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Elucidating the conservation status of Michigan's red-eared slider (trachemys scripta elegans): A phylogeographic approach

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Elucidating the Conservation Status of Michigan's Red-eared Slider (*Trachemys scripta elegans*): A Phylogeographic Approach

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ABSTRACT

The geographic origin of Michigan's *Trachemys scripta elegans* has been a contentious subject since its first description in 1934. At that time two explanations were proposed: 1) populations of *T. s. elegans* are native to Michigan and naturally expanded their range from Ohio and Indiana; or 2) populations are non-native and have been introduced by humans via the pet-trade from throughout the United States. To differentiate between these possibilities, I compare the genetic structure of six populations throughout Indiana, Michigan, and Ohio, using six microsatellite markers. No isolation-by-distance was detected and model-based statistics support two genetic clusters with five populations from Michigan, Ohio, and Indiana forming one cluster and a single Michigan population forming a second. These results indicate that some of Michigan's populations of *T. s. elegans* are composed of released pets from geographically distant sources, while others are either native relicts or have been introduced from nearby populations.

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INTRODUCTION

1.1 Alien Species and Phylogeography

The introduction of alien species from their native range to new environments is regarded as both a local and a global threat to biodiversity, economics, and environmental and public health (Lodge et al. 2006; Darling et al. 2008; Kikillus et al. 2010; Konečný et al. 2013). Further, the continuing expansion of human globalization and international trade exacerbates these threats (Lodge et al. 2006; Darling et al. 2008). In cases such as the introduction of the cane toad, *Bufo marinus*, to Australia (Phillips et al. 2007) or the black rat, *Rattus rattus*, to West Africa (Konečný et al. 2013), the translocation of alien species to new environments is obvious due to well documented introduction records and presence well outside the species' native range. However, in some instances, identifying the distinction between native or introduced range is less clear (Holman 1994; Vamberger et al. 2011; Suzuki et al. 2011) and because of the economic and environmental costs associated with alien species (Lodge et al. 2006), correctly defining these ranges, and thus a particular species' geographic origins, is essential.

Modern genetics offers an appropriate approach to discern between native and introduced populations in a given area by examining their phylogeographic structures (Avise 2000). Generally, this approach seeks to understand the relationship between genetic lineage and geographic distribution (Avise 2000) and has been used in several contexts, such as showing how past geological events have shaped current species' distributions (Starkey et al. 2003; Suzuki and Hikida 2010) and determining the geographic boundaries between cryptic subspecies (McGaugh et al. 2008). Phylogeography has also been used on several occasions to determine the native or non-native status of many species to a particular area (Taylor and

Keller 2007; Stepien et al. 2002; Hufbauer et al. 2004). Suzuki et al. (2011) and Vamberger et al. (2011), respectively, determined the Reeve's pond turtle, *Mauremys reevsii*, and the spur-thighed tortoise, *Testudo graeca*, to be introduced in areas where they were previously thought to be native. Snell et al. (2004) found that Norfolk, Great Britain populations of *Rana lessonae*, assumed to be introduced from Italy, shared similar haplotypes with northern European populations, indicating the species likely naturally colonized Norfolk through a post-glacial North Sea land bridge. Finally, Velo-Antón et al. (2011) dissected the impact of present and past trade activities in two widely traded European pond turtles (*Emys orbicularis* and *E. trinacris*) and found that non-native haplotypes were widespread throughout European populations. In each of these instances, distinctions between native and non-native ranges were obscured by geological events or human-mediated introductions. With the advent of the modern phylogeographic procedures employed by the researchers, such uncertainties were, at least partially, alleviated to such a degree that genetic and geographic lines became interpretable, thus allowing sound conclusions to be drawn upon the relevant taxon's indigenity to a region and displaying the applicability of the discipline to similar questions.

1.2 History of the Red-eared Slider, *Trachemys scripta elegans*

The red-eared slider, *Trachemys scripta elegans*, is a semi-aquatic turtle whose current range includes the central United States and south through Mexico and Central America to Columbia and Venezuela (Figure 1; Ernst et al. 2009). However, the turtle has been widely introduced by humans on every continent, except Antarctica (U.S. Geological Survey 2015), and is considered to be one of the 100 world's worst invasive alien species (IUCN SSC Invasive Species Specialist Group 2010). Within its introduced range, the turtle has been

documented competing with and displacing native species, disrupting local food-webs and acting as a vector for parasites and disease (Cadi and Joly 2004; Kikillus et al. 2010; Thomson et al. 2010). Incidentally, much of the success in this turtle's global invasion has been facilitated by humans, primarily through the pet trade (Williams 1999; U.S. Geological Survey 2015). *T. s. elegans* has been popular in this market since the 1930s, except during a brief lull in 1975 due to increased regulations of the United States Food and Drug Administration in response to a salmonella scare, and trade rose to its climax through the 1990s, following the popularity of the *Teenage Mutant Ninja Turtles* animated series (Williams 1999; U.S. Geological Survey 2015). Between 1989 and 1997 an estimated 52 million individuals were traded (Telecky 2001). Additionally, Asian food markets, where turtles are a popular food item, are suspected invasion vectors (Williams 1999).

In Michigan, the geographic origins of *T. s. elegans* has been controversial since Edgren (1943) first described the turtle from two specimens captured at the Owasippe Boy Scout camp in Muskegon County. Edgren (1943, 1948) hypothesized these specimens were introduced but later suggested the possibility of a natural expansion of this turtle's range from neighboring Ohio or Indiana. Through subsequent years, several more *T. s. elegans* individuals were recorded in Oceana, Ingham, Washtenaw, and Oakland Counties with the prevailing assumption that these were small populations resulting from multiple introductions of released pets (Gordon and Fowler 1961; Holman 1994; Harding 1997), and today the species has been recorded throughout much of the state's Lower Peninsula (Figure 2)**.** However, in 1994 Holman proposed that fossil evidence from Schultz Archaeological Site in Saginaw County, Michigan and another site in Wisconsin (Figure 3) support the hypothesis that at least some of Michigan's populations of *T. s. elegans* had been in the state for

approximately 6,000 years and were thus native (Adler 1968; Holman 1994). If fossils indicate natural presence since this time, the geographic distance between these fossil sites and the species' currently defined native distribution indicate that *T. s. elegans* underwent an intense range constriction in mid-Holocene times approximately 2,500 to 1,600 years before present (Adler 1968; Holman 2012). Further, Holman (1994) contended that *T. s. elegans* could have extended its range north during the warmer hypsithermal period of the mid-Holocene and then remained in refugia as cooling temperatures caused other populations to withdraw. Both scenarios would have resulted in geographically small populations of *T. s. elegans* in Michigan that were disjunct from the southern native range. Another species of reptile, the northern copper-bellied snake, *Nerodia erythrogaster neglecta,* is known to share this distribution and exists as relict populations in Michigan today (Holman 1994). Both Adler (1968) and Holman (1994) strongly contended that the fossil of *T. s. elegans* in Michigan originated from the Schultz Archaeological site and was not an object of intertribal trade. However, the evidence supporting their position does not exclude the possibility that populations of *T. s. elegans* underwent a local extinction event and were later reintroduced through anthropogenic means, nor does it exclude the possibility of a secondary colonization of Michigan from neighboring states.

1.3 Purpose and Hypotheses

Understanding whether populations of *T. s. elegans* are native or introduced to Michigan is an important conservation concern as its state-listed status as native or non-native has faced nearly as much ambiguity as its geographic origins. Currently, the species does not hold a specifically listed conservation status by the Michigan Department of Natural Resources (MDNR; Holman 2012). In publications put out by the agency and on their

website, the turtle is generally referred to as being found in the Muskegon County area and possibly being or thought to be introduced (Lagler 1954; State of Michigan 2015*a*). However, under Michigan's Natural Resources and Environmental Protection Act's legal definition of reptiles, “any turtle, snake, or lizard of the class reptilia”(Mich. Comp. Laws § 324.48701 [2008]), the turtle is considered property of the state and is protected to the same degree as known-native species in regards to take allowance and personal use (Mich. Comp. Laws § 324.48705 [1995]; Mich. Comp. Laws § 324.48702 [1996]), causing private practices to abide by the seasonal take regulations set by MDNR fisheries division (State of Michigan 2015*b*) and commercial practices to purchase an annual commercial reptile and amphibian license (Mich. Comp. Laws § 324.48705 [1995]).

Further, no apparent statewide surveys for the species appear to have been performed (Holman 2012) and the MDNR mentions that the history of all reptiles and amphibians in the state has been "sketchy" (State of Michigan 2015*c*). The MDNR has recently extended its efforts to monitor all reptiles and amphibians through the Michigan Herp Atlas Project, a researcher and citizen-science based, MDNR initiative administered through Herpetological Resource and Management (Mifsud 2014; State of Michigan 2015*c*). However, despite the relative robustness of MDNR's current records of *T. s. elegans* compared to those of a decade ago, it is still not possible to know if a particular population of *T. s. elegans* was established pre- or post- pet-trade.

The lack of an official conservation status and poorly documented locality records places Michigan's populations of *T. s. elegans* in potentially precarious situations in regards to their population health. For example, if external stressors are placed upon a population, such as the commercial development of a site, there is no state-listed conservation status to afford action

in aiding the population and neither is there knowledge of possible transplant populations if conservation action is approved. Further, the lack of a conservation status prohibits recourse should current protections under MDNR fishing regulations change. In the most extreme case, should management regulations in regards to this species change, populations of *T. s. elegans* could be euthanized upon capture as is the case in Ohio (Owen Lockhart, personal communication), except in two counties recorded well before the popularity of the turtle pet-trade (Wynn and Moody 2006).

While the former three examples are placed in the context of the turtle receiving a native state-listing, a listing of non-native would be equally important. Such a listing, as a non-native species, would likely initiate empirical investigations into the impacts of *T. s. elegans* on native Michigan species and ecosystems, a topic of research with very little documentation (Harding 1997; USGS 2015). Further, a non-native listing may spur a re-examination of Michigan's Animal Industry Act if *T. s. elegans* is found to be harmful to Michigan's native species. Specifically, this law prevents animals that may "…endanger native wildlife…" from being imported into the state (Mich. Comp. Laws § 287.731 [2004]). Such a measure would prevent pet stores from selling *T. s. elegans* or at least force them to obtain *T. s. elegans* individuals from local breeders.

Here, I take a phylogeographic approach to examine the phylogeographic origins of Michigan's populations of *T. s. elegans* and evaluate two competing hypotheses: 1) *T. s. elegans* is native and has naturally expanded its range north from the native range south of Michigan and 2) *T. s. elegans* is non-native and has been introduced by humans via the pet trade. I then discuss the genetic structure observed among populations in the region and make

recommendations regarding the management of the species and in the future work of this question.

MATERIALS AND METHODS

2.1 Field Sampling

Tissue samples of *T. s. elegans* were obtained from field sites throughout Michigan, Ohio, and Indiana (Figure 4). In Michigan, five sites were sampled: the Huron River (HR; *n* = 20; 42º16'33.93" N/ 83º41'51.40" W Gallup Park Nature Area), the Rouge River (RR; *n* = 9; 42º19'03.72" N/83º14'19.71" W University of Michigan Dearborn; 42º18'07.65" N/83º16'48.49" W Dearborn Heights Golf Course), the Lake St. Clair Metropark wetlands east of Lake St. Clair (*n* = 0; 42º34'41.73" N/83º48'20.00" W), the Cleveland Lake of the Owasippe Scout Reservation (*n* = 0; 43º25'41.43" N/86º13'17.20" W), and a small, urban pond within the Timber Lakes Apartment Complex in Lansing (TL; *n* = 7; 42º45'16.82" N/84º30'19.67" W). None of the Michigan sites have a putative population origin (i.e., native or non-native), and it should be noted that nine samples from the HR population came from a single clutch of eggs that were removed and incubated by Herpetologist David Mifsud after they were found being laid on site. In Indiana, sites were sampled: Dewart Lake (DL; *n* = 8; 41º22'17.28" N/85º46'28.28" W) and Hovey Lake (HL; *n* = 24; 37º48'25.84" N/87º56'41.53" W; *n* = 24). These Indiana sites are putatively native as they fall within the species' native range. In Ohio, four sites were sampled: the Ohio & Erie Canal, the Rocky River, and the Sunset and Strawberry ponds of the North Chagrin Cleveland Metropark (NEOH; *n* = 23; 41º43'61.20" N/81º66'22.60" W). *T. s. elegans* captured at the Ohio sites are considered to be non-native as they are outside the species' native range and were documented to the state after the popularity of the turtle pet-trade had been established.

Turtles were captured using Promar TR-502 funnel traps baited with either cat food or sardines. Traps were set overnight in shallow water or suspended between two poles with

enough space left for turtles to surface and breathe and were checked at least once every 24 hours. After capture, tissue samples were obtained by clipping approximately 1 cm from the tail and were then stored in a 95% ethanol solution. Re-sampling of individual turtles was prevented in two ways. First, since samples were taken by removing tissue from the tail, it was usually obvious when an individual had already been sampled. Thus, individuals with damaged tails were not sampled. Second, plastron and carapace width and length measurements were taken using calipers, sex was recorded, and any morphological anomalies, such as missing limbs or shell damage, were noted as well. If an individual was suspected of being previously sampled, these notes were consulted.

2.2 Extraction, Gel Electrophoresis, PCR

DNA was extracted from tissue samples using Quigen DNAeasy kits, following the manufacturer's instructions. To test that primers were annealing properly, DNA was amplified via polymerase chain reaction (PCR) with microsatellite markers (Xin et al. 2012, Simison et al. 2013; Table 1) and run on a 1% agarose electrophoresis gel. Microsatellites were distributed into four sets based upon their annealing temperatures: From Xin et al. (2012) TSE06, TSE09, and TSE21 composed primer set 1; TSE10, TSE14, and TSE80 composed primer set 2; TSE02, TSE03, and TSE78 composed primer set 3; and from Simison et al. (2013), TSC243, TSC260, and TSC263 composed primer set 4. Each sample tube was then prepared with 1 µl of DNA sample, 4 µl Multiplex Mix, 1 µl of forward primer, 1 µl of reverse primer, and 3 µl of H_2O. Primer sets then underwent PCR at various annealing temperatures to maximize annealing: Primer set 1 underwent PCR with annealing temperatures set at 63º and 66º C, samples with primer set 2 at 62º C, samples with primer set 3 at 58º, and 66º C and samples with primer set 4 at 55º C. PRC products were then viewed

on an electrophoresis gel under a Bio-Rad Molecular Imager ChemiDoc XRS+ imaging system using Image Lab 5.1 (Bio-Rad Laboratories 2014).

Once microsatellite primers were annealing properly, all DNA extractions were amplified via PCR. Each sample tube was prepared with 1 µl of DNA sample, 4 µl Multiplex Mix, 3 µl of H_2O, and 1 µl of a master mix from each primer set. For instance, primer set 1's master mix was composed of the forward and reverse primers of TSE06, TSE09, and TSE21. Samples with primer set 1 then underwent PCR with annealing temperatures set at 63º C, samples with primer set 2 at 62º C, samples with primer set 3 at 66º C, and samples with primer set 4 at 55º C. PRC products were then prepared for shipping to the Georgia Genomics Facility (GGF) by placing 2 µl of sample into each well of 96-well plates that were then covered with a paper towel, left out overnight to dry. The prepared wells were then shipped to the GGF for sequencing in the Applied Biosystems 3730xl 96 capillary DNA Analyzer.

