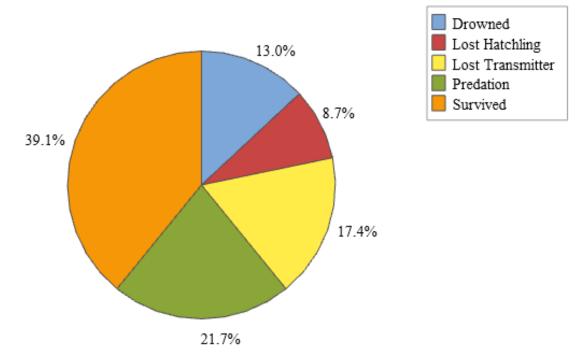
Figure 3.6 Hatchling Blanding's turtle (*Emydoidea blandingii*) nest site release locations and last known locations during the study at Camp Ripley. Map created by Arika Nyhus (2020).

Survivorship and Movement Patterns

From the hatchlings affixed with transmitters and released in Range Marsh and Goose Lake (n = 23), 39 % survived the duration of the study (n = 9). Five hatchlings were predated while three hatchlings were presumed deceased due to drowning. It is unknown the fate of 26 % of the monitored hatchlings, as four transmitters were thought to have fallen off the turtles and two hatchlings were lost due to transmitter malfunction or predation (see Figures 3.7 & 3.8). There was no significant difference in survival rates amongst wetland release sites (see Table 3.2; p = 0.637). Hatchlings released into wetlands traveled an average total distance of 104.84 m, with an average daily distance of 4.80 m (see Figure 3.9). There was no significant difference in distances traveled between hatchlings released in Range Marsh ($M = 104 \pm 34$ SD = 112) and hatchlings released in Goose Lake ($M = 105 \pm 46$ SD = 158), t(19) = 0.02, p = 0.983. From the hatchlings that were affixed with transmitters and released at the nest site (n = 20), 50 % survived the duration of the study (n = 10). There were five hatchlings presumed to be predated and it is unknown what happened to 25 % of the hatchlings, as three transmitters were found with no evidence of predation and two transmitters likely failed (see Figure 3.10). Hatchlings released at the nest site traveled an average total distance of 410.39 m, with an average daily distance of 24.60 m (see Figure 3.11).

From the hatchlings tracked and released in wetlands and at the nest site (n = 43), 44% of hatchlings survived the duration of the study (n = 19). Approximately 23% of hatchlings were considered predated (n = 10), 7% were presumed to be deceased due to drowning (n = 3), 16% of hatchlings had transmitters fall off (n = 7), and 9% of hatchlings were lost (n = 4; see Figure 3.12). There was no significant difference detected in survivorship between hatchlings released

in wetlands and hatchlings released at the nest site (see Table 3.3; p = 0.491). However, there was a significant difference in the total distance traveled between hatchlings released at the nest site (M = 410 SD = 207) and hatchlings released in wetlands (M = 162 SD = 161), t(27) = 3.79, p = 0.001 (see Figure 3.13).



Fate of Wetland Release Hatchlings

Figure 3.7 The fates of the hatchling Blanding's turtles (*Emydoidea blandingii*; n = 23) released in wetland complexes from 2017 - 2018 at Camp Ripley.

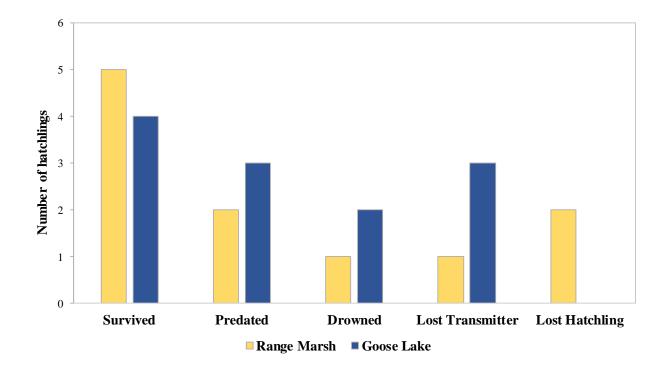


Figure 3.8 Hatchling Blanding's turtle (*Emydoidea blandingii*) fates between Goose Lake (n = 12) and Range Marsh (n = 11) at Camp Ripley.

