Wood Turtles (*Glyptemys insculpta*) in Northeastern Minnesota: An Analysis of GPS Telemetry and a Population Assessment

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Dedication

I dedicate this to my grandmother, Mary Fitts, who demonstrates everyday how to be a strong, intelligent, and kind woman, and who brought me to the Bay many years ago where I first developed my love for catching frogs.

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CHAPTER ONE

A Methodological Approach for Wood Turtle (*Glyptemys insculpta*) GPS Telemetry

Summary

Recent improvements in Global Positioning System (GPS) telemetry allow for advancements in our understanding of small vertebrate species behavior and ecology. We evaluated the positional data quality of snapshot GPS devices at a study area in northeastern Minnesota for use in freshwater turtle research. GPS stationary tests in four different cover types were used to evaluate location accuracy, fix success rate, and directional bias of GPS devices in Wood Turtle (*Glyptemys insculpta*) habitat. These tests demonstrated that positional data quality is reduced in closed canopy conditions. Utilizing the results for these tests, we developed a GPS screening procedure for turtle locations. We collected 122,657 locations and 399,606 temperatures readings from the carapace of 26 Wood Turtles from May to September 2015 and 2016. We removed locations with high horizontal dilution of precision values, locations farther from surrounding points than a predefined distance, biologically impossible movements, and then used moving averages to estimate turtle locations. The screening procedure removed 10% of GPS locations, and reduced mean location error from 26 m (SD = 33) to 11 m (SD = 12). We also developed a methodology to compare ambient temperature profiles from water, sunny, and shaded locations to the temperature of a turtle's carapace, to define a turtle's location as land or water. We estimated that Wood Turtles used land 65% of the time during their active season. Moreover, the fix success rate for all land locations was 37% (SD = 14), suggesting substantial use of hidden and brushy locations while on land. Our results suggest that snapshot GPS technology and temperature loggers provide temporally unbiased and abundant GPS data useful in describing spatial ecology and habitat use of semi-aquatic turtles.

Introduction

Global Positioning System (GPS) technology was originally developed for military operations in the 1970s. In the 1990s ecologists began using GPS technology to study animal movement. Initial deployments were on large animals such as moose (*Alces alces*). Over time lighter, miniaturized GPS devices were developed that could be deployed on smaller animals. Miniaturized GPS devices compromise power consumption to reduce weight so that smaller organisms are able to carry them. GPS devices have now been deployed on small mammals (Mcmahon et al. 2017), birds (Bridge et al. 2011), bumblebees (Hagen et al. 2011), and freshwater turtles (Christensen and Chow-Fraser 2014).

Tracking turtle movements with GPS technology has benefits. Traditional tracking methodology for turtles uses VHF telemetry, which is time consuming, logistically burdensome, and may disturb turtles from their natural patterns of behavior (Christensen and Chow-Fraser 2014). GPS devices are less invasive for study animals and less time-intensive for researchers. GPS devices record locations at pre-determined intervals day and night (Urbano et al. 2010). Despite the higher initial cost, GPS devices obtain more locations at a significant cost savings per location and without the temporal biases associated with weather and daylight (Beyer and Haufler 1994, Christensen and Chow-Fraser 2014).

Development of snapshot GPS technology further decreased the weight of GPS telemetry devices. Snapshot GPS devices reduce power consumption and weight by capturing raw satellite data and timestamp information during brief periods of data collection. Post-processing software uses satellite ephemeris data to calculate a location after the unit has been recovered from the animal (Tomkiewicz et al. 2010, Mcmahon et al. 2017).

Raw GPS datasets include both inaccurate and missing locations. Location errors decrease accuracy and precision of data (Moen et al. 2001, Rodgers 2001, D'Eon and Delparte 2005). Understanding species-specific GPS bias and accuracy, and developing methods to reduce bias and increase precision will help reduce habitat misclassification, biased movement path analyses, and inaccurate home range estimates. As failed location attempts are not random, understanding the reason for missing locations is critical for unbiased resource selection analyses. However, most analyses of habitat preference from GPS data ignore these effects (Rettie and McLoughlin 1999, Frair et al. 2004, 2010).

Location error (LE) is the difference between an animal's GPS location and its true location. Three-dimensional (3-D) fixes, estimated with > 4 satellites, provide the most accurate positional data (Lewis et al. 2007, Jiang et al. 2008). Traditional GPS devices attain locations with ≤ 30 m accuracy (Tomkiewicz et al. 2010, Recio et al. 2011). Limited data exists on LE for lightweight GPS devices. Mean LE for stationary tests with GPS devices used on small animals were 39.5 m (feral cats [*Felis catus*], Recio et al. 2011), 13.5 m (European hedgehog [*Erinaceus europaeus*], Glasby and Yarnell 2013), and 11.4 m for open and 12.7 m for closed canopy sites (Wood Turtle [*Glyptemys insculpta*], Elfelt and Moen 2014).

Fix success rate (FSR) is the percentage of successful GPS locations. Current GPS devices used on larger, terrestrial species have near 100% FSR (Cargnelutti et al. 2007, Lewis et al. 2007, Hebblewhite and Haydon 2010). Stationary FSR for comparable lightweight GPS devices was 89%, 85%, and 98% (Recio et al. 2011, Glasby and Yarnell 2013, Elfelt and Moen 2014). During deployment on European hedgehogs FSR decreased to a mean of 67%, with 38% FSR in woodland habitats, and 100% FSR in open pasture (Glasby and Yarnell 2013). The percentage of unrealized location attempts varied throughout the day because of animal behavior, with the highest FSR occurring between 0800 and 2000. In another study, overall FSR of a predominately aquatic freshwater species, the Blanding's turtle (*Emydoidea blandingii*), was 22% (Christensen and Chow-Fraser 2014).

One important reason for studying low FSRs and high LEs in GPS data is to identify cause of bias. The cause of bias could be associated with topographic features, vegetation, animal behavior, proximity to the ground, time since deployment, and antenna position. Before assuming the accuracy of location data, preliminary studies should test for features that affect GPS data collection. For example, leaves, water, or trees can limit the ability of GPS devices to receive enough data to calculate a position. Terrain and canopy coverage reduce the likelihood of a GPS device acquiring the satellite signals necessary to calculate a location (Rempel et al. 1995, Moen et al. 1996, 1997, Dussault et al. 1999). Large diameter trees, dense vegetation, and steep topography degrade reception of satellite signals in GPS devices of large terrestrial species (Rempel et al. 1995, Moen et al. 1996, 1997, Dussault et al. 1999, Glasby and Yarnell 2013). Burrow tunnels with ≤ 31 cm openings, vegetation, and horizontal dilution of precision (HDOP) most affected LE for pygmy rabbits (*Brachylagus idahoensis*) and snapshot GPS devices (Mcmahon et al. 2017). In the same study, snapshot GPS devices collected more consistent and accurate locational data > 9 hrs after deployment. Quantifying features that degrade GPS satellite signal for the specific conditions and seasons under study, and a sampling interval consistent with animal deployment, is necessary for unbiased GPS data analysis (Frair et al. 2004).

Previous Wood Turtle habitat analyses demonstrated the need for accurate land cover and locational datasets to define fine-scale habitat use patterns (Brown et al. 2016). Removing locations with large LE increases accuracy and precision of datasets (Bjorneraas et al. 2010). Similarly, the removal of locations with HDOP values remove erroneous GPS locations (Moen et al. 1997, D'Eon and Delparte 2005). Other screening procedures that can remove inaccurate locational data include sequential GPS locations with very acute turn angles and biologically impossible (large) distances between consecutive points, or locations located farther from the surrounding points than predefined distances (Bjorneraas et al. 2010).

Wood Turtles spend much of their time in locations that interfere or prevent GPS signals (such as water and mixed forest stands, Arvisais et al. 2004, Ernst and Lovich 2009). An additional tool for improving interpretation of GPS data is temperature loggers. Integrating temperature sensor data with a GPS unit should allow for better determination of aquatic vs. terrestrial habitat use, as a temperature logger attached to a turtle should reflect the temperature of the environment it occupies. Ambient environmental temperatures and both internal and external turtle temperatures are highly correlated (Grayson and Dorcas 2004). Previous freshwater turtle diel behavior analyses relocated individuals every 2 – 3 hrs (Ennen and Scott 2008). The use of both GPS and temperature loggers will eliminate the need to relocate animals frequently, and should improve habitat use estimates, particularly use of aquatic or terrestrial habitat.

Our first goal is to determine the accuracy, precision, and FSR of snapshot GPS devices that we deploy on Wood Turtles. Our second goal is to determine how vegetation, HDOP, time of day, animal behavior, and antenna position affect Wood Turtle GPS data bias. In order to include our GPS data in future resource selection and movement pattern studies, our third goal is to define an appropriate screening procedure for Wood Turtle GPS telemetry studies to reduce LE with minimal data loss. Finally, we will use environmental temperature profiles from land and water to help map a Wood Turtle's movement across the water/land interface.

Methods

Study Area

We conducted this study in northeastern Minnesota along a 40-km stretch of river occupied by Wood Turtles. The specific location is withheld in compliance with

Minnesota data practices law for species listed as endangered or threatened. The river is surrounded by 75% public land and 25% private land. More than 90% of the study area is forested, with the remainder in non-forest and water classes (Brown et al. 2016). Mesic forest types, which comprise 80% of the area, are dominated by aspen (*Populus* spp.), balsam fir (*Abies balsamea*), and paper birch (*Betula papyrifera*). Pine forest types are less common in the study area and found on sandy soils. Black spruce (*Picea mariana*), balsam fir, northern white cedar (*Thuja occidentalis*), and tamarack (*Larix laricina*) constitute over 90% of hydric forest species in the surrounding area. Non-forest vegetation consisting of lowland alder (*Alnus* spp.), grass/forb openings, oxbow lakes, and other non-flowing water features also occur in the study area (Brown et al. 2016).

