

DEMOGRAPHIC AND GENETIC STATUS OF THE WOOD TURTLE
IN THE LOWER PENINSULA OF MICHIGAN

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Real or not real, I dedicate this master's thesis
to all platypus, unicorns, dragons, *Tyrannasaurus rex*,
and narwhals that have ever, or will ever, exist.

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To list all individuals who have contributed to my project would require more pages than the actual thesis, so for the sake of brevity I'll only mention the chart-toppers. Firstly, I would like to extend a big thank you to Brad Swanson for his support and advise in my project and career, and for the daily humorous but all-too-truthful comments about life and work. (I probably would have quit this project 3 years ago if I had not feared the wrath of Takla Sarkar.) I would also like to give a special thanks to Tim Lewis who first introduced me to turtles and research, as well as camping and Maker's Mark. My life at Wittenberg (or any type of berg) and CMU would not have been as productive, interesting, or fun without Tim as a mentor and friend. I would also like to thank Scott McNaught for graciously serving as the final member of my committee. His comments on my initial design helped make the project the success it has become.

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ABSTRACT

DEMOGRAPHIC AND GENETIC STATUS OF THE WOOD TURTLE IN THE LOWER PENINSULA OF MICHIGAN

by Janna R. Willoughby

Populations of wood turtles, *Glyptemys insculpta*, have steadily decreased over the past 30 years due to the destruction and degradation of necessary habitat. We sampled wood turtles from three areas in Michigan, USA to determine the number of and distribution of populations, quantify demographic trends, and measure the effect of declining population size on genetic diversity. Wood turtle samples (n=68) were collected from 3 rivers in the Lower Peninsula of Michigan and analyzed at 9 microsatellite loci. The programs Structure and Baps identified 2 populations that split sampling sites between a North and South population. In both populations, MSVar analysis of genealogies estimated $r < 0$, indicating demographic decline. Although no evidence of a bottleneck was detected using the program Bottleneck ($p=0.303$ North, $p=0.290$ South), I did find some evidence of inbreeding (average North $F_{IS}=0.25$, average South $F_{IS}=0.23$). The relatively high genetic diversity observed in the North and South populations, an uncommon characteristic of declining populations, is likely due to immigration between the two populations ($F_{ST}=0.043$) coupled with the long lifespan of the wood turtle. The steady demographic decline observed and the slow loss of genetic diversity presents a unique conservation opportunity to improve populations demographically without the added cost and considerations necessary in the conservation of genetically depauperate populations.

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CHAPTER I

INTRODUCTION

Conservation has become a concern for many turtle species across a variety of habitats. While the decline in oceanic turtles, such as the Leatherback sea turtle, (*Dermochelys coriacea*) which declined 95% over the past 25 years (Spotila et al., 2000) is well known, less attention has been given to the similar decline seen in terrestrial turtles. The Eastern Box Turtle (*Terrapene carolina carolina*) has declined > 75% in the past 50 years (Hall et al., 1999; Donaldson and Echternacht, 2005), and Spotted Turtles (*Clemmys guttata*) are all but extirpated from Ohio wetlands (Lewis et al., 2004). Freshwater populations including Western Pond Turtles (*Actinemys marmorata*; Reese and Welsh, 1971), Blanding's Turtles (*Emydoidea blandingii*; Congdon and Sels, 1993), and Yellow-blotched Map Turtles (*Graptemys flavimaculata*; Moore and Seigel, 2006) are also all experiencing population declines. Despite the increased interest on global amphibian declines over recent years (Beebee, 2005; Hopkins, 2007; Xie et al., 2007), less concern has been voiced regarding the peril of turtle populations.

Multiple factors are responsible for the decline in turtle populations, including nest site predation (Daigle and Jutras, 2005) and decreased female fecundity (Garber and Burger, 1995). However, road-based habitat fragmentation may be especially hazardous to turtle populations; roads increase predation risk (Marchand and Litvaitis, 2004), collection by humans (Steen and Gibbs, 2004), and mortality (Gibbs and Shriver, 2002; Aresco, 2005), as slow movement makes turtles more vulnerable to traffic than more quickly moving species (Steen and Gibbs, 2004). In addition, the annual migration between water and nesting sites (Harding and Bloomer, 1979) increases turtles exposure to traffic, with deaths occurring in up to 98% of attempts to cross highways (Aresco, 2005). Because the probability of injury to turtles is positively related to

traffic volume (Gibbs and Shriver, 2002), the hazard for turtle survival is further heightened by the near doubling of vehicle travel on US roads, which has occurred over only the past 25 years (US Department of Transportation, 2006).

