POPULATION DECLINE IN A LONG-LIVED SPECIES: THE WOOD TURTLE IN MICHIGAN

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Abstract: Populations of Wood Turtles, *Glyptemys insculpta*, have steadily decreased over the past 30 yr because of habitat destruction and degradation. We sampled Wood Turtles from three areas in Michigan, USA, to characterize populations, quantify demographic trends, and measure the effect of declining population size on genetic diversity. Wood Turtle samples ($n = 68$) were collected from three rivers in the Lower Peninsula of Michigan and analyzed at nine microsatellite loci. Bayesian clustering programs identified two populations that split the three sampling sites into North and South populations. In both populations, analysis of genealogies estimated $r < 0$, indicating population decline. However, no evidence of a bottleneck was detected ($P = 0.30$ North, $P = 0.29$ South), and little evidence of inbreeding was observed (average North $F_{IS} = 0.25$, average South $F_{IS} = 0.23$), relative to other Emydidae populations. The high genetic diversity observed in the North and South populations is likely due to immigration between the two populations ($F_{ST} = 0.04$), coupled with the long life span of the Wood Turtle. The conflicting signals suggested from the genealogy models compared to the F_{IS} and bottleneck analysis suggests that coalescent models may be better suited to detect population decline than other measures of genetic diversity in long-lived species such as the Wood Turtle.

Key words: Coalescence; Conservation; Emydidae; Microsatellite; ONeSAMP

Conservation of turtle species has become a concern for populations across a variety of habitats. Populations of oceanic turtles such as the Leatherback Sea Turtle (*Dermochelys coriacea*) have declined by 95% over the past 25 yr (Spotila et al., 2000), the terrestrial Eastern Box Turtle (*Terrapene carolina carolina*) has declined more than 75% in the past 50 yr (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 in 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.

Although the cause of turtle population decline varies from nest-site predation and decreased female survivorship to increased dispersal barriers across a landscape (Garber and Burger, 1995; Steen and Gibbs, 2004; Daigle and Jutras, 2005), road-based habitat fragmentation may be especially hazardous to turtle populations. Roads increase predation risk, collection by humans, and mortality (Gibbs and Shriver, 2002; Marchand and Litvaitis, 2004; Steen and Gibbs, 2004; 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 increases turtles' exposure to traffic, with deaths occurring in up to 98% of attempts to cross highways (Harding and Bloomer, 1979; Aresco, 2005).

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 by decreasing connectivity and therefore

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dispersal between patches (Fahrig and Merriam, 1994; Stow et al., 2001; Van de Zande et al., 2007). Fragmentation results in restricted access to nesting sites and decreased likelihood of recolonization of empty patches (Hill et al., 2002). Diminished dispersal also results in reduced effective population size (Willi et al., 2006; Dixon et al., 2007) and inbreeding (Vila et al., 2003; Van Oosterhout et al., 2007). Increased inbreeding has numerous negative effects, including a decrease in disease resistance, an increase in the frequency of deleterious alleles, and a reduction in the ability of a population to adapt (Frankham, 1995; Calleri et al., 2006; Leberg and Firmin, 2008; Bakker et al., 2010). The combination of these effects decreases survival and reproduction, reducing fitness and ultimately decreasing time to extirpaton (Brook et al., 2002; Ryan et al., 2003).

Species with diverse habitat usage and large home-range sizes, such as the Wood Turtle, *Glyptemys insculpta*, may be more susceptible to local extirpation than other species. Although Wood Turtles hibernate, nest, and mate in and around rivers and streams, adjacent habitat is frequently used for foraging during summer months (Harding and Bloomer, 1979; Ernst et al., 1994). Home-range estimates vary from less than 1 ha in some localities to an average of 40.6 ha in others (Kaufmann, 1995; Remsberg et al., 2006). The diverse habitat usage and large home-range sizes leads to increased conflict with agricultural practices, compared to habitat-specialist species, which results in an increase in adult mortality accompanied by a decrease in growth and recruitment (Samure and Bider, 1998).