Stationary Tests

We conducted stationary tests with GPS devices to calculate 50% and 95% circular error probable (CEP) distance, mean location error (LE), fix success rate (FSR), and angular dispersion at each of four sites. Three different GPS devices were deployed at each location for at least 60 hrs. All devices were attached to the carapace of a dead Wood Turtle's shell to replicate placement on live turtles. For each stationary test, the mean of locations at a single test site was considered the true location for that test (Moen et al. 1997). We calculated the x- and y-error as the difference between the x- or y-coordinate for a single GPS location and the true location. We used the Pythagorean Theorem to calculate the distance in meters from each GPS location to the true location. Next, we calculated the radius of the 50% and 95% CEP using the percentile method, in which the 50% CEP is the radius of a circle centered at the true location which contains 50% of the GPS locations (Moen et al. 1997, D'Eon and Delparte 2005, Lewis et al. 2007). We calculated mean LE as the average difference between the true GPS location and the actual location for all data points. FSR was the total number of achieved locations divided by the total number of GPS attempts. We also calculated the direction and magnitude of angular dispersion for each dataset (Zar 1984). The magnitude of angular dispersion, r, is a measure of concentration of the angles, and is a value between 0 and 1, where a high r-value represents high concentration and therefore high directional bias.

We conducted stationary tests in four 2011 National Land Cover Dataset (NLCD) cover types, including woody wetlands, deciduous forest, developed/open space (hereafter referred to as open space) and evergreen forest (Homer et al. 2015). The evergreen forest test location was also located under substantial brush to replicate common hiding behavior by Wood Turtles (Figure 1.1, personal observations). All samples were collected during leaf out (14 July to 18 September 2016). These stationary test results helped us develop a set of procedural steps to create a screened GPS dataset.

Deployment on Turtles

We fit 26 turtles and three turtles, respectively, with tracking devices in 2015 and 2016. Nineteen of the turtles were adult females and 10 were adult males. We captured most turtles within or adjacent to areas of foraging and nesting habitat enhancement. We removed turtles from the field for < 24 hours to epoxy (EP-7, Protective Coating Company, Allentown, PA) a plated VHF telemetry unit (R1680; Advanced Telemetry Services [ATS], Isanti, MN) to the right middle costal scutes of turtles. We did not to place the unit on the top of the carapace in order to avoid impeding the mounting of females by males during mating or movement in tight spaces (Boarman 1998). The metal plate was attached to the first or second costal scute. We attached a removable GPS device (G10 UltraLITE; ATS) and a Thermochron iButton (DS1922L; Maxim Integrated, Dallas, TX) to the metal plate (Figure 1.2). The iButton was coated in Plasti Dip (Plasti Dip International, Blaine, MN) to protect it from weathering and water (Rasmussen and Litzgus 2010). VHF and GPS devices were coated with Scotchcast (3M, Maplewood, MN) for waterproofing by manufacturers. The weight of all three devices and epoxy was 38 g. Devices were only attached if they weighed < 5% of the turtle (Edge et al. 2010, Millar and Blouin-demers 2011). The plated VHF unit remained attached to the turtle's carapace until removal in late summer 2016. We attached the GPS device and temperature logger to each turtle from approximately May to September of both 2015 and 2016.

We deployed environmental temperature loggers (iButtons and Onset HOBO Pendant G loggers, part # UA-004-64; Bourne, MA) in aquatic and terrestrial habitats throughout the study area. iButton temperature data loggers measure air temperature from -10 to 65° C, with an accuracy of ± 0.50° C, and a response time of 130 s. HOBO Pendant temperature data loggers measure air temperature from -20 to 70° C, with an accuracy of ± 0.47° C, and a response time of 600 s. We recorded temperatures at ≤ 4 water locations (including a vernal pool in 2016), ≤ 3 shaded locations, and ≤ 7 sunny locations from May to September 2015 and 2016. All ambient temperature iButton loggers were coated in PlastiDip to replicate turtle deployment conditions.

Temperature loggers and GPS devices recorded data every 10 minutes. GPS devices recorded locations using snapshot technology. Snapshot devices only record 3-D locations, and only record a location when ≥ 6 satellites are in use. We set snapshot size of GPS devices to 512 ms (Elfelt and Moen 2014). We recaptured turtles approximately every 30 days to download data and replace GPS and iButton devices. We processed raw GPS data into latitude and longitude coordinates using UltraLITE Fixes software program (ATS). Then, using DNRGPS (Version 6.0, http://maps1.dnr.state.mn.us/dnrgps/), we converted coordinates into Universal Transverse Mercator (UTM) locations. We programmed and post-processed data from stationary tests and GPS devices affixed to live turtles in the same manner. Data collection occurred from 24 May 2015 to 3 October 2015 and then again from 1 May 2016 to 19 September 2016. Sampling and handling methods were approved by the University of Minnesota Duluth Institutional Animal Care and Use Committee (Protocol No. 1504-32514A), and permitted by the Minnesota Department of Natural Resources.

Stationary Test Data Analysis

We calculated the mean value for LE, 50% CEP, 95% CEP, FSR, and angular dispersion for each stationary test replicate of > 60 hrs locational data. We tested for the effect of cover type and GPS logger identity on LE, 95% CEP, and FSR using two-way ANOVA tests. We performed Tukey's *post hoc* tests to compare LE and FSR between cover types. We performed a Rayleigh z test for directional bias on all GPS data collected at each cover type.

Using each location as a replicate, we also tested the effect of time of day, time since deployment, HDOP, identity of GPS logger, and cover type on LE. We used backward stepwise selection to select the model with the lowest Akaike's information criteria (AIC) value (Akaike 1974). We used quantile-quantile plots to ensure the data satisfied assumptions of normality and homoscedasticity. To meet the assumption of normality we logarithmically transformed LE for all GPS test locations. Lastly, we regressed HDOP on LE to determine if HDOP is an accurate predictor variable for screening erroneous GPS locations. Significance for all statistical tests was $\alpha = 0.05$.

GPS Screening

We developed a corrected dataset from the uncorrected GPS dataset using GPS stationary test results and biologically feasible movement rates by Wood Turtles. Screening of poor quality GPS locations relied on distance, angle, and time between subsequent locations calculations. We calculated the Euclidean distance and turn angle between each set of locations using Geospatial Modeling Environment (Version 0.7.2.1, www.spatialecology.com). Initially, we removed all GPS locations that were within one hour of deployment. We then developed a GPS data screening methodology to reduce 50% and 95% CEP with minimal data loss, which included the removal of high HDOP values and removal of locations that were not biologically possible for Wood Turtle movement on land (maximum speed = 322m/hr; Woods 1945). To generate the screening procedure, we calculated the magnitude of data loss and subsequent reduction of location error for each step seperately and in combination. We ordered rules based on proven significance (e.g. high HDOP values) and then by ratio of locations to location accuracy.

Lastly, because Wood Turtles are sedentary for large periods of the day and active season, and have a small daily net displacement of about 100 m (Strang 1983), we tested if averaging a location with surrounding locations was an appropriate approach to reduce location error for this species. To do this we averaged each 10 minute location with its three nearest locations before and after it. If a location did not have surrounding locations near in time we were unable to average that location but did not remove it. To better understand the implications of averaging GPS locations we calculated net displacement per day, mean LE, and 95% CEP for both averaged-only GPS locations and all screened GPS locations. We also calculated the percentage of locations included in averaging, mean LE and 95% CEP for only locations that met criteria to be averaged, and the distance between averaged and original locations for each cover type.

Temperature Coupling

In order to match temperature and GPS locations, we rounded the time of each data point to the nearest 10 minutes. We averaged shade (T_{shade}), sun (T_{sun}), and water (T_{water}) temperature across all sample sites in the same environment because temperature differences within each set of shade, sun, or water loggers was minimal (SE water = 0.01° C, SE shade = 0.03° C, SE sun = 0.03° C). We included both T_{shade} and T_{sun} in the temperature rule set because we wanted to distinguish between use of open vs. closed canopies. Each 10 minute T_{turtle} reading was matched with its corresponding GPS location. We then classified a turtle in water if $T_{turtle} \approx T_{water}$. Cloacal temperatures of turtles are highly correlated with ambient environmental temperatures (mean difference = 0.2°C; Brown and Brooks 1991) and only small differences between internal cloacal temperature and external shell temperature have been previously recorded (Grayson and Dorcas 2004).

A sample of paired temperature readings were visually assessed (Microsoft Excel 2010, Microsoft Corporation, Redmond, Washington) to develop an automated protocol to define a turtle as on land or in water if environmental temperatures were similar. We used water as the default location due to the high specific heat of water, and because T_{water} (mean SD/day = 2.3^0 C) fluctuates less than T_{shade} (mean SD/day = 4.1^0 C) and T_{sun} (mean SD/day = 5.6^0 C) throughout the day. Location was switched from water to land if T_{sun} or T_{shade} were closer to T_{turtle} than $T_{water,}$ air temperatures crossed water temperature at the same time T_{turtle} did, or one land/water classification disagreed with the trend of locations surrounding it. Once satisfied with the accuracy of our land/water designation for each GPS location, we were able to calculate FSR for only those location attempts in which the turtle was on land, as GPS transmissions do not penetrate the water surface.

Results

Stationary Tests

We collected 5,112 locations from 11 stationary test deployments at four sites. Overall FSR was 77%, 50% CEP was 18 m, and 95% CEP was 69 m across all stationary tests. FSR for uncorrected stationary test data was 65%, 87%, 94%, and 36% for woody wetlands, deciduous forest, open space, and evergreen forest respectively (Table 1.1). Mean LE for uncorrected GPS data was 28 m, 29 m, 16 m, and 40 m for woody wetlands, deciduous forest, open space, and evergreen forest respectively.

Cover type significantly affected LE for uncorrected and corrected GPS stationary test data (uncorrected: $F_{3,6} = 8.28$, $p = 0.015$; corrected: $F_{3,6} = 15.25$, $p = 0.003$). LE was higher for evergreen forest than woody wetlands ($p = 0.013$), deciduous forest ($p = 0.003$), or open space ($p = 0.003$). Similarly, cover type significantly affected 95% CEP for uncorrected GPS stationary test data (uncorrected: $F_{3,6} = 8.13$, $p = 0.016$; corrected: $F_{3,6} = 15.88$, $p = 0.003$; Figure 1.3). The identity of GPS logger did not significantly affect either uncorrected or corrected LE (uncorrected: $F_{1,6} = 0.05$, $p = 0.83$; corrected: $F_{1,6} = 01.10$, $p = 0.33$). The identity of GPS logger also did not significantly affect 95% CEP (uncorrected: $F_{1,6} = 0.21$, $p = 0.66$; corrected: $F_{1,6} = 0.67$, $p = 0.44$). Evergreen forest increased 95% CEP in comparison to all other habitat types (Table 1.2).