In addition to road-based mortality, the location and quantity of other barriers influence the distribution of populations and individuals within a landscape (Alderman et al., 2005). Habitat fragmentation, caused by barriers to dispersal, negatively impacts species (Fahrig and Merriam, 1994) by decreasing connectivity and therefore dispersal between patches (Stow et al., 2001; Van de Zande et al., 2007). Fragmentation results in restricted access to nesting sites and decreased likelihood of re-colonization of empty patches (Hill et al., 2002). Diminished dispersal also results in reduced effective population size (Willi et al., 2006; Dixon et al., 2007), which leads to an increase in the intensity of selection (Willi et al., 2006; Dixon et al., 2007) and inbreeding in individuals (Vila et al., 2003; Van Oosterhout et al., 2007). Inbreeding has numerous negative effects, including a decrease in disease resistance (Frankham, 1995; Calleri et al., 2006), an increase in the frequency of deleterious alleles (Leberg and Firmin, 2008), and a reduction in the ability of a population to adapt (Bakker et al., 2010). The combination of these effects decreases survival and reproduction, reducing fitness (Ryan et al., 2003) and ultimately decreasing time to extinction (Brook et al., 2002).

Populations of Wood Turtles, *Glyptemys insculpta*, have decreased steadily over the past 30 years, due to habitat destruction and degradation (Arvisais et al., 2002). Anthropogenic activities including canalization, dam construction, agriculture, and urbanization (Litzgus and Brooks, 1996) have lead to an increase in nest site disturbance (Daigle et al., 2002) and gravid female (Garber and Burger, 1995) and juvenile (Congdon and Sels, 1993) mortality, in many Wood Turtle populations. Because of range-wide decline, the World Conservation Union

(IUCN) lists the Wood Turtle as an endangered species (van Dijk and Harding, 2011) and many states, including Michigan, list the Wood Turtle as a species of special concern (Lee, 1999).

The current range of the wood turtle is discontinuous through northern Virginia, New England, the Great Lakes area, and southeast Canada (Figure 1; Ernst and Zug, 1994; Amato et al., 2008). In Michigan, the wood turtle can be found throughout the Upper Peninsula and in areas of the northern half of the Lower Peninsula, but has been extirpated throughout much of the southern half of the Lower Peninsula (Lee, 1999). The loss of individuals in the south is likely due to the destruction and degradation of habitat (Remsburg et al., 2006) through the development of cities and roads that focused mainly in the Lower Peninsula (Price, 1982).

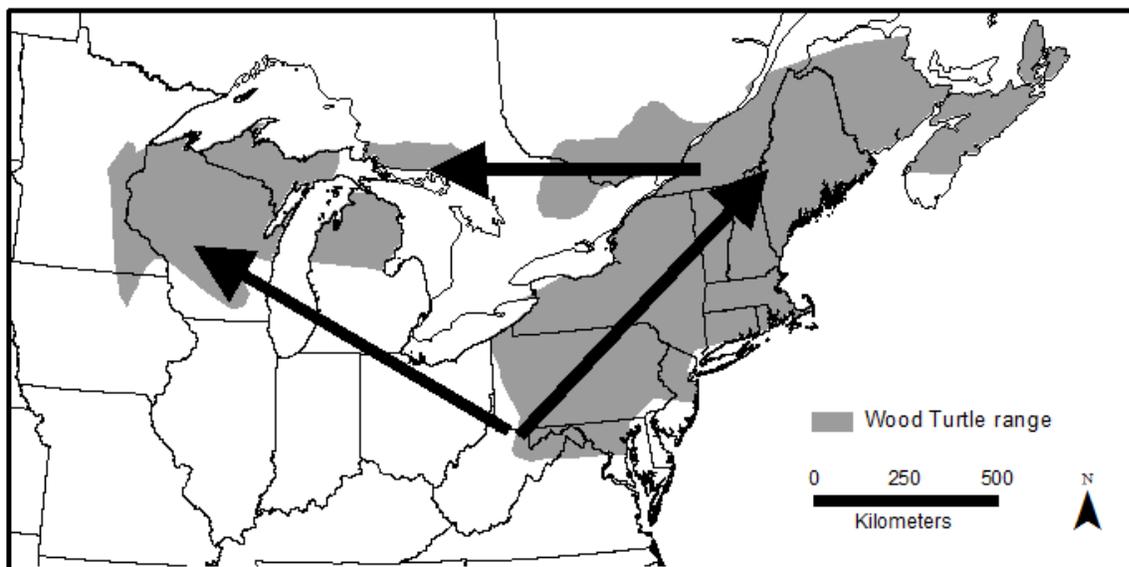


Figure 1. Current range and recolonization route of the Wood Turtle (*Glyptemys insculpta*). Wood Turtles recolonized their current range after the last ice age over two main routes; the first route was up the east coast, with a secondary dispersal event west through Canada, while the second route was northwest through Ohio and Indiana, and into Wisconsin. Figure reproduced from Amato et al. (2008).

Maintaining the genetic diversity of a species, such as the Wood Turtle, is important as it determines the evolutionary potential of that species (Mockford et al., 2007). Unfortunately, the effects of declining population size on turtle populations has been largely ignored in scientific

research (Rizkalla and Swihart, 2006), even though the delay in sexual maturity and low reproductive success of many turtle species may intensify the response. In this study, I address the effects of a range-wide demographic decline by examining wood turtles in the Lower Peninsula of Michigan. We hypothesized that population structure of the Wood Turtle in Michigan would reflect the east-west water drainage patterns into Lakes Huron and Michigan respectively, with little interpopulation dispersal. While the demographic trajectory of Wood Turtle populations in Michigan is unknown, given the range-wide decline of Wood Turtle populations and the recent listing as endangered on the IUCN red list, we hypothesized that identified populations would exhibit trends associated with declining populations.