The life-history characteristics of Wood Turtles coupled with habitat destruction and degradation are likely factors in the steady decrease in population size over the past 30 yr, resulting in their listing as endangered on the IUCN Red List (Arvisais et al., 2002; van Dijk and Harding, 2011). Anthropogenic activities including canalization, dam construction, agriculture, and urbanization have led to an increase in nest-site disturbance as well as gravid female and juvenile mortality in many Wood Turtle populations (Congdon and Sels, 1993; Garber and Burger, 1995; Litzgus

and Brooks, 1996; Daigle et al., 2002). The current range of the Wood Turtle is discontinuous through northern Virginia, New England, the Great Lakes area, and southeast Canada (Fig.1; Ernst and Zug, 1994; Amato et al., 2008). Largely because of the documented, range-wide decline, the World Conservation Union (IUCN) lists the Wood Turtle as an endangered species (van Dijk and Harding, 2011).

In Michigan, the Wood Turtle can be found throughout the Upper Peninsula and in areas of the northern half of the Lower Peninsula (Lee, 1999). However, the development of cities and roads, focused mainly in the lower half of the Lower Peninsula, and conversion of forest and grasslands to agriculture has lead to extirpation throughout much of the southern half of the Lower Peninsula (Lee, 1999; Price et al. 2005). As a result, the state of Michigan lists the Wood Turtle as a species of special concern (Lee, 1999).

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 to demographic declines. In this study, we 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 Lake Huron and Lake Michigan, respectively, with little interpopulation dispersal. Although the demographic trajectory of Wood Turtle populations in Michigan was unknown, given the range-wide decline of Wood Turtle populations, we hypothesized that identified populations would be isolated and exhibit trends associated with the occurrence of a recent bottleneck, have increased levels of homozygosity, and have a small effective population size.
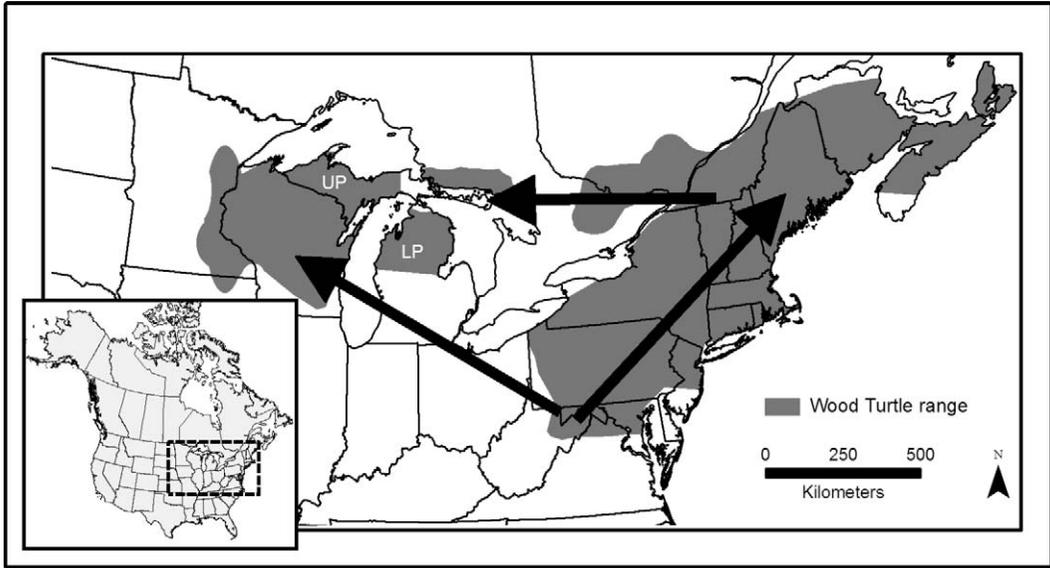
FIG. 1.—Current range and recolonization route of the Wood Turtle (*Glyptemys insculpta*). Wood Turtles recolonized their current range after the last ice age over two main routes; the first route was up the east coast, with a secondary dispersal event west through Canada, and the second route was northwest through Ohio and Indiana, and into Wisconsin. The Lower Peninsula (LP) and Upper Peninsula (UP) of Michigan are noted on the map. Wood Turtle range reproduced from Amato et al. (2008).