2.3 Genotyping

After processing at the GGF, microsatellite peaks were assigned using Geneious 6.1 (Kearse et al. 2012). Each sample's trim was set to 1600 base pairs and the ladder was set to GGF 500 with an upper ladder at 435 and a lower ladder set at 88. To maintain consistency in peak assignment, for loci that exhibited peak splitting, the farthest right, larger peak was always selected. After assignment, peaks were then exported to Microsoft Excel where they were binned as dinulceotide repeats (Xin et al. 2012). Binning was accomplished by identifying a pattern in how peak assignments were grouped and re-designating similar assignments into a single peak. For example, peak assignments at 227.3, 228.1, and 226.9 base pairs would have each been binned at 228 base pairs. After binning, data were then

reformatted for entry into genetic software using PDGSpider 2.0 (Lischer and Excoffier 2012).

2.4 Data Analysis

A. Microsatellite DNA Variation

Hardy-Weinberg equilibrium, linkage disequilibrium, and F-statistics for all population pairs were evaluated using on GENEPOP on the Web options 1, 2, and 6, respectively (Raymond & Rousset 1995; Rousset 2008). Option 1 was performed with a complete enumeration of alleles, per the program's suggested instructions, as some samples only contained allelic data for four or fewer loci. Options 2 and 6 were performed under default settings (Raymond & Rousset 1995; Rousset 2008). An isolation-by-distance (IBD) model was constructed using the genetic distances calculated in GENEPOP on the Web option 6 and pair-wise distances calculated in a point-distance analysis in ArcGIS 10.1 (ESRI 2012).

B. Population Structure

Population structure was evaluated using model-based clustering programs STRUCTURE 2.3 (Pritchard et al. 2000), GENELAND 4.0 (Guillot et al. 2012), and BAPS 6.0 (Corander and Marttinen 2006). In STRUCTURE, analyses were performed under two separate parameter sets, both of which were set to a burnin period of 5,000 with 10,000 Monte-Carlo Markov chain (MCMC) repetitions after burnin and advanced options selected to estimate the probability of the data under each *K* model and to print each individual's assignment within each cluster via the Q-hat analysis. The first parameter set was selected to perform under an admixture model with a LOCPRIOR model, which assigns individuals to a geographic population prior to the start of the analysis. Performing a STRUCTURE analysis under a LOCPRIOR model is recommended to assist in clustering when data sets are

composed of small population sizes or contain missing data (Hubisz et al. 2009). The second parameter set was selected to perform under an admixture model without the LOCPRIOR model. A simulation was then performed for 100 iterations, expecting 1 to 6 populations, and results were then entered into the web-based program STRUCTURE HARVESTER (Earl and vonHoldt 2012) to execute post-hoc evaluations using the Evanno et al. (2005) method, which further interprets STRUCTURE outputs to calculate ΔK based on the rate of change in the log probability of data between successive K-values.

In GENELAND, codominant marker, individual UTM coordinates and individual label files were analyzed for 10,000 iterations with a thinning of 10 under an uncorrelated allele frequency model, which accounts for unknown allele frequencies (The Geneland development group 2014), expecting K from 1 to 6. A spatial model was used at a resolution of 50 pixels for both X and Y axes and was performed at 50 burnins. A model accounting for null alleles was not selected as GENELAND interprets all missing allele data as null alleles (The Geneland development group 2014). K was assessed via a number of populations analysis set to a 500 burnin length period, and the probability of individual membership to population was assessed both spatially and non-spatially.

Finally, several clustering of individuals analysis in the program BAPS 6.0 (Corander and Marttinen 2006) were performed with upper bounds of K set to 2, 6, 25, 30, and 100. These analyses were then followed by respective admixture analyses using BAPS default parameters. The reasons for performing several analyses with various upper bounds of K is due to BAPS clustering method: rather than clustering individuals together at the population level, BAPS clusters individuals together based on similar haplotypes, thus several

haplotypes may be present within a small number of genetic clusters (Corander and Marttinen 2006).

C. Post-hoc Analyses without Locus TSE78

After analysis in GENEPOP on the web Option 2, loci TSE78 was found to be in linkage disequilibrium with TSE80. Due to this, the STRUCTURE, GENELAND, and BAPS analyses were performed again with TSE78 removed. Results were found to be qualitatively similar (see results section 3.3.D.), thus TSE78 was retained throughout all analyses.

RESULTS

3.1 Field Sampling & Genotyping

Field sampling and genotyping yielded successful data for 91 individuals (7 – 24 individuals/population) for six out of the twelve loci that were amplified (Table 1). Raw genotype data for these individuals are summarized in Table 2 and their associated population information in Table 3. From these 91 individuals, 93.96% of all allelic data was estimated: 77 are missing 0% of allelic data, 6 are missing 16% of allelic data, 2 are missing 33%, 3 are missing 50%, and 3 are missing 83% of allelic data. Individuals missing 83% of allelic data were not included in analyses.

3.2 Microsatellite DNA Variation

Hardy-Weinberg equilibrium (HWE) probability tests were performed in GENEPOP on the Web under the null hypothesis of a random union of gametes (Raymond & Rousset 1995; Rousset 2008). The Huron River, Deward Lake, and NE Ohio populations were out of HWE for three loci and the remaining populations for two loci. For locus comparisons, TSE02 was out of HWE for three populations, TSE10, TSE14, TSE78, and TSE80 for two populations and TSE03 for one population. Linkage disequilibrium probability tests showed only TSE80 and TSE78 to be in linkage disequilibrium ($p = 0.017$).

All populations share close genetic distances with the exception of pairings between the Timber Lakes population from Lansing, MI and all other populations. Fst-values ranged between -0.004 and 0.161 with an average Fst of 0.059 between all population pairs (Table 4). The Timber Lakes population from Lansing, MI appears to be well differentiated from all other sampled populations with pairwise Fst-values ranging from 0.106 to 0.161. When an

isolation-by-distance model was constructed, no correlation was detected between genetic distance and geographic distance ($p = 0.284$; $r^2 = 0.088$; Figure 5).

3.3 Population Structure

A. STRUCTURE

The Evanno et al. (2005) method executed in STRUCTURE HARVESTER (Earl and vonHoldt 2012) indicates that a model of $K = 2$ genetic clusters is most strongly supported for both the LOCPRIOR and non-LOCPRIOR parameters and that a $K = 4$ model was also supported for the non-LOCPRIOR parameter (Figure 6). For $K = 2$ models, HL, DL, the HR, and the RR appear to belong to cluster 1; TL appears to belong to cluster 2; and the NEOH population appears to belong to both cluster 1 and 2 (Figure 7). For the $K = 4$ models, TL appears to belong to its own, distinct cluster, while all other populations are composed of members from the other three clusters (Figure 7). At the individual level, both $K = 2$ models assigned individuals similarly with 64 individuals belonging to cluster 1 and the remaining 24 individuals belonging to cluster 2 under the LOCPRIOR parameter set, and 63 individuals belonging to cluster 1 and the remaining 23 individuals belonging to cluster 2 under the non-LOCPRIOR parameter set (Figure 8). Under the $K = 4$ model, 28 individuals belong to cluster 1, 23 to cluster 2, 10 to cluster 3, and the remaining 34 to cluster 4. The probability of each individual's assignment to each cluster, compared to the assignments given by GENELAND and BAPS, can be found in Table 5.

B. GENELAND

The number of populations analysis indicates that a model of $K = 2$ is most strongly supported (>60% of MCMC iterations) with the next closest model being $K = 3$ (20% of MCMC iterations) (Figure 9). A map of probability of population membership analysis under

the spatial model shows the TL population to belong to cluster 2 (90% of MCMC iterations) and all other populations to belong to cluster 1 (90% of MCMC iterations for each population) (Figure 10; Figure 11). The probability of each individual's assignment to each cluster, compared to the assignments given by STRUCTURE and BAPS, can be found in Table 5.

C. BAPS

The clustering of individuals analysis performed with an upper bound of $K = 6$ supports a model of $K = 6$ (100% of MCMC iterations). At the population level, a clear distinction is observable between the TL, Lansing population and all other populations (Figure 12). Further, the admixture analysis based on the previous clustering of individuals supports a model of $K = 5$, indicating widespread population admixture except in TL (Figure 13). Despite these respective *K*-values, a visual assessment of Figures 12 and 13 suggests two genetic clusters are present within the sampling area, and when BAPS is forced to assign individuals to a $K = 2$ model, the results are similar to both the STRUCTURE and GENELAND models. The probability of each individual's assignment to each cluster, compared to the assignments given by GENELAND and STRUCTURE under the $K = 2$ model, can be found in Table 5.

When the clustering of individuals analysis was performed with upper bounds of $K = 25$, 30, and 100, a model of either $K = 23$ or $K = 24$ was most strongly supported with a $K = 8$ after accounting for admixture. Results are qualitatively similar to Figures 12 and 13.

D. Post hoc Analyses without TSE78

The reassessment of the STRUCTURE, GENELAND, and BAPS results with TSE78 removed, due to the locus' linkage disequilibrium with locus TSE80, supported each of the *K*

models with TSE78 included (K = 2 in STRUCTURE, K = 2 in GENELAND, and K = 6 in BAPS) and similarly assigned individuals to each population (67.03% similarity in STRUCTURE, 81.32% similarity in GENELAND, and 95.60% similarity in BAPS).

DISCUSSION

4.1 Population Structure

Model-based clustering exhibit a clear congruence in population structure at the regional level with the Huron River (HR), Rouge River (RR), Dewart Lake (DL), Hovey Lake (HL), and NE Ohio (NEOH) populations belonging to a common genetic group and the Timber Lakes (TL) population distinct to a group of its own. Further, isolation-by-distance and pairwise Fst-values indicate that the HR, RR, DL, HL, and NEOH are relatively closely related to one another when compared to their relationship with TL. Due to their slow molecular evolution (Avise et al. 1992; FitzSimmons et al. 1995; Walker and Avise 1998), turtles tend to separate into genetic clusters at broadly regional levels (Walker et al. 1998; Weisrock and Janzen 2000; Starkey et al. 2003; Rosenbaum et al. 2007; Amato et al. 2008; Kimble et al. 2014). Since all populations except the TL population exhibit this structure, these results suggest the TL population was likely introduced to Michigan from a geographically distant source.

Unfortunately, the indigenity of the HR and RR populations from Michigan are more difficult to discern as their relationships to the remaining populations are more ambiguous. When considering the BAPS, GENELAND, and $K = 2$ STRUCTURE models, their clustering with the DL and HL populations from Indiana, which are within the native range of *T. s. elegans*, could suggest that these populations are Michigan natives. However, an introduction scenario from a regional source is just as likely. This is especially apparent when considering the cluster and pairwise Fst relationships of the HR and RR populations in Michigan with the NEOH population from Ohio, which is considered to be non-native (Wynn and Moody 2006; Conant 1951), but which also groups with DL and HL populations

from Indiana; although isolation-by-distance does group all NEOH pairwise comparisons together in a distinct, yet weak, cluster (Figure 5). The pairwise Fst between HR and RR itself is notable as one would expect its value to be relatively low if both populations are Michigan natives. In reality, however, other HR and RR comparisons, such as RR-DL, HR-DL, and especially RR-NEOH, are comparatively lower.

HR and RR indigenity becomes even more complicated when the $K = 4$ STRUCTURE model is considered (Figure 7). Under this model, the HR, RR, DL, HL, and NEOH appear to be highly admixed populations from three genetic clusters. While, HR, in particular, seems to have less genetic admixture, this may be due to nine samples being derived from the same clutch of eggs. Overall, the high amount of admixture among these populations indicates several genetic, hence geographic, origins for each population, which is altogether unsurprising as human-mediated introductions have always been known to be an important source of *T. s. elegans* in Michigan (Holman 1994, 2012; Harding 1997). However, the results of Figure 7 do not indicate that all genetic populations are of introduced origin. Instead, a scenario in which one of the genetic clusters is native, while the others are from non-native, human-mediated introductions, is just as likely.

Though model-based clustering clearly indicates population structure at the regional level, individual analyses suggest a history of introduction within these populations. This pattern is most obvious when only the $K = 2$ STRUCTRE models are examined, showing the HL, NEOH, and RR populations to contain individuals that belong to both genetic clusters (Figure 8). However, individual assignment analyses for STRUCTURE, BAPS, and GENELAND indicate that all populations, except TL, contain at least one individual that belongs to the genetic cluster opposite of most population members (Table 5). While these

results do not indicate the origin of a specific population, they do demonstrate a complex genetic structure within populations that is likely due to the influence of released pet-trade turtles, especially within the HL population, which is putatively native.

The results of this study are similar to those found in previous studies on the genetic structure of turtle populations, showing low genetic diversity over wide geographic regions (Walker et al. 1998; Weisrock and Janzen 2000; Starkey et al. 2003; Rosenbaum et al. 2007; Amato et al. 2008; Kimble et al. 2014). Among these, Kimble et al.'s (2014) evaluation of *Terrepene carolina carolina* revealed only two genetic clusters throughout the entire species' range in the eastern United States, each extending from the northern to southern limits of the range and separated by the Appalachian Mountains, and showed isolation-by-distance at ranges of 300 – 500 km. Walker et al. (1998) showed only one genetic population of *Chelydra serpentina* across ten states in the southern United States while Starkey et al. (2003) found only four unique clades in *Chrysemys picta* across the entire United States range.

Taken together, the above investigations suggest two interesting points regarding the genetic structure of *T. s. elegans* in Michigan, Ohio, and Indiana. First, molecular evolution has been shown to occur at reduced rates in Testudines compared to other taxa as slow base substitution rates for both mitochondrial and nuclear DNA have been observed in freshwater and marine turtles (Avise et al. 1992; FitzSimmons et al. 1995; Walker and Avise 1998). This point further supports the possibility that the TL population was introduced to Michigan from a distant genetic source as its high Fst-value would have taken a very long time to develop. Of course, the TL population is also small and isolated, which certainly would increase its Fst-value. Slow molecular evolution also would explain the similarity in the HR, RR, DL,

HL, and NEOH populations as they may not have been reproductively isolated long enough to yield a distinct cluster. Second, turtle populations in the northeastern United States exhibit lower genetic diversity and structure relative to those in the southern United States, which, interestingly, would be expected in a post-glacial recolonization scenario (Weisrock and Janzen 2000; Starkey et al. 2003). While the current study's range is simply not wide enough, perhaps a wider ranging dataset, including populations south of Indiana into the southern extent of *T. s. elegans*' native, United States range, could detect similar trends in genetic diversity and structure (Weisrock and Janzen 2000; Starkey et al. 2003). This happened to be the case in the sister genus to *T. s. elegans*, *Chrysemys picta*, when its range-wide phylogeography was evaluated (Starkey et al. 2003).