CHAPTER II

MATERIALS AND METHODS

Sample Collection, Extraction, and Amplification

Wood Turtle tissue samples were collected from three rivers in the Lower Peninsula of Michigan, including the Au Sable River in the northeast section of Michigan, the Manistee River in the west-central area of Michigan, and the Chippewa River in central Michigan along the southern boarder of the wood turtle's current range (Figure 2). Turtles were most often spotted while basking on the riverbank or on partially submerged logs, but were also found on the land adjacent to the rivers, and were captured by net or hand. Once captured, 3-4 small clippings were taken from the tips of the scales on the limbs from each individual (Tessier et al., 2005), and stored in a microcentrifuge tube until extraction of DNA could occur. All individuals were released immediately following tissue collection, at the site of capture (IACUC approval # 08-11).

Extraction of DNA from tissues followed published protocols (Qiagen 2006; Valencia, CA), and the amount of DNA extracted was quantified using a BioPhotometer (Eppendorf Westbury, New York). Samples were analyzed at ten microsatellite loci (GmuA32, GmuB21, GmuD16, GmuD28, GmuD40, GmuD55, GmuD87, GmuD88, GmuD93, GmuD95) designed for the Bog Turtle (*Glyptemys muhlenbergii*; King and Julian, 2004) and successfully used for the Wood Turtle (Tessier et al., 2005; Spradling et al., 2010). Amplification was conducted using PCR in an Eppendorf Mastercycler Gradient (Eppendorf, Westbury, New York) in 10 μ l reactions, an annealing temperature of 54°C for all primers, and following published protocol for Sahara Polymerase (Bioline USA Inc. Taunton, MA). All reactions contained 37.5 ng of

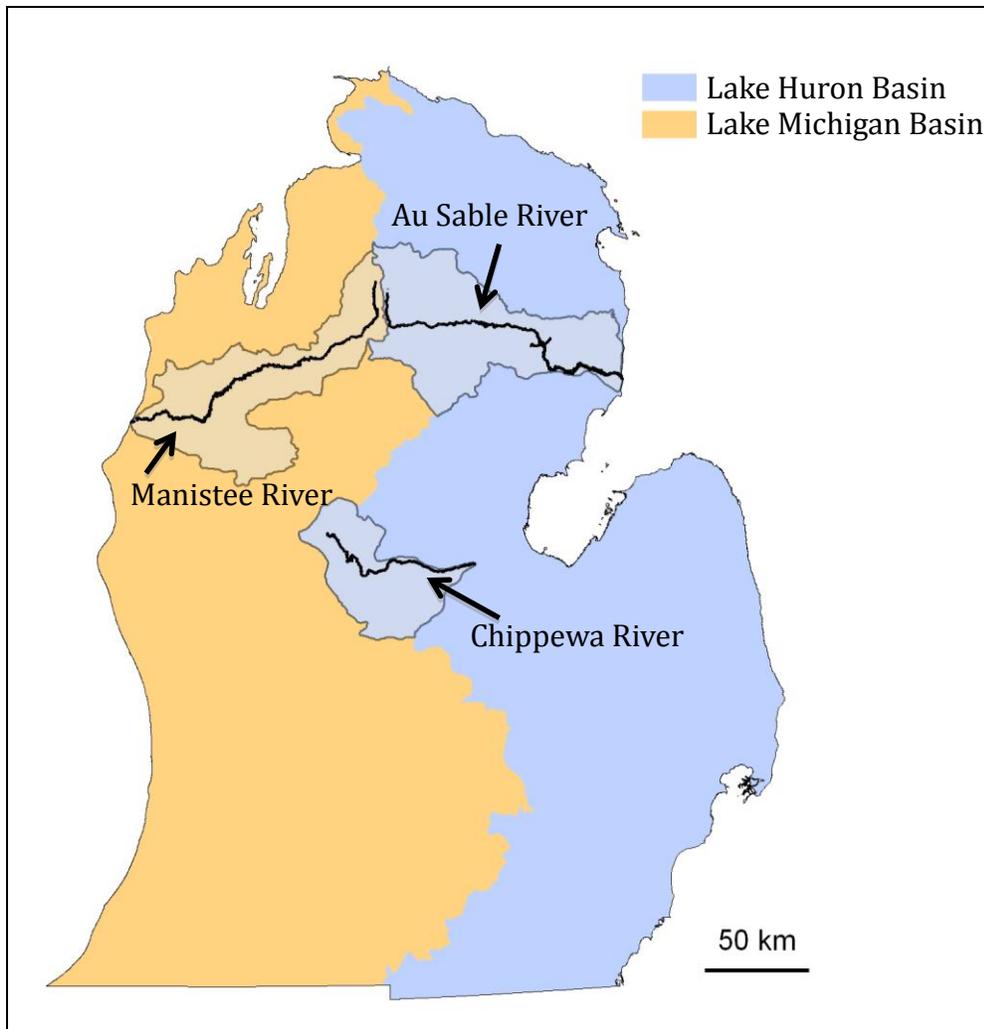


Figure 2. Rivers sampled in the Lower Peninsula of Michigan. Wood turtles (*Glyptemys insculpta*) were sampled from three different rivers, the Au Sable, Manistee, and Chippewa Rivers, which are located in the Au Sable, Manistee, and Chippewa watersheds, respectively. Watershed boundaries are indicated by light shading around the rivers. Precise sampling locations are not marked to prevent collection of turtles for the pet trade.

