

CONSERVATION PLAN *for the* WOOD TURTLE *in the* NORTHEASTERN UNITED STATES

Maine • New Hampshire • Vermont • Massachusetts • Connecticut • Rhode Island • New York • New Jersey • Pennsylvania • Delaware • Maryland • DC • Virginia • West Virginia



Photos by Mike Jones



Final Report

submitted to the Massachusetts Division of Fisheries and Wildlife,
the Northeast Association of Fish and Wildlife Agencies,
and the U.S. Fish and Wildlife Service



for the Competitive State Wildlife Grant:
Conservation Planning and Implementation for the Wood Turtle (*Glyptemys insculpta*)
and Associated Riparian Species of Greatest Conservation Need from Maine to Virginia

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Editors' Note

The partnerships that led to the development of this Conservation Plan extend back to August 2009, when the Northeast Wood Turtle Working Group first met in conjunction with the Northeast Partners in Amphibian and Reptile Conservation (NEPARC) meeting at Watkins Glen, New York.

The biology of the Wood Turtle precludes easy management applications. Successful conservation of representative and functional Wood Turtle populations will require the focused protection of optimal habitat—the relatively rare places where overwintering, nesting, foraging, and basking habitat are juxtaposed in relative isolation from development and regular human activity—and an emphasis on protecting the functional landscapes that appear, based on empirical evidence, to support self-sustaining Wood Turtle populations through dynamic fluvial processes. These uniquely functional landscapes form the core focus of this plan. To improve our chance of protecting these rare landscapes effectively, it was necessary to identify and confirm them in the field, which was only made possible through contributions of hundreds of collaborators since 2012. For example, 107 different people led nearly 400 surveyors in a total of 2,141 standardized field surveys in all thirteen northeastern States and the District of Columbia, an average of 20 surveys per lead observer, for a total of 4,611 Wood Turtle detections—a substantial investment of time and energy that will be difficult to replicate fully in future years without a federal or regional grant. About one-half of these surveys were funded directly by the CSWG. The other surveys were supported through state contracts, volunteer contributions, and a Regional Conservation Needs (RCN) award. It is important to note that in many cases, surveys were undertaken by volunteers who wanted to contribute in some way to the conservation of this unusual, iconic, and declining indicator of unfragmented river systems.

Conservation Plan for the Wood Turtle in the Northeastern United States provides a spatial framework and management outline for the prioritization and conservation of regionally important Wood Turtle streams and associated upland riparian areas, upland landscapes, and corridors. It is our hope that the new population information gathered through this process, and the prioritization framework that forms the basis of our Conservation Area Network, will provide guidance at the state level to accelerate appropriate land conservation and management efforts. However, throughout the conservation plan we emphasize the sensitivity of this particular species to human activity, and as a target of collectors. If regionally significant streams and Focal Core Areas become generally known, or are made public or widely distributed, the threat posed by increased awareness is likely to outweigh any practical benefit. So in addition to the range of challenges that accompany any land conservation effort in the Northeastern United States (large areas of private property, legacies of industrial and agricultural development,

expensive upland real estate, extreme fragmentation associated with urban, exurban, and suburban sprawl) we must also pursue the actions in this Plan with a keen awareness of the sensitivity of the regionally significant sites and continue to think creatively about how to share the spatial information about conservation priorities without further compromising priority populations, and hope that the current trends—of increasing commercial interest—diminish.

Executive Summary

The Wood Turtle (*Glyptemys insculpta*) has experienced dramatic population declines as a result of habitat loss, road mortality, detrimental anthropogenic land-use practices, and numerous other factors over the past century. At present, the Wood Turtle is listed as Endangered by the International Union for the Conservation of Nature (IUCN), as “G3 - Vulnerable” by NatureServe, and as Threatened under the Species at Risk Act (SARA) in Canada. The Wood Turtle is also listed in the State Wildlife Action Plans of all 13 states in the northeastern United States as a Species of Greatest Conservation Need (SGCN). As of this report (December 2018), the United States Fish and Wildlife Service (USFWS) is considering a petition by the Center for Biological Diversity, submitted in 2012, to list the Wood Turtle as Threatened under the Endangered Species Act. An earlier petition in 1994 was rejected.

In 2012, a collaborative project was funded through the Regional Conservation Needs program aimed at assessing the status of the Wood Turtle in the northeastern United States and addressing the paucity of population-level data throughout the species range. The result of this effort was a document entitled *Status and Conservation of the Wood Turtle in the Northeastern United States*, completed in 2015. Among other findings, this status assessment presented evidence that the species had undergone widespread population declines as well as pervasive range contraction and was in need of a robust, region-wide effort to identify and conserve representative populations of the species in the northeastern United States.

Conservation Plan for the Wood Turtle in the Northeastern United States represents the cumulative product of a multi-year, proactive effort among northeastern State Wildlife Agencies, and their partners, to articulate a strategic action plan to protect populations of Wood Turtles throughout the Northeast. This document is composed of six major components: (1) a standardized population assessment (Part II); (2) an analysis of regional population genetics (Part III); (3) a spatially-explicit, empirically-derived regional Conservation Area Network (Part IV); (4) a multi-scale Conservation Action Plan for priority sites (Part V); (5) an implementation framework modeled upon other regional conservation planning efforts including those for Blanding’s Turtle (Part V); (6) technical assistance materials for partners and key landowners (Appendices located at northeastturtles.org).

Part II. Regional Population Assessment

The overarching objectives of our regional population assessment were to (1) expand the network of standardized Wood Turtle study sites throughout the Northeast Region; (2) identify factors influencing Wood Turtle abundance at the regional scale; (3) quantify factors impacting Wood Turtle detection rates;

(4) identify regionally significant Wood Turtle populations; (5) monitor intensively studied sites to estimate population size, density, and demographic structure, and establish a baseline by which to evaluate population trends; and (6) conduct population assessments within data-deficient areas throughout the Northeast.

From 2012 to 2017, nearly 400 federal, state, university, and non-governmental biologists, students, and volunteers participated in 2,141 field surveys across 464 different stream segments throughout the 13 northeastern states and the District of Columbia. A total of 4,611 Wood Turtle detections were recorded across all surveys, averaging 2.15 turtles per survey ($sd = 3.81$). Approximately 50% of surveys were conducted in spring, 42% were conducted in fall, and 8% were conducted during the June-centered nesting period. Regionwide, catch per unit effort for the independent, lead observer during surveys averaged 1.52 turtles per survey during the spring and 1.2 turtles per survey during the fall. This seasonal pattern was consistent throughout much of the Northeast, except the southernmost states (MD, VA, and WV) where catch per unit effort for the lead observer was greater in fall than spring. Approximately 42% (894) of surveys conducted yielded zero turtles, and surveyors failed to detect a single turtle (across all surveys conducted) at 40% of sites regionwide.

Juveniles and subadults younger 14 years and under accounted for 16% of regional Wood Turtle detections. At the majority of sites, fewer than 25% juveniles were detected during surveys. Overall, the percent of detections that were juvenile did not increase with catch per unit effort. The average male:female ratio was 1.4:1 for sites with at least one female and >6 detections, but sex ratios varied considerably across states. The male:female ratio in the fall season was more than double that of spring (2.87:1 and 1.31:1, respectively) when all surveys with >6 detections were considered.

Land cover variables at the scale of 5500 m from the stream appeared in top N-mixture models of relative abundance, with percent undeveloped land (+), traffic rate (-), and percent agricultural cover (-) appearing as strong predictors across all datasets analyzed. These findings provide further support for the understanding that large-scale, landscape-level patterns play an important role in predicting Wood Turtle abundance and occurrence throughout the range and that broadscale planning and habitat protection is critical to the conservation of this species. The site-level 300-m scale was also an important predictive scale, with Wood Turtle abundance displaying a unimodal relationship with agricultural cover within 300 m, peaking at relatively low levels of agriculture (approx. 15%) and declining thereafter. This pattern should be interpreted cautiously because Wood Turtles can be attracted to agricultural conditions despite the negative impacts exerted on the population by machinery—a fact well established by research in Québec, Nova Scotia, New Hampshire, Massachusetts, and elsewhere.

Relative population density (not absolute population density) was estimated for 80 stream segments using Capture-Mark-Recapture (CMR) models across nine of 13 northeastern states: Maine, New Hampshire, Vermont, Massachusetts, Connecticut, New Jersey, Pennsylvania, Virginia, and West Virginia. It is important to note that these streams were selected nonrandomly by experts for intensive survey for a range of reasons and therefore likely represent at least some of the higher density populations in the region. Relative population density estimates varied significantly among segments with closed-population estimates (turtles pooled within seasons) ranging from 4–211 turtles/km (mean = 47.5 turtles, sd = 43.5). Twenty-five of 80 sites (32%) had pooled closed-population estimates >50 turtles/km and 8 of 80 sites (10%) had estimates >100 turtles/km. These analyses suggest that, even among sites that were selected by experts, large populations consisting of high-density stream segments appear to be rare within the northeastern United States. This observed pattern also highlights the clear tendency for Wood Turtles to reach high densities only within ideal landscape and microhabitat contexts.

Part III. Population Genetics of the Wood Turtle

The individual objectives of this regional Wood Turtle population genetics assessment were to: (1) describe and compare population genetic diversity (heterozygosity, allelic richness, private alleles); (2) identify the most likely number of population groups in the northeastern United States; (3) measure relative isolation by distance comparing genetic and geographic distances; (4) estimate contemporary migration rates; and (5) test population genetic assignment methods to identify the origin of confiscations from the illegal animal trade.

A total of 1,895 Wood Turtle tissue samples contributed to the regional population genetics analysis, with the large majority of samples collected in 2015 and 2016. Tissue samples were genotyped at 16 microsatellite markers for 1,244 individuals. Sample sizes ranged from 5 to 50 individuals (average $n=17.4$) collected from 62 sites. Heterozygosity and allelic richness did not suggest a loss of genetic diversity in any populations. The age-based test also did not indicate differences in genetic diversity across generations, but power to detect this trend was limited. A Bayesian genetic clustering analysis indicated that the Northeast Region likely consists of four major population groups (genetic clusters) representing northern Maine, the Potomac River, coastal Massachusetts, and New Jersey/New York populations, with sites in Pennsylvania and New Hampshire displaying admixture with the neighboring groups. Interpretation of rangewide findings led the authors to recommend that the Connecticut, Merrimack, and Kennebec basins should be treated as a fifth management unit. Allele frequency exact tests identified significant pairwise differences between 91% of the sites, indicating further fine-scale genetic structuring within clusters throughout the range.

Tests for full siblings indicated a maximum distance between family members of approx. 50 km, which is in line with several other studies that have failed to detect significant genetic differences among sites <50 km. This suggests that subpopulation boundaries may be as large as approx. 100 km. Pairwise F_{ST} and allele frequency tests indicated that the Wood Turtle is maintaining gene flow across drainage boundaries, emphasizing the importance of considering terrestrial connectivity within conservation efforts for the species. Euclidean distance provided a stronger correlation with F_{ST} than stream distances for two major population groups, further indicating that overland corridors are more likely connecting sites than pathways along the stream corridor (particularly in Potomoc populations).

The rate of successful assignment of individuals to their respective site of capture was low with only 52% placed correctly (only slightly higher than random chance). Relatively high assignment success (>75% correct) was achieved for coastal Massachusetts, northern Maine, the Potomac, and a single New Hampshire site. Overall, the findings of this study suggest that the application of these methods for repatriation of confiscated Wood Turtles is limited. A transition to next generation genomics would likely improve population genetic assignments.