Table 3.2

Wetland Release					
	Goose Lake	Range Marsh	All		
Alive	4	5	9		
% Alive	44.44	62.5	52.94		
Dead	5	3	8		
% Dead	55.56	37.5	47.06		
All	9	8	17		
		Fisher's Exact Test	0.637		

Fisher exact results for differences in survival rates amongst wetland release sites.

Excludes unknown fates

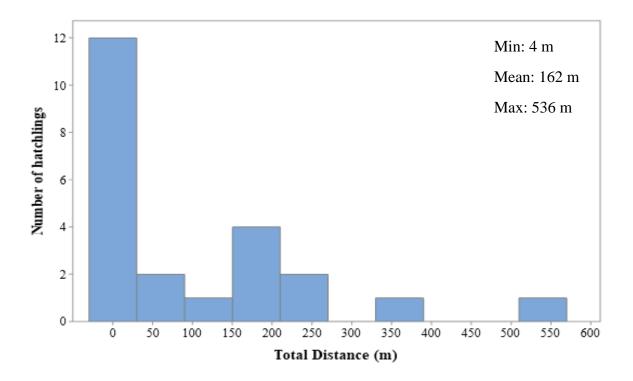


Figure 3.9 Distribution of the distances traveled (m) by hatchlings Blanding's turtles (*Emydoidea blandingii*; n = 23) released in wetland complexes at Camp Ripley from 2017 - 2018.

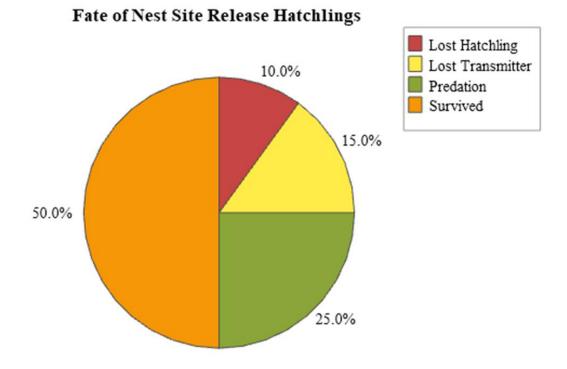


Figure 3.10 The fates of the hatchling Blanding's turtles (*Emydoidea blandingii*; n = 20) at the nest site in 2019 at Camp Ripley.

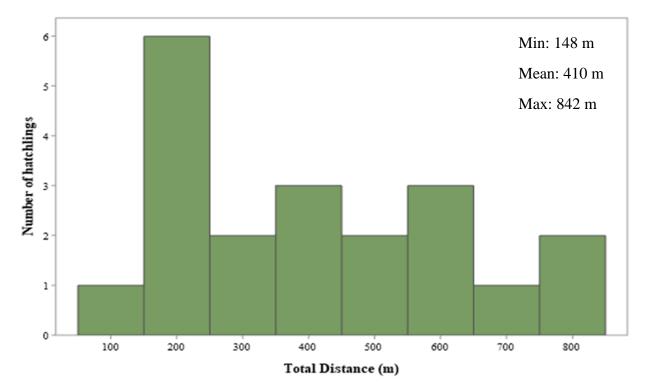
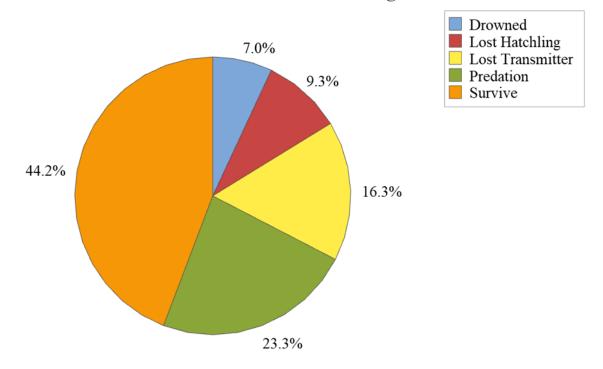


Figure 3.11 Distribution of the distances traveled (m) by hatchlings Blanding's turtles (*Emydoidea blandingii*; n = 20) released at the nest site in 2019 at Camp Ripley.