Alleviating a few limitations of this study could reveal more on geographic origins of *T. s. elegnas* in Michigan despite the influence of slow molecular evolution. The benefits of obtaining a range-wide dataset of samples has already been explained above but are mentioned here as this author recommends it in high regard. Larger sample sizes for each population would further increase genetic clarity as this study's sample sizes ranged from 7 – 24 individuals and it has been suggested that 25 – 30 individuals per locality is optimal in microsatellite-based population genetic studies as measures of allele frequency and expected heterozygosity within the individuals sampled change minimally with additional individuals above this range (Hale et al. 2012). Last, increasing the number of loci might also increase genetic clarity. It has been empirically demonstrated that increasing the number of loci by even a moderate degree can be beneficial (Koskinen et al. 2004) and modern genetic population studies tend to use at least nine microsatellite markers (Inoue et al. 2013; O'Leary et al. 2014; Willis et al. 2015) compared to this study's six. I did attempt to amplify twelve

loci, but six failed entirely across all sampled individuals (Table 1). Perhaps performing PCR on these failed loci at higher annealing temperatures will allow them to anneal properly.

4.2 Management Implications

Making inferences to whether a single population is native or introduced to a small geographic area may not be possible due to the low genetic diversity exhibited among turtle populations (Walker et al. 1998; Weisrock and Janzen 2000; Starkey et al. 2003; Rosenbaum et al. 2007; Amato et al. 2008; Kimble et al. 2014). Even when individuals within a population can be broadly assigned to either a nearby or distant region, managing a population at the individual level for a species that is essentially morphologically identical across its range would be a daunting task. However, it may still be possible to manage populations appropriately by taking land-cover into consideration as land-cover models are able to predict genetic patterns (Greenwald et al. 2009) and predictors of species introductions may be landscape dependent, such as in cases of past disturbance (Brown et al. 2008). From this investigation's results, concise conclusions regarding land-cover are difficult to make; however, it is qualitatively evident that the isolated and heavily urbanized, artificial apartment pond of the putatively non-native TL population (Figure 14) greatly differs from larger, rural/agricultural area lake of the DL population (Figure 15), and the riverine landscapes of the agricultural area HL population (Figure 16), and urban HR, RR, and NEOH populations (Figure 17). While such a qualitative assessment hardly warrants any substantial management recommendations, it certainly beckons further, empirical analysis of the relationship between land-cover and non-native introductions of *T. s. elegans*.

Of course, population management is only necessary if potentially introduced populations of *T. s. elegans* are, in fact, impacting Michigan's native species or affecting native

ecosystems. Pertaining to the latter, very little has been documented despite *T. s. elegans* establishment as a world-wide invader (USGS 2015), although the turtle's diet does include a variety of aquatic plants (Harding 1997), hence it could be affecting the habitat structure through grazing. In Michigan, however, *T. s. elegans* is known to prey upon non-native vegetation as well, which is considered to promote ecosystem health (Mifsud 2014).

Parasite and disease introductions from introduced populations of *T. s. elegans* are also a warranted concern. The turtle is known to be a host to parasites, namely nematodes (Hidalgo-Vila et al 2008), and is suspected to be a host for a number of diseases, including *Ranavirus* spp., *Herpesvirus* spp., *Mycoplasma* spp. and *Salmonella* spp. (Silbernagel et al. 2013), the latter of which is also a public health concern (Harris et al. 2010). Despite *T. s. elegans* being an available host, however, researchers have failed on multiple occasions to document increased parasite and disease frequency in native species that are sympatric with introduced *T. s. elegans* (Hidalgo-Vila et al 2008; Silbernagel et al. 2013), although instances of turtle-to-human infections of *Salmonella* spp. have increased recently, including at least three multistate outbreaks since 2006 (Harris et al. 2010). It is well documented that commercial pet-trade rearing and shipping practices promote the spread of *Salmonella* spp. among hatchling turtles, despite increased efforts by farmers to raise "Salmonella-free" turtles since increased United States Food and Drug Administration regulations in 1975 (Harris et al. 2010). While the known instances of turtle-to-human *Salmonella* spp. infections are between people, mostly children and their pets (Harris et al. 2010), one can imagine how the release of these infected, pet-trade turtles into native ecosystems could result in isolated instances of potential consequence to human health either indirectly by spreading into sport fish through

the food web or directly through contact with surviving pathogens in sediments or the water column (Gaertner et al. 2008).

As far as directly impacting Michigan's native species, the turtle is well known to feed on amphibian larvae, but as to the effects of this dietary habit, little has been documented (Harding 1997; Ernst et al. 2009; Holman 2012). Accounts from the introduced populations of *T. s. elegans* in California and in Europe suggest *T. s. elegans'* relatively large size allows it to outcompete native species for basking habitat (Cadi and Joly 2004; Thomson et al. 2010). If this is the case in Michigan, *C. picta*, *Graptemys geographica*, and the state-listed (Special Concern) *Emyoidea blandingii*, Michigan's known-native basking turtles, would be most affected by non-native populations of *T. s. elegans*, although *G. geographica* is of similar size to *T. s. elegans* and their habitat overlap would be minimal (Harding 1997). One study, suggesting that *T. s. elegans* outcompetes *C. picta* for basking sites, does exist (McKenna 2001); however, the study utilizes a mesocosm design, making it difficult to infer how turtles would interact in a natural environment where they could simply swim to new basking locations if basking sites are not limited. Another study, utilizing fossil evidence, in southwest Indiana indicates that *T. s. elegans* replaced *E. blandingii* from the Pleistocene to the Holocene (Holman and Richards 1993). However, it is difficult to say if this result is due to competition between the two species as *E. blandingii* and *C. picta* share habitats today, and *C. picta* does not seem to contribute to *E. blandingii's* decline (Holman 2012).

However, *T. s. elegans* is larger than *C. picta* and is able to use its larger size to more effectively compete for basking sites with *E. blandingii* (Harding 1997). Despite these two accounts, if basking sites are the limiting factor in a particular area, simply installing

additional basking sites would likely fix the issue and would enhance habitat quality for turtles as well as other herpetofauna (Mifsud 2014).

Another recent study on effects of invasive populations of *T. s. elegans* on native turtles in Pennsylvania, United States suggests that juvenile *T. s. elegans* are able to outcompete other juvenile native species for food (Pearson et al. 2015). Such a finding could be a concern in habitats shared by juvenile *T. s. elegans* and *E. blandingii*, the latter of which is required to maintain relatively high rates of juvenile recruitment compared to other turtle species, along with high levels of adult recruitment, to maintain population stability (Congdon et al. 1993).

However, the experimental design chosen by Pearson et al. (2015) utilized mesocosms only differing in their assemblages of species and number of individuals. Because immature turtles do not travel far from where they hatched (Bodie and Semlitsch 2000), such a design could accurately reflect food competition in natural systems among juvenile turtles that hatched from the same area but fails to account for other selective forces, such as predation.

4.3 Future Research

Subsequent investigations to this study should focus on increasing the genetic resolution of sample data by utilizing more microsatellite markers, increasing the number of individuals sampled per population, and expanding the geographic range of sampling sites. While the utility of increasing the number of microsatellite markers was discussed above, due to the regional similarities in microsatellite variation observed in this study, obtaining samples from the species' range outside of the Great Lakes Region may indicate different genetic clustering patterns than the current dataset allows. Of particular interest would be obtaining samples from commercial turtle farms or purchasing turtles from local pet stores as genetic comparisons with such individuals would be considerably revealing to the origins of

Michigan's populations of *T. s. elegans*. Currently, breeders for the commercial pet-turtle trade are mostly based in Louisiana (Harris et al 2010). Collaboration with researchers in California, United States to obtain samples would be similarly useful as introductions of *T. s. elegans* have been well documented along with Sacramento River Basin.

If possible, there are limited number of populations within Michigan and Ohio that should be sampled as they hold the highest possibility of being native populations to the region. In Michigan, the Owasippi Boy Scout Reservation holds the earliest records of *T. s. elegans* in Michigan (Edgren 1943). During the course of my second field season, I visited this area and observed several *T. s. elegans* individuals but was unsuccessful at capturing them (see Figure 18 for precise location). Perhaps adopting a trapping technique using basking traps or nets with lead-lines would be more successful. In Ohio, two populations may still exist along the Scioto River, one in Pickaway County, and another in Ross County, well south of the NE Ohio population sampled in this study. These populations were observed as early as 1928, well before the popularity of the turtle trade (Wynn and Moody 2006). However, Conant (1951) noted that their current status is unknown.

4.4 Conclusions

This analysis of Michigan's populations of *T. s. elegans* has empirically indicated, through genetic methods, that at least some of Michigan's populations are composed of released pets from geographically distant sources, while others could be native relicts or could be introduced from geographically nearby populations. The former point is unsurprising as human-mediated introductions have always been suspected to be important for the spread of this species into Michigan (Edgren 1943; Harding 1997; Holman 2012; State of Michigan 2015*b*). Unfortunately, the limitations of this study, compounded with

relatively slow evolutionary rates of the taxon, proved discerning between the latter two possibilities, which should certainly be the focus of future investigations, to be difficult. Despite this shortcoming, the results of this study clearly show that Michigan's populations of *T. s. elegans* have a shared ancestry with populations from the nearby native range in Indiana and thus, do not disprove J. Alan Holman's (1994) hypothesis that some population's of *T. s. elegans* could have re-colonized Michigan during the warmer mid-Holocene climate and remained in refugia as other populations were driven out by cooling temperatures.

Currently, no substantial evidence exists to suggest that *T. s. elegans* is harming Michigan's native species or ecosystems (Harding 1997; USGS 2015); thus, managers will have to make their own decisions in regards to the management of this species. While the release of unwanted pets into ecosystems should not be tolerated, as they may act as vectors for parasites and diseases, especially *Salmonella* spp. (Hidalgo-Vila et al 2008; Harris et al 2010), perhaps the best approach is to treat already naturalized populations of *T. s. elegans* as if they were native populations and to seek to improve a sites overall habitat quality to reduce any competition the turtle may have with other native species.

Finally, if future evaluations of the indigenity of Michigan's populations of *T. s. elegans* are explored, they should seek to obtain samples of high genetic resolution through collecting large sample sizes, trapping over an extensive geographic range that includes potentially native populations from Muskegon County, Michigan and Ross and Pickaway Counties, Ohio and through to the species' southern United States, native range, and genotyping individuals with at least nine microsatellite markers. Researchers should also consider how land-cover can be used to identify between introduced and native localities.

TABLES

Table 1. Microsatellite primers used to assess population structure.

Locus ID	**Primer sequence (5'-3')**	**R. motif**	T_a	***A***	H_O	H_E	***P* Value**
TS243	GCAAAACCTGGAGATTTTCAA	$(ATAG)_{20}$	55	17	1.00	0.94	$0.97^{(BH)}$
TS260	TGCAAATGGAGTTGCAAGA	$(ATCT)_{16}$	55	17	0.91	0.93	$0.94^{(BH)}$
TS263	TGTGCACGGGAGTTGTATG	$(GATA)_{10}$	55	15	0.87	0.92	$0.90^{(BH)}$
TSE02	**TCAGACGTGGCCTTCCTCAATCAAACGCTGCTCCCT**	**$(AC)_5(AT)_7$**	**66**	**10**	**0.90**	**0.85**	**0.01**
TSE03	**TGGGCCACATGGCTAATCAAAGCACCAGCTCGTTCA**	**$(AC)_{19}$**	**66**	**7**	**0.97**	**0.84**	**0.00**
TSE06	ACCCTGACATCTGCCGACAGAGACCTTCCGCTGCTGC	$(AC)_{43}$	68	11	0.86	0.88	0.07
TSE09	ACGGAGGACACTGCTTGATTGCTTGGCTAAGGTGGA	$(AC)_6$	64	12	0.67	0.83	0.58
TSE10	**TTTCAAACACCCCTCCAGCACCTAGCACCATTTTCC**	**$(GT)_{12}N(GT)_6$**	**60**	**5**	**0.43**	**0.45**	**0.11**
TSE14	**CTGTCGGTGTCTTGTCCCTGAGCCCAGAAGTAGTGATG**	**$(CT)_{12}N(AC)_{19}$**	**64**	**9**	**0.76**	**0.82**	**0.29**
TSE21	GGAACCGCAAGGAGGAAAGCCATGCAACTGAGCACC	$(GT)_8$	66	6	0.77	0.77	0.36
TSE78	**AAGGCAGCACAAATGGAGACAGAATGTGGCAGGGAC**	**$(GT)_6(GA)_{14}$**	**66**	**7**	**0.79**	**0.67**	**0.21**
TSE80	**AGACAGTTGCTTCCTTGACATCCCCTTGCTTTTAGT**	**$(GT)_{11}$**	**60**	**6**	**0.43**	**0.61**	**0.02**

Bold font denotes successful markers used in analyses; R. motif abbreviates Repeat Motif; *T* annealing temperature in Celsius; *A* number of alleles; H_O observed heterozygosity; H_E expected heterzygosity; $^{(BH)}$ Benjamini & Hochberg false discovery rate.

Table 2. Raw genotype data for all amplified individuals. Missing Loci refers to the percentage of loci data that did not amplify.

		Loci					
Individual ID	**Missing Loci**	**TSE10**	**TSE14**	**TSE80**	**TSE2**	**TSE3**	**TSE78**
DL-01	16	000000	302302	280280	277277	228248	202218
DL-02	33	305305	340340	280282	285285	000000	000000
DL-03	0	289289	334334	280282	277277	228246	202202
DL-04	16	293297	294340	276280	279287	246246	000000
DL-05	0	309309	334340	280282	285325	248254	202202
DL-06	0	299299	322334	284284	285285	228254	200218
DL-07	0	299299	294360	278286	277287	228254	198202
DL-08	0	299299	294294	278286	285285	228254	198202
HR-01	0	299299	320326	284284	283301	224248	202202
HR-02	0	289295	318322	280280	285303	224252	198198
HR-03	0	299299	326334	282284	277295	228246	200200
HR-04	0	291291	334344	280284	295325	224224	198210
HR-05	0	297329	346360	276280	285285	236246	202202
HR-06	83	000000	298298	000000	000000	000000	000000
HR-07	50	295297	294294	280290	000000	000000	000000
HR-08	0	295299	322338	276280	325325	242246	202202
HR-09	0	297297	320346	278280	303325	246246	202206
HR-10	0	295299	302326	280280	285285	224228	198202
HR-11	0	291303	294294	278280	285285	252254	198218
HR-12	0	297299	302320	280284	293293	246262	210224
HR-13	0	289299	294334	280284	293293	224260	210224
HR-14	0	289299	294320	276280	285325	246262	198210
HR-15	0	289299	294334	276280	285325	246260	198198
HR-16	0	289299	294320	276280	285285	224224	198224
HR-17	0	297299	294320	280284	293325	224224	210224
HR-18	16	289299	294334	276280	000000	224224	198198
HR-19	33	289299	302320	276280	000000	224224	000000
HR-20	0	297299	294334	276280	285325	224262	198198
HL-01	83	000000	000000	000000	275295	000000	000000
HL-02	0	291293	294294	280282	285295	248250	202202
HL-03	0	299299	276340	276276	285325	232242	198202
HL-04	0	277321	310324	267274	325327	236246	202222
HL-05	0	295297	324340	278280	285285	242246	198202
HL-06	0	297299	294340	278282	285325	232246	216220
HL-07	0	253293	342342	284284	277299	228246	198222
HL-08	0	295299	320334	276280	285325	224252	198222
HL-09	0	293293	294294	276276	277311	224246	202220
HL-10	0	289295	294342	278282	277285	224250	222222
HL-11.1	0	297299	294342	284284	301307	228248	200202

Table 2 continued. Raw genotype data for all amplified individuals. Missing Loci refers to the percentage of loci data that did not amplify.