Results from these analyses should be interpreted cautiously as the current Wood Turtle genome may not adequately reflect current patterns and processes in the landscape. Specifically, given the longevity and generation time (~50 years) of this species, contemporary genetic signals may reflect conditions that existed approximately 100 years ago. Landscapes have changed substantially in the study area during this period, and processes such as fragmentation may not be detected for several more Wood Turtle generations.

Part IV. A Conservation Area Network for the Wood Turtle in the Northeastern United States

This section of the Conservation Plan is made up of three distinct components: (1) an overview of fundamental concepts and theory underpinning many contemporary conservation planning efforts; (2) a brief review of major single-species Conservation Area Networks in existence; and (3) a complete description of the Conservation Area Network that was developed for the Wood Turtle in the northeastern United States.

The Northeast Wood Turtle Conservation Area Network (CAN) represents the core concept underpinning the Conservation Plan. Our fundamental objective in developing this CAN was to protect the evolutionary potential of the species by ensuring the persistence of functional, ecologically viable, and representative populations of Wood Turtles throughout the Northeast Region. Due to the evident expense of meaningful conservation work for this species, the Northeast Wood Turtle CAN—a collection of sites

with the greatest conservation value throughout the region—serves as a means by which to maximize the effectiveness of limited available resources.

The Northeast Wood Turtle CAN is founded on fundamental concepts within the field of conservation planning, and its design was based not only on the extent and quality of Wood Turtle habitat and the integrity of the landscape context, but also on actual, observed abundance, demographic structure, and genetic traits of populations that were sampled between 2012 and 2017 using a standardized assessment protocol. The design process primarily followed an automated and repeatable, quantitative selection process that was informed by Wood Turtle experts and tailored to reflect the unique natural history and ecology of the species. Automation of the ranking and stratified selection process provided a high degree of objectivity, while expert opinion ensured the incorporation of professional judgment in the final site selection process. Ecological, political, hydrographic, and genetic stratification was used as the primary means of guaranteeing adequate representation.

Wood Turtle CAN sites fall under two major tiers: high-priority Focal Core Areas and lower-priority Management Opportunity Sites. Focal Core Areas represent the highest priority sites that, when considered together, are critical to the long-term persistence and evolutionary potential of the species in the northeastern United States. These sites represent not only the most robust Wood Turtle populations in the region, but also sites that represent geographic, ecological, and genetic variation throughout the species range. Management Opportunity Sites represent lower priority areas/subpopulations that are ideal targets for agricultural mitigation programs (e.g., Natural Resources Conservation Service Working Lands for Wildlife), federal engagement (i.e., National Wildlife Refuges), and/or international collaboration. Finally, the CAN also identifies priority HUC8 Connectivity Basins that represent basins with regionally significant sites and existing landscape structure that is highly conducive for connectivity among Wood Turtle populations; these basins are meant to guide regional efforts to promote large-scale landscape conservation partnerships and initiatives. Site pseudonyms are used to protect sensitive locations.

Part V. Conservation Action Plan & Implementation Framework

The culmination and final section of this plan, a Conservation Action Plan and Implementation Framework, serves as our proposed framework and methodology for executing and maximizing the effectiveness of the Conservation Area Network. This section of the plan provides a structured path forward with respect to the conservation of Wood Turtle populations for the foreseeable future. It integrates current field and analytical techniques through an adaptive management framework that emphasizes continual reevaluation of methods, progress, and conservation benchmarks at regular

intervals—thereby providing opportunities for redirection of the conservation strategy in the face of underperformance and/or uncertainty. This section of the plan is made up of (1) a proposed personnel management structure for overseeing future Wood Turtle conservation efforts, (2) a summary of critical conservation actions needed to address the complex array of threats facing Wood Turtle populations at multiple scales, and (3) our proposed strategy for the implementation of these actions.

The Conservation Action Plan (CAP) and Implementation Framework will be guided by the Wood Turtle Council (Appendix XIII), a formal Working Group made up of members that represent conservation-focused entities throughout the Northeast Region. The central component of the CAP, a Site Action Tracking Database, is intended to facilitate the assessment and management of CAN sites by tracking 64 site-specific variables within 17 broad categories (e.g., nesting habitat quality and status, site protected status, and technical assistance needs). The database, in its current form, is meant to serve as a basic framework from which more detailed site-specific management plans and spatially-explicit geodatabases can eventually be developed, but also as a guiding document for prioritization and implementation of near-term (<5 years) actions. The Conservation Action Plan also includes site-level management guidelines, Connectivity Basin actions, state-level recommendations, as well as regional and federal recommendations. Due to the fact that large populations of Wood Turtles are most commonly associated with unfragmented forested blocks, the primary action required for the conservation of the species is **land protection in perpetuity**. Additionally, we strongly recommend the development of a comprehensive and creative anti-poaching strategy, application of best habitat management practices, mitigation of road mortality hotspots, strategic population monitoring and data collection, riparian restoration and management of invasive species at targeted locations, large-scale landscape connectivity initiatives, providing technical assistance, and minimizing recreational access within CAN sites.

Part I. Background and Rationale

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Conservation Context

The North American Wood Turtle (*Glyptemys insculpta*) presents a unique combination of conservation challenges in the northeastern United States, the contiguous core of its formerly expansive range. A successful Wood Turtle Conservation Plan must consider several life history, ecological, and behavioral characteristics particular to this unusual species. For example, the Wood Turtle's generation time is among the longest of any North American terrestrial vertebrate at over 35 years, and it is iteroparous, reproducing throughout its long lifespan (van Dijk and Harding 2011, Jones and Willey 2015). Adult Wood Turtles exhibit pronounced fidelity to key habitat features—overwintering sites, natural and anthropogenic nesting areas, terrestrial and aquatic basking sites, and upland foraging sites—over years or decades, with minimal rates of inter-annual home range drift or outright dispersal away from familiar areas (Compton 1999, Compton et al. 2002, Jones 2009). Rare among North American turtles, Wood Turtles exhibit an amphibious seasonal ecology, spending winters in oxygenated, coldwater streams but primarily foraging in open and forested landscapes up to several hundred meters from water during the spring, summer, and fall. Other North American turtles with fluvial habitat requirements, such as Western Pond Turtles (*Actinemys marmorata*), Sonora Mud Turtles (*Kinosternon sonoriense*), and Flattened Musk Turtles (*Sternotherus depressus*), do not forage extensively on land or congregate in agricultural fields and working forests for weeks or months at a time as do Wood Turtles (Ernst and Lovich 2009). And by contrast, terrestrial North American species such as the Box Turtles (*Terrapene* spp.) and Gopher Tortoises (*Gopherus* spp.) have a definable range of optimal upland habitat characteristics similar to the Wood Turtle, but their suitable habitats are not further constrained by fluvial geomorphology.

High-density, demonstrably viable subpopulations of Wood Turtles tend to occur in discrete patches of appropriate habitat, often where there is a pronounced convergence of suitable stream geomorphology, stream substrate and nesting site availability, stable overwintering sites, up-watershed basin characteristics, and relatively low-density development and agriculture in the surrounding uplands. Thus, while individual Wood Turtles may be found in a wide variety of stream conditions from Maine to Virginia, it is a mistake to presume that Wood Turtles can be easily conserved through general applications of habitat management practices within streams where they have been documented to occur. Rather, it is critical to identify the necessary juxtapositions of suitable stream habitat, reliable nesting habitat, and diverse mosaics of upland cover types where it is still possible to permanently minimize the annual risk of road mortality, machinery mortality, and collection through land protection, conservation easements, and relative isolation.

Unless and until these factors are addressed directly and effectively, we expect that the Wood Turtle will continue to decline. Strategic protection of functional core habitats and surrounding upland must remain the priority for regional conservation partnerships, at the expense of intensive population management or short-term habitat management.

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Conservation Plan for the Wood Turtle in the Northeastern United States represents the cumulative product of a multi-year, proactive effort among northeastern State Wildlife Agencies, and their partners, to articulate a strategic action plan to protect representative populations of Wood Turtles. Our fundamental objective is to ensure the persistence of functional, ecologically viable, and representative populations of Wood Turtles throughout the Northeast Region in order to protect the evolutionary potential of the species. To do so, it is necessary to stabilize and reverse population declines. Because of the evident expense of meaningful conservation work for this species, it is necessary to establish a spatially-explicit, stratified Conservation Area Network that prioritizes sites based on best available population, landscape, and genetic data.

Our specific methodology is outlined in the subsequent parts of this conservation plan. Our overall approach is summarized here. To acknowledge the unique biological traits of the species (outlined above), we designed the Conservation Plan around the core concept of a Conservation Area Network, which in turn was heavily influenced not only by the extent and quality of Wood Turtle habitat and the integrity of the landscape context, but on actual, observed abundance, demographic structure, and genetic traits of populations that were sampled between 2012 and 2017 using a standardized rapid assessment protocol. To ensure adequate representation, Focal Core Areas for the Conservation Area Network were

selected for each State, Watershed (HUC4), and Ecoregion (EPA Level III), and further informed by genetic structure (Part III). Because of recent increased interest in Wood Turtle conservation in the Northeast, we identified and included special Management Opportunity Sites, which encompass both lands administered by the U.S. Fish and Wildlife Service (USFWS) as well as sites identified as ideal targets for programs administered by the U.S. Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) and other land management agencies and organizations. For both Focal Core Areas and Management Opportunity sites, we established a matrix of site condition and necessary management actions, resulting in a spatially-explicit Conservation Action Plan for sites identified in the Conservation Area Network. From the Conservation Action Plan, we specify regional targets for implementation and effectiveness. Finally, we specify aspatial conservation actions to minimize declines both within and outside of the Conservation Area Network, and establish an adaptive implementation framework to ensure and track progress toward regional objectives.

The Conservation Plan builds directly upon a Status Assessment (2015) funded by State Wildlife Grants (SWG) through the Regional Conservation Needs (RCN) program of the Northeast Association of Fish and Wildlife Agencies (NEAFWA). *Status and Conservation of the Wood Turtle in the Northeastern United States* (Jones and Willey 2015) provided a review of the species ecology, regulatory status, historic distribution, detectability, and estimate of range contraction, and a bibliography. The ecology and bibliography sections are included here in expanded form as Appendices to the Conservation Plan. In other areas, the Conservation Plan builds upon the Status Assessment by (1) expanding the database of standardized population assessments; (2) providing information from data-deficient areas through the region; (3) assessing genetic structure; (4) hosting the first rangewide conservation symposium for the species; (5) providing technical assistance to key landowners; (6) specifying Focal Core Areas and supporting habitats for conservation; and (7) establishing a Conservation Action Plan for priority sites.

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Biology and Ecology of the Wood Turtle

The biology, ecology, and regulatory status of the Wood Turtle (*Glyptemys insculpta* LeConte 1830; Fig. 1.1) was summarized in detail by Jones and Willey (2015; updated versions are provided in Appendix VII; Appendix XI). Here, we provide a brief overview of important components of the biology and ecology of the Wood Turtle to provide context for the interpretation of this Conservation Plan.