Fate of Wetland and Nest Site Release Hatchlings

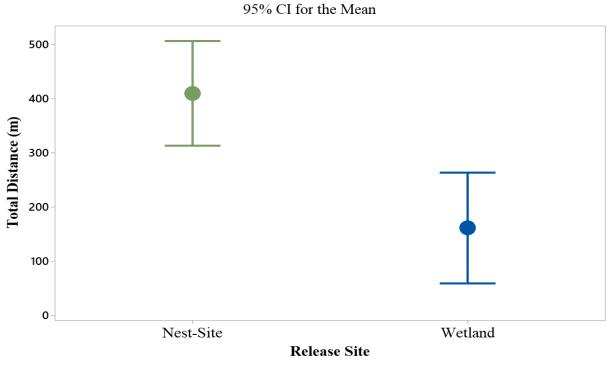
Figure 3.12 Fate of all hatchling Blanding's turtles (*Emydoidea blandingii*; n = 43) released at Camp Ripley from 2017 - 2019.

Table 3.3

Fisher exact results for differences in survival rates among hatchling release strategies.

	Release Site					
	Nest Site	Wetland	All			
Alive	10	9	19			
% Alive	66.67	52.94	59.38			
Dead	5	8	13			
% Dead	33.33	47.06	40.63			
All	15	17	32			
		Fisher's Exact Test	0.491			

Excludes unknown fates



Individual standard deviations are used to calculate the intervals.

Figure 3.13 Confidence intervals showing group means for total distance traveled (m) between hatchling Blanding's turtles (*Emydoidea blandingii*) released at the nest site and hatchlings released in wetlands.

Third Order Habitat Selection (Macro-Scale)

From the hatchlings released in Range Marsh and Goose Lake, there was a total of 14 hatchlings that were monitored for 10 or more days that were included in the analysis (see Table 3.4). The analysis of distances revealed that hatchling locations to wetlands (M = 6.2 SD = 10.8) were significantly different from the random location distances to wetlands (M = 29.7 SD = 50, t(139) = -5.44, p = 0.0001). Hatchling locations to uplands (M = 5.2 SD = 8.77) were also significantly different from random locations (M = 14.4 SD = 25.9, t(139) = -3.99, p = 0.0001). Examination of distance ratios indicated that hatchlings used edges of wetlands (M = 0.259 SD = 0.414, t(13) = -6.69, p = 0.0001) and uplands (M = 0.361 SD = 0.407, t(13) = -5.88, p = 0.0001) more than expected based on habitat availability. There was no evidence that one habitat was significantly preferred over the other (M = 0.102 SD = 0.640, t(13) = 0.60, p = 0.562).

From the hatchlings released at the nest site, there was a total of 15 hatchlings that had 10 or more locations that were included in the analysis (see Table 3.5). The analysis of distances revealed that hatchling locations to wetlands (M = 79 SD = 104) were not significantly different from the random location distances to wetlands (M = 61 SD = 110, t(149) = 1.50, p = 0.135). However, hatchling locations to uplands (M = 2.25 SD = 7.27) were significantly different from random locations (M = 25.9 SD = 42.2, t(149) = -6.75, p = 0.0001). Examination of distance ratios indicated that hatchlings used wetlands (M = 0.10 SD = 0.190, t(14) = -18.41, p = 0.0001) significantly more than expected based on habitat availability. When hatchlings were released at the nest site, uplands were more preferred when compared to wetlands (M = -1.543 SD = 2.059, t(14) = -2.90, p = 0.012).

When analyzing wetland release and nest site release hatchlings together, the analysis of distances revealed that hatchling locations to wetlands (M = 43.9 SD = 83.1) were not significantly different from the random location distances to wetlands (M = 45.6 SD = 87.7, t(289) = -1.76, p = 0.804). However, hatchling locations to uplands (M = 3.67 SD = 8.15) were significantly different from random locations (M = 20.3 SD = 35.7, t(289) = -7.75, p = 0.0001). Examination of distance ratios indicated that hatchlings used wetlands randomly (M = 0.97 SD = 1.603, t(28) = -0.09, p = 0.930) while hatchlings used uplands (M = 0.22 SD = 0.34, t(28) = -12.43, p = 0.0001) significantly more than expected based on habitat availability. Ultimately, uplands were more preferred when compared to wetlands (M = -0.749 SD = 1.734, t(28) = -2.33, p = 0.028).