Cover type ($F_{3,6} = 11.93$, $p = 0.06$) and the identity of GPS logger ($F_{1,6} = 0.41$, $p = 0.54$) did not significantly affect FSR for uncorrected GPS stationary test data. However, cover type was significant in a one-way ANOVA test without GPS identity included (Figure 1.4). Evergreen forest reduced FSR compared to other cover types (Table 1.3). The mean angle of dispersion ($\bar{a}$) was slightly biased in the N/NE direction for uncorrected GPS tests and in the S/SE direction for corrected GPS tests (Table 1.4). However, the magnitude (r) was small for both uncorrected locations (range 0.02 – 0.12) and corrected locations (range 0.05 – 0.31). The evergreen forest site, where the unit was located under a brush pile, had the largest directional bias for both corrected and uncorrected GPS data. Open space also had a slightly biased mean angle, although magnitude was small.

With each location as a replicate, cover type (uncorrected: $F_{3,5115} = 164.3$, $p < 0.001$; corrected: $F_{3,4568} = 231.0$, $p < 0.001$) and HDOP (uncorrected: $F_{1,5115} = 294.9$, $p < 0.001$; corrected: $F_{1,4568} = 46.8$, $p < 0.001$) significantly affected LE for all GPS stationary test locations. GPS logger identity ($F_{1,4568} = 24.1$, $p < 0.001$), time of day ($F_{1,4568} = 4.1$, $p = 0.04$) and time since deployment ($F_{1,4568} = 4.1$, $p = 0.04$) significantly affected LE for corrected locations. However, they were insignificant for all uncorrected GPS stationary test locations (GPS logger identity: $F_{1,5113} = 1.3$, $p = 0.26$; time of day: $F_{1,5113} = 0.9$, $p = 0.34$; time since deployment: $F_{1,5113} = 0.4$, $p = 0.52$). Akaike's information criteria (AIC) supported the model with all five predictor variables (cover type, HDOP, time of day, time since deployment, and GPS identity); a model without time since deployment and time of day also ranked well (Table 1.5).

GPS screening reduced 50% CEP from 18 m to 8 m and 95% CEP from 70 m to 27 m (Table 1.6; Figure 1.5). Mean LE decreased from 26 m to 11 m. We reduced mean LE for corrected GPS data to 12 m, 9 m, 7 m, and 25 m for woody wetlands, deciduous forest, open space, and evergreen forest, respectively. We screened an additional 13%, 11%, 3%, and 16% of locations for each cover type, respectively. Data screening reduced the number of GPS locations by 10%, for a total of 109,865 Wood Turtle locations.

Wood Turtle GPS and Temperature Data

Four Wood Turtles died during the study, three < 30 days and one > 1 yr after release; the cause of death was not determined in all cases. We collected 122,657 Wood Turtle locations out of 464,415 location attempts (mean = 61,328 locations/turtle, SD =

26,508). This included a total of 3,748 turtle days with GPS data (mean = 150 days/turtle, SD = 76). Both GPS and iButton devices had missing data or failed to record any data on 22% of all 30-day deployments due to battery failure, programming errors, or unknown causes. A total of 399,606 temperatures were collected (mean = 15,369 readings/turtle, SD = 7,179), of which 320,758 were matched with GPS data.

Overall FSR for all turtle land locations was 37% (SD = 14; Table 1.7). FSR was greatest in May (55%) and lowest in September (30%). FSR for all attempted fixes (e.g. whether turtle was in land or water) was 25% (SD = 11).

GPS Screening

The overall effect of data screening was to remove 10% of turtle GPS locations. HDOP significantly affected LE ($F_{1,5118} = 437.1$, $p < 0.001$; Figure 1.6). We eliminated < 0.005% of location attempts with HDOP > 4. Second, we removed a location if it was not within 50 m of the mean x and y coordinate calculated from a moving window of four locations before and four locations after. To calculate the moving window all four locations must have ocurred within 60 min of surrounding locations. This removed 6.8% of Wood Turtle GPS locations. Third, we removed “spikes” in movement paths that indicate unrealistically fast movement away and back towards the same location (Bjorneraas et al. 2010). To accomplish this we screened locations with incoming and outgoing speeds ≥ 5 m/min and a turn angle ≥ 100°. We only included locations if they occurred within 40 min of surrounding locations. This removed 2.1% of Wood Turtle

GPS locations. Similarly we removed locations with a movement rate ≥ 8 m/min in 10 mins. This removed 1.8% of Wood Turtle GPS locations.

Last, we averaged each remaining GPS location with the three locations before and after it if all locations were within 40 mins from surrounding locations. Mean net movement per day for screened Wood Turtle GPS locations was 64 m (SD = 74) for un-averaged locations and 57 m (SD = 77) for locations after averaging. Fifty eight percent of Wood Turtle GPS locations were averaged. Forty two percent of screened locations were unable to be averaged but remained in the dataset. Eighty six percent of woody wetland stationary test locations, 99% of deciduous forest locations, 99% of open space locations, and 45% of evergreen forest locations were averaged (Table 1.8). For stationary test locations that were averaged, mean distance between averaged and un-averaged GPS locations was 12 – 22 m (SE = 2). Averaging locations reduced mean LE for all cover types, but did not reduce 95% CEP for evergreen forest locations. However, if we were to change our screening protocol to only include averaged locations, 95% CEP for evergreen forest stationary test locations would be reduced from 73 m to 25 m.

Temperature Coupling

When T_{turtle}, T_{water},T_{shade} and T_{sun} were all available, the environmental location of each turtle was identified with our automated protocol defined below. When T_{shade} was not available, T_{sun} was the only air temperature used in analysis. All values are in degree Celsius.

Rule 1: Turtles classified in water if $|T_{turtle} - T_{water}| \leq 1$ (example: Figure 1.7).

Rule 2: Turtles classified in water if $|T_{turtle} - T_{water}| \leq 2$; $(T_{turtle} - T_{shade}) > 4$; and $(T_{turtle} - T_{sun}) > 4$.

Rule 3: If a GPS location was attained in any of the above scenarios (1 - 3) the turtle ≠ water.

Rule 4: Turtles were classified in water if $|T_{turtle} - T_{water}| \leq 2$ and the two locations before and after are both in water.

If $|T_{shade} - T_{turtle}| \leq 1$ or $|T_{sun} - T_{turtle}| \leq 1$ and $|T_{water} - T_{turtle}| \leq 1$, then further rules are applied. Rules 5 to 7 were applied to 5.9% of T_{turtle} data points.

Rule 5: If $|T_{shade} - T_{turtle}| < |T_{water} - T_{turtle}|$ and $|T_{sun} - T_{turtle}| < |T_{water} - T_{turtle}|$ and $|T_{sun} - T_{water}| > 0.2$ and $|T_{shade} - T_{water}| > 0.2$, then the turtle was classified on land.

Rule 6a: If $|T_{sun} - T_{water}| > 0.2$ both 50min before and after a location; $T_{sun} > T_{water}$ 50min before and $T_{sun} < T_{water}$ 50min after or $T_{sun} < T_{water}$ 50min before and $T_{sun} > T_{water}$ 50min after; and the turtle was classified on land 50min before/after, then the turtle was classified on land (this scenario is also true if T_{shade} had same relationship as T_{sun} above) (example: Figure 1.8).

Rule 6b: If $|T_{shade} - T_{water}| > 0.2$ both 50min before and after a location; $T_{shade} > T_{water}$ 50min before and $T_{shade} < T_{water}$ 50min after or $T_{shade} < T_{water}$ 50min before and $T_{shade} > T_{water}$ 50min after; and the turtle was classified on land 50min before/after, then the turtle was classified on land.

Rule 7: If $|T_{sun} - T_{turtle}| < |T_{water} - T_{turtle}|$ or $|T_{shade} - T_{turtle}| < |T_{water} - T_{turtle}|$, and the two locations before and after were on the land then the turtle was classified on land.

In total, turtles were classified on land 65% of the time, and these percentages did not considerably change from step to step (Table 1.9).

Discussion

Our results indicate that miniaturized GPS devices are able to collect large quantities of temporally unbiased GPS locations that can be post-processed to estimate Wood Turtle locations with fairly high resolution. Snapshot GPS devices deployed on Wood Turtles had lower FSR in comparison to GPS devices on larger, terrestrial organisms. This reduced FSR is an impact of Wood Turtle behavior, in particular use of partially covered and riverine habitats. It is also indicative of the reduced abilities of miniaturized GPS devices to have the same FSR as larger units. The greater memory capacity and time to collect satellite information per location attempt (up to 90 s in typical GPS devices in comparison to 512 ms for snapshot) of larger devices, likely explains why FSR for smaller, snapshot GPS devices in open areas is only 94%. However,despite the unavoidable challenges of tracking a small animal in dense and wet habitats, GPS devices in our study collected valuable spatial information.

There are benefits to tracking aquatic instead of terrestrial animals. A GPS signal does not penetrate water, and as a result combining last known GPS locations with failed location attempts informs researchers about aquatic movements and aquatic habitat use. Temperature loggers enhance this ability. Using biologically-driven rules to match the exterior temperature of a turtle to its current ambient environment improved our understanding of Wood Turtle habitat use patterns. This methodology allowed us to characterize FSR for land-only GPS location attempts. Instead of an overall FSR of 25%, similar to the Blanding's turtle (Christensen and Chow-Fraser 2014), we were able to identify FSR on land to be 37%. Frequent, unsuccessful location attempts in terrestrial habitat specify Wood Turtle use of closed canopy sites whereas frequent, successful location attempts specify Wood Turtle use of open canopy sites. Increasing the number and spatial distribution of ambient temperature loggers to include vernal pools and varying stream depths would also allow us to further define local habitat use.