The Wood Turtle is a medium-sized semi-aquatic riverine and riparian species within the family Emydidae, subfamily Emydinae, together with the genera *Clemmys*, *Terrapene*, *Emydoidea*, *Emys*, and

Actinemys (Fig. 1.2). The current distribution of *G. insculpta* ranges from Nova Scotia to northeastern Minnesota, south to Iowa and northern Virginia (Fig. 1.3). The Northeast Region, for the purposes of this Conservation Plan, encompasses thirteen states from Maine to Virginia. This geographic area supports a majority of the Wood Turtle's extent of occurrence within the United States. Wood Turtles are known to have occurred historically in all thirteen northeastern states and the District of Columbia. Although many of the major northeastern streams have been degraded by agriculture, textiles, industry, deforestation, and habitat fragmentation, potentially viable populations of Wood Turtles can be found in many areas throughout the region.

Habitat

A comprehensive summary of Wood Turtle habitat requirements is provided in Appendix VII. Wood Turtle populations are typically associated with sections of clear, cold, medium-sized streams and rivers (3–20 m wide) that are often situated within a mosaic of mature forest and early-successional habitats (Fig. 1.4; Saumure 2004; Akre and Ernst 2006; Ernst and Lovich 2009; Jones and Willey 2015). These streams are generally characterized by sand, gravel, cobble, and/or bedrock substrates and significant accumulations of within-stream woody structure such as fallen trees, branches, and root-masses that play a critical role in providing overwintering sites, basking areas, cover, and stability during periods of elevated flows (Jones and Willey 2015). Although single individuals and small populations may be found with regularity throughout the species range, it is clear that robust, demographically stable populations are generally found within landscapes and stream systems that sustain dynamic fluvial, geomorphic, and biological processes (e.g., seasonal flooding, meandering stream channels, and/or periodic Beaver [*Castor canadensis*] activity) that allow for frequent deposition of nesting material and maintenance of ephemeral early-successional habitats.

Although Wood Turtles require streams for overwintering and mating, they also rely upon adjacent terrestrial habitats, spending much of the warm months, from late spring to early fall, in the surrounding landscape, sometimes hundreds of meters from their overwintering stream. Terrestrial habitat preferences vary by geographic region and season, but Wood Turtles will typically occupy a mosaic of habitats including mature forest and early-successional cover types. Ecotones (i.e., transitional zones between adjacent habitats) appear to play an important role for Wood Turtles by providing opportunities to balance both thermoregulation and food requirements. Ephemeral pools (especially within river floodplains), springs, seeps, and temporary wetlands appear to serve as complementary habitat, but do not support overwintering activity over most of the range.

Generally, Wood Turtles require well-drained, elevated, and exposed areas of sand and/or gravel for nesting, but preferred nesting conditions likely vary across the species range. Wood Turtles typically select nesting sites in coarse alluvium, poorly graded sand, or fine to medium gravel, and sandy loam associated with a wide range of natural and anthropogenic settings. Documented natural nesting features include sandy point bars on the inside of river bends, cutbanks on the outside of river bends, sand and gravel bar deposits in the stream channel (associated with stream obstructions, constrictions, or directional changes in flow), areas of overwashed sand in open floodplains, and dry stream beds. Documented anthropogenic nesting features include sand and gravel pits, gravel boat ramps, exposed areas along powerline/pipeline corridors and rights-of-way, roadsides, unpaved farm roads near streams, railroad beds, gravel piles in waste areas such as junkyards, golf course sand traps, and nesting areas created specifically for turtles (see Appendix VII).



Figure 1.1. Wood turtle appearance varies with age, sex, habitat, and geographic location. Eight male wood turtles of different ages and from different streams are pictured. Photographs by Mike Jones / MassWildlife.



Figure 1.2. The freshwater turtle subfamily Emydinae is comprised of about 12 to 14 species distributed primarily in the USA and Mexico, including the small and/or monotypic genera *Glyptemys* A & B); *Emydoidea* (C); *Actinemys* (D); *Emys* (E); *Clemmys* (F); and *Terrapene* (G, H), which diverged an estimated 17 to 30 million years ago. Photographs by American Turtle Observatory and Mike Jones / MassWildlife.

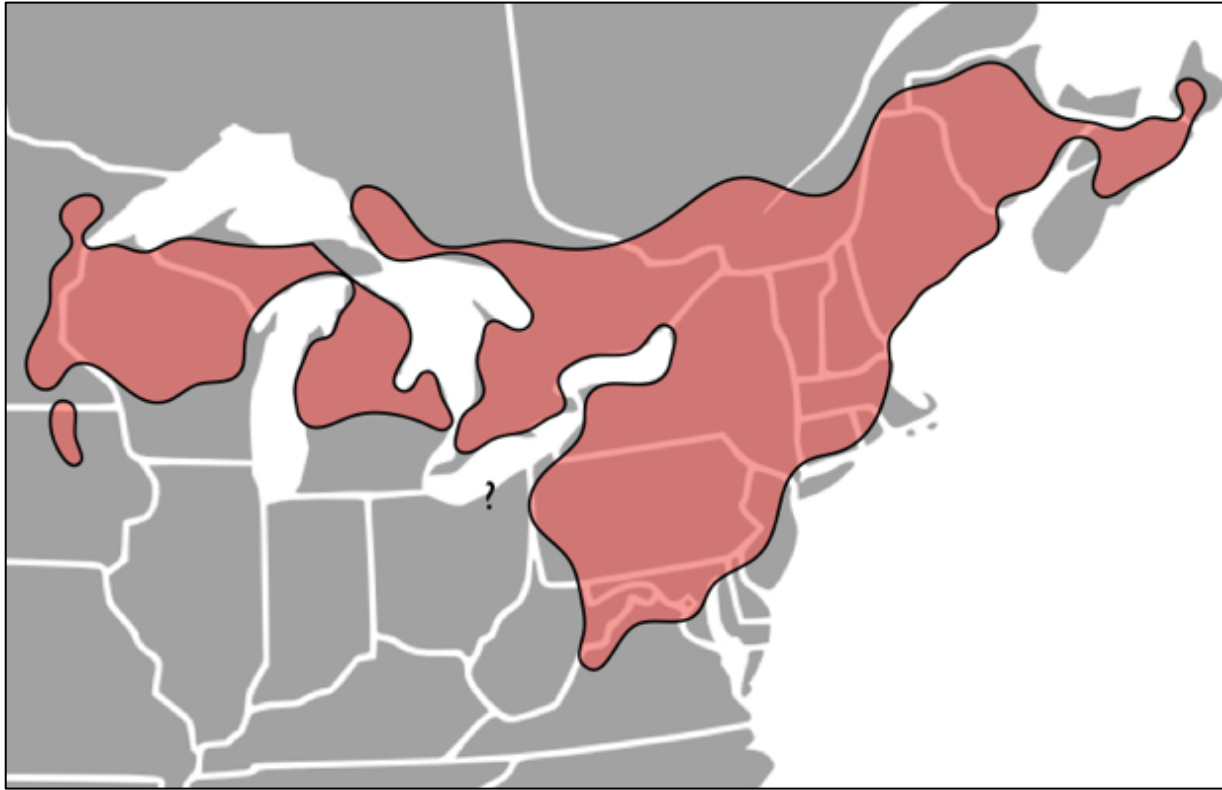


Figure 1.3. Approximate current geographic range of the Wood Turtle in North America.



Figure 1.4. Throughout their northeastern range, Wood Turtles are fluvial specialists, associated with defined sections of slow-flowing sections of sand- and gravel-bottomed streams in mosaics of forested and non-forested uplands from Nova Scotia to Virginia. Photographs by American Turtle Observatory and Mike Jones / MassWildlife.

Movements

A comprehensive, referenced summary of Wood Turtle seasonal movements is provided in Appendix VII. An adequate understanding of the local spatial ecology and seasonal habitat selection of the Wood Turtle is necessary in order to appreciate and address the unique vulnerability of the Wood Turtle to existing threats (see below) as well as effective conservation planning, management, and environmental regulation for the species. Although movement patterns among populations vary, broad characterizations of movement and space-use is valuable when contextualizing many of the challenges associated with conserving the Wood Turtle. The Wood Turtle “active period” refers to the portion of the year that Wood Turtles are active and can be found both in streams and on the surrounding upland landscape. The length of this period varies with latitude and elevation (i.e., may be longer in warmer regions), but generally spans from April to October. The active period can be subdivided into five distinct periods: emergence and pre-nesting, nesting, post-nesting, pre-hibernation, and overwintering. In some streams, especially where winter ice cover is low or nonexistent, Wood Turtles may be detected year-round even where activity may be minimal in mid-winter.

The emergence and pre-nesting period begins during March and April throughout the species range, and in northern populations, spring activity may be determined by ice-out. During this the earliest weeks of the emergence period, Wood Turtles are typically found 0–10 m from streams and exhibit elevated rates of basking and thermoregulatory behavior. Nesting primarily occurs in June, but can occur from mid-May to mid-July. Females will frequently make use of instream features for nesting, but may travel large distances to nest when suitable nesting habitat is unavailable. Documented nest locations in the northeastern United States have ranged 0.2–600 m from streams, with a median distance of 25.6 m in New England (Jones 2009; Steen et al. 2012). Studies in New Jersey (C. Osborn, pers. comm.) and Maine (Compton 1999) have observed nesting movements >1 km from typical home ranges.

The post-nesting period begins when nesting has concluded and spans from approximately July to late September. During these warm months, Wood Turtles spend much of their time on the surrounding landscape and can typically be found 0–90 m from streams (Parren 2013), with the large majority (>95%) of movements occurring within 300 m of streams (Arvisais et al. 2002; Jones 2009; Parren 2013). Large-scale aquatic and terrestrial dispersal movements of >16 km have been documented throughout the range (T. Akre, unpubl. data.; Jones 2009), but the general frequency of these events among subpopulations is unknown. Pre-hibernation typically begins in October or November when environmental temperatures start to decline, but may begin earlier in colder areas and later in warmer areas. At this point, Wood Turtles retreat to streams and eventually settle into overwintering locations within the stream channel. The overwintering period occurs during the coldest months of the year from November or December to

March or April. Wood Turtles remain largely immobile while overwintering, but may make occasional small underwater movements.

The annual home range, linear range (greatest annual distance between recorded locations), and stream range (greatest annual distance between locations within stream) is generally larger for males than females. Among thirteen studies examined by Jones and Willey (2015), the average mean annual home range was 18.2 ha (0.3–32.2 ha) for males and 11.6 ha (0.5–29.4 ha) for females. Overall, annual home ranges appear to be larger in northern populations than southern populations (Smith 2000; Arvisais et al. 2002). The averaged mean annual linear range from studies examined by Jones and Willey (2015) was 1028 m (481–1531 m) for males and 647 m (435–866 m) for females. From a sample of 123 adult turtles in Massachusetts and New Hampshire, Jones (2009; unpublished data) reported a male stream range of 1422 ± 1295 m (221–6304 m) and female stream range of 757 ± 814 m (62–5537 m).

Demography

Similar to many emydine turtle species, Wood Turtles display delayed sexual maturity (12–20 years), relatively small clutch sizes (7–11 eggs/nest range-wide), and low nesting frequencies (ranging from 0.33–0.9; Jones and Willey 2015). In addition, Wood Turtle populations typically suffer high nest predation and juvenile mortality rates even without the presence of anthropogenic pressures. These factors are only offset by their longevity (>70 years in the wild), high adult survival rates, and sustained reproduction into old age (generation time = approx. 45 years; Jones and Willey 2014). It is clear, from studies of related species with similar life history characteristics (e.g., Congdon et al. 1993), that even very small increases in the adult Wood Turtle mortality rate can lead to the rapid decline and functional extirpation of populations. The intrinsically precarious balance of its life history traits, coupled with their highly terrestrial nature, have made the Wood Turtle particularly susceptible to the broad array of anthropogenic threats affecting streams throughout the Northeast.