MATERIALS AND METHODS

We collected wood turtle tissue samples from three rivers in the Lower Peninsula of Michigan, including the Au Sable River (northeast locality), the Manistee River (northwest locality), and the Chippewa River (southern locality), which is located along the southern border of the Wood Turtle's current range. Samples came from approximately 20 km on each river, and from localities that were approximately 120 km from each other and formed an equilateral triangle. The northeast and northwest localities were separated by largely forested land, with a few major roads and few agricultural interruptions. However, agriculture and major roads, and very little forested land, separated the northeast and northwest localities from the southern locality (Fig. 2). Typically, we spotted turtles that were basking on the riverbank or on partially submerged logs, but we also found individuals on the land adjacent to the rivers. We captured all turtles by net or hand, and took three–four small clippings from the tips of the scales on the limbs of each individual (Tessier et al., 2005). We stored the collected tissue in

a microcentrifuge tube until DNA extraction could occur. We released all individuals immediately following tissue collection and at the site of capture. Our capture and sampling procedures followed current ASIH–HL–SSAR Guidelines for Use of Live Amphibians and Reptiles in Field Research (Michigan Scientific Collector's Permit Issued 2008–2010; IACUC approval 08–11).

We extracted DNA with the use of Qiagen DNeasy kits (Valencia, CA, USA) following published protocols and quantified DNA concentration with the use of a BioPhotometer (Eppendorf Westbury, New York, USA). We used nine microsatellite loci designed for the Bog Turtle (GmuA32, GmuB21, GmuD16, GmuD28, GmuD40, GmuD55, GmuD87, GmuD88, GmuD93; *Glyptemys muhlenbergii*; King and Julian, 2004) and successfully used for the Wood Turtle (Tessier et al., 2005; Spradling et al., 2010) to analyze our samples. We conducted 10-µl PCR reactions with the use of an Eppendorf Mastercycler Gradient (Eppendorf, Westbury, New York, USA) and an annealing temperature of 54°C for all primers, and followed the published protocol for Sahara
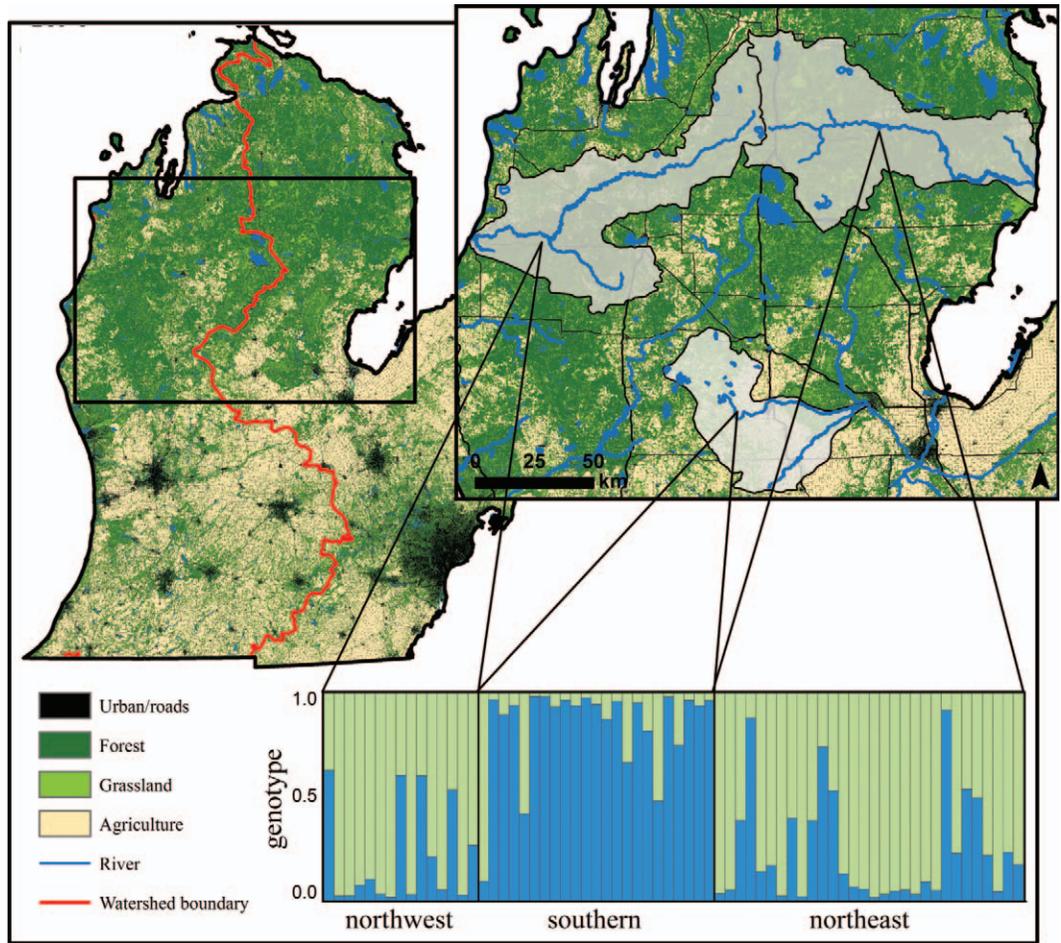
Fig. 2.—Land cover, roads, and rivers sampled in the Lower Peninsula of Michigan. Wood turtles (*Glyptemys insculpta*) were sampled over approximately 20 km from three different rivers, the Au Sable (northeast locality), Manistee (northwest locality), and Chippewa Rivers (southern locality), which are located in the Au Sable, Manistee, and Chippewa watersheds, respectively. Sampled localities were approximately 120 km from each other and formed an equilateral triangle. However, precise sampling locations are not marked to prevent collection of turtles for the pet trade. The light grey shading indicates watershed boundaries around each of the sampled rivers. The plot in the lower right corner indicates the genetic assignment of individuals by sampling locality, where each bar represents one individual. The percent of the genome assigned to the North population is indicated by green (light) shading, and blue (dark) shading denotes the percent assigned to the South population. Color figure available in on-line version.