template DNA, 10 mM MgCl₂, 0.063 mM Bioline dNTP mix, 2.5 uM forward and reverse primers, 1x Sahara reaction buffer, and 0.4 u Sahara DNA polymerase. An Applied Biosystems 310 Automated DNA Sequencer (Applied Biosystems Foster City, CA) was used for visualization of the DNA fragments, and the programs Genescan Analysis 3.1.2 and Genotyper 2.0 Software (Applied Biosystems Foster City, CA) were used to size each allele. The program

Microchecker (Van Oosterhout et al., 2007) was used to test for scoring errors, null alleles, and allelic dropout.

Population Distribution

The programs Baps 5.2 (Corander et al., 2008) and Structure 2.3.3 (Pritchard et al., 2000) were used to identify the number of populations (K) and the most likely population origin for each individual. Baps was used to determine the probabilities of K = 1 - 6, as the hierarchical approach to Structure interpretation is unable to evaluate K = 1 (Evanno et al., 2005), and a single population was possible given that the three sites sampled cover a small geographic range. Clustering of groups of individuals was run in Baps for ten replicates for K = 1 - 6, using no spatial prior. The number of populations was suggested by Baps as the combined maximum likelihood and highest posterior probability estimates for the number of populations over all ten replicates. Once the number of populations was identified using Baps, Structure was used to examine admixture between identified populations (Pritchard et al., 2000). Structure was run for 10 independent runs using the most likely number of populations indicated by Baps (K = 2), and the assumptions of admixture and correlated allele frequencies with a burn-in period of 100,000 steps and 100,000 replicates (Falush et al., 2003). Admixed individuals were identified as individuals with $q < 0.75$, which represents the equivalent of a single, grandparent ancestor from outside the identified population. Finally, the FullSearch option in the program Clumpp 1.1.2 was used to align clusters across all ten replicates (Jakobsson and Rosenberg, 2007).

The program Genalex 6.4 (Peakall and Smouse, 2006) was used to quantify population structure via F_{ST} , and significance was assessed using AMOVA permutations. F_{ST} was calculated between populations and between sampling sites to investigate movement between and within

populations, respectively. A total of 9999 permutations, per population, were conducted to assess the likelihood of obtaining the observed F_{ST} . Significant differences between the calculated F_{ST} and the F_{ST} of randomly assigned groups were assessed using AMOVA.

Demographic and Genetic Trends

Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were tested with Genepop 4.1 (Rousset, 2008), using a Bonferroni corrected alpha (Rice, 1989). Standard summary statistics including observed heterozygosity, expected heterozygosity, and allelic diversity were also computed using Genepop 4.1 (Rousset, 2008). All programs were run using the clusters suggested by the Bayesian clustering programs.

Although populations of wood turtles are declining throughout the range, current demographic trends in Michigan remain unknown. In order to determine if population size has remained constant, increased, or decreased, the program MSVar was used to model population size change (Beaumont, 1999; Storz and Beaumont, 2002). Parameter estimates for mutation rate, rate of change of the population, and time to total coalescence were made by examining initial posterior distribution for a subset of the data, the most variable locus, as suggested in the program notes. After running the program for 10^9 steps using the default parameters, values at the extreme edges of each posterior distribution for mutation rate, rate of change of the population, and time to total coalescence were chosen as starting the starting value for subsequent simulations. The identified parameters were then used in five sequential runs of 2×10^9 steps utilizing all loci. After discarding the first 10% of each run, the marginal distributions were plotted using CODA (Best et al., 1995) in the statistical package R (Ihaka and Gentleman, 1996), to assess convergence of the model via the Gelman-Rubin statistic (Best et al., 1995).

Finally, the last half of each run was combined in to a single data set, and the relative likelihood of competing models was determined via the Bayes Factor. The Bayes Factor was estimated by dividing the number iterations in which the population contracted by the number of iterations in which the population expanded, where a factor greater than e^2 represented a significant model (Storz and Beaumont, 2002). To prevent division by zero, one was added to both the numerator and the denominator before calculating the Bayes Factor.