		Loci					
Individual ID	**Missing Loci**	**TSE10**	**TSE14**	**TSE80**	**TSE2**	**TSE3**	**TSE78**
HL-12.1	0	299299	294294	278284	277285	244250	222222
HL-13	0	297299	324324	276282	277277	246284	192222
HL-14	0	293299	294346	284290	285285	244246	198200
HL-16	0	293295	334362	276276	325325	224246	198202
HL-17	16	279289	000000	272284	293325	256284	198218
HL-18	0	299299	334356	280280	295325	232246	210220
HL-19.1	50	293297	320334	288288	000000	000000	000000
HL-20	0	283297	304318	276284	307325	246248	200222
HL-21	0	293299	294318	284284	325325	228284	198202
HL-22	0	293299	342358	284284	285295	232246	200222
HL-23	0	289297	326340	282284	277281	240248	198200
HL-24	0	291293	294334	276284	277285	228246	200222
HL-25	0	333335	294340	280280	277325	246250	202202
OH13-01	0	279287	306306	268280	277299	240248	198200
OH13-02	0	293299	312326	278280	285325	246268	214224
OH13-03	0	289297	320338	280290	215215	186192	348358
OH13-04	0	281281	294294	282284	297303	224248	204226
OH13-05	0	299299	312342	280282	321321	246250	202218
OH13-06	0	291291	312326	276290	293293	224228	198206
OH13-07	0	291295	294294	280286	197237	192194	356364
OH13-08	0	283283	292324	271280	287303	246266	202202
OH13-10	0	297299	318372	280290	325325	210210	200206
OH13-11	0	293295	294334	280280	215285	174186	348358
OH13-12	0	295297	326338	278280	273273	228268	222224
OH13-14	0	291299	334346	280282	283323	224228	202220
OH13-15	0	293293	312320	284286	285285	246250	198198
OH13-17.1	0	299299	294340	276280	285325	240246	178200
OH13-18	0	297299	320344	284284	285285	232252	198218
OH13-19	0	289293	328338	278278	277277	236246	198202
OH13-21	0	299299	338344	276276	285285	232246	178198
OH13-22	0	297297	320340	280280	285325	232242	178198
OH13-23	0	295299	326334	290290	277325	246250	198210
OH13-24	0	299309	322334	278280	277285	228254	198202
OH13-25	0	289295	330346	280280	283285	238254	202210
OH13-26	0	291291	294294	276280	285285	252254	198202
OH13-27.1	0	315315	328330	278286	277285	228238	198198
R-01	0	293297	312328	280304	275325	248252	200202
R-02	0	289291	284296	276286	287299	232236	204222
R-03	0	293299	334334	280280	283295	236244	200200

Table 2 continued. Raw genotype data for all amplified individuals. Missing Loci refers to the percentage of loci data that did not amplify.

Individual ID	Missing Loci	Loci					
		TSE10	TSE14	TSE80	TSE2	TSE3	TSE78
R-05	16	295295	000000	280280	285285	248260	198210
R-06	0	299327	326334	280280	285285	228274	198210
R-07	0	291293	330338	278278	285285	228252	198200
R-08	0	293293	320334	280282	285285	228252	198200
R-09	83	000000	000000	000000	000000	000000	202202
TL-01	16	299299	320334	280290	171171	000000	198198
TL-02	50	289295	294294	280288	000000	000000	000000
TL-03	0	289299	294294	280290	235235	296296	278278
TL-04	0	295309	326340	280284	235235	298298	280280
TL-05	0	289299	294334	288290	235235	304304	286286
TL-06	0	295299	294322	276282	235235	304304	286286
TL-07	0	299315	320326	282290	235235	300300	282282

Table 3. Locality and sample size information. Coordinates are expressed as the average latitude and longitude of sampling sites.

Site ID	Location	Coordinates	*n*
HR	Gallup Park, Washtenaw Co., MI	42.2706° N, 83.6835° W	20
RR	University of Michigan Dearborn & Huron Hills Golf Course, Wayne Co., MI	42.3095° N, 83.2579° W	7
TL	Timber Lakes Apartment Complex, Ingham Co., MI	42.7547° N, 84.5053° W	9
DL	Quaker Haven Camp, Koscuisko Co., IN	41.3670° N, 85.7604°	8
HL	Hovey Lake Fish and Wildlife Area, Posey Co., IN	37.8167° N, 87.9333° W	24
NEOH	Rocky River & Ohio and Erie Canal, Cuyahoga Co., OH & N. Chagrin, Lake Co., OH	41.4642° N, 81.6370° W	23

n sample size

Table 4. Pairwise Fst-values between sampled populations.

	DL	HR	HL	NEOH	RR
HR	0.05285323	-	-	-	-
HL	0.03263114	0.0399334	-	-	-
NEOH	0.01419878	0.0136847	0.0152284	-	-
RR	0.01368474	0.038206	0.0384216	0.003587086	-
TL	0.16076611	0.149954	0.1432491	0.106806862	0.1646867

Table 5. Comparison of individual population assignments among STRUCTURE, GENELAND, and BAPS. Values indicate proportional assignment from 0.00 to 1.00. Comparisons are considered to be congruent if assignment to the same population is equal to or greater than 0.5. No ID directly corresponds to numbers outside parentheses in Figure 8.

		STRUCTURE		GENELAND		BAPS		
No. ID	**Individual ID**	**Pop 1**	**Pop 2**	**Pop 1**	**Pop 2**	**Pop 1**	**Pop 2**	**Discrepant**
1	DL-01	0.945	0.055	1	0	1	0	-
2	DL-02	0.8399	0.1601	0.954	0.046	0	1	BAPS
3	DL-03	0.9591	0.0409	1	0	1	0	-
4	DL-04	0.8637	0.1363	0.948	0.052	1	0	-
5	DL-05	0.9332	0.0668	1	0	1	0	-
6	DL-06	0.9766	0.0234	1	0	1	0	-
7	DL-07	0.9038	0.0962	1	0	1	0	-
8	DL-08	0.9647	0.0353	1	0	1	0	-
9	HR-01	0.9728	0.0272	1	0	1	0	-
10	HR-02	0.9754	0.0246	1	0	1	0	-
11	HR-03	0.9905	0.0095	1	0	1	0	-
12	HR-04	0.9942	0.0058	1	0	1	0	-
13	HR-05	0.9558	0.0442	1	0	1	0	-
14	HR-06	0.7194	0.2806	0.104	0.896	0	1	STRUCTURE
15	HR-07	0.9519	0.0481	0.276	0.724	0	1	STRUCTURE
16	HR-08	0.9888	0.0112	1	0	1	0	-
17	HR-09	0.9725	0.0275	1	0	1	0	-
18	HR-10	0.9937	0.0063	1	0	1	0	
19	HR-11	0.9831	0.0169	1	0	1	0	-
20	HR-12	0.9876	0.0124	1	0	1	0	-
21	HR-13	0.9925	0.0075	0.988	0.012	1	0	-
22	HR-14	0.9931	0.0069	1	0	1	0	-
23	HR-15	0.9938	0.0062	1	0	1	0	-
24	HR-16	0.9946	0.0054	1	0	1	0	-
25	HR-17	0.9933	0.0067	0.996	0.004	1	0	-
26	HR-18	0.9942	0.0058	1	0	1	0	-
27	HR-19	0.9914	0.0086	0.996	0.004	1	0	-
28	HR-20	0.9949	0.0051	1	0	1	0	-
29	HL-01	0.4921	0.5079	0.568	0.432	1	0	STRUCTURE
30	HL-02	0.8839	0.1161	1	0	1	0	-
31	HL-03	0.8435	0.1565	1	0	1	0	-
32	HL-04	0.1403	0.8597	0.108	0.892	1	0	BAPS
33	HL-05	0.8461	0.1539	1	0	1	0	-
34	HL-06	0.7313	0.2687	1	0	1	0	-
35	HL-07	0.4707	0.5293	1	0	1	0	STRUCTURE
36	HL-08	0.9176	0.0824	1	0	1	0	-
37	HL-09	0.7964	0.2036	0.998	0.002	1	0	-

- denotes no discrepancy among analyses.

Table 5 continued. Comparison of individual population assignments among STRUCTURE, GENELAND, and BAPS. Values indicate proportional assignment from 0.00 to 1.00. Comparisons are considered to be congruent if assignment to the same population is equal to or greater than 0.5. No ID directly corresponds to numbers outside parentheses in Figure 8.

		STRUCTURE		GENELAND		BAPS		
No. ID	**Individual ID**	**Pop 1**	**Pop 2**	**Pop 1**	**Pop 2**	**Pop 1**	**Pop 2**	**Discrepant**
38	HL-10	0.6792	0.3208	1	0	1	0	-
39	HL-11	0.6118	0.3882	1	0	1	0	-
40	HL-12	0.7923	0.2077	1	0	1	0	-
41	HL-13	0.3335	0.6665	0.956	0.044	1	0	STRUCTURE
42	HL-14	0.8886	0.1114	1	0	1	0	-
43	HL-16	0.8619	0.1381	1	0	1	0	-
44	HL-17	0.3051	0.6949	0.98	0.02	1	0	STRUCTURE
45	HL-18	0.7749	0.2251	1	0	1	0	-
46	HL-19	0.5178	0.4822	0.334	0.666	0	1	STRUCTURE
47	HL-20	0.3395	0.6605	0.982	0.018	1	0	STRUCTURE
48	HL-21	0.8039	0.1961	1	0	1	0	-
49	HL-22	0.661	0.339	1	0	1	0	-
50	HL-23	0.4413	0.5587	0.998	0.002	1	0	STRUCTURE
51	HL-24	0.8884	0.1116	1	0	1	0	-
52	HL-25	0.6508	0.3492	0.992	0.008	1	0	-
53	OH13-01	0.0663	0.9337	0.76	0.24	1	0	STRUCTURE
54	OH13-02	0.5658	0.4342	1	0	1	0	-
55	OH13-03	0.0642	0.9358	0	1	0	1	-
56	OH13-04	0.091	0.909	0	1	1	0	BAPS
57	OH13-05	0.484	0.516	1	0	1	0	STRUCTURE
58	OH13-06	0.7691	0.2309	1	0	1	0	-
59	OH13-07	0.0648	0.9352	0	1	1	0	BAPS
60	OH13-08	0.0862	0.9138	0.36	0.64	1	0	BAPS
61	OH13-10	0.1561	0.8439	0.912	0.088	1	0	STRUCTURE
62	OH13-11	0.1941	0.8059	0.012	0.988	0	1	-
63	OH13-12	0.2375	0.7625	0.756	0.244	1	0	STRUCTURE
64	OH13-14	0.7552	0.2448	1	0	1	0	-
65	OH13-15	0.9049	0.0951	1	0	1	0	-
66	OH13-17.1	0.8601	0.1399	1	0	1	0	-
67	OH13-18	0.9281	0.0719	1	0	1	0	-
68	OH13-19	0.6466	0.3534	1	0	1	0	-
69	OH13-21	0.9258	0.0742	1	0	1	0	-
70	OH13-22	0.9095	0.0905	1	0	1	0	-
71	OH13-23	0.8269	0.1731	1	0	1	0	-
72	OH13-24	0.934	0.066	1	0	1	0	-
73	OH13-25	0.7408	0.2592	1	0	1	0	-

- denotes no discrepancy among analyses.

Table 5 continued. Comparison of individual population assignments among STRUCTURE, GENELAND, and BAPS. Values indicate proportional assignment from 0.00 to 1.00. Comparisons are considered to be congruent if assignment to the same population is equal to or greater than 0.5. No ID directly corresponds to numbers outside parentheses in Figure 8.

		STRUCTURE		GENELAND		BAPS		
No. ID	**Individual ID**	**Pop 1**	**Pop 2**	**Pop 1**	**Pop 2**	**Pop 1**	**Pop 2**	**Discrepancy**
74	OH13-26	0.9607	0.0393	1	0	1	0	-
75	OH13-27.1	0.3595	0.6405	0.972	0.028	1	0	STRUCTURE
76	R-01	0.4099	0.5901	1	0	1	0	STRUCTURE
77	R-02	0.0951	0.9049	0.018	0.982	1	0	BAPS
78	R-03	0.8875	0.1125	0.994	0.006	1	0	-
79	R-04	0.9669	0.0331	1	0	1	0	-
80	R-05	0.9507	0.0493	0.998	0.002	1	0	-
81	R-06	0.8411	0.1589	1	0	1	0	-
82	R-07	0.9387	0.0613	1	0	1	0	-
83	R-08	0.973	0.027	1	0	1	0	-
84	R-09	0.8478	0.1522	0.856	0.144	1	0	-
85	TL-01	0.3642	0.6358	0.974	0.026	1	0	STRUCTURE
86	TL-02	0.1537	0.8463	0.102	0.898	0	1	-
87	TL-03	0.0231	0.9769	0	1	0	1	-
88	TL-04	0.0224	0.9776	0	1	0	1	-
89	TL-05	0.0233	0.9767	0	1	0	1	-
90	TL-06	0.0267	0.9733	0	1	0	1	-
91	TL-07	0.0177	0.9823	0	1	0	1	-

- denotes no discrepancy among analyses.