Polymerase (Bioline USA, Inc., Taunton, MA, USA). All reactions contained 37.5 ng of template DNA, 10 mM $MgCl_2$, 0.063 mM Bioline dNTP mix, 2.5 μM forward and reverse primers, 1x Sahara reaction buffer, and 0.4 u Sahara DNA polymerase. We visualized PCR products with an Applied Biosystems 310 Automated DNA Sequencer (Applied Biosystems Foster City, CA), and used Genescan Analysis 3.1.2 and Genotyper 2.0 Software (Applied Biosystems Foster City,

CA) to size each allele. Finally, we used Microchecker (Van Oosterhout et al., 2007) to test for scoring errors, null alleles, and allelic dropout.

Population Distribution

We used Baps 5.2 (Corander et al., 2008) and Structure 2.3.3 (Pritchard et al., 2000) to identify the number of populations (K) and the most likely population of origin for each individual. Specifically, we used Baps to

determine the probabilities of K = 1–6, because 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 sampled localities covered a small geographic range. We ran Baps for 10 replicates of K = 1–6, with the use of clustering of groups of individuals and no spatial prior. Baps suggested the number of populations based on the combined maximum likelihood and highest posterior probability estimates over all 10 replicates. Once we identified the number of populations in our data set, we used Structure to examine admixture between identified populations (Pritchard et al., 2000). We ran Structure for 10 independent runs with the use of 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). We identified all individuals with $q <$ 0.75, which represents the equivalent of a single, grandparent ancestor from outside the identified population, as admixed individuals. Finally, we used the FullSearch option in the program Clumpp 1.1.2 to align clusters across all 10 replicates (Jakobsson and Rosenberg, 2007). We ran all subsequent programs with the use of the clusters suggested by the Bayesian clustering programs.

We used the program Genalex 6.4 (Peakall and Smouse, 2006) to quantify population structure via F_{ST}, and assess significance with the use of analysis of molecular variance (AMOVA) permutations. F_{ST} was calculated between populations and between sampling localities to investigate movement between and within populations, respectively. We conducted 9999 permutations, per population, to assess the likelihood of obtaining the observed F_{ST} and significance between the calculated F_{ST} and the F_{ST} of randomly assigned groups with AMOVA.

Demographic and Genetic Trends

We tested for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium with Genepop 4.1 (Rousset, 2008), with the use of a Bonferroni-corrected alpha (Rice, 1989). Genepop 4.1 also calculated standard summary statistics, including observed heterozygosity, expected heterozygosity, and allelic diversity (Rousset, 2008).