Wood turtle populations were tested for evidence of a recent bottleneck using the program Bottleneck (Piry et al., 1999). Bottleneck was run using a two-phase model, as the model more accurately imitates microsatellite mutations than a strict step-wise model (Ohta and Kimura, 2007). The two-phase model parameters were set at 95% for single-step mutations occurrence and 5% for multiple step mutations, with a variance of 12, which has been shown to be appropriate for microsatellite in turtles (Kuo and Janzen, 2004). Excess heterozygosity was interpreted using a Wilcoxon sign rank test (Rooney et al., 1999), and a loss in rare alleles was examined by looking for a mode shift in allele frequency classes (Luikart et al., 1998). The impact of recent bottlenecks in the focal populations was assessed using F_{IS} and relatedness. I calculated F_{IS} in each population using FSTAT (Goudet, 1995) and relatedness using the Queller and Goodnight (1989) 'r' in the program Genalex 6.4 (Peakall and Smouse, 2006). Bootstrapping was conducted to calculate the 95% confidence interval around the mean relatedness value, and significant differences between relatedness and zero for both populations was assessed using 99 permutations.

Effective population size was measured using the program ONeSAMP, which creates simulated populations based on allelic data provided, draws individuals from the created population, and compares summary statistics of the simulated sample and the actual data. After

50,000 simulated populations, the program determines the most likely effective population size (Tallmon et al., 2008). Samples were run using the populations identified by Baps and assuming a minimum effective population size of 2 and a maximum effective population size of 2000, which is equal to a maximum census size of 4,000 – 20,000 individuals. The input priors, which limit the population sizes, were varied to look for consistency of results (Tallmon et al., 2008). Tested priors include all combinations of minimum population sizes of 2 and 10, and maximum sizes of 1000, 2000 and 5000 individuals.

CHAPTER III

RESULTS

Population Distribution

Baps suggested the most likely K was 2 ($L(K) = -2529.1$; $p = 1.0$), with individuals from the southern sampling site clustering into a single group, and individuals from the northeast and northwest sampling sites clustering into the other group. The program Structure revealed admixed individuals ($q < 0.75$) from all three sampling sites, and accounted for 29% of sampled individuals in the North population and 17% of individuals in the South population (Figure 3). Additionally, 2 individuals captured in the northeast sampling site genetically clustered with the south site, and 1 individual captured in the south clustered with the northern sampling sites. These 3 individuals are likely recent immigrants between the observed populations (Figure 3).

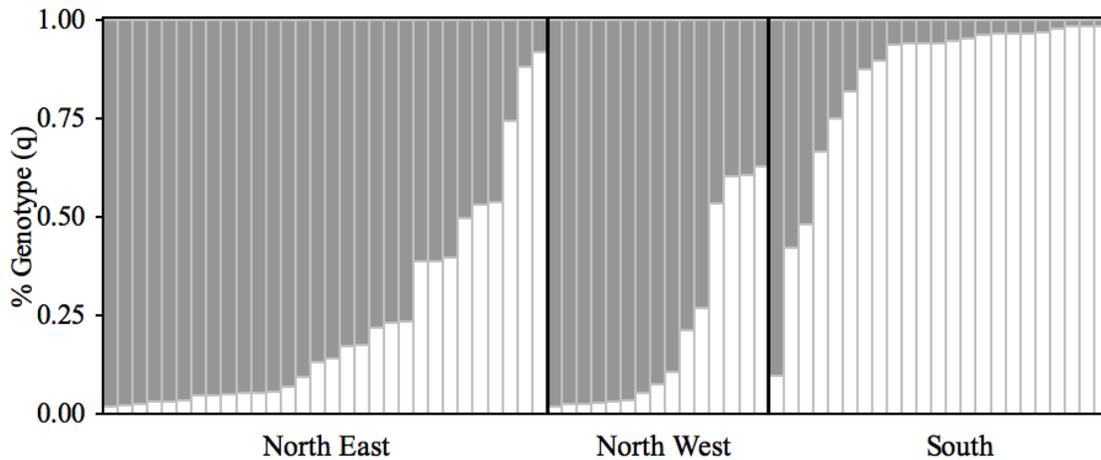


Figure 3. Percent of each Wood turtle (*Glyptemys insculpta*) genotype assigned to the north and south populations. The grey shading indicates assignment to the North population while the white indicates assignment to the South population. Samples were collected from three locations, which are denoted by the dark, vertical bars. Individuals within each sample site are sorted by q -values.

Population structure, as measured by F_{ST} , was significantly different between populations and randomly drawn groups, as well as between populations (Table 1). The lowest differentiation

was observed between the northeast and northwest sampling sites and is indicative of the amount of isolation by distance within the population. The highest differentiation was observed between the North and the South populations.

Table 1. Results F_{ST} comparisons between sample sites (Au Sable, Manistee, and Chippewa Rivers) as well as North and South populations of Wood Turtles (*Glyptemys insculpta*) in Michigan. Calculated F_{ST} values (below diagonal) were compared to 9999 F_{ST} values of randomly constructed populations within the data set, using an AMOVA. The p-values from this comparison are listed above the diagonal.