FIGURES

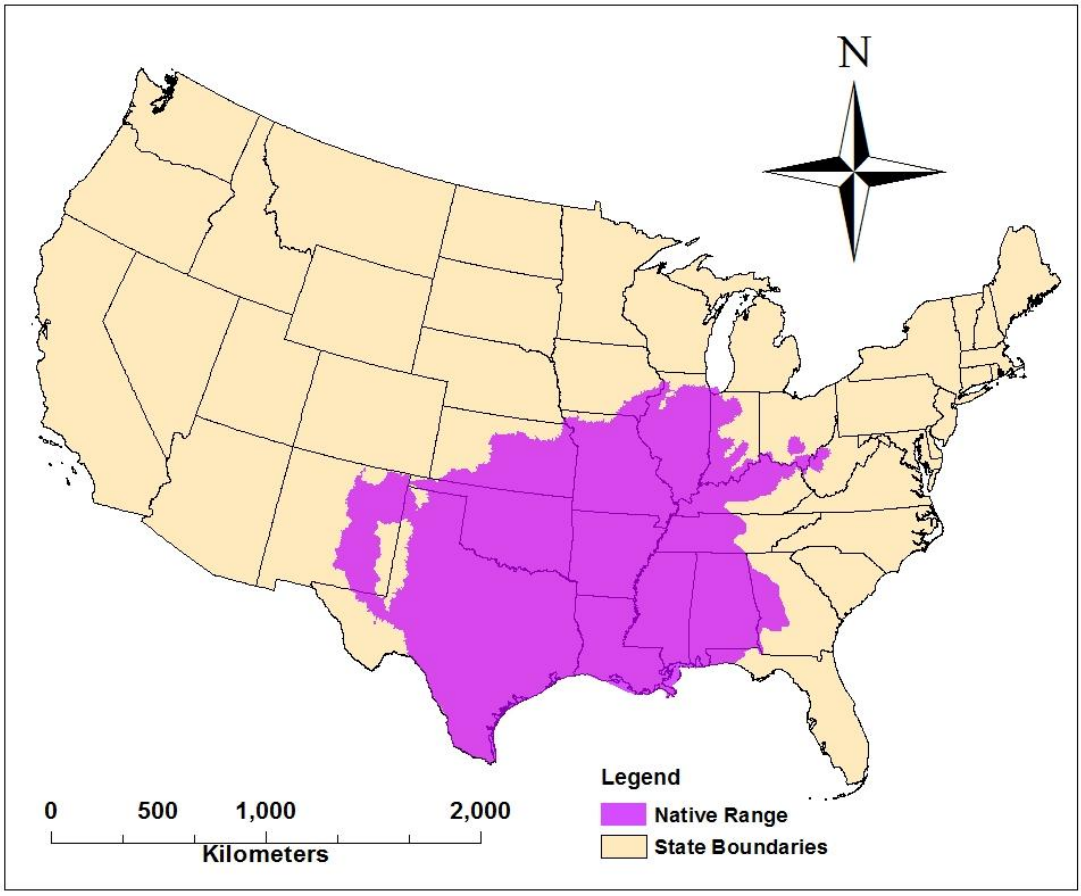

Figure 1. Currently defined native range of *Trachemys scripta elegans* in the United States. While Michigan is not officially considered to within this species' native range, the possibility has in consideration since the species was described to the state in 1943 (Edgren 1943).

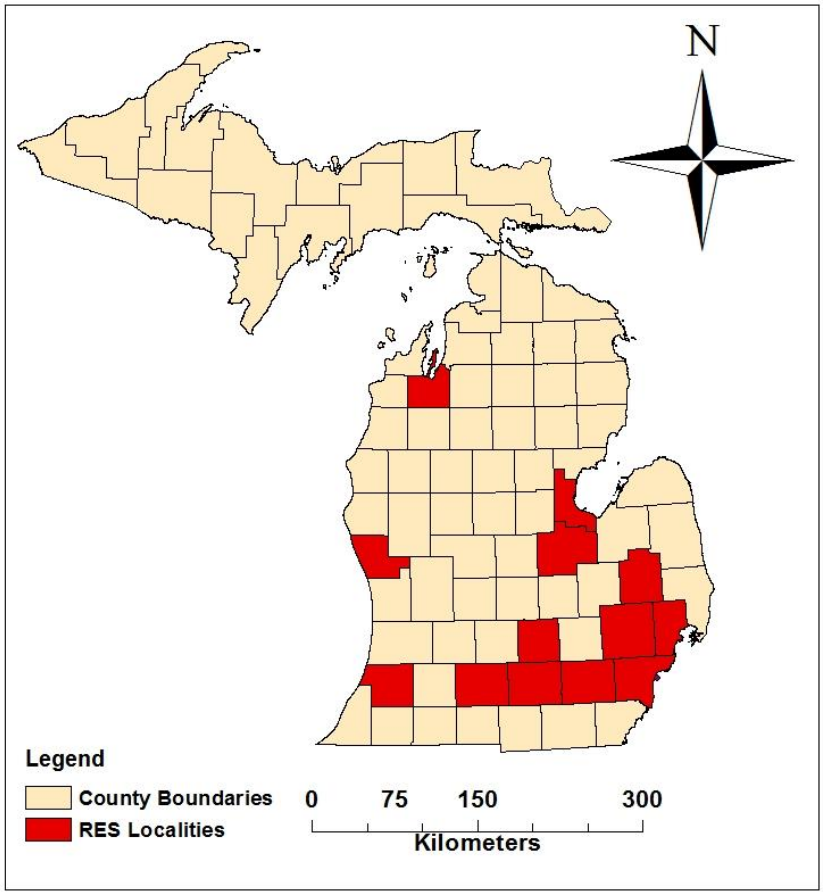

Figure 2. *Trachemys scripta elegans* observations in Michigan. RES Localities refers to observations of *T. s. elegans* at the county level. Data disseminated from Herpetological Resource and Management, LLC and Michigan Herpetological Atlas point lever observational accounts for Red-eared Sliders in Michigan.

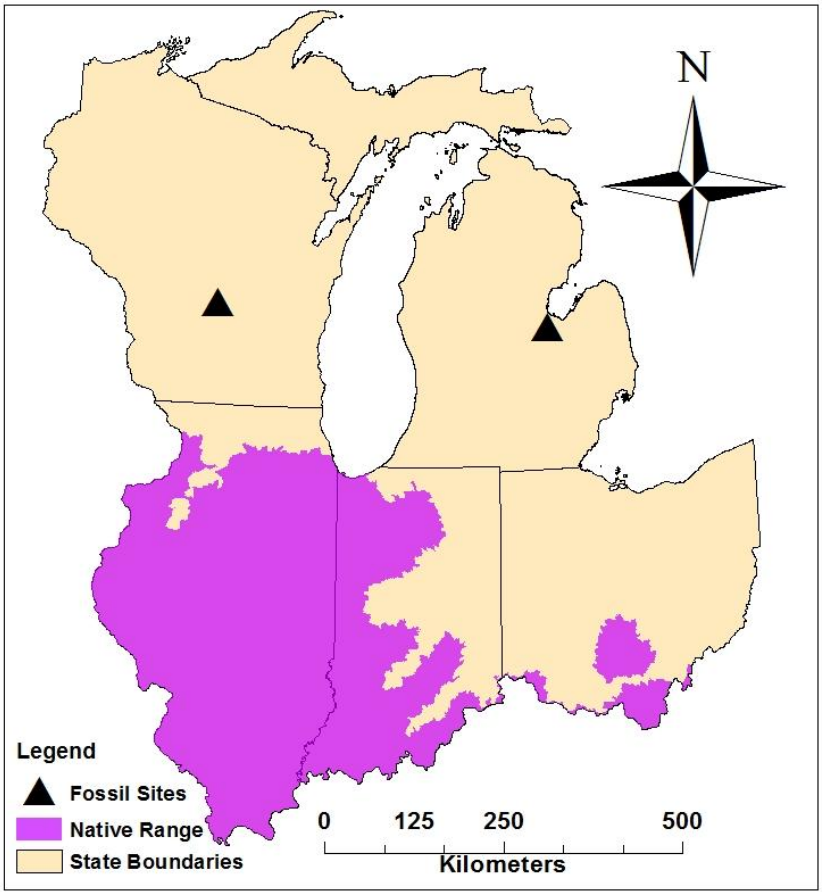

Figure 3. Proposed *Trachemys scripta elegans* fossil sites. Note that the fossil sites occur at similar latitudes. Fossil sites documented in Adler 1968.

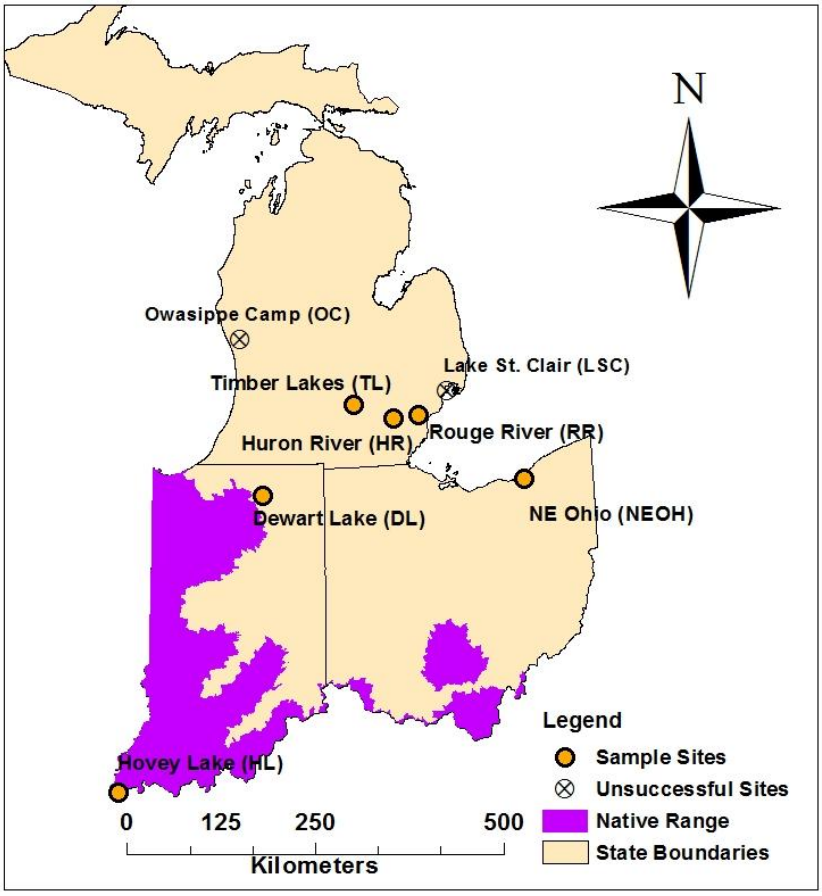

Figure 4. Sampling localities throughout Indiana, Michigan, and Ohio. The OC sampling site is considered unsuccessful as no *Trachemys scripta elegans* individuals were captured. The LSC sampling site is considered unsuccessful as DNA from the 4 individuals captured did not amplify.

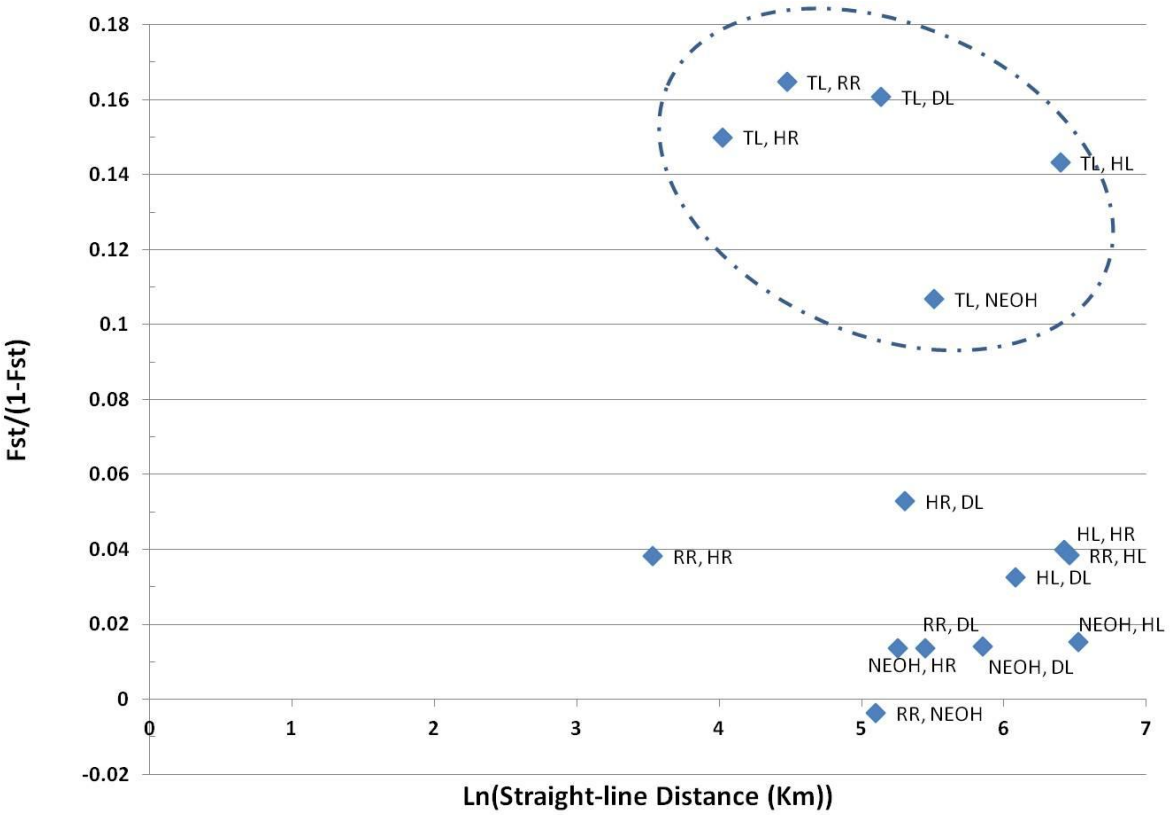

Figure 5. Isolation-by-distance model for sampled populations. No isolation-by-distance is observable ($r^2 = 0.088$). Note the grouping of all TL pairwise comparisons away from all other pairwise comparisons, indicating that the population constitutes a different genetic source.

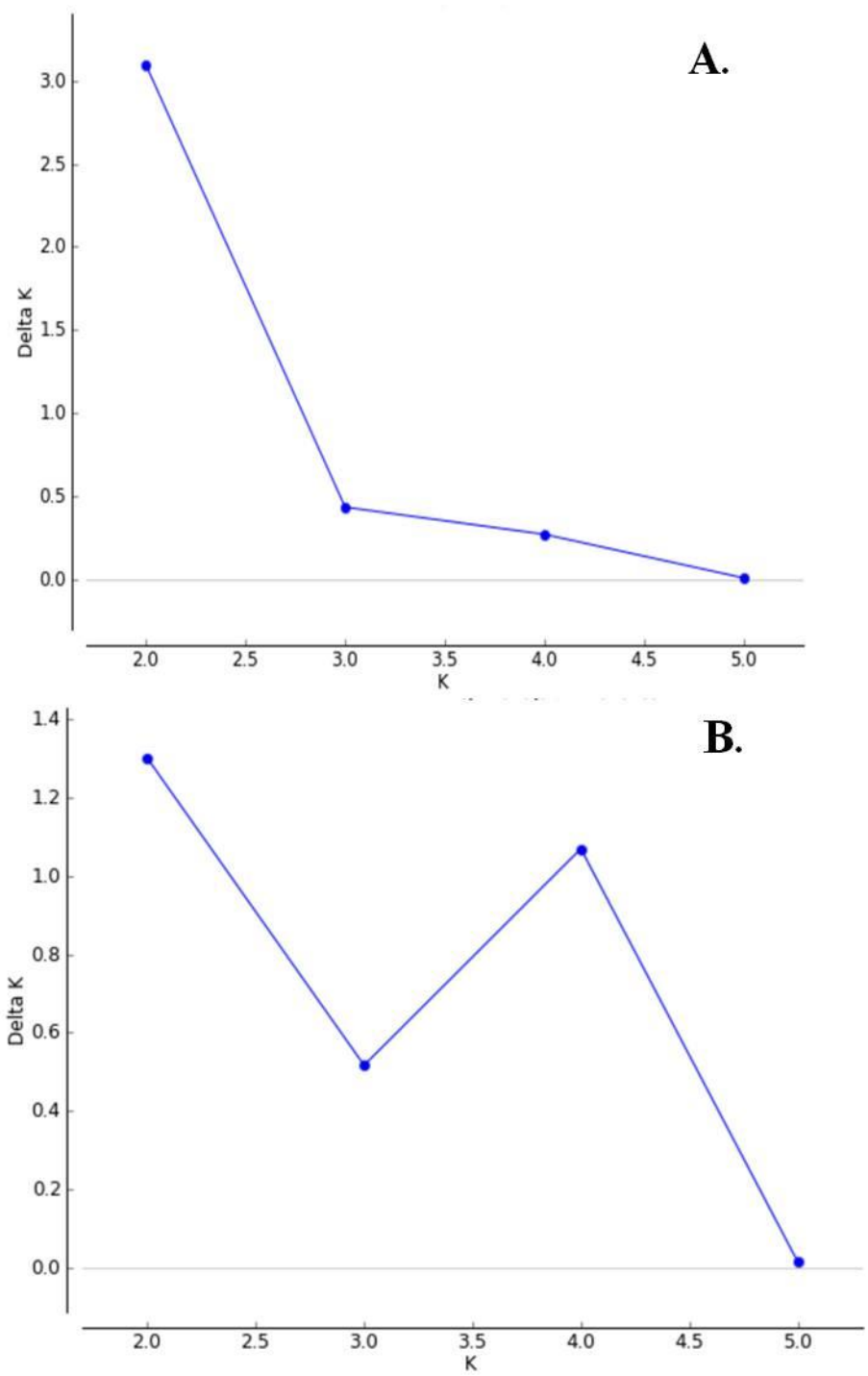

Figure 6. Evanno et al. (2005) post-hoc evaluation of STRUCTURE output. Note that the Evanno et al. (2005) method is not capable of assessing a $K = 1$ model. Evaluation was performed in STRUCTURE HARVESTER (Earl and vonHoldt 2012). (A.) The post-hoc evaluation for the LOCPRIOR parameter indicates that a $K = 2$ model is best supported by the dataset. (B.) The post-hoc evaluation for the parameter performed without priors indicates that either a $K = 2$ or $K = 4$ model are supported by the dataset.