Based on published data (van Dijk and Harding, 2011), populations of Wood Turtles are declining throughout the range. However, current demographic trends in Michigan remain unknown. We used the program MSVar to look for evidence of increasing or decreasing population size in Michigan Wood Turtle populations (Beaumont, 1999; Storz and Beaumont, 2002). We estimated parameters for mutation rate, rate of change of population size, and time to total coalescence with the use of MSVar program notes. Briefly, we ran the program for 10^9 steps with the default parameters and the most variable locus, and chose values at the extreme edges of each posterior distribution for mutation rate, rate of change of population size, and time to total coalescence, as starting values for subsequent simulations. Next, we used the identified parameters in five sequential runs of 2×10^9 steps with all loci used. We discarded the first 10% of each run and plotted the marginal distributions with CODA (Best et al., 1995) in the R statistical package (R Development Core Team, http://www.R-project. org, accessed last 25 August 2011) and assessed convergence of the model via the Gelman–Rubin statistic (Best et al., 1995). Finally, we combined each run into a single data set and computed the relative likelihood of competing models with the use of the Bayes factor. We estimated the Bayes factor by dividing the number of iterations in which the population contracted by the number of iterations in which the population expanded, and considered a Bayes factor greater than 10 to be strong evidence against an equal number of population contraction and expansion genealogies (Kass and Raftery, 1995; Storz and Beaumont, 2002). We added 1 to the numerator and the denominator before calculating the Bayes factor to prevent division by 0.

We tested for evidence of a recent bottleneck in identified populations with the use of the program Bottleneck (Cornuet and Luikart, 1997; Piry et al., 1999). We ran Bottleneck with the use of a two-phase model, as the two-phase model more accurately imitates micro-

satellite DNA mutations than a strict stepwise model (Ohta and Kimura, 2007). We set the two-phase model parameters at 95% for single-step mutations and 5% for multiple-step mutations, with a variance of 12, as is appropriate for microsatellites in turtles (Kuo and Janzen, 2004). We interpreted excess heterozygosity with the use of a Wilcoxon signed-rank test (Rooney et al., 1999), and looked for a mode shift in allele frequency classes to assess the loss of rare alleles (Luikart et al., 1998). We investigated the impact of recent bottlenecks in each population with the use of F_{IS} and relatedness. We calculated F_{IS} with the use of FSTAT (Goudet, 1995) and relatedness using the Queller and Goodnight (1989) 'r' in the program Genalex 6.4 (Peakall and Smouse, 2006). We used bootstrapping and 99 permutations to calculate the 95% confidence interval around the mean relatedness value and to assess significance between relatedness and zero for both populations.

We measured effective population size with the use of the program ONeSAMP, which creates simulated populations based on user-provided allelic data, 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). We ran ONeSAMP assuming a minimum effective population size of 2 and a maximum effective population size of 2000. We varied the input priors, which limit the population sizes, to examine the consistency of ONeSAMP results (Tallmon et al., 2008). Tested priors included all combinations of minimum population sizes of 2 and 10, and maximum sizes of 1000, 2000, and 5000 individuals.

Results

We successfully amplified an average of 8.5 microsatellite loci in each of the collected Wood Turtle tissue samples ($n = 68$). We observed between 5 and 16 alleles per locus (Table 1), and found no evidence of scoring errors, null alleles, or large allele dropout.

Baps suggested the most likely K was 2 ($L[K] = -2500$; $P = 1.0$), with individuals from the southern sampling locality clustering into a single group, which we have termed the South population, and individuals from the northeast and northwest sampling localities clustering into a second group, termed the North population. Structure revealed admixed individuals ($q < 0.75$) from all three sampling localities, and accounted for 29% of sampled individuals in the North population and 17% of individuals in the South population. Additionally, two individuals captured in the northeast sampling locality genetically clustered with the South population, and one individual captured in the South with the North population. These three individuals are likely recent immigrants between the observed populations.

Population structure, as measured by F_{ST}, was significantly different between populations and randomly drawn groups, as well as between populations. The lowest differentiation was observed between the northeast and northwest sampling localities and is indicative of the amount of isolation by distance within the population. We found the highest differentiation between the North and the South populations (Table 2).