Selected hatchling Blanding's turtles (Emydoidea blandingii) released in wetlands included in the macrohabitat selection analysis that had 10 or more locations and their corresponding fates.

Hatchling ID	Hatchling Release	Fate
H_PW01	Range Marsh	Survived
H_PW02	Goose Lake	Survived
H_PW04	Goose Lake	Survived
H_PW06	Range Marsh	Survived
H_ACY01	Goose Lake	Survived
H_ACY02	Range Marsh	Survived
H_ACW02	Range Marsh	Lost Hatchling
H_ACW03	Range Marsh	Lost Transmitter
H_ACW05	Goose Lake	Predation
H_ACW07	Range Marsh	Survived
H_ACW13	Goose Lake	Survived
H_ADU01	Goose Lake	Predation
H_ADU02	Range Marsh	Survived

Selected hatchling Blanding's turtles (Emydoidea blandingii) released at the nest site included in the macrohabitat selection analysis that had 10 or more locations and their corresponding fates.

Hatchling ID	Hatchling Release	Fate
H_BH01	Nest Site	Lost Hatchling
H_BH02	Nest Site	Survived
H_ACQ01	Nest Site	Predation
H_ACQ02	Nest Site	Predation
H_ACQ04	Nest Site	Survived
H_AJK01	Nest Site	Survived
H_AJK02	Nest Site	Survived
H_AJK03	Nest Site	Lost Transmitter
H_AJK04	Nest Site	Survived
H_ADW01	Nest Site	Survived
H_ADW02	Nest Site	Survived
H_ADW03	Nest Site	Survived
H_ADW04	Nest Site	Survived
H_AKY01	Nest Site	Lost Transmitter
H_AKY02	Nest Site	Survived

Fourth Order Habitat Selection (Micro-Scale)

Hatchlings that were released in wetland complexes and located in water throughout the duration of the study were found in significantly shallower water depths (p = 0.0001) with more floating vegetation (p = 0.041) than the corresponding random locations. When using these two variables, a model was accurately able to predict a turtle location over a random location 61.7% of the time. For every 1 cm increase in water depth, there was a 4.4% decrease in the odds of a hatchling choosing that microhabitat. For every 1% increase in floating vegetation, there was a 2.1% increase in the odds of a hatchling choosing that microhabitat. For every 1% increase in floating vegetation, there was a 2.1% increase in the odds of a hatchling choosing that location. Hatchlings that were released in wetland complexes and not located in water were found in significantly greater substrate depths (p = 0.001) with significantly less woody vegetation (p = 0.052) when compared to random locations. When using these two variables, a model was accurately able to predict a turtle location over a random location 73.2% of the time. For every 1 cm increase in substrate depth, there was a 31.7% increase in the odds of selection. For every 1% increase in woody vegetation, there was a 6.8% decrease in the odds of a hatchling choosing that location (see Table 3.6).

Hatchlings that were released at the nest site and found in water had significantly more detritus cover (p = 0.0001) than the corresponding random locations. When using only this variable, a model was accurately able to predict a turtle location compared to a random location 65.2% of the time. For every 1% increase in detritus cover, there was a 2.3% increase in the odds of a hatchling choosing that location. It was found that hatchlings not located in water had significantly greater substrate depths (p = 0.033) with more sphagnum vegetation cover (p = 0.025) than the corresponding random locations. When using these two variables, a model was accurately able to predict a turtle location compared to a random location 57.1% of the time. For

every 1 cm increase in substrate depth, there was a 5.4% increase in the probability of finding a hatchling in that location. Additionally, for every 1% increase in moss vegetation within the 1 m² quadrat frame, there was a 1.5% increase in the chance of a hatchling selecting that site (see Table 3.7).

Table 3.6

Akaike's information criterion for small samples (AICc) rankings, fitted parameter estimates, and odds ratios for paired logistic regression models for microhabitat selection by hatchling Blanding's turtles (Emydoidea blandingii) released in wetlands at Camp Ripley.

	Wetland Release						
Coefficients (β)							
Model	AICc	Water Depth	Floating Vegetation	Substrate Depth	Woody Vegetation	Percentage Correct	
Aquatic	211.71	-0.0452	0.0206			61.7	
Terrestrial	125.59			0.2753	-0.0705	73.2	
Odds Ratio		0.9559(1%)	1.0208(1%)	1.3169(1%)	0.9319(1%)		

Table 3.7

Akaike's information criterion for small samples (AICc) rankings, fitted parameter estimates, and odds ratios for paired logistic regression models for microhabitat selection by hatchling Blanding's turtles (Emydoidea blandingii) released at the nest site at Camp Ripley.