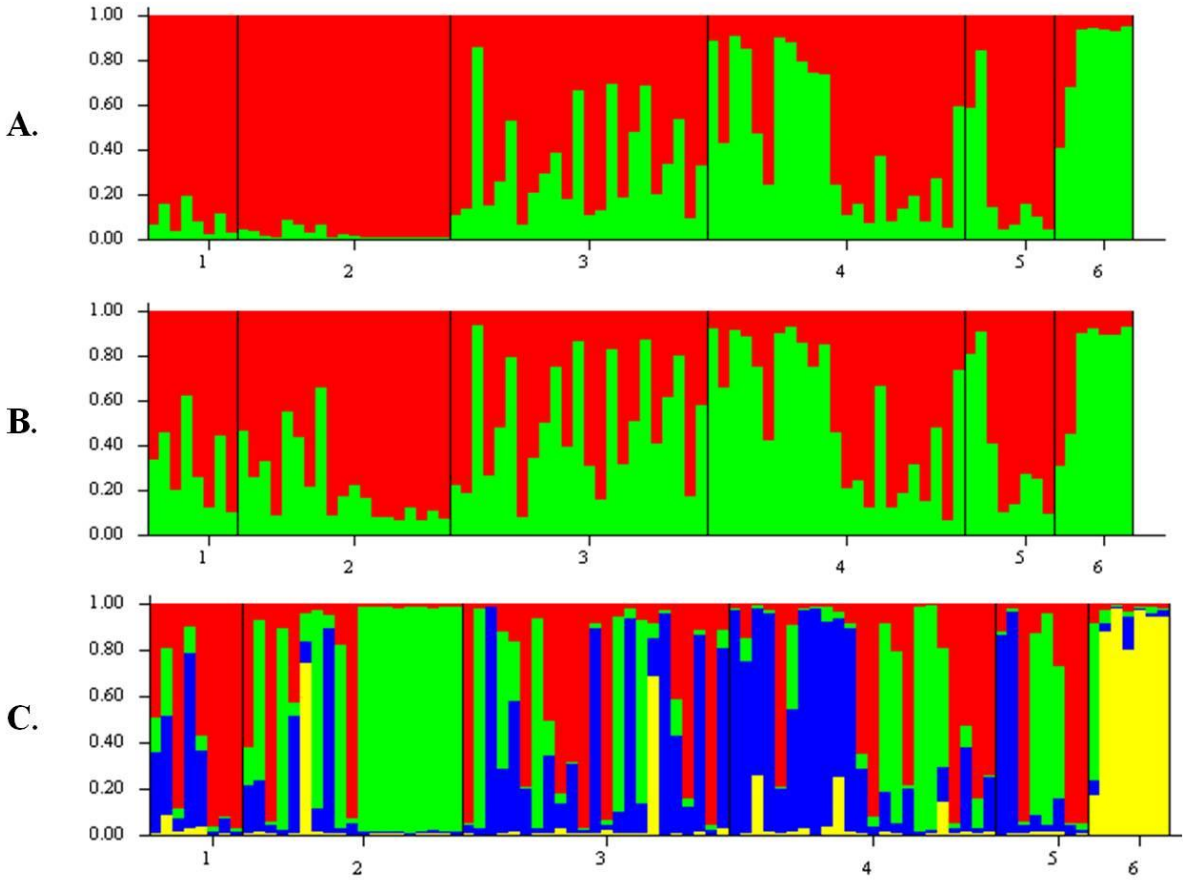

Figure 7. The estimated ancestry coefficients (*Q*) for *Trachemys scripta elegans* individuals from STRUCTURE output by population. Numbers below graphs correspond to populations: 1-DL, 2-HR, 3-HL, 4-NEOH, 5-RR, 6-TL. (A.) Output from the LOCPRIOR parameter set. A *K* =2 model, cluster 1 being red and cluster 2 being green, with apparent admixture is supported by the dataset. (B.) *K* = 2 output from the parameter set performed without the LOCPRIOR parameter set. Results are similar to Figure 7A. with cluster 1 being red and cluster 2 being green, with apparent admixture, being supported by the dataset. (C.) *K* = 4 output from the parameter set performed without the LOCPRIOR parameter set. Even though 4 genetic clusters are supported by the dataset, the TL population still forms a distinct cluster.

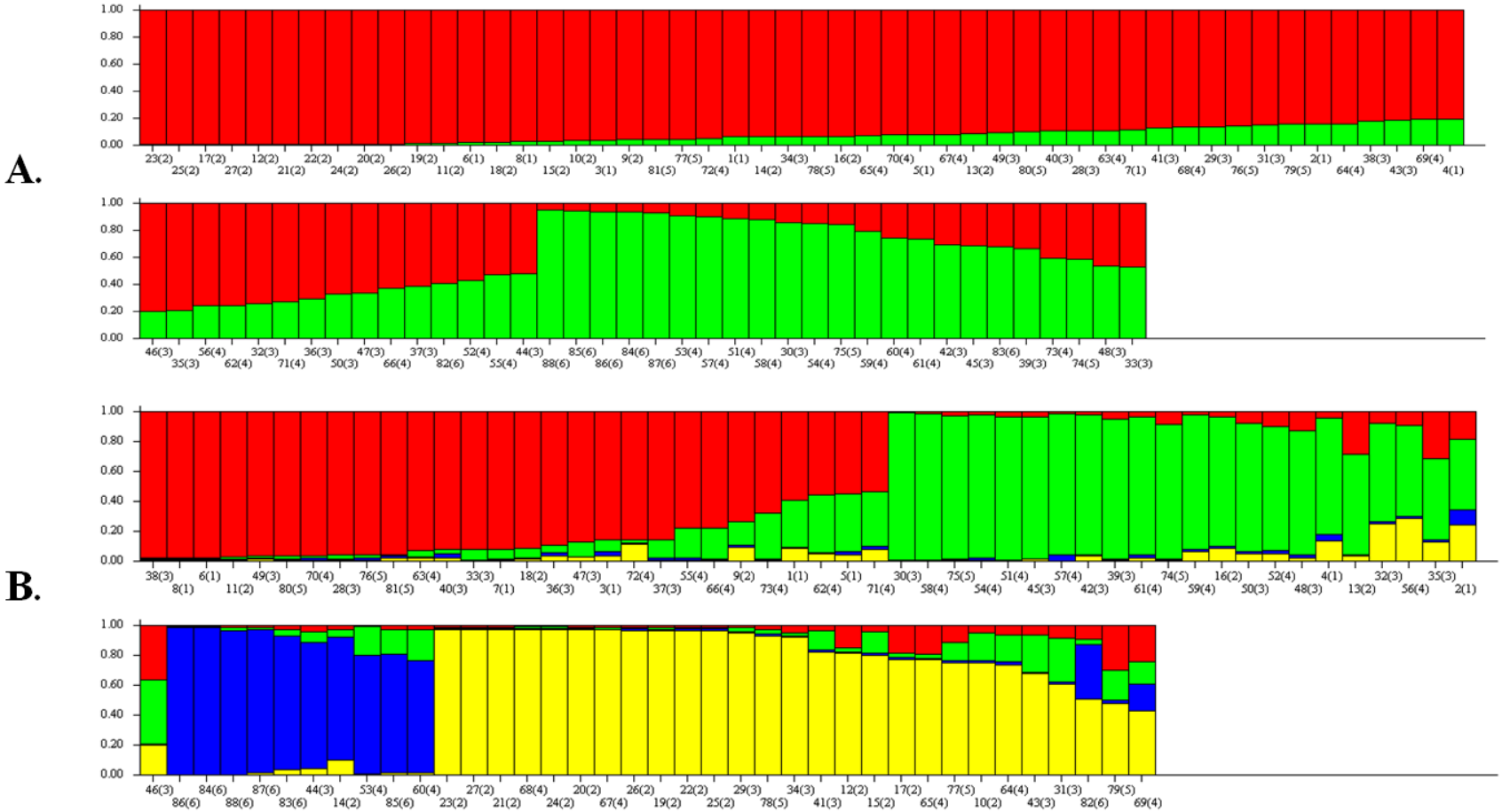

Figure 8. The estimated ancestry coefficients (*Q*) for *Trachemys scripta elegans* individuals from STRUCTURE output by individual. Numbers outside parentheses directly correspond to No. IDs in Table 5. Numbers with parentheses below output correspond to populations: 1-DL, 2-HR, 3-HL, 4-NEOH, 5-RR, 6-TL. (A.) Displays the individual assignments to each genetic population under the $K = 2$, LOCPRIOR model. The non-LOCPRIOR is similar, with only a single individual differing between clusters.. (B.) Displays the individual assignments to each genetic population under the $K = 4$, non-LOCPRIOR model.

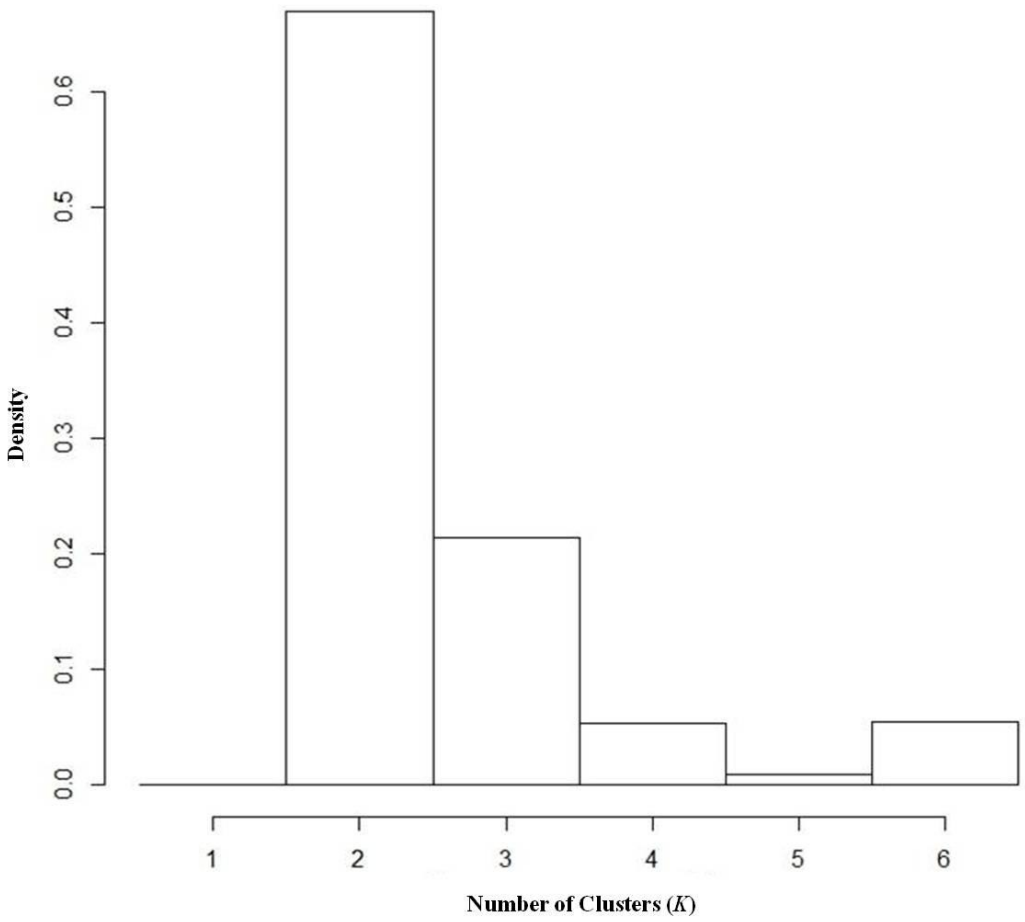

Figure 9. The number of populations (*K*) analysis from GENELAND output. The analysis indicates that a model of $K = 2$ is most strongly supported (>60% of MCMC iterations) with the next closest model being $K = 3$ (20% of MCMC iterations). The x-axis refers to the number of clusters (*K*) along all Markov-chains after a burnin of 500, while the y-axis refers to the proportional support of each *K* model.