Because we studied biases in Wood Turtle GPS telemetry, it is now possible to decide how and when to use and interpret results derived from Wood Turtle GPS data. Bias against closed canopy habitats needs to be addressed for future freshwater turtle movement or resource selection analyses. One alternative is to reduce the size of the GPS dataset and include only averaged freshwater turtle locations, and thus reduce expected LE for locations within both closed and open habitats. This alternative reduces expected 95% CEP in closed canopy sites from 75 m to 25 m but has high data loss. Alternatively, our approach of not removing locations that cannot be averaged without neighboring locations accepts higher LE, especially for closed canopy habitats, but does not remove limited closed canopy locations at a disproportionate rate compared to open canopy locational data. For example, only keeping locations that meet averaging criteria screens an expected 55% of evergreen forest locations in comparison to 1% of locations for the open site. This removal of locations that do not meet averaging criteria is in combination with the low effective FSR after GPS screening of only 20% for evergreen forest locations. The two approaches are a tradeoff between higher GPS accuracy and loss of GPS locations, especially in closed canopy habitats.

The most influential rules in reducing LE for GPS stationary test locations were Rule 2, screening locations outside of an average location calculated from a moving window of surrounding locations and Rule 5, averaging a GPS location with its nearest locations in time. The two additional rules to screen biologically impossible movements (Rule 3 and 4) screened few locations that had not previously been eliminated by rule two. Screening high HDOP values decreased LE, but few locations were found to violate these rules. We were unable to remove locations that did not meet screening criteria despite the fact that they may have had high LE. We found stationary test GPS data to have a slight statistical angular dispersion bias, but it would not be biologically impactful. Although not needed from a biological perspective, future tests should ensure stationary test platforms are oriented along North/South and East/West axes equally.

Our study provides a foundation to understand the benefits and challenges inherent in freshwater turtle GPS telemetry. Our systematic approach to define and enhance GPS data accuracy provides a foundation for future Wood Turtle diel movement and habitat analyses. While dense cover reduces the ability of devices to attain fixes, this lack of locational data still informs us about the types of habitats Wood Turtles prefer for various thermoregulatory, protective, and foraging needs. We demonstrate that GPS technology is feasible for studying Wood Turtle spatial ecology. Future studies should consider GPS devices for Wood Turtles, or similar freshwater turtles, if researchers cannot commit to time-intensive VHF-tracking, researchers want an unbiased 24-hr snapshot of locations, or there is need for a large spatial dataset.

Table 1.1. Summary of Global Positioning System (GPS) stationary test data from four National Land Cover Database (NLCD) cover types in northeastern Minnesota. Data include number of successful locations, 50% and 95% circular error probable (CEP), mean location error (LE), and standard deviation for both uncorrected and corrected GPS data. Table also includes GPS fix success rate (FSR) (uncorrected data) and effective FSR (corrected data).

Cover Type	Locations (n)	50% CEP (m)	95% CEP (m)	Mean LE (m)	SD LE	FSR (%)
Uncorrected						
Woody Wetlands	1711	21	72	28	29	65
Deciduous Forest	1991	20	77	29	42	87
Open Space	943	12	37	16	31	94
Evergreen Forest	475	28	104	40	46	36
Corrected						
Woody Wetlands	1483	9	29	12	10	52
Deciduous Forest	1778	8	18	9	7	76
Open Space	916	6	13	7	6	91
Evergreen Forest	398	17	73	25	28	20

Table 1.2. P-values for pair-wise comparisons (Tukey HSD) of 95% circular error probable (CEP) for (A) uncorrected and (B) corrected GPS data from four National Land Cover Database (NLCD) cover types in northeastern Minnesota (each test site was sampled three times for > 60 hrs/sample).

A.

Cover Type	Woody Wetlands	Deciduous Forest	Open Space
Deciduous Forest	0.717		
Open Space	0.091	0.311	
Evergreen Forest	0.155	0.038♦	0.006♦

B.

Cover Type	Woody Wetlands	Deciduous Forest	Open Space
Deciduous Forest	0.429		
Open Space	0.345	0.982	
Evergreen Forest	0.011*	0.002♦	0.003♦

♦Significant $p < 0.05$ for both uncorrected and corrected GPS data.
* Significant $p < 0.05$ for only corrected GPS data.

Table 1.3. P-values for pair-wise comparisons (Tukey HSD) of fix success rate (%) for GPS data from four National Land Cover Database (NLCD) cover types in northeastern Minnesota (each test site was sampled three times for > 60 hrs/sample).

Cover Type	Woody Wetlands	Deciduous Forest	Open Space
Deciduous Forest	0.178		
Open Space	0.150	0.980	
Evergreen Forest	0.070	0.004*	0.005*

♦Significant $p < 0.05$ for both uncorrected and corrected GPS data.
* Significant $p < 0.05$ for only corrected GPS data.

Table 1.4. Mean angle of dispersion (ā), the magnitude of dispersion (r), and Rayleigh's *z* test statistic for uncorrected and corrected GPS locations at four National Land Cover Database (NLCD) cover types in northeastern Minnesota. When magnitude is large (e.g. r = 1) data are concentrated in the same direction (not uniformly distributed around a circle). Rayleigh's test investigates if locations are randomly dispersed around a circle.

Location	n	Mean Angle, ā (deg)	Magnitude (r)	Rayleigh's *z* test statistic
Uncorrected				
Deciduous Forest	1991	35.35	0.02	0.6
Woody Wetlands	1711	24.69	0.03	1.21
Open Space	943	9.6	0.09	7.75◊
Evergreen Forest	475	24.23	0.12	6.68*
Corrected				
Deciduous Forest	1778	186.01	0.05	3.69*
Woody Wetlands	1483	177.98	0.10	16.28◊
Open Space	916	165.43	0.18	30.91◊
Evergreen Forest	309	157.88	0.31	29.16◊

*Significant at $p < 0.05$, ◊Significant at $p < 0.001$

Table 1.5. Multi-factor ANOVA model selection results to determine which covariates strongly influence location error (LE) for Global Positioning System (GPS) stationary test data in northeastern Minnesota in July – September 2016 based on Akaike's Information Criterion (AIC) results. The covariates we tested included turtle identity, horizontal dilution of precision (HDOP), GPS identity, time of day (TOD), and time since deployment (TSD). We included the number of covariate parameters (k), change in AIC (ΔAIC), and model weight.

Covariates	**k**	**ΔAIC**	**w**
Turtle ID, HDOP, GPS ID, TOD, TSD	5	0.00	6.80
Turtle ID, HDOP, GPS ID, TOD	4	2.11	0.24
Turtle ID, HDOP, GPS ID	3	4.27	0.08
Turtle ID, HDOP	2	25.28	0.00

Table 1.6. Summary of screening procedure for Global Positioning System (GPS) stationary test and Wood Turtle (*Glyptemys insculpta*) data, including the total number of locations included in each step and percent of total data kept during screening. Other columns include 50% and 95% circular error probable (CEP), mean location error (LE) and standard deviation for stationary test GPS data because true GPS location was known.

Screening Procedure:	**STATIONARY TEST**					**TURTLE**	
	Locations (n)	**Locations (%)**	**50% CEP (m)**	**95% CEP (m)**	**Mean LE (SD)**	**Locations (n)**	**Locations (%)**
All Locations	5112	100	18	69	26 (± 33)	122657	100
HDOP > 4	5105	100	18	69	25 (± 32)	122511	100
> 50 m from average of surrounding locations	4707	92	16	47	20 (± 17)	114219	93
Turn angle ≥ 100°	4647	91	16	47	20 (± 16)	111874	91
Movement rate ≥ 8 m/min	4575	90	16	46	20 (± 15)	109865	90
Averaged with surrounding locations	4575	90	8	27	11 (± 12)	109865	90

Table 1.7. Fix rate for all 2015 and 2016 Wood Turtles (*Glyptemys insculpta*) in northeastern Minnesota. (A) Includes land-only calculations for mean fix rate, standard deviation, total deployed time, total number of locations, and number of turtles. (B) Includes land and water calculations.

A.

Month	Mean Fix Rate	SD Fix Rate	Time (days)	Locations (n)	Turtles (n)
May	0.55	0.13	272	21551	13
June	0.37	0.09	977	52550	23
July	0.35	0.11	1767	90098	23
August	0.34	0.13	1034	51105	21
September	0.30	0.20	117	5093	6
All	**0.37**	**0.14**	**4091**	**220,397**	**25**

B.

Month	Mean Fix Rate	SD Fix Rate	Time (days)	Locations (n)	Turtles (n)
May	0.28	0.05	1068	42674	13
June	0.25	0.07	2179	78473	23
July	0.26	0.11	3186	121007	23
August	0.25	0.12	1961	70368	21
September	0.14	0.21	573	11267	6
All	**0.25**	**0.11**	**8830**	**323,789**	**25**

Table 1.8. Summary of 95% circular error probable (CEP) and mean location error (LE) for final steps in Global Positioning System (GPS) screening procedure from stationary test GPS data from four National Land Cover Database (NLCD) cover types in northeastern, MN from July – September, 2016. Includes summary data for all screened GPS data for step four and step five in screening procedure, and includes summary data for screened GPS data for step five if only averaged locations are included in final analysis. Table includes a column for the percent of locations that were averaged from each cover type and the mean distance between x and y coordinates for averaged-only locations from step four to step five.

Cover Type	95% CEP (m)			Mean LE (m)			Averaged Only Locations	
	All Locations		Averaged Only	All Locations		Averaged Only		
	Step 4	Step 5	Step 5	Step 4	Step 5	Step 5	%	Moved (m)
Woody Wetlands	47	29	21	21	12	9	86	19
Deciduous Forest	44	18	18	19	9	8	99	16
Open Space	33	13	13	14	7	6	99	12
Evergreen Forest	73	73	25	30	25	11	45	22

Table 1.9. Percentage of all Wood Turtle locations classified as land or water for each step of the temperature coupling protocol. The last column is the percentage of locations that change designations each step.

Step	Land (%)	Water (%)	Affected Locations (%)
1	67	33	NA
2	62	38	5.1
3	64	36	2.2
4	65	35	0.1
5	64	36	0.2
6	65	35	0.7
7	65	35	0.1

Figure 1.1. Evergreen forest Global Positioning System (GPS) stationary test site in northeastern Minnesota (GPS unit deployed on a turtle shell is under the brush pile and wrapped in orange tape).