	Northeast	Northwest	South	North
Northeast	--	0.0012	0.0001	NA
Northwest	0.021	--	0.0001	NA
South	0.039	0.074	--	0.0001
North	NA	NA	0.044	--

Demographic and Genetic Trends

Individuals (n=68) were genotyped at an average of 8.5 loci and at a minimum of 6 loci per individual. The number of alleles observed ranged from 5 to 16 per locus (Table 2). The program Microchecker found no evidence of scoring errors, null alleles, or large allele dropout. In both populations, loci GmuB21 and GmuA32 deviated from Hardy Weinberg equilibrium and, in the North population, loci GmuD93 and GmuD88 also deviated from Hardy Weinberg equilibrium. All other loci were in Hardy Weinberg equilibrium (all $p > 0.05$ following Bonferroni correction). No significant linkage was found between any loci in either population (all $p > 0.05$). The average observed and expected heterozygosity was higher in the North population and compared to the values for the South (Table 2).

The results of the population size models (MSVar) suggest that there has been a demographic decline for both the North and South populations. The 97.5% confidence interval for the Gelman-Rubin statistic ranged from 1.00 – 1.08 for all parameters, and averaged 1.03 in

the north population and 1.02 in the south populations indicating that appropriate variable values were chosen for the population simulations (Brooks and Gelman, 1998). In both populations, all iterations suggested a decline in population size, resulting in a Bayes Factor of 9000. Because the Bayes Factor was greater than e^2 the model indicates decline within the North and South populations.

Table 2. Number of alleles, number of amplified individuals, expected heterozygosity and observed heterozygosity for each locus, for the North and South populations of Wood Turtles (*Glyptemys insculpta*) in Michigan. Average values for each population are also listed. * = locus out of Hardy Weinberg equilibrium.

	Locus	Number of Alleles	Number of Individuals Amplified	Expected Heterozygosity	Observed Heterozygosity
North Population	GmuB21*	7	43	0.78	0.28
	GmuD16	15	45	0.91	0.76
	GmuD40	14	45	0.91	0.82
	GmuD87	10	44	0.80	0.71
	GmuD93*	12	43	0.83	0.45
	GmuA32*	9	45	0.82	0.44
	GmuD28	12	44	0.84	0.75
	GmuD55	5	45	0.37	0.37
	GmuD88*	12	44	0.91	0.48
	Average	10.67	44.22	0.80	0.56
South Population	GmuB21*	4	23	0.64	0.23
	GmuD16	14	23	0.91	1.00
	GmuD40	11	23	0.88	0.87
	GmuD87	5	23	0.72	0.52
	GmuD93	4	23	0.53	0.41
	GmuA32*	6	22	0.77	0.41
	GmuD28	7	22	0.56	0.55
	GmuD55	2	22	0.41	0.27
	GmuD88	11	22	0.86	0.61
	Average	7.11	22.56	0.70	0.54

Due to the demographic decline observed via the population size model (MSVar), I expected to find genetic evidence of a recent bottleneck. However, a recent bottleneck signal was

not detected in either population. The observed heterozygosity did not differ from that expected under drift-mutation equilibrium in either the North ($p = 0.500$) or the South population ($p = 0.730$). Similarly, no mode-shift was detected in either population with the distribution of allele frequency classes showing L-shaped distributions (Figure 4).

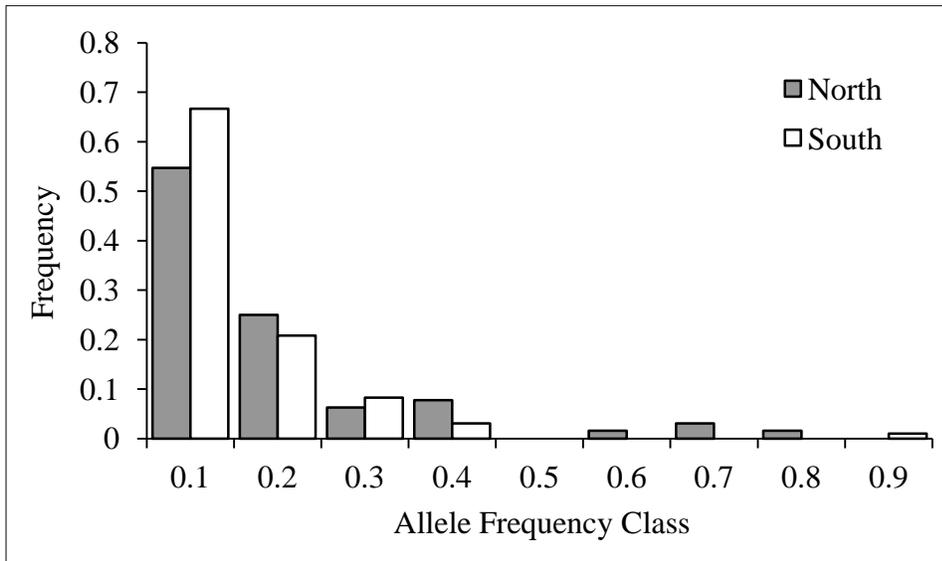


Figure 4. Frequency of alleles in each allele frequency class in the North and South populations. The normal, L-shaped distribution observed in both populations, suggests that no bottleneck has occurred.

Observed F_{IS} values in both populations covered a wide range; in the North population, F_{IS} ranged from -0.090 to 0.480 (mean = 0.294) over all nine loci, and from -0.099 to 0.476 (mean = 0.230) in the South. The mean relatedness value in the North population was -0.023 (95% CI = -0.013 to -0.032) and was significantly different than zero as shown by the non-overlapping permutation test confidence interval. The mean relatedness in the South population 0.166 (95% CI = 0.190 to 0.142) and was also significantly different than zero (Figure 5).