Nest Site Release						
		Coefficients (β)				
AICc	Detritus	Moss Vegetation	Substrate Depth	Percentage Correct		
268.08	0.02247			65.2		
626.59		0.01447	0.0525	57.1		
	1.023(1%)	1.015(1%)	1.054(1%)			
	268.08	AICc Detritus 268.08 0.02247 626.59	AICcDetritusMoss Vegetation268.080.022470.01447	AICcDetritusMoss VegetationSubstrate Depth268.080.022470.014470.0525		

Chapter 4: Discussion

Survivorship

One of the objectives of this thesis project was to quantify survivorship of hatchling Blanding's turtles based on release strategy (wetland release vs. nest site release) following nest emergence at Camp Ripley. The alternative hypothesis stated that survivorship would be lower for hatchlings released at the nest site compared to hatchlings released in wetlands. From the hatchlings tracked in this study (n = 43), there was no significant difference in survival rates based on release strategy (see Table 3.3). The alternative hypothesis that survival rates will be lower in nest site release hatchlings was not supported. In total, 44 % of monitored hatchlings survived (n = 19) the duration of the study, despite the population being located at a military training base with heavy tank traffic (see Figure 3.12). These results are close to the survival rate of 42 % documented for hatchling Blanding's turtles in Ontario (Paterson et al., 2012). Our study found that predation rates of hatchling Blanding's turtles at Camp Ripley were much lower (23 %) than predation rates documented for hatchling wood turtles (56 %; 52 %), a closely related species (Paterson et al., 2012; Dragon, 2015). Differences in predation between the two species could be due to habitat availability and selection of habitat. Additionally, our estimates of predation could be much lower than the actual rate of predation if the lost hatchlings (9%) were subject to avian predators taking them out of the telemetry reception range. Future studies should focus on identifying important predators that impact hatchling survival. Methods to achieve this could include trail cameras at nest sites (Riley & Litzgus, 2014), identification of predators from attack marks on clay models (Low, Sam, McArthur, Posa, & Hochuli, 2014), or through DNA

extraction from hatchling carcasses if funding is available (Dawson, Crawford, Huston, Adams, & Fleming, 2017).

Movement Patterns

The second objective of this thesis project was to quantify the distances traveled by hatchling Blanding's turtles and to determine whether distances would be significantly different depending on release strategy at Camp Ripley. The alternative hypothesis stated that hatchlings released at the nest site would travel significantly farther than hatchlings released in wetlands. It was found that hatchlings released at the nest site did travel significantly farther than hatchlings released in wetlands (see Figure 3.13), so the alternative hypothesis was supported in this study. The maximum path length traveled by hatchlings released in wetlands was 536 m while the maximum path length traveled by hatchlings released at the nest site was 842 m. The maximum path length distance traveled by hatchling Blanding's turtles in Ontario was 449 m (Paterson et al., 2012). Female nest site selection may contribute to the large distances traveled by hatchlings at Camp Ripley. Additionally, Pappas, Brecke, and Congdon (2000) concluded that adults at Camp Ripley travelled farther than other Blanding's turtle populations. They reported that this difference could be due to large distances between resources, or extensive but dispersed suitable habitat. When hatchlings are released at the nest site, individuals may be required to travel great distances like their adult counterparts to reach suitable habitat for overwintering. Though survivorship was not significantly different between release strategies and no road mortalities occurred in the present study, tank traffic could potentially become a significant source of mortality for hatchlings if all future clutches are released at the nest site.