Threats

Individual Wood Turtles face numerous threats that are directly or indirectly associated with anthropogenic development. Habitat loss, fragmentation, and degradation due to development, road mortality, and human land use (e.g., agriculture) are widely considered the primary causes of population declines throughout the range (Saumure 2004; van Dijk and Harding 2011). However, Wood Turtles are also vulnerable to incidental and commercial poaching, invasive plant species, pathogens, human-subsidized predators, pollution, and stream bank stabilization. These factors, which affect Wood Turtle

populations in varying combinations and degrees of severity, have—as a whole—contributed to the overall decline of the Wood Turtle throughout the global species range and the Northeast (van Dijk and Harding 2011; Jones and Willey 2015). For a complete review of threats facing the Wood Turtle in the Northeast, see Jones and Willey (2015).

As a result of perceived rarity, documented population declines, and localized extirpations, the Wood Turtle has received listing designations by agencies and organizations throughout the species range. Wood Turtle is listed in the State Wildlife Action Plans (SWAP) of all 13 northeastern states (and are only considered “secure” by two states: Maine and Maryland—the latter of which does not seem to reflect the extensive range contraction in distribution in the Piedmont and Coastal Plain), listed as “G3 - Vulnerable” by NatureServe (NatureServe 2017), listed as “Endangered” by the International Union for the Conservation of Nature (IUCN; van Dijk and Harding 2011), and listed as “Threatened” under the Species at Risk Act (SARA) in Canada. In 1995, the United States Fish and Wildlife Service (USFWS), in a response to a petition for listing under the federal Endangered Species Act, rejected a status listing of “Threatened” because of “...the inadequacy of existing data to support the contention that the wood turtle has undergone rangewide decline or that the threats identified in the petition are affecting wood turtle populations across all or a significant portion of its range to the extent that the species is likely to become an endangered species in the foreseeable future” (Amaral 1995). The USFWS is currently considering a proposal by the Center for Biological Diversity (2012) to list the Wood Turtle as Threatened.

Status Assessment

To address the paucity of population-level data throughout the species range, a collaborative project to assess the status of the species in the Northeast Region was funded in 2012 through the Regional Conservation Needs program (a cooperative grant program established with State Wildlife Grant funds by the Northeast Association of Fish and Wildlife Agencies). The result of this effort was a document entitled *Status and Conservation of the Wood Turtle in the Northeastern United States* (Jones and Willey 2015; referred to as the “Status Assessment” in this document), which was completed in 2015. As a part of the Status Assessment, cooperators and partners completed a Literature Review (an updated version is available in expanded form as an appendix [Appendix VIII] to this Conservation Plan), conducted a regional threat assessment, developed a standardized and field-tested monitoring protocol, conducted standardized population assessments in all thirteen States and the District of Columbia, began to gather and corroborate occurrence information, built Species Distribution Models, and assessed the apparent extent of range contraction throughout the region.

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Conservation Plan Overview

In 2014, prior to the completion of the Status Assessment, eight states—Maine, New Hampshire, Massachusetts, Connecticut, Pennsylvania, New Jersey, Maryland, and Virginia—and their partners were awarded a regional USFWS Competitive State Wildlife Grant (CSWG) to develop and implement a conservation plan for the Wood Turtle in the northeastern USA, entitled “Conservation Planning and Implementation for the Wood Turtle (*Glyptemys insculpta*) and Associated Riparian Species of Greatest Conservation Need from Maine to Virginia.” The Northeast Wood Turtle Working Group (NEWTWG)—a partnership of representatives from thirteen state wildlife agencies, universities, land managers, and researchers in association with the Northeast Partners for Amphibians and Reptile Conservation (NEPARC)—has worked cooperatively to fulfill the obligations of this grant and to lead the conservation effort for the Wood Turtle in the Northeast.

This conservation plan—the product of eight years (including the Status Assessment) of coordination among agencies, universities, and individual biologists throughout the Northeast Region of the United States—represents the primary outcome of the CSWG-funded project and builds upon the groundwork of the 2015 Status Assessment. This Plan is intended to be a living document, updated at regular intervals based on new assessments of population and landscape condition that provides a prioritized framework for Wood Turtle conservation from the St. John Basin of Maine to the Potomac Basin in Virginia.

Our overall objective in developing this Conservation Plan is to ensure the **regional persistence** and **evolutionary potential** of the Wood Turtle from northern Maine to northern Virginia, including its major ecological associations, local (basin- and ecoregion-level) adaptive pressures, basin-level distribution, and genetic structure (i.e., clusters and connectivity). This means that we have directed our major planning decisions toward evolutionary timescales and attempted to avoid an unsustainable plan based upon intensive intervention—such as population augmentation through headstarting—for near-term population gains. We have emphasized the protection and restoration of landscapes capable of supporting Wood Turtles for the foreseeable future through natural or encouraged isolation and natural disturbance regimes.

As a necessary part of this objective, we developed a Conservation Area Network (CAN) of prioritized sites. We selected these priority Wood Turtle areas for their extent, rigor, genetic distinctiveness, genetic diversity, and/or probability of persistence through a precautionary of stratified levels (Level III Ecoregion, 4-digit HUC basin, major genetic structure cluster, and state) to ensure adequate representation across the species’ range in the Northeast. We utilized prioritization metrics in order to

account for vulnerability to both projected climate change and land conversion to development, the intended consequence of which is to identify high-risk sites of high conservation value while otherwise maximizing the resiliency of the streams in the CAN.

Several additional overarching themes have guided the development of this Conservation Plan. For example, we have emphasized the independence of our assessment and have attempted to minimize our preconceived decisions about the species' status. In this way we have placed strong emphasis on intensive **empirical sampling**.

The conservation planning process of this grant was made up of six distinct elements: (1) a regional, hierarchical population assessment using standardized protocols, which occurred from 2012 to 2017; (2) an analysis of regional population genetics; (3) development of habitat management guidelines and technical assistance to key landowners; (4) a Conservation Area Network; (5) a Conservation Action Plan for priority sites; (6) an implementation framework modeled on other regional conservation efforts. These components have been compiled as chapters or appendices to form this Conservation Plan.

The culmination and final chapter of this plan, a Conservation Action Plan (CAP) and Implementation Framework, serves as our proposed framework and methodology for executing and maximizing the effectiveness of the CAN. The CAP provides a structured path forward with respect to the conservation of Wood Turtle populations for the foreseeable future. It aims to integrate current field and analytical techniques through an adaptive management framework that emphasizes continual reevaluation of methods, progress, and conservation benchmarks at regular intervals—thereby providing opportunities for redirection of the conservation strategy in the face of underperformance and/or uncertainty. The CAP will be guided by the Wood Turtle Council (Appendix XIII), a formal Working Group made up of members representative of conservation-focused entities throughout the Northeast Region. Though it is clear that Wood Turtle populations have declined markedly from pre-Industrial levels across much of its northeastern range—and today face a range of seemingly intractable threats—it is also clear that with strategic land conservation informed by empirical sampling, important and representative populations may be conserved in many key areas of the original range.

Part II. Regional Population Assessment

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Summary

The individual objectives of this Regional Population Assessment were to (1) expand the network of study sites throughout the Northeast Region, (2) identify factors influencing Wood Turtle abundance at the regional scale, (3) quantify factors impacting Wood Turtle detection rates, (4) identify regionally significant Wood Turtle populations, (5) monitor intensively studied sites to estimate population size, density, and demographic structure, and establish a baseline by which to evaluate population trends, and (6) conduct population assessments within data-deficient areas throughout the Northeast.

From 2012 to 2017, nearly 400 federal, state, and university biologists and students participated in 2,141 field surveys across 464 different stream segments throughout the 13 northeastern states and the District of Columbia. A total of 4,611 Wood Turtle detections were recorded across all surveys, averaging 2.15 turtles per survey (sd = 3.81). Approximately 50% of surveys were conducted in spring, 42% were conducted in fall, and 8% were conducted during the nesting period. Regionwide, catch per unit effort for the lead observer during surveys averaged 1.52 turtles per survey during the spring and 1.2 turtles per survey during the fall. This seasonal pattern was consistent throughout much of the Northeast, except the southernmost states (MD, VA, and WV) where catch per unit effort for the lead was greater in fall than spring. Approximately 42% (894) of surveys conducted yielded zero turtles, with surveyors failing to detect a single turtle (across all surveys conducted) at 40% of sites regionwide.

Juveniles made up 16% of regional Wood Turtle detections, with the majority of sites consisting of <25% juveniles. Overall, the percent of detections that were juvenile did not increase with catch per unit effort. The average male:female ratio was 1.4:1 for sites with at least one female and > 6 detections, but sex ratios varied considerably across states/provinces. The overall within-season male:female ratio in fall was more than double that of spring (2.87 and 1.31 respectively) when all surveys with >6 detections were considered.

Land cover variables at the 5500 m scale appeared in top N-mixture models of relative abundance, with percent undeveloped land (+), traffic rate (-), and percent agricultural cover (-) appearing as strong predictors across all datasets analyzed. These findings provide further support for the understanding that large-scale landscape-level patterns play an important role in predicting Wood Turtle abundance and occurrence throughout the range. The site-level 300-m scale was also an important, with Wood Turtle abundance displaying a unimodal relationship with agricultural cover within 300 m, peaking at relatively low levels of agriculture (approx. 15%) and declining thereafter. This pattern should be interpreted cautiously because Wood Turtles can be attracted to agricultural conditions despite the negative impacts exerted on the population by machinery.

Relative population density (not absolute population density) was estimated for 80 stream segments using Capture-Mark-Recapture (CMR) models across nine of 13 northeastern states: Maine, New Hampshire, Vermont, Massachusetts, Connecticut, New Jersey, Pennsylvania, Virginia, and West Virginia. Relative population size estimates varied dramatically among segments with closed-population estimates (turtles pooled within seasons) ranging from 4–211 turtles/km (mean = 47.5 turtles, sd = 43.5). Twenty-five of 80 sites (32%) had pooled closed-population estimates >50 turtles/km and 8 of 80 sites (10%) had estimates >100 turtles/km. These analyses suggest that, even among sites that were selected by experts, large populations appear to be rare within the northeastern United States. This observed pattern also highlights the clear tendency for Wood Turtles to reach high densities only within ideal landscape and microhabitat contexts.

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Introduction

As a cryptic species occurring primarily at low densities throughout the northeastern portion of its range, the Wood Turtle presents several distinct challenges as the subject of a quantitative, regional monitoring program. Relatively low population densities throughout much of the species range and highly variable detection rates make it difficult to efficiently detect viable and regionally significant populations or

quantify meaningful population trends across broad geographic areas. In most areas, Wood Turtles are most easily detected while basking or nesting; however, these are seasonal behaviors that are determined by complex relationships between temporal and environmental factors (e.g., temperature, solar exposure, humidity) that vary both daily and geographically, thus making the precise prediction of ideal survey conditions difficult. Further, Wood Turtles utilize a wide range of habitats, from stream channels and floodplain forests to surrounding upland habitats including fields, shrublands, and mature forests, each of which vary in extent among sites and foster different detection rates. These factors compound to make survey results highly variable and at least partially dependent on the experience-level of the surveyors involved. As a result, Wood Turtle studies have often employed survey protocols tailored to address the specific challenges associated with detecting Wood Turtles at their respective study sites. For this reason, it has proved difficult to compare survey returns among studies and make inferences about the species over broad geographic scales. This, in part, contributed to the lack of data needed for the U.S. Fish and Wildlife Service (USFWS) to make a federal listing determination over two decades ago (Amaral 1995), which, in turn, highlighted the need to develop a standardized monitoring protocol that could be easily applied to the entire species range.