The estimates of effective population size provided by ONeSAMP for the North and South populations were not significantly different, regardless of the minimum and maximum population size priors used (Figure 6). In the North population, the estimate with the largest

credible limit was 97 (95% credible limit = 48 - 334) while in the South population the widest estimate was 37 (95% credible limit = 23 - 102).

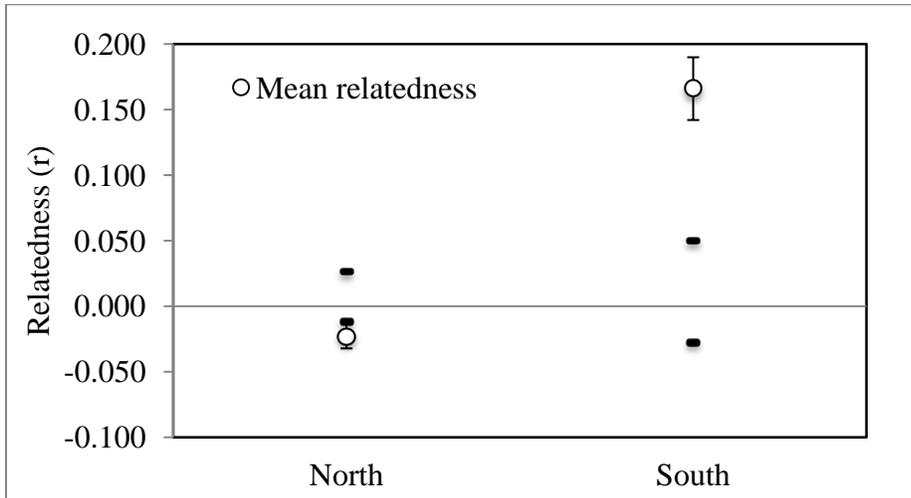


Figure 5. Mean relatedness values of the North and South populations of Wood Turtles (*Glyptemys insculpta*). Mean relatedness values are denoted with open circles, and associated errors bars. The dark, black dashes note the upper and lower permutation confidence intervals (North 95% CI = 0.026 to -0.012, South 95% CI = -0.028 to 0.050).

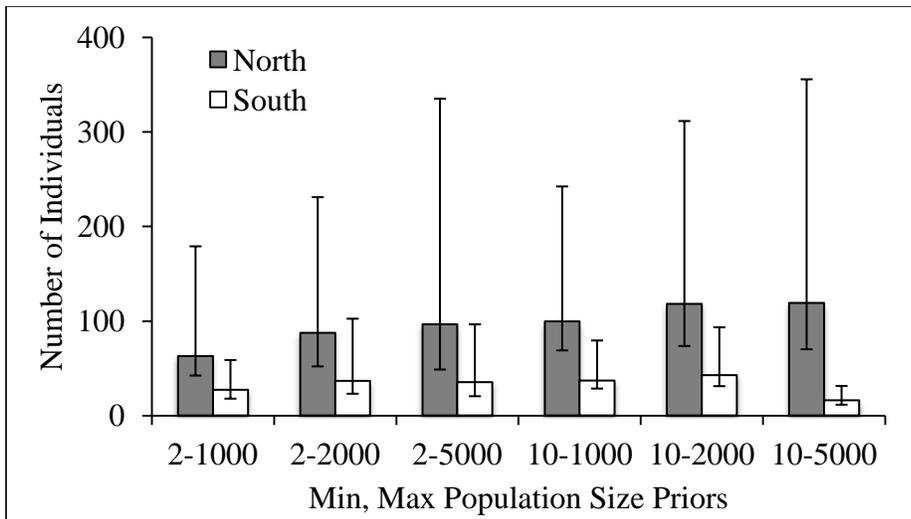


Figure 6. Effective population size estimates of the North and South populations using multiple priors. Although the mean effective size estimated in the North population was always appears larger than the mean estimated in the South, there is no significant difference, as illustrated by the overlapping 95% credible limits (error bars).

CHAPTER IV

DISCUSSION

Wood turtles in the Lower Peninsula of Michigan exist as two populations a North population and a South population (Figure 2). Both populations show evidence of demographic decline but do not yet show the associated loss of genetic diversity commonly observed in small, declining populations.

Rivers and other waterways appear to be natural dispersal corridors, especially for riparian species. Accordingly, I expected the wood turtle population distribution to follow a split along the water basin boundary, resulting in an east-west split in the Lower Peninsula of Michigan. The observed north-south split combines two sampling locations that occur in different basins, and may be a result of the glacial history in the area, as recolonization is known to impact wood turtle population structure (Tessier et al., 2005). Following the glacial retreat approximately 10,000 years ago, trees, shrubs, grasses began recolonizing the northern portion of North America (Pielout, 1999), facilitating the movement of animals from a southern refugia (Delcourt and Delcourt, 1991). The wood turtle, trailing the changing habitat, recolonized via a large dispersal corridor up the east coast, followed by two smaller, alternative routes: some individuals proceeded north and then west through Canada, while others moved west through Ohio, Illinois, and Wisconsin (Amato et al., 2008). It is possible that individuals moving westward into Illinois and Wisconsin recolonized the south portion of Michigan, while the north portion of the Lower Peninsula was recolonized by individuals traveling west through Ontario and Quebec, and eventually south across the exposed land bridge between the Upper and Lower Peninsulas of Michigan, causing the north-south population split observed.