Due to the discontinuation of model R1614 and shipping issues in 2018, some wetland release hatchlings were outfitted with a heavier radio transmitter series. Therefore, there was a significant difference in percent body weight of transmitters used on hatchlings released at the nest site (M = 3.983 SD = 0.233) compared to hatchlings released in wetlands (M = 5.06 SD =1.63), t(23) = -3.15, p = 0.004. However, the mean percent body weight of transmitters for hatchlings released in wetlands was not significantly far from 5 % ($M = 5.065 \pm 1.629$, t(22) =.19, p =0.850). Conservative approaches recommend restricting the transmitter weight to ≤ 5 % of an animal's body weight (Gottwald et al., 2019) and more liberal approaches recommend no more than 10 % of an animal's body weight (Beaupre et al., 2004). When reviewing freshwater turtle literature, transmitters have weighed anywhere from 5 - 13.4 % of a hatchling's body mass (Forsythe, Flitz, & Mullin, 2004; Tuttle & Carroll, 2005; Paterson et al., 2012; Dragon, 2015). Though this study falls within the range of the weights used for transmitters in previous studies, no literature has been found reporting the effect of radio transmitters on the survival, movement, or overwintering selections of hatchling freshwater turtles. Because several studies have been completed regarding these variables, future studies should focus on understanding the possible effects radio transmitters have on hatchling freshwater turtles.

Third Order Habitat Selection (Macro-Scale)

Another important objective of this thesis project was to identify whether hatchlings were selecting wetlands or uplands for overwintering following nest emergence at Camp Ripley. It was predicted that hatchlings would select habitat based on release strategy. Therefore, hatchlings released in Goose Lake and Range Marsh would remain in those wetlands for hibernation and hatchlings released at the nest site would overwinter on land. To recall, hatchlings released in wetlands were found significantly closer to wetland and upland edges than random locations, suggesting that hatchlings were selecting habitat non-randomly. Although, no habitat was significantly preferred over the other when distance ratios were compared between uplands and wetlands. Hatchlings released at the nest site were significantly closer to uplands than random points, however, hatchlings were not significantly closer to wetlands than random points. Therefore, hatchlings used uplands non-randomly while wetland habitat was used randomly, and upland habitat was most preferred. Because wetlands were not significantly preferred over uplands when hatchlings were released in Goose Lake and Range Marsh, the alternative hypothesis was not supported.

It has been assumed that hatchlings must retreat to aquatic habitats to reduce predation and desiccation following nest emergence (Ultsch et al., 2007). When hatchlings from both release strategies were analyzed together, our results concluded that hatchlings ultimately preferred upland habitat and used wetlands randomly. Hatchling Blanding's turtles also displayed random movement with respect to water in Nova Scotia (Standing et al., 1997). They mentioned that while several turtles entered water, others overtly avoided it. From the hatchlings released in wetlands and at the nest site (n = 43), only 37 % (n = 16) were last located in wetlands (see Figures 3.1 – 3.6). McNeil, Herman, and Standing (2000) released hatchling Blanding's turtles at wetland edges in Nova Scotia and claimed that the hatchlings were repelled by the water and traveled upland away from the wetlands. From the hatchlings released in wetlands and at the nest site (n = 43), 67 % (n = 27) were last located in uplands and 52 % (n =14) of hatchlings last located in uplands were within the riparian zone of a wetland. The transitional area between aquatic and upland habitat is defined as the riparian zone and often consists of habitat elements of both aquatic and terrestrial ecosystems (Semlitsch & Bodie, 2003). These habitat elements include high water tables, hydric soils, and various vegetation compositions. Riparian zones play a critical role in many taxa including mammals, birds, reptiles, and amphibians (Semlitsch & Bodie, 2003). Riparian zones provide more habitat niches than any other habitat and offers the most critical habitat components: food, water, and shelter (Oakley, Collins, Everson, Heller, Howerton, & Vincent, 1985). These zones have been considered pertinent in the conservation and management of freshwater turtles, as most species rely on both upland and wetland habitat to complete their life cycles (Burke & Gibbons, 1995). Additionally, some species select riparian zones during certain seasons of the year. Burke & Gibbons (1995) reported that wetland protection alone is not adequate for the conservation of freshwater turtles, as they found several species depend on habitat adjacent to wetlands for overwintering. They suggested incorporating a 73 m buffer zone to protect 90 % of hibernation sites and a 275 m buffer zone to protect 100 % of hibernation sites.