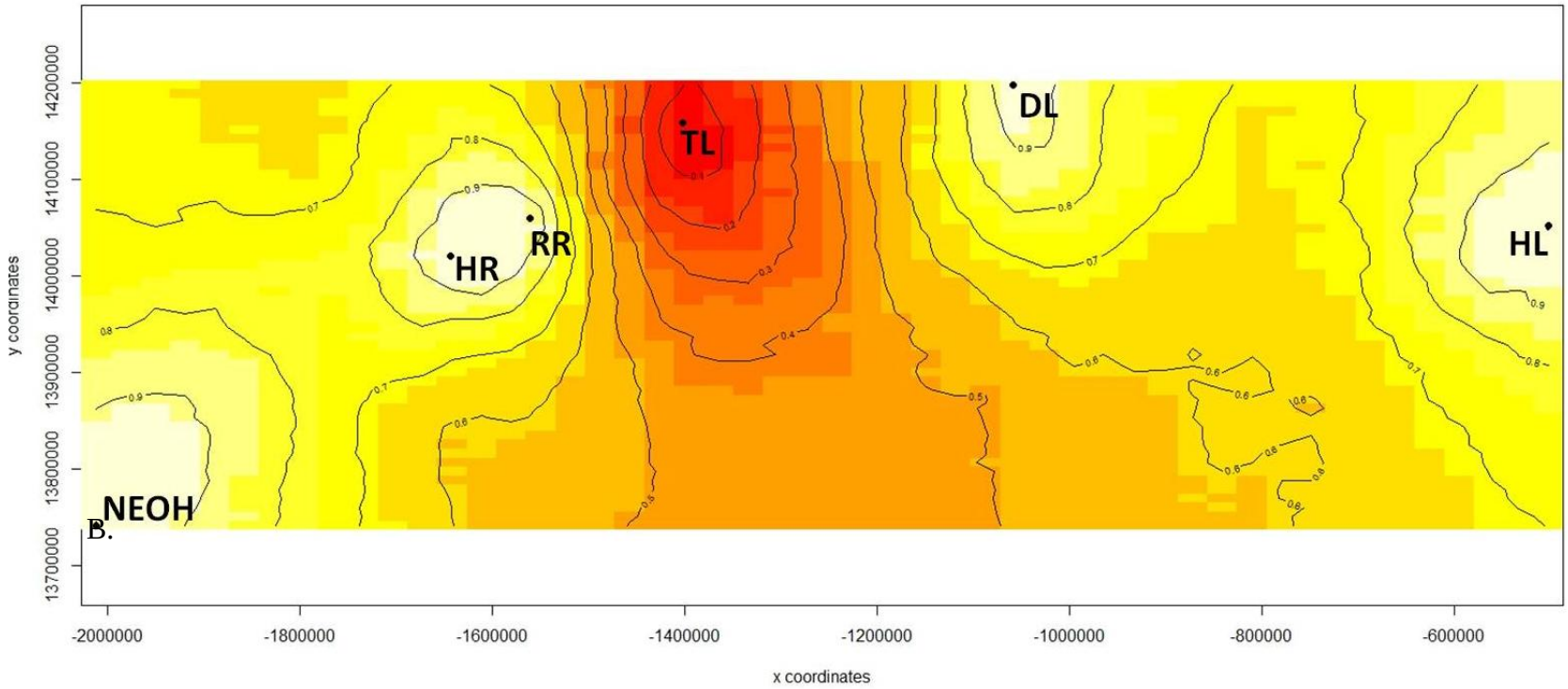

Figure 10. Map of probability of population membership analysis to cluster 1 under GENELAND spatial model. Topographic lines and coloration of shading indicates the degree to which a particular population belongs to a particular cluster. The analysis shows NEOH, HR, RR, TL, DL, and HL to belong to cluster 1 (90% of MCMC iterations for each population), while TL has <10% probability of belonging to cluster 1.

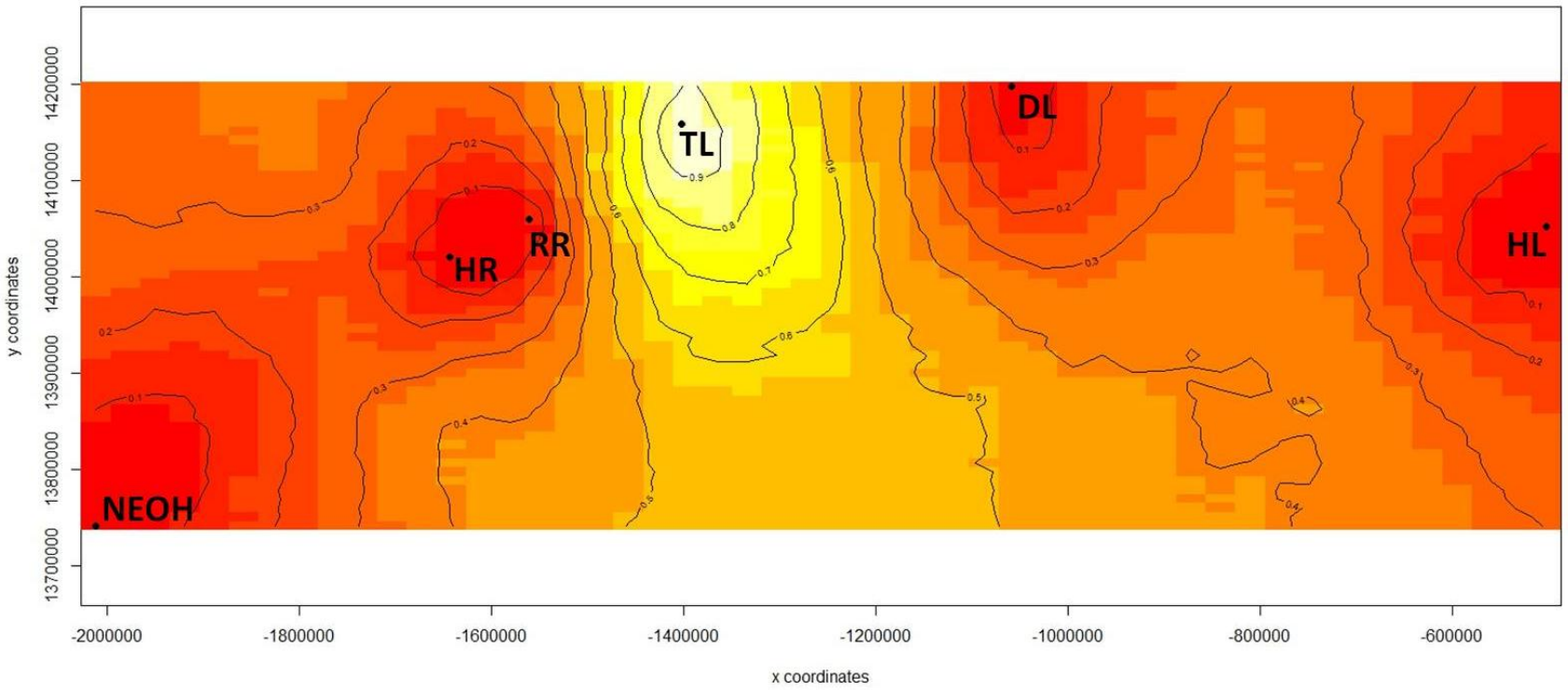

Figure 11. Map of probability of population membership analysis to cluster 2 under GENELAND spatial model. Topographic lines and coloration of shading indicates the degree to which a particular population belongs to a particular cluster. The analysis the TL population to belongs to cluster 2 (90% of MCMC iterations), while all other populations have a <10% probability of belonging to cluster 2.

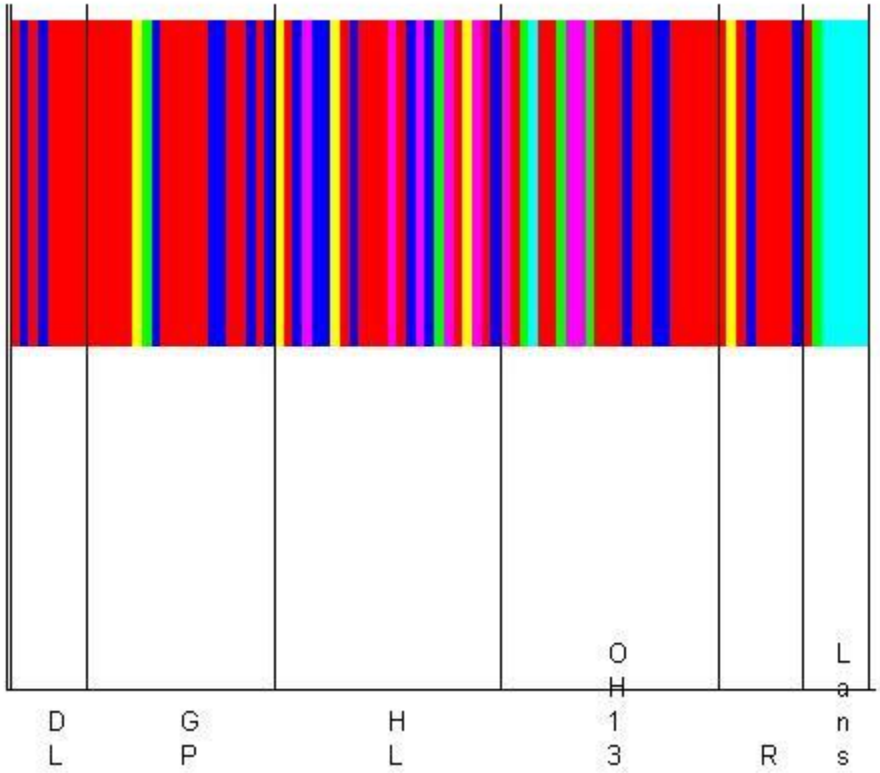

Figure 12. The clustering of individuals analysis from BAPS output. A $K = 6$ model was supported (100% of MCMC iterations) within the six geographic populations. A visual assessment of the figure indicates that the TL population forms one distinct cluster, while the remaining populations appear to form another with apparent admixture. Text below graph correspond to populations: DL-DL, GP-HR, HL-HL, OH13-NEOH, R-RR, Lans-TL. Each bar represents a single individual.

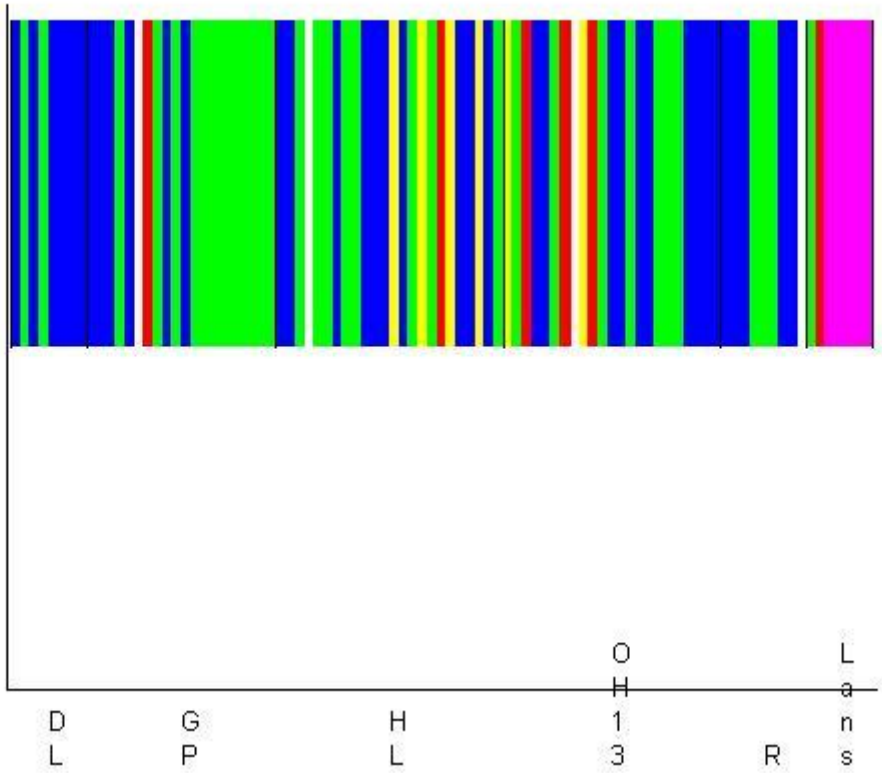

Figure 13. Admixture analysis based on clustering of individuals analysis from BAPS output. A $K = 5$ model was supported by the dataset. A visual assessment of the figure indicates that the TL population forms one distinct cluster, while the remaining populations appear to form another with apparent admixture. Text below graph correspond to populations: DL-DL, GP-HR, HL-HL, OH13-NEOH, R-RR, Lans-TL. Each bar represents a single individual.

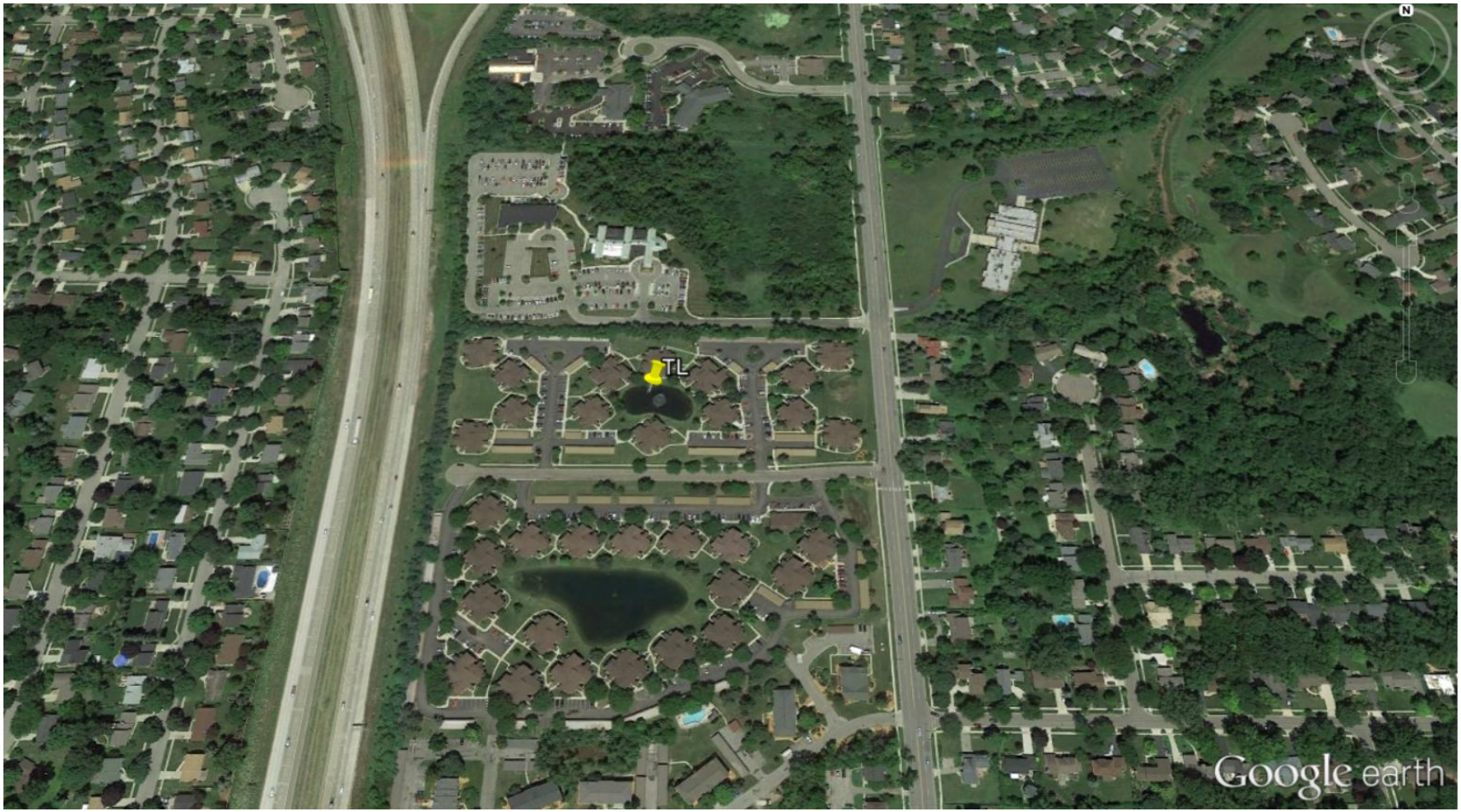

Figure 14. Aerial photograph of TL sampling site. The aerial photograph displays the urbanized land-cover and isolated landscape surrounding the TL population sampling site. Photograph was captured at approximately a 1.0 Km elevation from Google Earth (Google Inc. 2015*a*).