In 2011, as a part of the regional Wood Turtle Status Assessment (Jones and Willey 2015), the Northeast Wood Turtle Working Group (NEWTWG) developed a regional monitoring protocol (of which an updated version is provided as Appendix V) that could be applied to Wood Turtle populations throughout the Northeast Region. The protocol was intended to be flexible, robust, and repeatable, and reflect an acceptable methodology with respect to competing research, inventory, and monitoring objectives throughout the region. The initial objectives underpinning the development of this protocol were to allow for (a) the identification and characterization of covariates influencing detection probability, occupancy, and abundance, (b) the identification of potentially viable and robust populations of regional significance, and (c) the establishment of a baseline of abundance in order to detect long-term population trends and to evaluate the effectiveness of conservation actions.

Between 2012 and 2013, partners established a broad network of sites throughout the northeastern United States and Canada where the regional monitoring protocol was implemented. Sites were identified by Wood Turtle experts from each state and primarily represented known or historic populations or data-deficient areas. This standardized, multi-year effort provided valuable insight into ideal detection conditions. By combining historical occurrence data, Species Distribution Models (SDM), and survey and monitoring data, it was estimated that >50% of potential Wood Turtle stream habitat in the Northeast Region is situated within potentially impaired landscape contexts. The Status Assessment effort also found that most historical sites that are now likely extirpated are situated within human-dominated

landscapes, suggestive of widespread population declines due to habitat loss, fragmentation, and degradation.

Upon the completion of the Status Assessment, the NEWTWG identified the continuation and expansion of this monitoring effort as a priority for a subsequent conservation planning phase for the Wood Turtle in the northeastern United States (funded through a Competitive State Wildlife Grant; CSWG). The specific objectives of this Regional Population Assessment were to (1) expand the network of study sites throughout the Northeast Region, (2) identify factors influencing Wood Turtle abundance at the regional scale, (3) quantify factors impacting Wood Turtle detection rates, (4) identify regionally significant Wood Turtle populations, (5) monitor intensively studied sites to estimate population size, density, and demographic structure, and establish a baseline by which to evaluate population trends, and (6) conduct population assessments within data-deficient areas throughout the Northeast Region.

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Methods

Population Monitoring

Survey Protocol

All CSWG partners (including state agency, university, and independent biologists) throughout the Northeast followed a single set of recommended, standardized survey guidelines. The CSWG protocol (Appendix V) was updated from a pilot protocol established in 2012 by the Northeast Wood Turtle Working Group as a component of the Regional Conservation Needs (RCN) Wood Turtle Status Assessment (Jones and Willey 2015), and it provides detailed survey guidelines intended to maximize consistency and compatibility among entities in different states tasked with collecting population data. The guidelines were designed to be flexible in a wide range of environmental and logistical conditions, with the understanding that they cannot be applied perfectly to all stream segments throughout the range, and the recognition that unforeseen circumstances may arise during some surveys, which can make strict adherence to the guidelines difficult. The overarching objective of the standardized survey protocol was to detect robust and potentially viable populations with a rapid, flexible, and quantitative methodology.

Site selection.—To focus survey efforts most effectively, the survey guidelines specified four categories of eligible fluvial habitats: (1) existing priority conservation sites (i.e., sites that are known to have high densities of Wood Turtles or a relatively large Wood Turtle population, excellent landscape context,

ongoing conservation efforts, or supportive/engaged landowners); (2) sites that are existing long-term study locations; (3) sites situated within regionally data-deficient areas; and (4) randomly selected sites (from Classification and Regression Tree [CART] habitat suitability analyses; Jones and Willey 2015). Once a general section of stream was identified by either the state agency team leader or the state project manager, a specific one-kilometer portion of meandering stream was measured in Google Earth, following the stream centerline (Fig. 2.1). Start and end coordinates were recorded in decimal degrees. Before conducting an official survey, surveyors were encouraged to perform at least one reconnaissance visit in order to confirm the ideal access location, identify potential logistical issues, and confirm access permission with landowners.

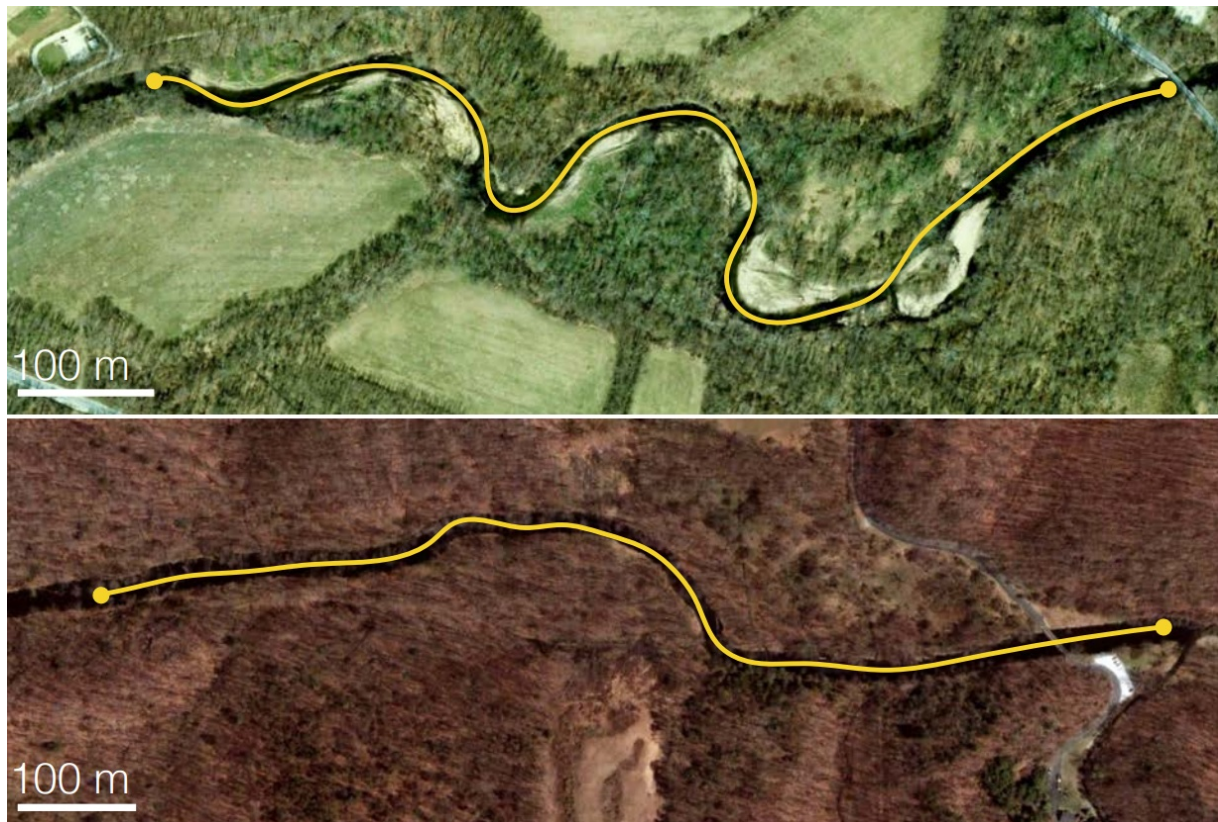


Figure 2.1. Example delineations of two standardized one-kilometer survey segments within different landscape contexts (agriculture and forested) using leaf-off imagery in Google Earth.

A concerted effort was made to stratify site selection across a variety of environmental gradients. Sites were identified at the state level and official stream segments were selected with the goal of maximizing geographic dispersion and watershed representation and representing gradients in various land cover

classes (e.g., mature forest, human development, agriculture). Sampling effort was largely determined by available resources within the respective states.

Observers.—Surveys were completed by 1–4 observers whenever feasible. Prior to each survey event, surveyors were instructed to give each participant a number that corresponded to their respective position during the survey. Observer 1 (“lead observer”) led each survey, walking ahead of all other observers, such that turtles were not disturbed by any other, trailing observers, ensuring an independent survey event for Observer 1 but influencing the availability of turtles for the subsequent observers. This stipulation ensured that the number of Wood Turtles found by Observer 1 was independent and could be directly compared to Observer 1 returns from other surveys throughout the region, irrespective of number of observers per survey. Surveyors were encouraged to survey as many different sites as possible and to frequently alternate lead observers to avoid observer bias. Single surveys at one site by an individual were discouraged.

Search area.—Surveyors were instructed to generally search the stream channel, bank, and surrounding floodplain (but not upland areas away from the evident flood-influenced zone) along the 1 km meandering stream centerline at a rate of approximately 1 km/hr. Surveyors could search riparian features such as oxbows, braided streams, sidestreams, pools, and overflow channels. Observers were instructed to focus search effort on features likely to harbor Wood Turtles, including open herbaceous- and shrub-dominated areas, banks with high solar exposure, deep pools (especially in fall), rootmasses, fallen trees, woody, debris, and logjams (Harding and Bloomer 1979; Akre and Ernst 2006). Greater emphasis was placed on searching aquatic habitats on colder days (air temperature <9°C).

Seasonal survey windows.—Biological seasons were predefined based on our contemporary knowledge of Wood Turtle spatial ecology and behavior (Fig. 2.2). Pre-nesting or “spring” was defined as the period between emergence and May 28. Nesting was defined as May 29–July 8. Summer was defined as July 9–September 1 or October 1 (variable throughout the Northeast). Fall was defined as September 1 or October 1 to brumation. Spring surveys were encouraged because previous analyses have shown that surveys conducted before May 29 yield approximately twice as many turtles as those conducted in the fall (Jones and Willey 2015). Fall surveys were encouraged at long-term reference sites (see Section 2.1.1). Summer surveys were avoided because Wood Turtles typically move away from the floodplain landscape during this period.

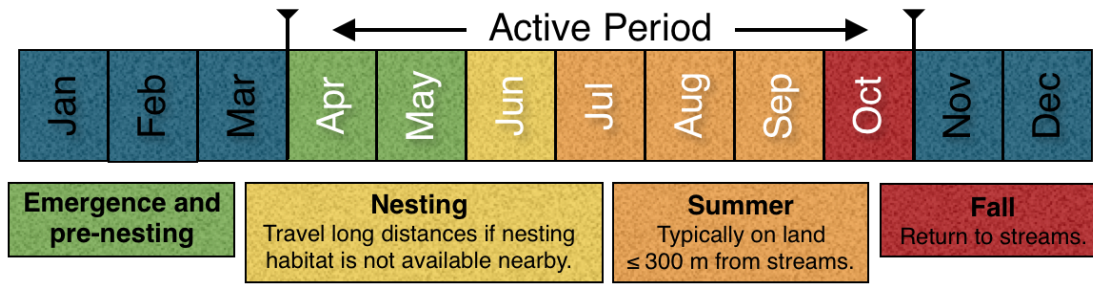


Figure 2.2. The approximate Wood Turtle active period in the northeastern United States.