In both the North and the South 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). We found no significant linkage disequilibrium between any loci (all $P > 0.05$). The average observed and expected heterozygosity was higher in the North population compared to the values for the South population (Table 1).

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 1.0 to 1.1 for all parameters, and averaged 1.0 in the North population and 1.0 in the South population, 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

TABLE 1.—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	Number of alleles	Number of individuals	Expected heterozygosity	Observed heterozygosity
North population				
GmuB21[a]	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[a]	12	43	0.83	0.45
GmuA32[a]	9	45	0.82	0.44
GmuD28	12	44	0.84	0.75
GmuD55	5	45	0.37	0.37
GmuD88[a]	12	44	0.91	0.48
Average	10.67	44.22	0.80	0.56
South population				
GmuB21[a]	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[a]	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

[a] Locus out of Hardy–Weinberg equilibrium.

Bayes factor was greater than 10, the model indicates decline within the North and South populations.

Although we observed a decline via the population-size model (MSVar), we found no evidence of a bottleneck (program Bottleneck). The observed heterozygosity did not differ from that expected under drift-mutation equilibrium in either the North ($P = 0.50$) or the South population ($P = 0.73$). Similarly, we observed a typical L-shaped distribution of allele frequency classes, suggesting no mode shift or loss of rare alleles in either population (Fig. 3).

Observed F_{IS} values in both populations covered a wide range; in the North popula-tion, F_{IS} ranged from −0.09 to 0.48 (mean = 0.29) over all nine loci, and from −0.10 to 0.48 (mean = 0.23) in the South. The mean relatedness value in the North population was −0.023 (95% confidence interval [CI] = −0.013 to −0.032) and was significantly different than 0, as shown by the nonoverlapping confidence interval from the permutation test. The mean relatedness in the South population was 0.17 (95% CI = 0.19–0.14), and was also significantly different from 0.

ONeSAMP provided estimates of effective population size for the North and South populations that were not significantly different, regardless of the minimum and maximum population-size priors used (Fig. 4). In the

TABLE 2.—F_{ST} comparisons among sample sites (northeast, northwest, and southern) 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, with the use of AMOVA. The *P* values from this comparison are listed above the diagonal.

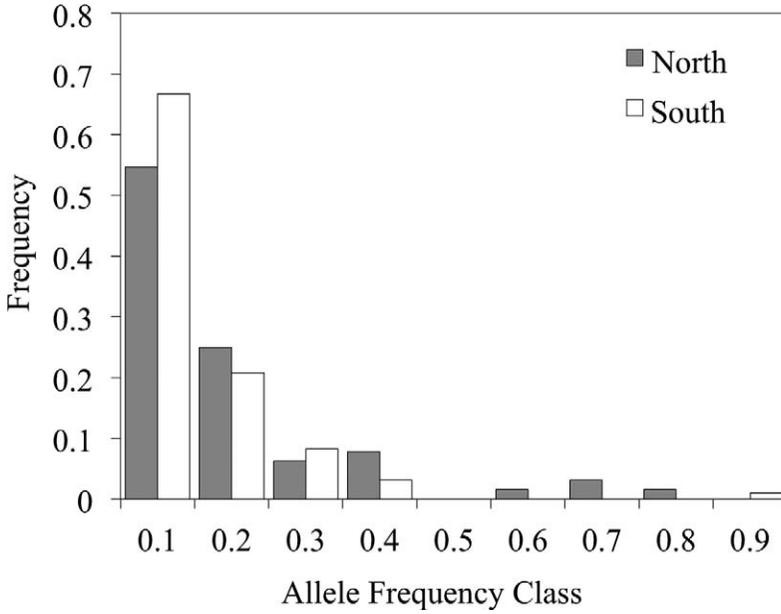|	Northeast	Northwest	Southern/South	North
Northeast	–	0.0012	0.0001	NA
Northwest	0.021	–	0.0001	NA
Southern/South	0.039	0.074	–	0.0001
North	NA	NA	0.044	–