Figure 1.2. Telemetered Wood Turtle (*Glyptemys insculpta*) with epoxied VHF transmitter, GPS device, and temperature logger.

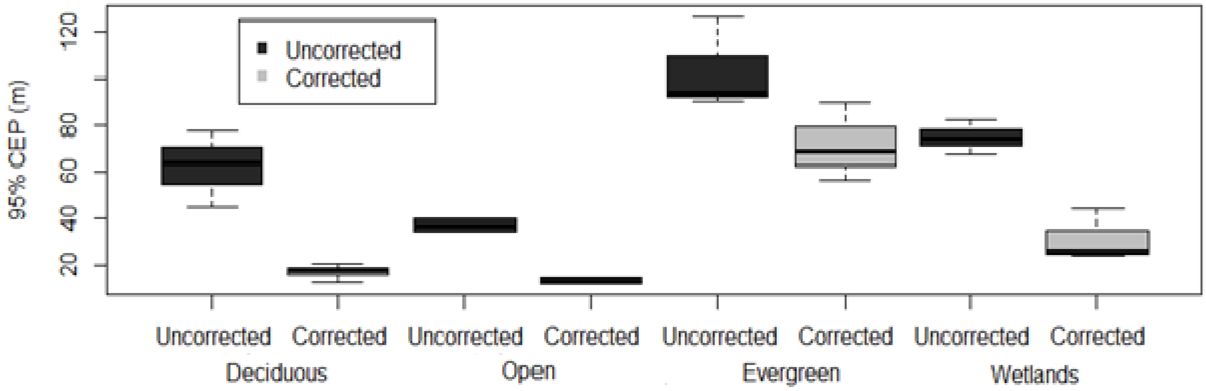


Figure 1.3. Boxplot comparing 95% circular error probable (CEP) for uncorrected and corrected GPS data from four National Land Cover Database (NLCD) cover types (deciduous forest, open space, evergreen forest, and woody wetlands) in northeastern Minnesota (each test site was sampled three times for > 60hrs/sample). Uncorrected GPS one-way ANOVA statistics: $F_{3, 7} = 9.17$, $p = 0.008$. Corrected GPS one-way ANOVA statistics: $F_{3, 7} = 16.66$, $p = 0.001$.

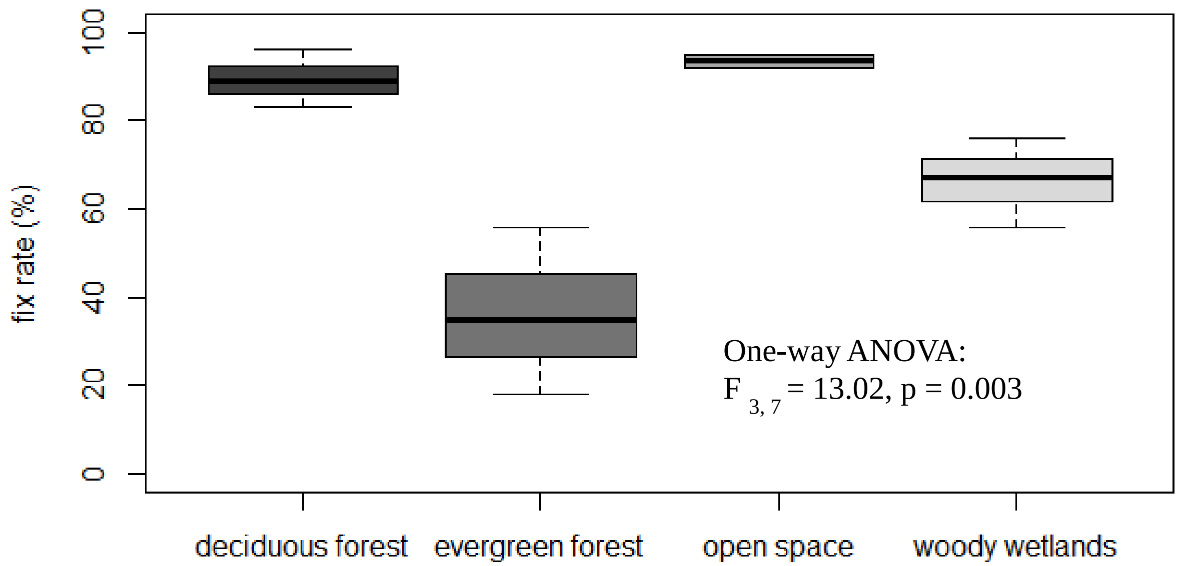


Figure 1.4. Boxplot comparing the fix success rate (%) for GPS data at four National Land Cover Database (NLCD) cover types in northeastern Minnesota (each test site was sampled three times for > 60 hrs/sample). Includes one-way ANOVA F-statistic and p-value.

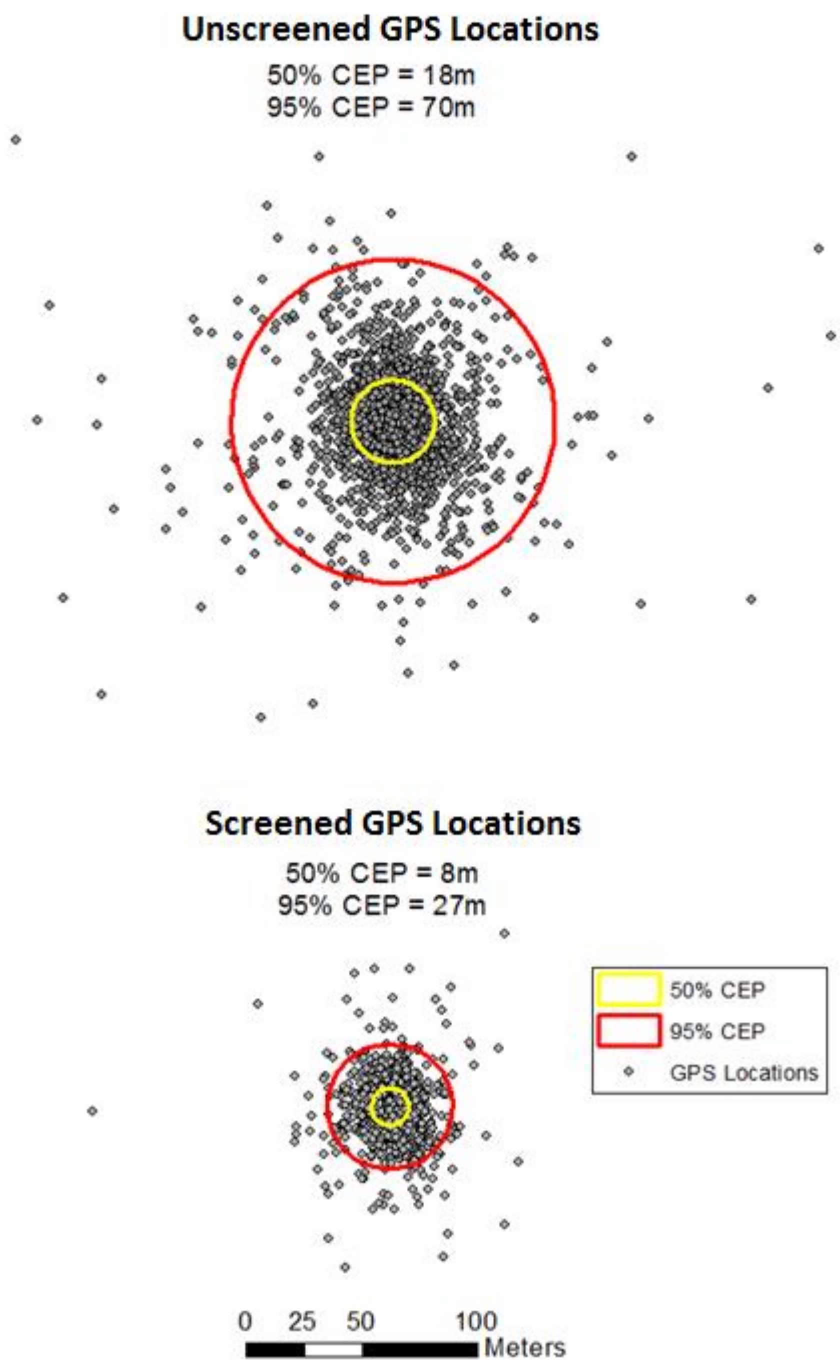
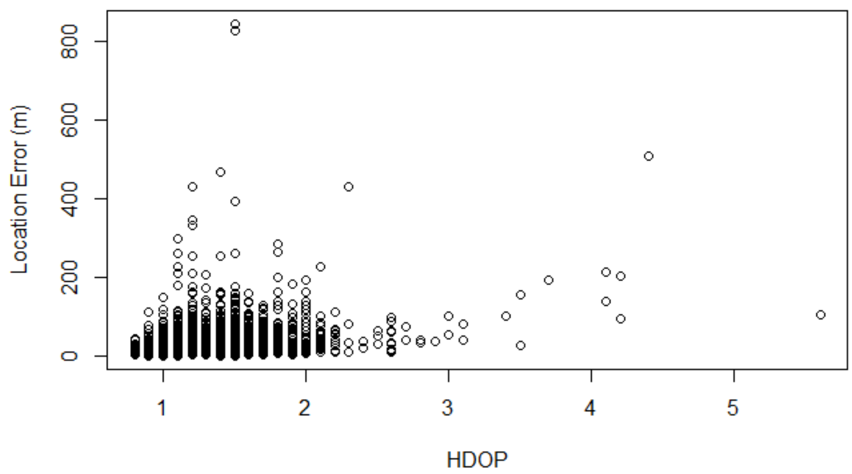


Figure 1.5. Map comparing uncorrected and corrected Global Positioning System (GPS) locations from GPS stationary tests at a closed canopy site (deciduous forest). The yellow circles indicate the 50% (inner) and red circle 95% (outer) circular error probable (CEP).

A.



B.