Recommended survey conditions.—Ideal survey conditions were daytime in the Spring, late Summer, and Fall when air temperatures were 9–24°C and water temperatures were 7–20°C, but surveys were also conducted outside of these windows. Surveying sites in the same day or on consecutive days was discouraged unless accessing the site multiple times in a season was logistically unfeasible. In these cases, obtaining three surveys during a single season was prioritized over the need to separate surveys by >1 day.

Processing turtles.—An Individual Turtle Field Form (see Appendix V) was completed for each Wood Turtle captured. Coordinates were recorded for each turtle found. Morphometrics (straight carapace length, carapace width, plastron length, plastron width and shell height) were measured using a 300-mm dial caliper. Mass was measured using a digital or spring-loaded scale (e.g., 2kg Pesola). We also recorded sex, shell wear condition (not worn, partly worn [$<50\%$]), >50 worn, and >90 worn), and the number of visible plastral annuli. Each turtle was assigned a unique identity (within the respective state) signified by notching marginal scutes (Cagle 1939) using a triangular file and following either Ernst et al. (1974) or a local notching system particular to a given state. Ernst et al. (1974) was generally followed in ME, NH, MA, and PA. Modified versions of Cagle (1939), already in use, were used in CT, NJ, MD, and VA. All turtles >1 yr old were individually notched. To provide a secondary mode of identification, the carapace and plastron of each turtle was photographed in full sun or full shade (Fig. 2.3). Tissue samples were collected by trained or qualified observers following the Tissue Collection Protocol (Appendix VI; see Part III).



Figure 2.3. Examples of carapace (left) and plastron (right) photos taken during a standardized Wood Turtle survey. Note that the turtle is photographed with a dry shell, in even light. Photos by Mike Jones / MassWildlife.

Survey-specific data.—Surveyors recorded the time of day, weather (clear skies, partly cloudy, mostly cloudy, overcast, light rain, or heavy rain), air temperature (in shade), and water temperature (at the surface in the fastest current accessible) at the start and end of each survey. Surveyors also recorded the number of minutes not spent searching (e.g., due to processing turtles), predominant stream-bottom substrate (e.g., sand, silt, clay, gravel, rock), the predominant surrounding land cover/use (e.g. agriculture, mature forest), water visibility (e.g., clear, turbid, tannic, zero visibility), streamflow conditions (low-flow, elevated flow, bank full, flood), survey direction (upstream, downstream, or both directions), survey method (on foot, kayak/canoe, motorboat, snorkel/scope, or nesting survey), and stream size (small [$<7\text{m}$ wide], medium [$7\text{--}15\text{m}$ wide], large [$>15\text{m}$ wide]). At the end of each survey, participants summarized survey returns by recording: (a) the number of Wood Turtles found on land and in water; (b) the number of male, females, and juveniles found; (c) the number of Wood Turtles each observer detected; and (d) the identity (sex and notch code) of all turtles found. Data were recorded on a standardized data form for each survey (Appendix V).

Visual aids.—Polarized lenses, which facilitate detection of submerged turtles, were recommended for all surveyors. Underwater bucket scopes were used in Virginia. Snorkel equipment was used at a selection of long-term sites.

Decontamination.—Decontamination of equipment and field gear using the most recent NEPARC and SEPARC guidelines was strongly recommended for all participants (Miller and Gray 2009), but this was not actively tracked as part of the regional data analysis.

Additional considerations.—Boats (canoes, kayaks, and inflatable boats), though not formally a part of the recommended survey protocol, were allowed to facilitate surveys along large rivers. However, the Lead Observer would ideally search for turtles on foot >100 m ahead of the boat to avoid the disturbance of turtles.

Monitoring Framework

Our monitoring methodology constituted a nested framework in which there were two levels of sampling intensity: Rapid Assessment sites (low intensity) and Long-Term sites (high intensity). This framework provided a relatively expedient means of assessing new sites while simultaneously and intensively assessing the status and demography of select sites of conservation importance. LT sites required three surveys (see Section 2.1.2 below) during each of three separate seasons, totaling at least nine surveys, while RA sites only needed a minimum of three surveys in a single season—thus, LT sites consisted of replicates of RA sites. We selected RA sites in two ways: (1) opportunistically, targeting high quality habitats, potential populations, known populations, and data-deficient areas, or (2) randomly, from segments drawn from GIS-based models of stream habitat suitability. LT sites were selected non-randomly and, while any site with a known Wood Turtle occurrence could be chosen, LT sites were often strategically selected to represent previous long-term Wood Turtle monitoring sites or populations of regional conservation significance.

Data Reporting and Management

Surveyors submitted survey and individual data by using a centralized, password protected online data-entry portal (www.northeastturtles.org) or via email in the form of standardized, template Excel (.xls) or comma-separated values (.csv) files. Surveyors reported data seasonally or annually and the regional survey results were pooled into a single spreadsheet.

Site Delineation and Data Collection

Survey Segment Delineation

To delineate survey sites in GIS, we first converted start and end locations for stream survey segments from ArcGIS (version 10.5; Environmental Systems Research Institute, Redlands, CA, USA) Shapefiles to Google Earth Keyhole Markup Language (KML) files and hand-delineated the intervening stream segment using aerial photographs as guides. Working in Google Earth allowed for more accurate, streamlined, and rapid delineation of stream segments than ArcGIS-based delineation or the use of USGS National Hydrologic Dataset (NHD) flowlines. We mapped the vast majority of segments using the most recent leaf-off imagery available in Google Earth (typically from 2016). To maintain a high degree of accuracy in capturing the shape of stream segments, a concerted effort was made to map the exact centerline of all stream segments whenever possible. Occasionally segments were too narrow to be viewed using the most recent imagery, or foliage obscured aerial views of certain portions of segments. To address this issue, we used Google Earth's most recent historical imagery that provided sufficient leaf-off views of the actual stream channel.

Data Collection

We identified a range of spatial data that reflected factors known (e.g., impervious surface [Jones and Willey 2015]) or hypothesized (e.g., Index of Ecological Integrity [McGarigal et al. 2017]) to influence Wood Turtle abundance. We prioritized datasets that had been consistently measured across the entire 13-state region. We utilized North Atlantic Landscape Conservation Cooperative (NALCC) Designing Sustainable Landscapes (DSL) datalayers (http://www.umass.edu/landeco/research/dsl/products/dsl_products.html) for most covariates of interest, with the exception of roads, for which we used U.S. Census Bureau TIGER/Line® Shapefiles. NALCC DSL data took the form of raster layers that encompassed the entire Northeast Region (i.e., ME, NH, VT, MA, CT, RI, NY, NJ, PA, DE, MD, DC, VA, WV) using a 30-m cell size. Road data were downloaded as vector shapefiles by county and compiled for the entire region.

Table 2.1. Summary and descriptions of spatial data collected for the Regional Population Assessment.

Data layer	Description/calculation
Elevation	Digital Elevation Model (DEM)
July temperature	Mean annual daily temperature in July
January temperature	Mean annual minimum temperature in January
Heat index	Sum of daily mean temperatures above 35°C
Growing season degree days (GDD)	Sum of mean daily temperatures above 10°C
Incident solar radiation	The amount of sunlight reaching a given location. Derived from elevation using two algorithms. See www.umass.edu/landeco/research/dsl/products/ for details.
Precipitation	Mean annual precipitation
Flow accumulation	The amount of stream that flows into any given stream location
Land cover	Land cover map derived from The Nature Conservancy's Northeast Habitat Classification Map (Ferree and Anderson 2013; Anderson et al. 2013; Olivero and Anderson 2013; Olivero-Sheldon et al 2014).
Imperviousness	Percentage of the ground surface area that is impervious to water infiltration
Traffic rate	Estimated probability of an animal crossing the road being hit by a vehicle given the mean traffic rate
Index of Ecological Integrity (IEI)	A measure of relative intactness (i.e., isolation from adverse human modifications and disturbance) and resilience to environmental change (i.e., capacity to recover from or adapt to changing environmental conditions driven by human land use and climate change)
Roads	All roads classification types provided by U.S. Census Bureau TIGER/Line®

Site Covariate Extraction and Calculation

In ArcGIS, we converted KML files of survey segments into polyline shapefiles, making sure to preserve unique identification codes for each segment. We used the Buffer Analysis Tool in ArcGIS to create 300-m and 5500-m buffer polygon shapefiles around each survey segment, which represented “site” and “landscape” scales respectively. We used 300 m for the site scale because most studies of Wood Turtle spatial ecology have found that the large majority of movements occur within this distance of streams (Harding and Bloomer 1978; Compton et al. 2002; Saumure 2004). We used 5500 m for the landscape scale because this distance has been shown by Jones and Willey (2015) to be effective at predicting Wood Turtle abundance. We clipped (using the Clip Analysis Tool) road polyline shapefiles with buffer shapefiles. Road layers at both the 300-m and 5500-m scales were then rasterized using the Polyline to Raster Conversion Tool.

Land cover variables.—We calculated variables related to land cover in the R software environment (R version 3.1.1, <http://r-project.org/>, accessed 4 March 2017). We used the Maptools package (Bivard 2017) to read polyline buffer files and raster files into R. We extracted mean traffic rate, IEI, and imperviousness values from raster files at both 300-m and 5500-m buffer zones using the Raster package in R. We calculated road density and percent cover of land cover classes by dividing the number of cells within each class by the total number of cells within each buffer. We used the Formation and Ecosystem classification categories within the NALCC DSL land cover layer to represent general land cover variables. The Formation category consists of broad land cover classifications (e.g., “Developed”) while the Ecosystem category provides more specific classifications (e.g., “Motorway” or “Developed-low intensity”). We combined Northeastern Upland Forest and Boreal Upland Forest Formation types to represent forest cover. We used the Grassland and Shrubland Formation to represent early-successional cover. We used the Agriculture Formation to represent agricultural cover. We created a “Primary Wood Turtle Habitat” category by extracting all Wetland Ecosystem types that coincided with corroborated Wood Turtle occurrence (Jones and Willey 2015) locations throughout the Northeast. Primary Wood Turtle Habitat included 15 ecosystem types at the 5500-m scale:

- Laurentian-Acadian Alkaline Conifer Hardwood Swamp
- Laurentian-Acadian Freshwater Marsh
- Laurentian-Acadian Large River Floodplain
- Laurentian-Acadian Wet Meadow Shrub Swamp
- North Central Appalachian Acidic Swamp
- North Central Appalachian Large River Floodplain
- North Central Interior and Appalachian Rich Swamp

North Central Interior Large River Floodplain
North Central Interior Wet Flatwoods
North Atlantic Coastal Plain Basin Swamp and Wet Hardwood Forest
North Atlantic Coastal Plain Pitch Pine Lowland
Northern Appalachian Acadian Conifer Hardwood Acidic Swamp
Ruderal Shrub Swamp
Southern Piedmont Small Floodplain and Riparian Forest
North Atlantic Coastal Plain Basin Peat Swamp

Primary Wood Turtle Habitat at the 300-m scale included the same ecosystem types except North Atlantic Coastal Plain Basin Peat Swamp.