At Camp Ripley, 35 % (n = 8) of hatchlings released in Goose Lake and Range Marsh (n = 23) retreated to the edge of wetland and upland habitat. From the hatchlings released at the nest site (n = 20), 30 % (n = 6) of hatchlings were last located on the edge of wetland and upland habitat. For this study, habitat was broadly categorized into two groups: upland and wetland habitat. Since riparian zones were classified under upland habitat, our findings may overvalue habitat preference for upland habitat and undermine the importance of riparian zones. Hatchlings released into wetlands were significantly closer to wetland and upland edges than random locations. This may suggest that hatchling Blanding's turtles also prefer riparian zones for

hibernation as Burke & Gibbons (1995) found in other freshwater turtle species. Though selecting riparian zones for hibernation has never been confirmed, several publications have speculated terrestrial overwintering in moist environments in hatchling Blanding's turtles (Standing et al., 1997; McNeil et al., 2000; Dinkelacker et al., 2004). Future studies should include riparian zones as a habitat category when investigating overwintering habitat selection in hatchling Blanding's turtles.

Fourth Order Habitat Selection (Micro-Scale)

The next important objective of this thesis project was to detect whether hatchlings were selecting for certain micro-habitat characteristics following nest emergence at Camp Ripley. It was predicted that hatchlings would select micro-habitat characteristics with less open water and more emergent vegetation cover when compared to random sites. It was found that that when hatchlings were released in wetlands and found in water, hatchlings were found in shallower water depths with more floating vegetation. This floating vegetation was often characterized by floating mats consisting of moss. Hatchlings released in wetlands and found not in water were found in standing water were found in significantly more detritus cover than random sites. Hatchlings released at the nest site and found not in water were found in significantly greater substrate depths with more moss vegetation. The alternative hypothesis that less open water and more emergent vegetation would be preferred was not supported as evidence showed preference for deeper substrate depths and more moss vegetation between both hatchling release strategies.

These findings are like what Butler and Graham (1995) found in their study. They reported that hatchlings preferred more muck and *Sphagnum* following nest emergence in

Massachusetts. McMaster and Herman (2000) reported that *Sphagnum* was the most important micro-habitat characteristic in their Blanding's turtle (ages 1 - 20) study and claimed *Sphagnum* could help predict juvenile Blanding's turtle habitat. McNeil and colleagues (2000) did not statistically identify moss as being preferred, however, they did report finding juveniles buried 4 – 5 cm in *Sphagnum* mats. Similarly, Standing and associates (1997) described hatchling Blanding's turtles using *Sphagnum* for protection following nest emergence.

It is possible that hatchling Blanding's turtles use *Sphagnum* moss as an olfactory or visual cue following nest emergence to help orient themselves to moist environments (Butler & Graham, 1995; Standing et al., 1997; McNeil et al., 2000). Most moss species grow in wet regions of habitat with high amounts of shade. While mosses can grow in almost any soil type, most mosses prefer clay soil which retain the most amount of water, making moss species a great indicator for wetlands (Stapanian, Schumacher, Gara, Adams, & Viau, 2016). Shifting or unpredictable winds would make olfactory cues for *Sphagnum* moss impossible as a sole orientation mechanism. However, an olfactory gradient in the air in conjunction with other orientation mechanisms such as a sun compass could help lead hatchlings to critical habitat (Tuttle & Carroll, 2005; Pappas et al., 2009; Congdon et al., 2015). Future studies should investigate the possibility that *Sphagnum* moss influences hatchling movements following nest emergence.

As water holding capacity increases, so do the apparent soil depths of the environment. Unfortunately, soil moisture was not one of the microhabitat characteristics collected in this study. Noble and Breslau (1938) found that hatchling painted and snapping turtles selected humid environments over dry environments and claimed that humidity is a principal orienting cue. I believe that if soil moisture had been included, we would see a preference for soil moisture as opposed to soil depth, as this variable is an indicator of soil moisture. Future research concerning micro-habitat selection in hatchling freshwater turtles should consider the addition of soil moisture as one of the micro-habitat characteristics to quantify.