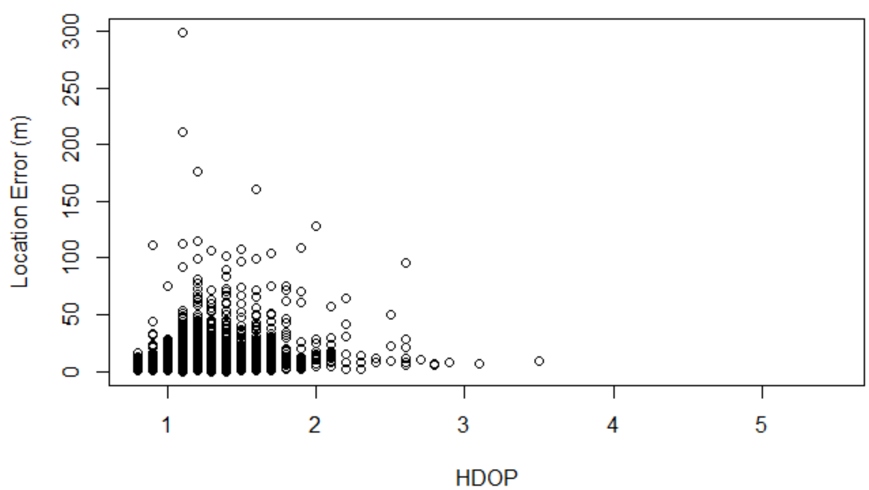


Figure 1.6. Relationship between horizontal dilution of precision (HDOP) and location error (m) for (A) uncorrected and (B) corrected Global Positioning System (GPS) data from all stationary test data in northeastern Minnesota.

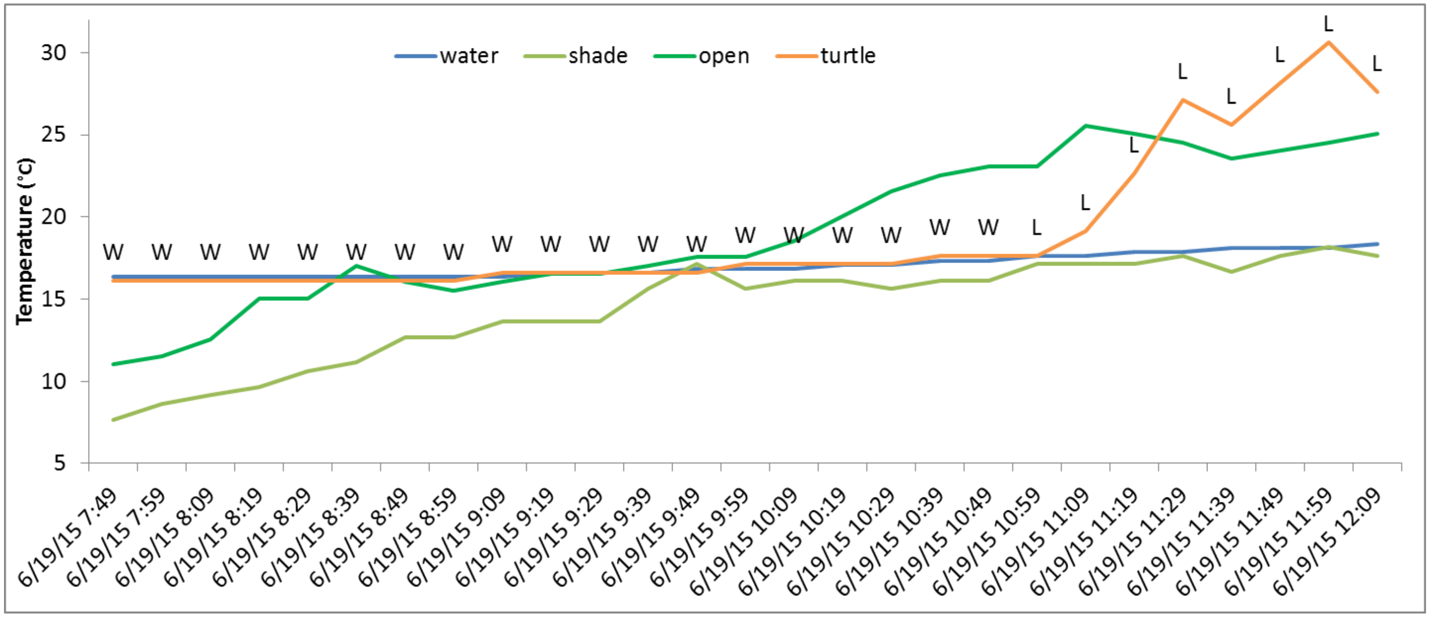


Figure 1.7. Temperature profile for a turtle (T_{turtle}) and the surrounding water (T_{water}), shade (T_{shade}) and sun (T_{sun}). The turtle is classified either as on land (L) or in water (W) every 10 mins based the proximity of T_{turtle} to the nearest ambient temperature (T_{sun}, T_{shade} or T_{water}). This example shows a turtle in water and emerging as the sun warms the air above T_{water} during the day.

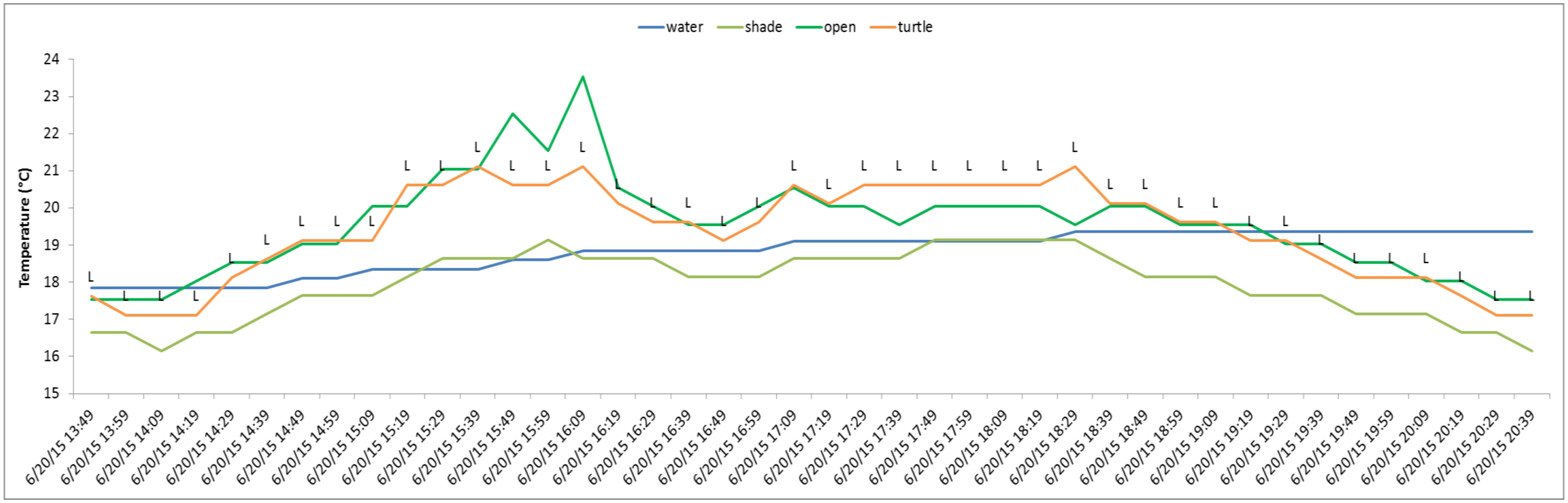


Figure 1.8. Temperature profile for a turtle (T_{turtle}) and the surrounding water (T_{water}), shade (T_{shade}) and sun (T_{sun}). The turtle is classified either as on land (L) or in water (W) every 10 mins based the proximity of T_{turtle} to the nearest ambient temperature (T_{sun}, T_{shade} or T_{water}). Generally if $|T_{turtle} - T_{water}| \leq 1$ then the turtle is classified by default as "W." However, this example demonstrates the relevance of step 5 to dictate that a turtle is on "L" when the T_{turtle} temperature profile closely matches T_{sun} as it crosses water's temperature profile.

CHAPTER TWO

Status of a Wood Turtle (*Glyptemys insculpta*) Population in Northeastern Minnesota

Summary

Wood Turtle (*Glyptemys insculpta*) populations have experienced declines across their North American extent of occurrence and are listed as a state threatened species in Minnesota. To improve our understanding of the current conservation status of the species in northeastern Minnesota, we performed a snapshot comparison study using data from population surveys in 1990 and 2015. Our snapshot comparison indicated relative abundance, adult sex ratio, and juvenile-adult ratio did not differ between years. Thus, we found no evidence of Wood Turtle population change in a 40 km river system in northeastern Minnesota over the last 25 years. Intermittent Wood Turtle capture data collected from 1997-2014 supported a lack of change in population status in the years between 1990 and 2015. The surveyed population exists in a forested landscape with predominantly public ownership and little development pressure. The large amount of suitable habitat and limited human exposure in this watershed have likely allowed this population to avoid many of the stressors impacting populations in other regions.

Introduction

The Wood Turtle (*Glyptemys insculpta*) is a semi-aquatic freshwater turtle endemic to northeastern North America. In many parts of its range Wood Turtles have experienced population declines (Harding and Bloomer 1979, Garber and Burger 1995, Daigle and Jutras 2005, Willoughby et al. 2013). It is listed as a threatened species in the state of Minnesota (Moriarty and Hall 2014), considered endangered globally (International Union for Conservation of Nature 2016), and is currently under review for listing under the Endangered Species Act in the United States (U.S. Fish and Wildlife Service 2016).

Wood Turtles in Minnesota represent the westernmost populations in the species' range. The distribution of Wood Turtles in northeastern Minnesota occurs uncommonly across midsize rivers of at least two watersheds that flow through forest areas (Moriarty and Hall 2014). The northeastern population is likely isolated from Wood Turtle populations to the south and east in Minnesota and Wisconsin because of the disjunct distribution of sandy glacial outwash, which comprises optimal riverine habitat for this species in the northern Great Lakes Region (Buech et al. 1997). Isolated populations and those at distributional limits are typically more vulnerable to extirpation than connected populations and those in core parts of its distribution (Henle et al. 2004, Cushman 2006, Yackulic et al. 2011). Despite its overall threatened status in Minnesota and likely added vulnerability to extirpation due to its isolation from southern Minnesotan and Wisconsin populations, the status of the Wood Turtle population in northeastern Minnesota is unknown.