FIG. 3.—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.
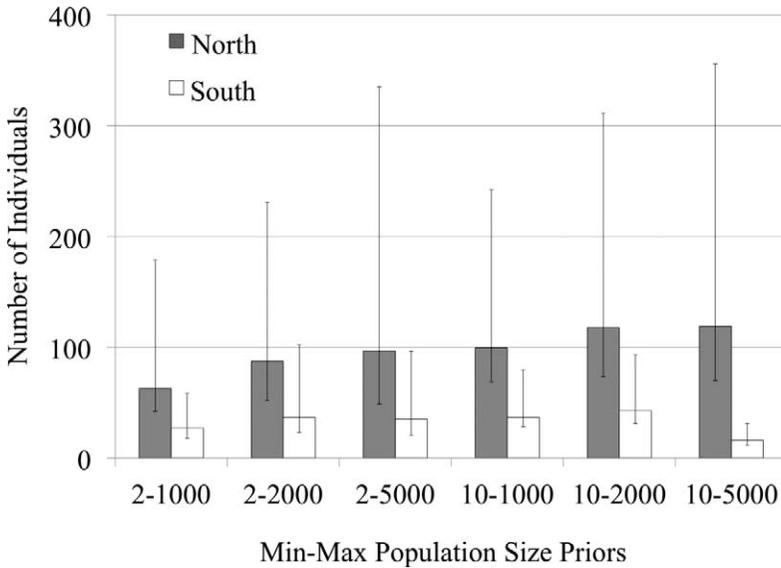

FIG. 4.—Effective population-size estimates of the North and South populations using multiple priors. Although the mean effective size estimated in the North population always appears larger than the mean estimated in the South, there is no significant difference between the populations, as illustrated by the overlapping 95% credible limits (error bars).

North population, the estimate with the largest credible limit, which is analogous to a confidence interval, was 97 (95% credible limit = 48–334) whereas in the South population the estimate with the largest credible limit was 37 (95% credible limit = 23–102).

DISCUSSION

Wood Turtles in the Lower Peninsula of Michigan exist as two populations, referred to as a North population and a South population (Fig. 2). Although both populations showed evidence of decline with the use of a model based on coalescent theory, loss of genetic diversity was not detected using the more typical measures of genetic decline including F_{IS} and bottleneck.

Rivers and other waterways appear to be natural dispersal corridors, especially for riparian species. Accordingly, we 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. We observed a north–south split that combines two sampling locations from different drainage 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 yr ago, trees, shrubs, and grasses began recolonizing the northern portion of North America (Pielout, 1991), 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, and others moved west through Ohio, Illinois, and Wisconsin (Fig. 1; Amato et al., 2008). It is possible that individuals moving westward into Illinois and Wisconsin recolonized the south portion of Michigan, whereas 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 (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 because of 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 suggest 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 observed genetic diversity 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 ($F_{ST} = 0.04$) is 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 into 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 yr (van Dijk and Harding, 2011), which means that a maximum of three 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).

The utilized population-size model suggested a decline in population size for both the north and the south populations, as evidenced by an estimated Bayes factor of greater than 10 (Kass and Raftery, 1995; Storz and Beaumont, 2002). However, a bottleneck and significant inbreeding was not detected in either population. Although the effective population-size credible limits ranged from 48 to 334 in the north and from 23 to 102 in the south (Fig. 3), there was no evidence of a recent bottleneck, and the average observed heterozygosity was near 0.5 in both populations. However, in 17 of 18 comparisons, the observed heterozygosity is lower than what was expected, suggesting population decline (Table 1). The conflicting signals suggested from the MSVar model and the F_{IS} and bottleneck analysis suggests that coalescent models may be able to detect declining populations in fewer generations than other measures of genetic diversity in long-lived species.

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 (U.S. Department of Transportation, 2006), we expect to see genetic evidence of demographic decline in future populations. The observed average F_{IS} value for both populations (mean = 0.23; 95% CI = 0.13–0.45) suggests the populations are losing alleles. Other turtle populations of species of lesser conservation concern 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), whereas 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*, Ra-

feliarisoa et al., 2006). Additionally, we observed a small but statistically significant increase in the average relatedness in the South population compared to the North population, which is associated with the start of an extinction vortex (Blomqvist et al., 2010). 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. Although our results suggest that a timely intervention is needed to prevent dramatic decreases of genetic diversity in populations of Wood Turtles in Michigan, additional data are required to search for demographic signatures of population decline, such as reduced recruitment and male-biased sex ratios, to elucidate the impact of urbanization fully. 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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