During the duration of the study, a total of four hatchlings were successfully relocated in the spring to assess the fates of the hatchlings. H_AKY02 overwintered in upland habitat with no standing water was found desiccated in the last known location from the previous fall (see Figure 4.1). Dinckelacker and colleagues (2004) suggested hatchlings that may overwinter on land need to seek moist substrates where they can burrow to avoid severe water loss and minimize freezing. H_ACY02 overwintered in a small vernal pool within the forest adjacent to Range Marsh but appeared to have drowned (see Figure 4.2). Dinckelacker and coworkers (2005) proposed that if hatchling Blanding's turtles overwinter in water, they must overwinter in highly oxygenated micro-habitats. Lack of highly oxygenated water could have played a role in the mortality fate of this hatchling. In the spring of 2020, two hatchlings were successfully relocated from their overwintering locations and found alive. H_ADW01 overwintered on land near a wetland within the riparian zone (see Figure 4.3) while H_ADW02 overwintered in a wooded swamp (see Figure 4.4) and neither hatchling overwintered in standing water. Our results support the notion that hatchlings can successfully overwinter on land but are limited to moist environments (Dinckelacker et al., 2004).



Figure 4.1 Hatchling H_AKY02 relocated in the spring of 2019 after overwintering upland under detritus. It was concluded that this hatchling was deceased due to desiccation.



Figure 4.2 Hatchling H_ACY02 relocated in the spring of 2019 after overwintering in a vernal pool. After efforts were put in to warm up the hatchling, it was concluded that the hatchling was deceased due to drowning.



Figure 4.3 Hatchling H_ADW01 was successfully relocated alive in the spring of 2020 after overwintering on land near a wetland within the riparian zone at Camp Ripley.



Figure 4.4 Hatchling H_ADW02 was successfully relocated alive in the spring of 2020 after overwintering in a wooded swamp.

Management Implications

The final objective of this thesis project was to determine the most effective hatchling release strategy; either a) release hatchlings into the nearest wetland complex or b) release hatchlings directly at the nest site. It was predicted that releasing hatchlings in the nearest wetland complex is the most effective hatchling release strategy and the results of this project support the alternative hypothesis.

The results from this project showed that hatchlings released at the nest site travel a significantly farther distance than those released in wetlands, though survivorship was not affected based on release strategy. When released in wetlands, hatchlings were selecting the edges of upland and wetland habitat. When released at the nest site, hatchlings used wetlands randomly and preferred uplands to wetlands. Unfortunately, riparian zones were not included in the macrohabitat analysis. However, our findings suggest that riparian zones may be an important overwintering habitat for hatchlings following nest emergence, as it has been reported to be critical during hibernation for other freshwater turtles (Burke & Gibbons, 1995). No matter the release strategy, hatchlings selected for great substrate depths and showed some preference for moss vegetation. The importance of substrates and moss vegetation to hatchlings has also been reported in other Blanding's turtle populations (Butler & Graham, 1995; Standing et al., 1997; McMaster & Herman, 2000; McNeil et al., 2000).

Due to these findings, it is suggested that Camp Ripley Training Center continue to escort hatchlings to wetlands following nest emergence. However, wetland release sites should be characterized by waterlogged substrates and an abundance of moss species with minimal open water, as opposed to the current management strategy of releasing them in the nearest body of water. At Camp Ripley, resources and wetlands are greatly dispersed (Pappas et al., 2000) so releasing hatchlings near wetlands would ultimately reduce risks of predation, water loss, and road mortality (Pappas, Congdon, & Brecke, 2017). Additionally, releasing hatchlings near wetlands would significantly reduce the distance hatchlings have to travel to find great substrate depths and riparian zones if hatchlings are truly selecting these areas for hibernation. Hatchlings that randomly use wetlands do not need to search for it and hatchlings that prefer uplands are close to riparian zones which have the necessary moisture and soil depth hatchlings need to avoid water loss and freezing over the winter (Dinkelacker et al., 2004).

It is also recommended that Camp Ripley Training Center incorporates a terrestrial buffer zone surrounding wetland habitats at Camp Ripley. As previously mentioned, hatchlings significantly preferred upland habitat no matter the release strategy. Whether hatchlings are selecting for upland habitat or truly selecting riparian zones, including a terrestrial buffer zone around wetlands would not only increase the protection of hatchling Blanding's turtles but increase the protection of many taxa that depend on riparian areas to complete their life cycles (Semlitsch & Bodie, 2003). Initially, land management practices should be updated to include a minimum of a 73 m buffer radius around all wetlands, as this radius has been reported to protect 90 % of hibernation sites in other freshwater turtle species (Burke & Gibbons, 1995). However, it is advised that future research be conducted to establish an appropriate buffer width specific to the population at Camp Ripley for the protection of hatchling Blanding's turtles following nest emergence.

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