Previous Wood Turtle population assessments do not exist for northeastern Minnesota. As a species with low fecundity and delayed reproductive maturity, the loss of only one or two reproductive adults from a small population each year can precipitate extirpation in isolated populations (Compton 1999). Terrestrial habitat loss (e.g., land-use conversion and human recreation), habitat degradation (e.g., mesopredator population increase, fire suppression), and direct human impacts (e.g., road mortality) negatively impact Wood Turtle populations across the range (Harding and Bloomer 1979, Garber and Burger 1995, Buech et al. 1997). The scale and influence of these threats in northeastern

Minnesota is unknown. While not previously recorded in the region, collection for the commercial pet trade (Maya Hamady, Minnesota Department of Natural Resources, pers. comm.) can also contribute to Wood Turtle population declines (Levell 2000). Threats found in other parts of the range from altered stream flow and sandbar availability during nesting season are not currently present in Minnesota's northern watersheds (Lenhart et al. 2013). However, the anticipated effects of climate change include an increase in storm frequencies and more pronounced flood and drought intensities that can negatively impact adult survival, nest success, and habitat quality (Wisconsin Department of Natural Resources 2016).

State agencies in the Upper Midwest (i.e., Minnesota, Wisconsin, Michigan, and Iowa) are currently engaged in research and management actions to identify threats to Wood Turtle populations, increase Wood Turtle recruitment and survivorship, and enhance Wood Turtle habitat suitability. As part of this larger conservation initiative, we sought to determine if relative abundance, adult sex ratio, and adult-juvenile ratios have changed in the largest known Wood Turtle population in northeastern Minnesota. We replicated a 1990 population survey in 2015 to investigate changes in population size and structure over the last 25 years. We used intermittent Wood Turtle monitoring data from 1997–2014 to indicate population trends in intervening years. In addition, we wanted to determine if habitat changes relevant to Wood Turtles have occurred in the study region over the past 2–3 decades. To answer this question we quantified changes in forest size class and cover, developed land, and wetland habitat cover within the watershed.

Methods

Study site

Wood Turtle population survey sites were located along a 40 km stretch of river and tributaries in a section of northeastern Minnesota (specific locations withheld in compliance with state of Minnesota data practices law) characterized by mean human density of 4.4 people/mile^2 (ESRI 2012). The elevation of the survey sites ranges from 1,500 to 1,700 feet above sea level. Mean temperature in May is 6.4^0 C, while monthly average precipitation is 5.0 inches (National Oceanic and Atmospheric Administration 2017). This study area includes one of two major watersheds that constitute one of the two main populations in Minnesota (Moriarty and Hall 2014). It is unknown if Wood Turtle populations in the two northern watersheds are connected (Gaea Crozier, Minnesota Department of Natural Resources, pers. comm.), with mean distance between main river channels of approximately 35 km.

The 12 survey sites were chosen in 1990 to include representative riparian habitat for the entire northern Minnesota region, a range of stream sizes occupied by Wood Turtles, and included sites with public lands and private ownership (Buech et al. 1990). We surveyed seven sites on the main river and five sites on smaller tributaries. The river and tributaries are located within the Laurentian Mixed Forest ecological province (Minnesota Department of Natural Resources 1999). More than 90% of the surrounding land is forested, with the remainder in non-forest and aquatic habitat classes. About 75% of the area is in public ownership. Mesic forest types, which comprise 80% of the area, are dominated by aspen (*Populus* spp.), Balsam Fir (*Abies balsamea*), and Paper Birch

(*Betula papyrifera*). Although pine forest types (*Pinus* spp.) are less common in the surrounding landscape, they are present in sandy soils adjacent to some nest sites at river cutbanks. Black Spruce (*Picea mariana*), Balsam Fir, Northern White Cedar (*Thuja occidentalis*), and Tamarack (*Larix laricina*) comprise over 90% of hydric forest types in the surrounding area. Non-forest vegetation consists of lowland alder (*Alnus* spp.) and grass/forb openings. Oxbow lakes and other non-flowing water features also occur in the study area (Brown et al. 2016).

Snapshot comparison data

We originally conducted population surveys at 12 sites in May and early June of 1990 as part of a larger study investigating Wood Turtle nesting habitat characteristics and individual habitat use patterns (Buech et al. 1990, Brown et al. 2016). Detailed capture records, aerial imagery with marked capture locations, and field notes were available from May 1990 surveys. We replicated surveys of these sites in May of 2015. To standardize search area between years, one of the original researchers delineated surveyed boundaries from 1990 using detailed field notes and marked aerial imagery. This allowed us to standardize the area surveyed and search protocol in 2015 with 1990 efforts. We ensured the same area was surveyed in 1990 and in 2015 by loading survey boundaries into handheld GPS units. Two to four observers surveyed each side of river and we recorded total survey time. We did not attempt to standardize search rate due to variable density of brush at different sites.

In both years novice observers with ≤ 1 y Wood Turtle field experience completed a single-observer visual encounter survey of each site. Surveyors walked approximately 15 m apart from one another traversing all potential Wood Turtle habitat within the site. The size of each site varied from 0.63–3.37 km stretches of river (mean = 1.47 km). The survey area at each site included the shallow edges of the river up to ca. 100 m inland, with both sides of the river surveyed. We could not survey the interior of the river due to the natural turbidity of the water.

In 1990, we completed a single survey at seven sites and two surveys at five sites. In 2015, we completed a single survey at all 12 sites. We attempted to survey sites under similar air temperature and day of year as initial surveys in 1990. We matched 1990 surveys to 2015 surveys of the same site that most closely matched the weather conditions of the 2015 survey.

In both years, each turtle found was measured and individually marked using carapace notches (Cagle 1939). Data recorded in both years included sex, plastron annuli count, and location (pin point capture locations on an aerial photograph [flown in July, 1981; 1:15,800 scale] in 1990; Garmin Etrek 30X GPS units in 2015). We classified individuals as juveniles when CL was ≤ 170 mm (Harding and Bloomer 1979).

Population trend data

From 1997–2014, we surveyed for Wood Turtles at nesting sites and pre-nesting staging areas within six of the sites surveyed in 1990. Intermittent surveys occurred from 28 April to 17 July, with most surveys in late May and early June. We performed 179 surveys across all six sites. Sites varied in size but surveyed area was consistent across years. We did not record search effort or number of observers (range 1–3 observers). The same observers surveyed each site each year. Twelve surveys from 2013–2014 were surveyed with the help of a dog. We recorded sex, age, mark number, and location of detected individuals, and marked new individuals using carapace notches. We obtained mean temperature for each survey day from a central station (National Oceanic and Atmospheric Administration 2014). Over the 17-year period, the number of sites surveyed per year ranged from 1–6 (mean = 3.1). During years when sites were surveyed, number of survey replications per site ranged from 1–11 (mean = 3.4).

Habitat data

To determine if habitat changes relevant to the Wood Turtle have occurred in the study region over the past 2–3 decades, we quantified changes in forest size class and cover, developed land, and wetland habitat cover within the study area watershed (ca. 2,000 km^2). We estimated changes in forest size class using U.S. Forest Service Forest Inventory and Analysis (FIA) 5-y and 20-y forest stand age data representing the period 1977–2013 (Miles et al. 2016). We calculated mean forest stand age by weighting each age class by the proportion that class occupied out of the total area of the watershed. We estimated changes in forest, wetland, habitat cover, and developed land using the National Oceanic Atmospheric and Administration Coastal Change Analysis Program (C-CAP) land cover data from 1996 and 2010 (Department of Commerce et al. 2013).

Statistical analyses

To assess changes in population size and structure between 1990 and 2015, we calculated relative abundance (i.e., number of captures), relative abundance standardized by survey effort (i.e., number of captures per survey time), adult sex ratio, and adult-juvenile ratio at each site for each survey year. We used paired randomization tests with 10,000 iterations to determine if these population metrics differed between years. Specifically, we paired captures, captures per survey time, adult sex ratio, and adult-juvenile ratio from 1990 and 2015 from each site. Each year the values for each site were randomized, and the difference in value between years was computed. For example, we randomized the mean number of captures per site across all 12 sites, for both years 10,000 times, and the mean difference between captures at each site across years was calculated. The P-values represent the proportion of trials resulting in a mean difference in total captures per site between sampling years greater than the one obtained in our study (Sokal and Rohlf 1995). For each metric tested, we only included sites with data in both 1990 and 2015, which ranged from 8 sites (adult sex-ratio) to 12 sites (relative abundance).

To understand population dynamics between the 1990 and 2015 snapshot survey dates, we used intermittent annual capture data, collected from 1997 to 2014. We calculated the mean number of individuals detected per survey across those 17 years, and then tested for a significant trend (i.e., deviation of the slope from 0) for captures per survey using Generalized Linear Models (GLM) with a Gaussian distribution (Zuur et al. 2009). To develop this model we assessed the effects of covariates including help of dog, mean temperature, site, day of year, and year on mean captures per survey. We used quantile-quantile plots to ensure the data satisfied assumptions of normality and homoscedasticity.

Leverage and Cook's distance were used to assess for influential observations, and no observations had extreme leverage (≥ 0.4) or Cook's distance (≥ 0.5) values. Variance inflation factors (VIF) were used to assess for multi-collinearity, and no observations had VIF > 1.6. We used backward selection to determine the preferred GLM model. We performed these analyses using R version 3.2.1 (The R Foundation for Statistical Computing, Vienna, Austria, 2013) and inferred significance at $\alpha = 0.05$.

Results

We captured 44 and 50 Wood Turtles during snapshot population surveys in 1990 and 2015, respectively (Table 2.1). Relative abundance did not differ between years (P = 0.58). Relative abundance standardized with survey effort did not differ between years (P = 0.11). Relative abundance in 1990 per site was 0.50 (SD = 0.46) and relative abundance in 2015 was 0.32 (SD = 0.26). The adult sex ratio (P = 0.22) and adult-juvenile ratio (P > 0.99) did not differ between years. In 1990, the overall adult ratio was 1.1 adults: 1 juvenile, whereas in 2015 it was 1.4 adults: 1 juvenile. In 1990, the adult sex ratio was 1.8 female: 1 male, whereas in 2015 it was 1.4 female: 1 male. The mean SCL for all turtles was 187.5 mm (±61.9) in 1990 and 188.6 mm (±35.6) in 2015. The minimum SCL was 76 mm and 66 mm, and the maximum SCL was 233 mm and 229 mm in 1990 and 2015 respectively.