Environmental and stream covariates were determined in ArcGIS. We used the Extract Values to Points Spatial Analyst Tool to extract July temperature, minimum temperature, heat index, incident solar radiation, precipitation, elevation, growing-degree-days, and flow accumulation from the mid-points of survey segments. We measured segment length using Calculate Geometry in ArcGIS. We calculated gradient by dividing the change in elevation from start to end of each segment by the length of the segment. We calculated a relative measure of sinuosity by dividing the segment length by the straight-line distance between the start and end of each segment.

Data Exploration and Statistical Analyses

Data Summary and Exploration

We examined environmental, temporal, and political (i.e., U.S. state) patterns in survey effort, survey returns, and detection using a range of graphical techniques including standard graphs, barplots, histograms, and boxplots. We examined detection locations (land vs. water) to illustrate Wood Turtle behavioral patterns in relation to various environmental factors.

Demography

We measured within-subpopulation age structure by calculating the percent of all detections that were juveniles or subadults ≤ 14 years old. We calculated male:female ratios to assess potential sex imbalances or demographic stress. For both age structure and sex ratios, we examined surveys with >6 turtles detected to reduce the impact of survey effort and observer experience on estimates.

Because juveniles generally exhibit relatively lower detection and recapture rates than adults, we estimated the approximate regional average juvenile percentage obtained through standardized surveys that would reflect a true juvenile percentage of 0.25—a proportion considered to reflect a minimum stable stage distribution (Willey and Jones 2014). To calculate this recapture-rate-corrected juvenile percentage, we first calculated the average juvenile and adult recapture rates for all sites with >8 surveys across three seasons (e.g., sites for which we calculated capture-mark-recapture estimates; see below). We used these sites to ensure that adequate survey effort as well as annual and seasonal variation was accounted for. We then averaged the overall percent difference in recapture rates (adult recapture rate divided by juvenile rate) across all sites with at least eight surveys and one juvenile recapture. Finally, the ideal minimum juvenile percentage, 0.25, was divided by overall percent difference in recapture rate to obtain an approximate estimate of relative representativeness of juveniles in the population.

Estimation of Relative Abundance Using Hierarchical Models

Survey data preparation.—Before beginning analyses, we examined the master survey dataset for missing data, outliers, and other factors that may influence analyses. We removed a single survey segment in New Brunswick, Canada from the analysis because we were unable to acquire environmental variables from that portion of the species range. We replaced four missing survey dates with the median date with respect to the first and last survey of the season for the survey’s respective state and year. We replaced 39 missing start-weather (weather at the beginning of the survey), 19 missing total search times (length of survey minus the time spent processing turtles), five missing total number of observer values, 12 missing start times, and 55 missing starting air temperature values with the median value for each respective variable. We excluded nine survey variables (starting water temperature, end water temperature, end air temperature, end weather, survey direction, survey method, water visibility, streamflow, and stream size) from consideration in models due to large numbers of missing values (>225). We changed start-weather values that were reported as “light rain” or “heavy rain” to “overcast” because they made up a small portion of the surveys (n=46). We then converted start-weather to a continuous numerical variable representing the relative approximate amount of cloud cover, where “clear” = 0, “partly cloudy” = 1, “mostly cloudy” = 2, and “overcast” = 3. We converted survey date to ordinal date (e.g., January 1 = 1, December 31 = 365). We represented time of day as a proportion of 24 hours. We scaled all continuous covariates such that mean = 0 and SD = 1 in order to facilitate model convergence.

We identified varying degrees of geographic overlap for several survey segments throughout the range. If greater than two thirds of either segment was overlapping another segment, they were combined and treated as the same segment. In situations such as these, we chose to use the survey segment that was surveyed the most. If less than one third of either segment was overlapping, we treated each segment as a

separate site. If greater than one third, but less than two thirds of the segment were overlapping, we dropped the site with fewer surveys or that which minimized the number of segments that would need to be dropped nearby (e.g., survey segments along a river in NY were all overlapping by approximately one half).

Modeling approach.—We used hierarchical N-mixture models (Royle 2004) to relate relative Wood Turtle abundance to stream, environmental, and land cover variables. This modeling approach uses repeated surveys at sites to account for bias associated with detection (Thompson 2002) to produce relative measures of abundance. We used closed population N-mixture models, which assume that no deaths, emigration, or immigration occur within a sampling period. We were unable to completely meet these assumptions because surveys were conducted over enough time that deaths may have occurred and individuals likely immigrated and emigrated from 1-km segments, even within a single season. We fit models using the Unmarked package (Fiske and Chandler 2011) in R. We included all surveys at sites that were surveyed at least 3 times. Covariates considered for inclusion in models are provided in Table 2.2.

To assess the impact of including all surveys (which varied in number and spanned multiple seasons and years per site) within models, which represents a violation of the model assumptions, we compared results to those using data that only included sites with three surveys during the spring season. We removed sites with fewer than three surveys and randomly selected surveys from sites with >3 surveys in a given spring season. We treated each year as a separate sampling unit and included year as a site covariate in all models. We removed surveys from 2014 and 2017 to reduce the variable–sample size ratio because we conducted relatively few surveys in those years. This reduced dataset still violated the model assumptions (because deaths, immigration, and emigration are still possible within a single spring season), but to a lesser degree than when using all surveys.

Table 2.2. Detection and site covariates considered for inclusion in N-mixture models. We examined site covariates within three different conceptual categories: environmental, stream, and land cover.

Detection Covariates	Site Covariates		
	Environmental	Stream	Land Cover (300 and 5500 m)
Time spent searching	July temperature	Segment length	% undeveloped
Time of day	Solar incidence	Gradient	% moderately developed
Cloud cover	Precipitation	Sinuosity	% highly developed
Air temperature	January temperature	Flow accumulation	Mean imperviousness
Number of observers	Heat index		Road density
Ordinal date	Elevation		Traffic rate
Growing-degree-days			% forest cover
Date*GDD interaction			% early-successional cover
Season			% agricultural land
			% primary habitat
			Mean IEI

Model selection.—We compared negative binomial and zero-inflated Poisson error distributions (Fig. 2.4, stage 1) by comparing the performance of models containing all observation covariates as well as a selection of six land cover covariates at the 300-m scale (wetland/riparian cover, forest, early-successional, traffic rate, agriculture, and impervious surface score). We included only a subset of the site covariates in order to avoid overfitting. A negative binomial error distribution performed considerably better than zero-inflated Poisson distribution with regard to Akaike’s Information Criterion (AIC) and was used for all subsequent models. We performed an additional comparison of distributions after the final model was selected to confirm that negative binomial was indeed the most appropriate distribution for the data. We used AIC to compare models (Burnham and Anderson 2002) and identify a single, best performing model. We employed a multi-stage model building process (Fig. 2.4; similar to that conducted by Roberts and King [2017]). We began by determining the best performing detection covariates. To do this, we fit all subsets of detection covariates using the MuMIn package (Barton 2015) in R. We included six arbitrarily selected land cover variables at the 300-m scale (primary habitat, forest, early-successional, traffic rate, agriculture, and impervious surface score) that were relatively uncorrelated ($r < 0.7$) as fixed

covariates in all detection candidate models. We kept detection covariates for inclusion in subsequent models that were statistically significant ($P \leq 0.05$) and appeared in models that performed better than the null model and had a $\Delta AIC < 2$ (Smetzer et al. 2014).

We then used the same selection criteria to determine the best performing covariates within each of three site covariate categories: stream, environmental, and land cover (Fig. 2.4). July temperature, minimum temperature, and heat index were highly correlated ($r > 0.7$), leading us to exclude July temperature and minimum temperature from consideration because heat index performed best in single variable models (based on AIC). From earlier analyses (Jones and Willey 2015), we understood that Wood Turtle occurrence may be greatest at intermediate elevations regionally (though generally lower in the northern populations and higher in the southern populations); therefore, we included a quadratic term for elevation in the environmental variable selection process. Because there were a large number of land cover variables under consideration, we divided the selection process into sub-stages, where the best performing variables were first identified at the 300-m and 5500-m scales (using the previously described criteria), which were then used to determine the best overall land cover covariates in a final selection process. We considered quadratic terms for non development-related land cover covariates: agriculture, early-successional cover, forest, and wetland/riparian habitat. Lastly, we conducted a final model determination process where the best performing model among all subsets of the previously identified covariates was selected. We performed a parametric bootstrap in the “unmarked” package (Fiske and Chandler 2011) in R to test for goodness-of-fit.

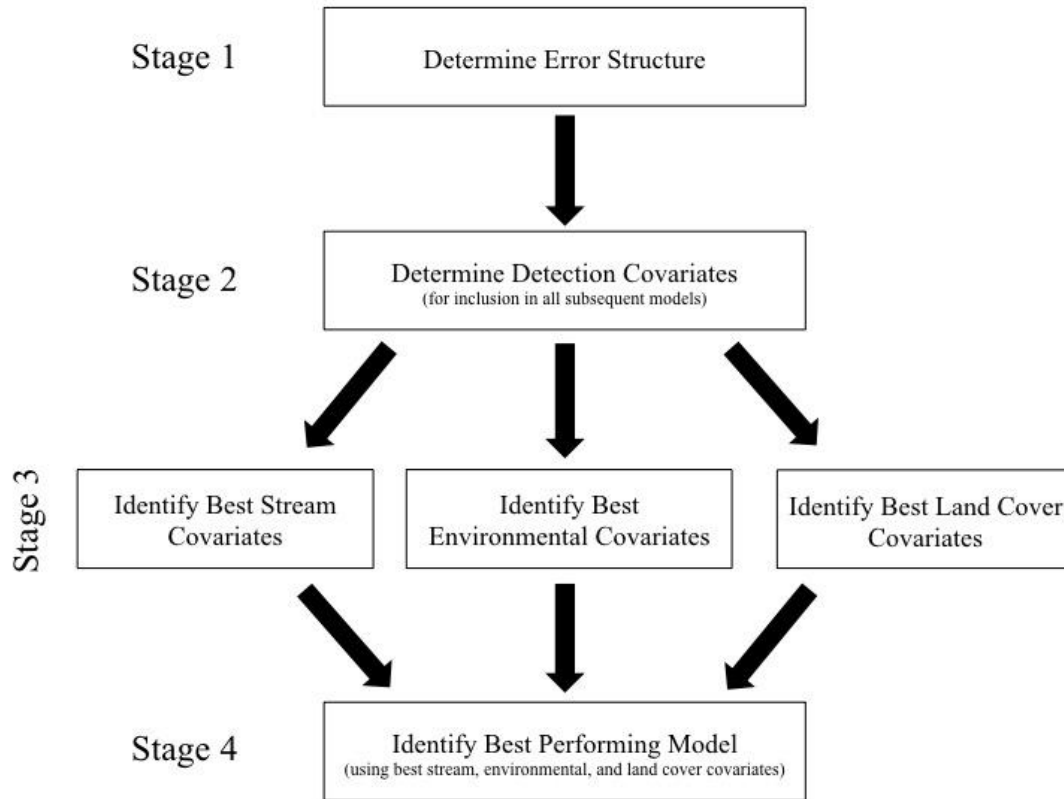


Figure 2.4. Conceptual model depicting the model building process used to identify the best performing N-mixture model of Wood Turtle abundance.

To help understand the effects and potential benefits of using different subsets of data, we examined the results for three variations of response variable: (1) total Wood Turtles detected during spring only, (2) lead observer (Observer #1) detections during spring only, and (3) total Wood Turtles detected throughout the year. The number of lead observer detections was missing for 64 surveys, therefore we imputed the average proportion of turtle detections by the lead observer per state per respective number of observers.