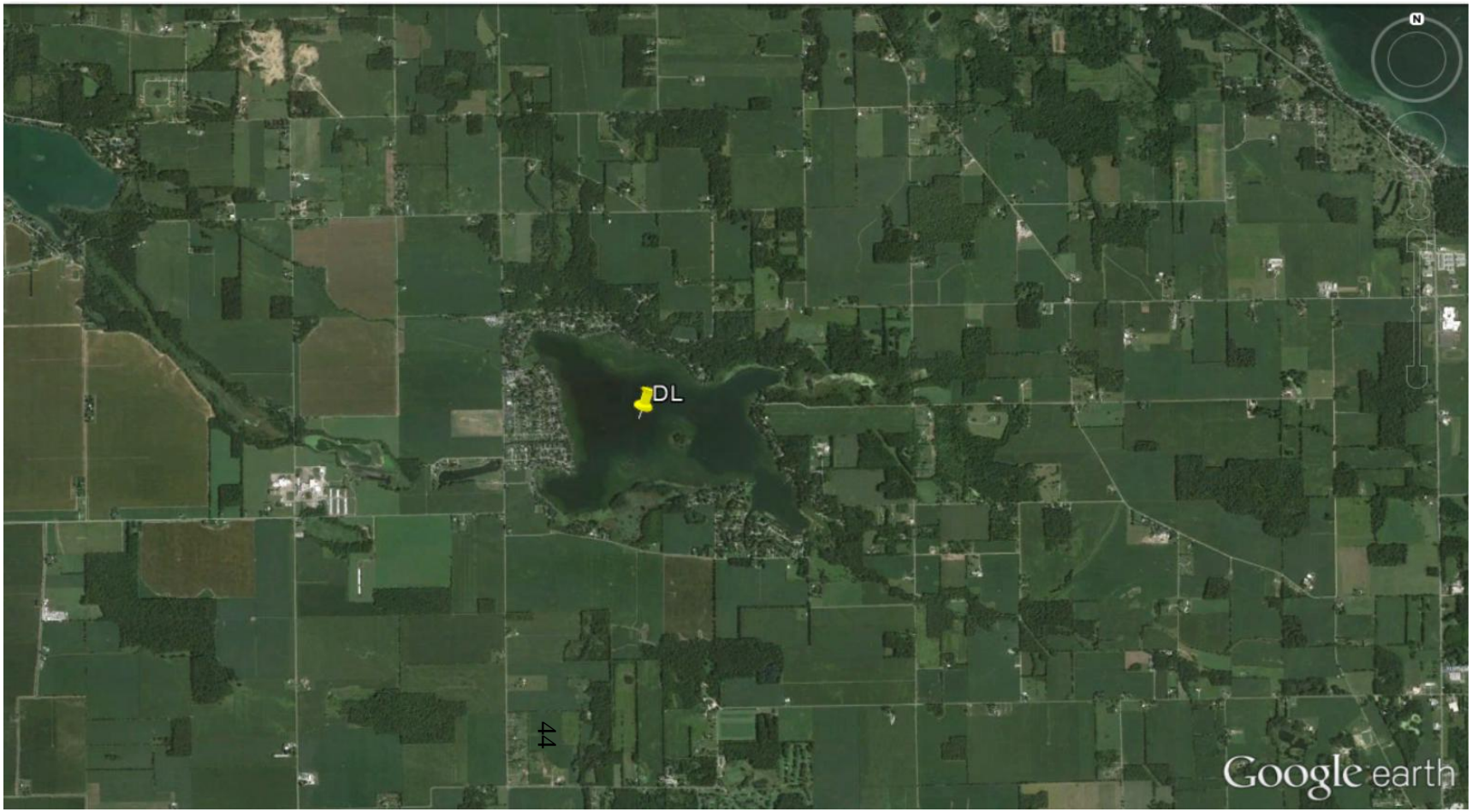

Figure 15. Aerial photograph of the DL sampling site. The aerial photograph displays the mixed agricultural/rural land-cover and isolated landscape surrounding the DL population. Photograph was captured at approximately a 1.0 Km elevation from Google Earth (Google Inc. 2015*b*).

Figure 16. Aerial photograph of HL sampling site. The aerial photograph displays the agricultural land-cover and riverine landscape surrounding the HL sampling site. Photograph was captured at approximately a 15.0 Km elevation from Google Earth (Google Inc. 2015*c*).

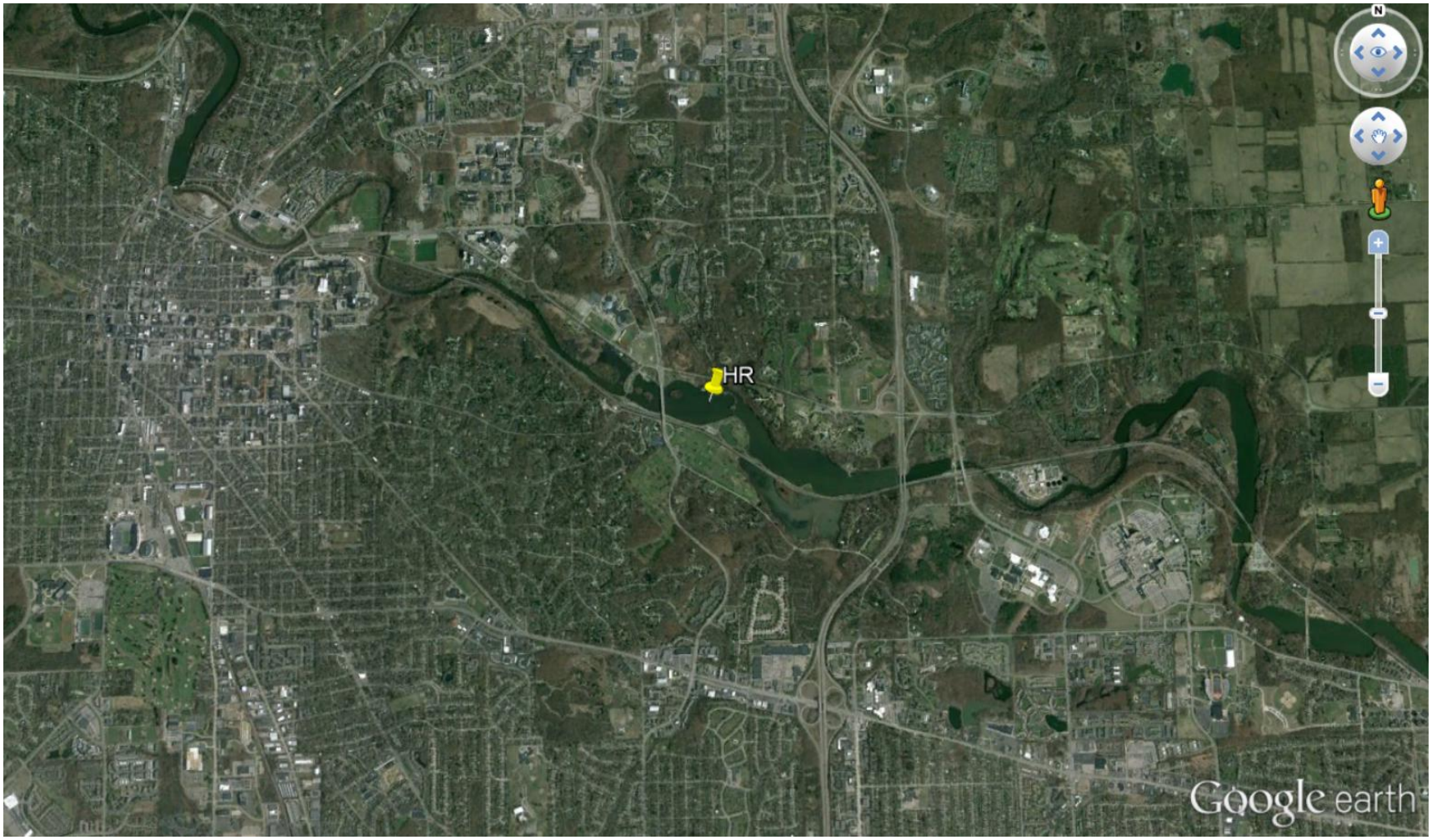

Figure 17. Aerial photograph of HR sampling site. The aerial photograph displays the mixed urban/natural area land-cover and riverine landscape surrounding the HR sampling site. The RR and NEOH sampling areas are qualitatively similar. Photograph was captured at approximately a 10.0 Km elevation from Google Earth (Google Inc. 2015*d*).

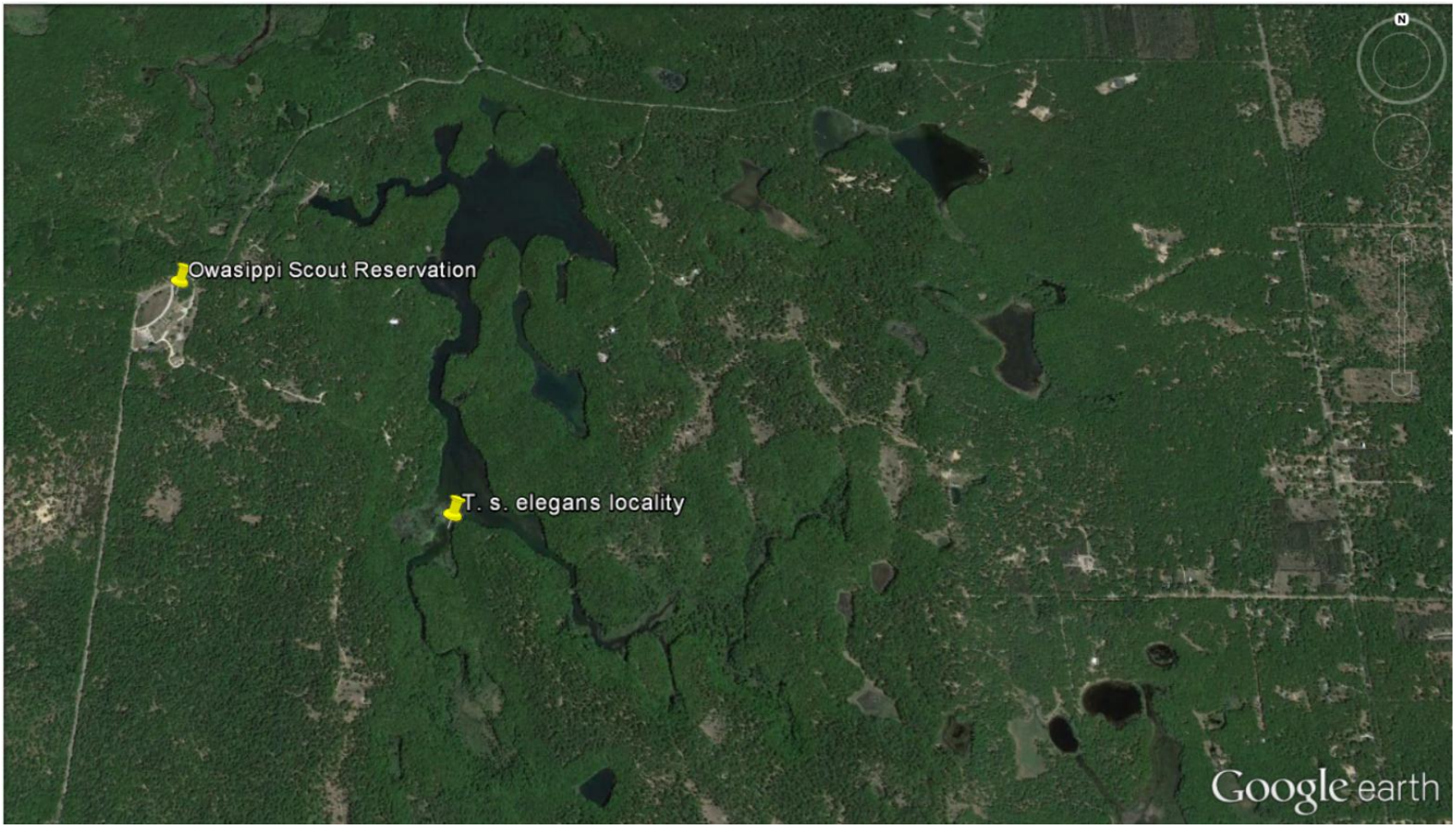

Figure 18. Aerial photograph of the *Trachemys scripta elegans* locality at the Owasippi Boy Scout Reservation in Muskegon County, Michigan. The locality lies within a water-lily, fringe marsh on the southwestern bend of Cleveland Lake. The other segments of Cleveland Lake, as well as several of the other lakes in the surrounding landscape, were thoroughly surveyed; however, no other *T. s. elegans* individuals were observed. Photograph was captured at approximately a 5.0 km elevation from Google Earth (Google Inc. 2015*e*).

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Goolge Inc. 2015*e*. Google earth V 7.1.1557. June 1, 2015. Owasippi Scout Reservation, Muskegon Co., MI. 43.4327° N 86.1962° W. Eye alt. 220 m. http://www.earth.google.com [September 19, 2015].

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APPENDIX

Addendum 1. Eastern Michigan University's Institutional Animal Care and Use Committee Approval Notification.

APPROVAL NOTIFICATION

EASTERN MICHIGAN UNIVERSITY
Office of Research Development
Institutional Animal Care and Use Committee
Starkweather Hall, 2nd Floor
487-3090

Date: **April 9, 2013**

To: **Katherine Greenwald**

From: Susan Campbell
ex officio

Eastern Michigan University's Institutional Animal Care and Use Committee (IACUC) has reviewed your *Application To Use Animals In Research or Instruction* referenced below. This project has been approved. The proposed animal use procedures are in compliance with University guidelines, State and Federal regulations and the standards of the "Guide for the Care and Use of Laboratory Animals."

When communicating with the IACUC office, please refer to the Approval Number referenced below. The appropriate Approval Number must accompany all requisitions for animals and pharmaceuticals. No research, testing or instructional use of vertebrate animals may be initiated without an Approval Number.

The Approval Period for your Approval Number is also indicated below. However, the United States Department of Agriculture (USDA) requires an annual review of applications to use animals. Therefore, each year of this application, prior to the anniversary of its approval date, you will receive a short Annual Review Form. Your continued animal use approval is contingent upon the completion and return of this form. You will also be notified prior to the expiration of the Approval Period so that any renewal application can be prepared, submitted and reviewed in a timely manner and an interruption in the approval status of this project avoided.

Committee approval must be obtained prior to changes in procedures that could affect the humane use of animals. If changes are contemplated, a revised Animal Use Form (with the changes highlighted) must be submitted and approved prior to initiation of the modified procedures. Contact this office for more information.

Title: **Elucidating the Status of Michigan's Red-eared Slider, Trachemys scripta elegans**

Approval Period: **04/10/2013 to 04/09/2016**

IACUC Approval No.: **2013-057**

cc: Committee