A total of 553 Wood Turtles and 433 unique captures were recorded at the six monitoring sites from 1997–2014. The percentage of females captured per year ranged from 60 to 94%, with an overall sex ratio of 1 male: 7.7 females from 1997–2014. The percentage of adults ranged from 57 to 100%, with an overall adult-juvenile sex ratio of 1 juvenile: 8.3 adults. The GLM model indicated that the use of a dog in surveys had a positive influence on capture rates (β = 4.63, t = 4.0, df = 1, P = 0.0002), so we reran the model with and without the 91 captures from 12 surveys in 2013–2014. The GLM model without the use of a dog indicated that average temperature (*P* = 0.20), site (*P* range = 0.32 to 0.98), and year (*P* = 0.84) did not significantly influence captures per survey. Day of year (*P* = 0.02) and number of surveys (*P* = 0.05) significantly affected capture rates. The best-fit GLM model including day of year (*t* = 2.14, df =1, *P* = 0.04) and number of surveys (*t* = -1.74, df =1, *P* = 0.09), found no significant trend in mean number of Wood Turtles per survey across years (*t* = 0.13, df =1, *P* = 0.89), and the slope coefficient was slightly positive (β = 0.01). A model with year as the only predictor variable similarly found no significant trend in mean captures per survey (*t* = -0.20, df =1, *P* = 0.85), but the slope coefficient was slightly negative (Figure 2.1: β = -0.01). The best-fit GLM model including use of dog (*t* = 4.62, df = 1, *P* = 0.00002), day of year (*t* = 2.29, df = 1, *P* = 0.03), and average temperature (*t* = -1.46, df = 1, *P* = 0.15), found no significant trend in mean Wood Turtles per survey across years (*t* = 0.87, df = 1, *P* = 0.87), and the slope coefficient was slightly positive (β = 0.008)

Average age of forest stands in the watershed increased slightly from 1977–2013 (slope of trendline = 3.6 and 3.8 for 5-y and 20-y age class stand calculations, respectively). The mean age of forest stands based on 5-y age classes was 40.8 y and 55.4 y in 1977 and 2009–2013, respectively. Between 1996 and 2010, there was a 4.8% net increase in developed land, such that in 2010 0.6% of the watershed was developed. Overall, 14.1%

of land in the watershed changed cover classes between 1996 and 2010. Net forest cover decreased by 1.7%, and net wetland cover decreased by 0.2%.

Discussion

In contrast to many other regions (e.g., Burger and Garber 1995, Daigle and Jutras 2005, Willoughby et al. 2013), and despite being located at a distribution edge, we found no evidence of a decline in this Wood Turtle population in northeastern Minnesota over the last 25 years. We hypothesize that Wood Turtle in this watershed did not show strong evidence of population decline due to low human population densities, minimal development and habitat alteration, and large tracts of suitable habitat in public ownership. We demonstrate that even if there is a large interval between abundance surveys, a snapshot population assessment provides a test for change in population size and structure over time.

Despite little evidence for a population decline in the last 25 years, we do not completely understand the implications of all potential threats to the population of Wood Turtles in the region. Therefore, we suggest a conservative assessment of the health of the population in northeastern Minnesota. While we documented little change to the amount of forested, wetland, or developed land, we were not able to assess the impact of habitat degradation, specifically the impact of mesopredator release on the population. For instance, we recently discovered that American Badgers (*Taxidea taxus*) are a common nest predator in northeastern Minnesota, destroying nests at most of the large nesting sites (Cochrane et al. 2015). Similarly, while we found little evidence of annual vehicular mortality near key nesting areas (Cochrane et al. 2017), which is below the 2 –3 % additive mortality needed to limit positive population growth rates (Gibbs and Shriver 2002), significant use of road nesting sites present challenges to adult and nest survival. Moreover, while we demonstrate recruitment (i.e., no difference in the adult-juvenile ratio compared to 1990), "ghost" populations representing populations in which adults are surviving from year to year but have no successful reproduction can result in a gradual but inevitable population decline (Compton 1999, Bowen and Gillingham 2004). Fluctuating temperatures and sporadic weather events may also be challenging adult survival in the region. Thus, we recommend additional research to determine the extent of road use, unsustainable nest predation, climate change, among other unknown threats influencing recruitment and adult survival in the study area.

Our area of inference is restricted to a 40 km stretch of habitat in one of two major river systems currently inhabited by Wood Turtle in northeastern Minnesota. While our study area includes representative riparian habitat and stream sizes occupied by Wood Turtles in northern Minnesota, a population assessment within both watersheds would provide data across a larger gradient of human density and private land ownership. Small capture numbers from the early 1990s provide challenges to the inferential power of a comparable snapshot survey in other areas (Buech et al. 1997), thus additional methods would be needed to determine the status of the population in an adjacent watershed.

Repeating surveys at 25-year intervals is inherently limited in inferential power due to a lack of long-term rigorous population monitoring. We do not know if the population could have declined prior to 1990 or declined and increased between 1990 and 2015. However, in the absence of long-term monitoring, standardized snapshot comparison studies provide valuable quantitative assessments of the status of populations (Dodd et al. 2007, Brown et al. 2012, Foster et al. 2013). Similarly, while our annual survey data was limited in its inferential power and scope, we were able to use available abundance data to track Wood Turtle population trends between 1990 and 2015.

Although the Wood Turtle population in this 40-km river system in northeastern Minnesota appears to be stable, a sustained conservation commitment is still needed to ensure long-term persistence. We encourage continued investment in a long-term monitoring initiative to allow for rapid detection of a population decline, increasing the ability to determine the causal factor and appropriate response strategies. A monitoring program would also allow managers to link current conservation actions (e.g., nest protection, roadside barriers) to population trends, thus informing future management decisions. An additional survey and analysis protocol to assist with long-term population monitoring in the Upper Midwest should help address this issue in the future (Brown et al. 2017).

Table 2.1. Summary of Wood Turtle (*Glyptemys insculpta*) abundance (captures), relative abundance (captures/total search time), adult ratio (adult/total captures), and percent female (females/total adult captures) at 12 population survey sites in northeastern Minnesota, USA, in 1990 and 2015. For the first summary statistic, we only included sites with data in both 1990 and 2015, which ranged from 8 sites (adult ratio) to 12 sites (relative abundance). For the second summary statistic, we included global values or global mean value for all terms.

	Abundance		**Relative Abundance**		**Adult Ratio**		**Female Ratio**	
Site	**1990**	**2015**	**1990**	**2015**	**1990**	**2015**	**1990**	**2015**
Site 1	0	1	0.00	0.17	NA	0.00	NA	0.00
Site 2	2	2	0.33	0.17	0.00	1.00	NA	1.00
Site 3	5	8	0.63	0.31	1.00	0.75	0.40	0.83
Site 4	10	4	0.50	0.12	1.00	0.75	0.50	1.00
Site 5	2	9	0.33	0.82	1.00	0.67	0.50	0.67
Site 6	6	9	1.50	0.82	0.83	0.89	0.40	0.63
Site 7	3	2	0.43	0.11	0.67	0.50	0.50	0.00
Site 8	4	5	0.44	0.42	0.75	1.00	0.33	0.60
Site 9	3	4	0.75	0.23	1.00	0.75	0.33	0.33
Site 10	9	4	1.13	0.44	1.00	1.00	1.00	1.00
Site 11	0	0	0.00	0.00	NA	NA	NA	NA
Site 12	0	2	0.00	0.27	NA	0.50	NA	1.00
Mean (SD) - sites with replicate captures	3.7 (±3.3)	4.2 (±3.1)	0.50 (±0.46)	0.32 (±0.26)	0.81 (±0.33)	0.81 (±0.17)	0.50 (±0.22)	0.63 (±0.34)
Total / Mean (SD) - all data	44	50	0.56	0.33	0.89	0.72	0.56	0.72

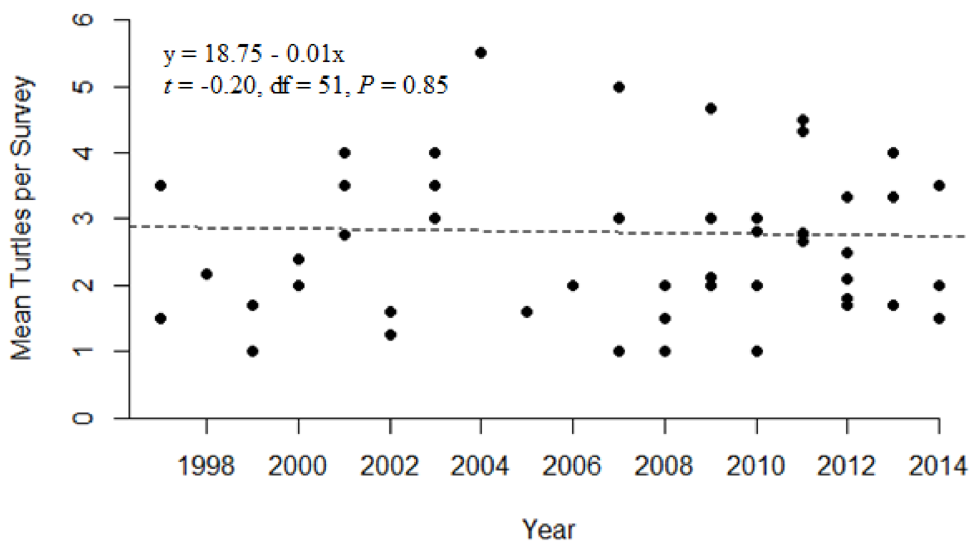


Figure 2.1. Wood Turtle (*Glyptemys insculpta*) capture trend from 1997-2014 in northeastern Minnesota, USA, based on visual encounter surveys at six population monitoring sites (total turtles included = 462). Mean number of individuals captured per survey from six different sites (n = 1–11 surveys per site/year). Includes regression trendline for mean captures per survey and slope estimate (with associated slope, t-statistic, and P-value) over time.

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