The delineation of two populations, instead of a population for each sampled river, suggests that either urbanization and the associated fragmentation is not severe enough to isolate individuals to single river systems as may have been expected (Aresco, 2005), or that the time since the rivers have been isolated is not sufficient to have measurable genetic differentiation (Marsack and Swanson, 2009). Previous studies have found potentially misleading results due to the long and overlapping generation of turtles, and diverse habitat needs (Ernst and Zug, 1994; Harding and Davis, 1999; Converse et al., 2005). Ornate Box Turtles (*Terrapene ornate*) found in Nebraska and Illinois showed evidence of dispersal between populations (Kuo and Janzen, 2004), as did populations of Arizona Desert Tortoise (*Gopherus agassizii*) that were up to 186 km apart (Edwards et al., 2004). These results suggests that the genetic effects of habitat degradation are slow to develop in turtle species (Mockford et al., 2007; Marsack and Swanson, 2009) implying that current distributions may be artifacts from previous management procedures (Mockford et al., 2007) and genetic effects of habitat destruction may not be evident for decades (Ernst and Zug, 1994). However, previous work on Wood Turtles has shown significant genetic differences between populations separated by a major barrier, such as the St. Lawrence River (Tessier et al., 2005), suggesting that the Wood Turtle is not immune to the genetic consequences of isolation and that if roads isolate rivers in Michigan, migration rates and population structure may be altered.

The population size model utilized suggested a decline in population size for both the north and the south populations, as evidenced by an estimated Bayes Factor of greater than e^2 (Storz and Beaumont, 2002). However, the genetic signals often associated with declining populations were not yet apparent in either population. Although the effective population size credible limits ranged from 48 – 334 in the north and 23 – 102 in the south (Figure 4), there was

no evidence of a recent bottleneck, and the average observed heterozygosity was near 0.5 in both populations (Table 1), suggesting that the genetic diversity of surviving individuals is high, which is reflected in the retention of heterozygosity despite demographic declines.

The genetic diversity observed in the North and South populations is likely due to a combination of immigration and long life span of the wood turtle. Immigration between the two populations ($F_{ST}=0.043$) appears to be high, which is known to facilitate retention of heterozygosity within declining populations (Agudo et al., 2011). Migration may be particularly useful in introducing new alleles in evolutionarily distinct populations, as is likely the case for Wood Turtles in the Lower Peninsula of Michigan. Finally, a long generation time can act as a reservoir of genetic variability within surviving individuals (Hailer et al., 2006; Marsack and Swanson, 2009). The generation time of the wood turtle is between 36 and 47 years (van Dijk and Harding, 2011), which means that only 2 - 3 generations have passed since the onset of widespread urbanization and road construction in Michigan, and that the genetic diversity observed may likely reflect movement of individuals in a less fragmented landscape (Marsack and Swanson, 2009).

Given the recent listing of the Wood Turtle as endangered on the IUCN Redlist (van Dijk and Harding, 2011), and the increase in rate of urbanization in Michigan (US Department of Transportation, 2006), we should expect to see genetic evidence of demographic decline in future populations. The observed, average F_{IS} value for both populations (mean = 0.230; 95% CI = 0.133 - 0.455) suggests the populations are already approaching homozygosity levels of concern. Other turtle populations of species of lesser conservation concern often have F_{IS} values on the low end of the range we observed ($F_{IS} = 0.16$ in *Graptemys geographica*, Bennett et al. 2010; $F_{IS} = 0.13$ in *Terrapene ornata ornata*, Richtsmeier et al., 2008) while species of higher

concern have inbreeding levels similar to the values observed in this study ($F_{IS} = 0.29$ in *Terrapene carolina triunguis*, Buchman et al., 2009; $F_{IS} = 0.22$ in *Erymnochelys madagascariensis*, Rafeliasoa et al., 2006), indicating the endangered IUCN listing is likely appropriate. Additionally, we observed a small but statistically significant increase in the average relatedness in the South population compared to the North population (Figure 5). Although the difference may be due to confounding variables, the pattern observed may also be due to the higher urbanization in the southern portion of Michigan compared to the north half of the state and is consistent with the smaller effective population size found in the South population.

The Wood Turtle populations in Michigan should be of concern from a conservation standpoint because increase in relatedness of individuals is associated with the start of an extinction vortex (Blomqvist et al., 2010). Our results suggest that a timely intervention is needed to prevent dramatic decreases of genetic diversity in populations of Wood Turtles in Michigan and turtles in the southern portion of the Lower Peninsula are likely to be in more danger than turtles in the north.

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