Relative Population Size Estimates

Modeling approach.—We estimated population sizes using two Capture-Mark-Recapture (CMR) based models: (1) Jolly-Seber loglinear open-population models (Cormack 1985, 1989) and (2) closed-population loglinear models corrected for bias (Rivest and L’evesque 2001). We used the Rcapture package (Rivest and Baillargeon 2014) in the R to perform analyses. To reduce the influence of autocorrelated recaptures in the same season, we compared two strategies for conducting closed population estimates: (1) using each survey as a separate capture event within models and (2) pooling captures within seasons to form a single capture event per season.

Jones and Willey (2015) suggested that the target sampling regime to estimate population size should be three surveys conducted within each of at least three seasons (i.e., minimum nine surveys) across no more than two years. This sampling regime was meant to reflect temporal and seasonal variation in capture rates while striving to achieve model assumptions and allowing for logistic flexibility for surveyors. For this analysis, using all surveys, across all years represented a clear violation of closed population model assumptions because sites likely experienced substantial immigration and emigration and potentially deaths over what was in some cases a four-year period. However, we determined that there was value in understanding relative population sizes even if they were not absolute population size estimates. Therefore, we decided to calculate CMR estimates—for sites with sufficient sampling effort—using all available survey information, with the understanding that they would represent *relative* population size, not absolute population size estimates.

To examine the extent to which violating the guidelines of Jones and Willey (2015) impacted relative population estimates, we calculated estimates using two different datasets: (1) all surveys at sites that had been surveyed at least nine times across at least three different seasons, irrespective of year and (2) sites with an “ideal” sampling regime (three surveys conducted within each of at least three seasons across no more than two years). For sites that had >3 surveys within a given season, but otherwise met the ideal requirements, we randomly selected three surveys from those seasons.

In an effort to examine the relationship between population estimates and survey effort, we calculated bias-corrected closed-population size and standard error with each successive survey conducted at each site. To visualize this relationship, we plotted select sites with standard error values as well as all sites together (without standard error for visualization purposes).

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Results

Survey Summary

Standardized surveys were conducted across all northeastern states, the District of Columbia, and New Brunswick from spring of 2012 to spring of 2017 (Table 2.3). In total, we obtained standardized data from 2141 surveys at 464 stream segments across all states except Rhode Island (where surveys were conducted, but not reported to the regional team). We conducted 835 surveys in 2012 and 2013 as a part of the RCN status assessment, 168 surveys between the two major phases of this effort in 2014, and 1138 surveys after the start of CSWG in 2015 (Fig. 2.5). Massachusetts was the most intensively sampled state

(Table 2.3, Fig. 2.6) with 489 surveys conducted, followed by Maine (400), and New Hampshire (347). The most survey segments were established in Maine with 115, followed by 88 in Massachusetts, and 66 in New Hampshire (Table 2.3). Sites were sampled 1–31 times (highly sampled sites were typically targets for genetic material, see Part III), averaging 4.6 surveys per site. West Virginia had the most intensively sampled sites with 7.5 surveys per site, followed by Vermont, Pennsylvania, and Massachusetts. Approximately 50% of surveys (1074) were conducted in spring, 42% (897) were conducted in fall, and 8% (169) were conducted during the nesting period (Table 2.3, Fig. 2.7, Fig. 2.8). The proportion of surveys conducted in the spring ranged from 12–97% across political boundaries (excluding states and provinces with <4 surveys). The average number of observers per survey ranged from 1–3.09 across states and provinces, averaging 1.95 observers per survey throughout the region. A total of 392 different federal, state, and university biologists and students participated in surveys, ranging 2–69 per state/province (Table 2.3).

Overall, surveys were more likely to yield a detection in spring (Table 2.3). Overall Wood Turtle detections (Fig. 2.9) as well as detections on land per survey (Fig. 2.10) were greater during spring than fall. Surveys were conducted in air temperatures ranging from -2–32°C and water temperatures ranging from -1.5–25°C. Ninety percent of Wood Turtle detections on land occurred at air temperatures >8.9°C (Fig. 2.11). The percent of surveys that yielded a detection on land was 39% during clear skies, 38% when partly cloudy, 34% when mostly cloudy, and 29% when overcast. Wood Turtles were more likely to be detected on land during cold temperatures (<9°C) during the spring when skies were clear (Fig. 2.12). Surveys tended to yield more land detections earlier in the day when there were clear skies and there was a greater difference between start and end air temperatures (Fig. 2.13). The percentage of surveys that yielded a detection was nearly identical for surveys conducted moving both down and upstream (59% and 58% respectively).

In total, we recorded 4611 Wood Turtle (1958 males, 1773 females, and 727 juveniles) detections across all surveys, including recaptures, averaging 2.15 turtles per survey (sd = 3.81), 1.39 (sd = 2.41) of which were detected by the lead observer (Observer 1; Table 2.3). Regional catch per unit effort (CPUE) for observer 1 was 1.52 turtles/survey during spring and 1.2 turtles/survey during fall. This seasonal pattern was consistent throughout much of the Northeast, except the southernmost states (MD, VA, and WV) where CPUE for Observer 1 was greater in fall than spring. Zero turtles were detected during 894 surveys—approximately 42% of all surveys conducted (Table 2.3). All states/provinces except Virginia had a greater percentage of surveys with zero detections in the fall than spring. Surveyors failed to detect a turtle at 187 of 464 stream segments (40.3%).

Table 2.3. Summary of standardized Wood Turtle surveys conducted throughout the northeastern United States from 2012–2017.

Variable	CT	DC	DE	MA	MD	ME	NB	NH	NJ	NY	PA	VA	VT	WV	Total
Surveys	70	2	2	489	30	400	4	348	196	41	247	265	17	30	2141
Segments (sites)	15	2	2	88	10	115	1	67	51	14	44	48	3	4	464
Average observers/survey	1.56	1	2	1.27	2.07	2.18	2.75	1.64	1.95	2.02	2.19	3.09	1.12	2.9	1.95
Survey participants	19	2	3	33	8	45	8	38	67	16	62	69	4	18	392
Surveys in spring	40	0	1	310	29	119	0	186	71	5	140	156	6	12	1075
Proportion of surveys conducted in spring	0.57	0	0.5	0.63	0.97	0.29	0	0.53	0.36	0.12	0.57	0.59	0.35	0.4	0.50
Surveys in nesting season	0	0	0	32	0	95	3	20	9	0	10	0	0	0	169
Proportion of surveys conducted in nesting season	0	0	0	0.07	0	0.24	0.75	0.06	0.05	0	0.04	0	0	0	0.08
Surveys in fall	30	2	1	147	1	186	1	142	116	36	97	109	11	18	897
Proportion of surveys conducted in fall	0.43	1	0.5	0.3	0.03	0.46	0.25	0.41	0.59	0.88	0.39	0.41	0.65	0.6	0.42
Total turtles	69	0	0	514	409	934	20	641	232	41	484	1098	25	144	4611
Turtles in spring	42	0	0	385	388	228	0	425	140	16	334	662	7	40	2667
Turtles in fall	27	0	0	117	21	308	0	196	81	25	143	436	18	104	1476

Variable	CT	DC	DE	MA	MD	ME	NB	NH	NJ	NY	PA	VA	VT	WV	Total
CPUE	0.99	0	0	1.05	13.63	2.34	5	1.84	1.18	1	1.96	4.14	1.47	4.8	2.15
CPUE standard deviation	1.53	0	0	1.59	9.41	3.61	5.77	2.52	1.9	1.64	2.69	6	3.34	5.97	3.81
CPUE in spring	1.05	0	0	1.24	13.38	1.92	0	2.28	1.97	3.2	2.39	4.24	1.17	3.33	2.49
CPUE in fall	0.9	0	0	0.8	21	1.66	0	1.38	0.7	0.69	1.47	4	1.64	5.78	1.65
CPUE observer 1	0.73	0	0	0.93	7	1.47	2.25	1.5	0.77	0.98	1.16	2.19	1.12	2.5	1.39
CPUE observer 1 in spring	0.65	0	0	1.08	6.97	1.21	0	1.82	1.27	3.2	1.26	1.71	1.17	2.33	1.52
CPUE observer 1 in fall	0.83	0	0	0.74	8	1.11	0	1.15	0.47	0.67	1.12	2.88	1.09	2.61	1.20
Surveys with zero turtles	40	2	2	252	0	149	2	130	110	24	90	77	8	8	894
Proportion of surveys with zero turtles	0.57	1	1	0.52	0	0.37	0.5	0.37	0.56	0.59	0.36	0.29	0.47	0.27	0.42
Surveys with zero turtles in spring	23	0	1	145	0	46	0	61	29	2	46	52	2	3	410
Proportion of spring surveys with zero turtles	0.57	0	1	0.47	0	0.39	0	0.33	0.41	0.4	0.33	0.33	0.33	0.25	0.38
Surveys with zero turtles in fall	17	2	1	83	0	74	1	60	76	22	39	25	6	5	411
Proportion of fall surveys with zero turtles	0.57	1	1	0.56	0	0.4	1	0.42	0.66	0.61	0.4	0.23	0.55	0.28	0.46

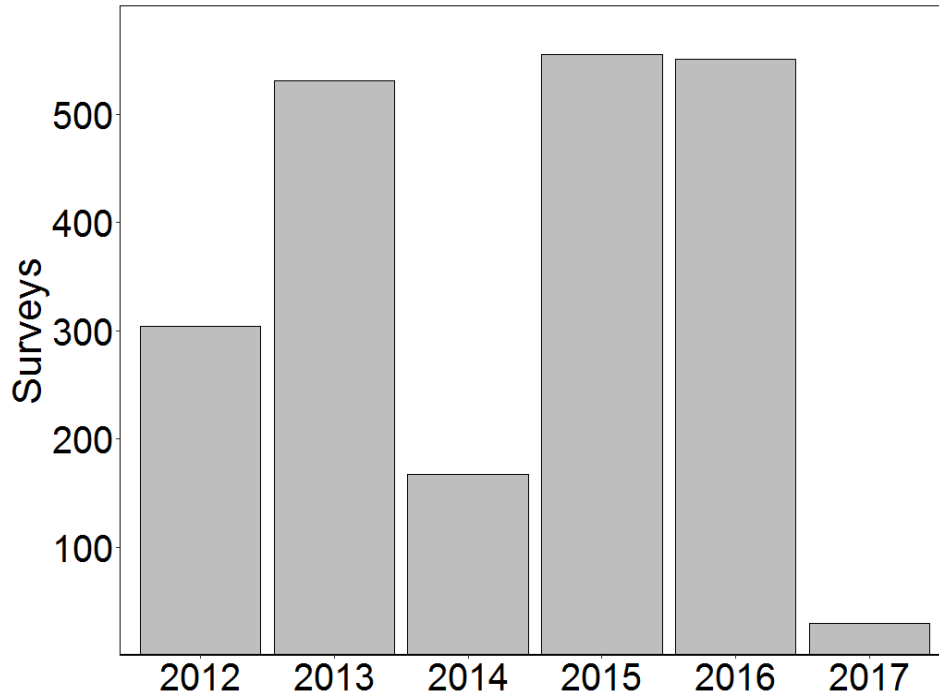


Figure 2.5. Number of standardized Wood Turtle surveys conducted per year throughout the northeastern United States (Virginia to Maine).

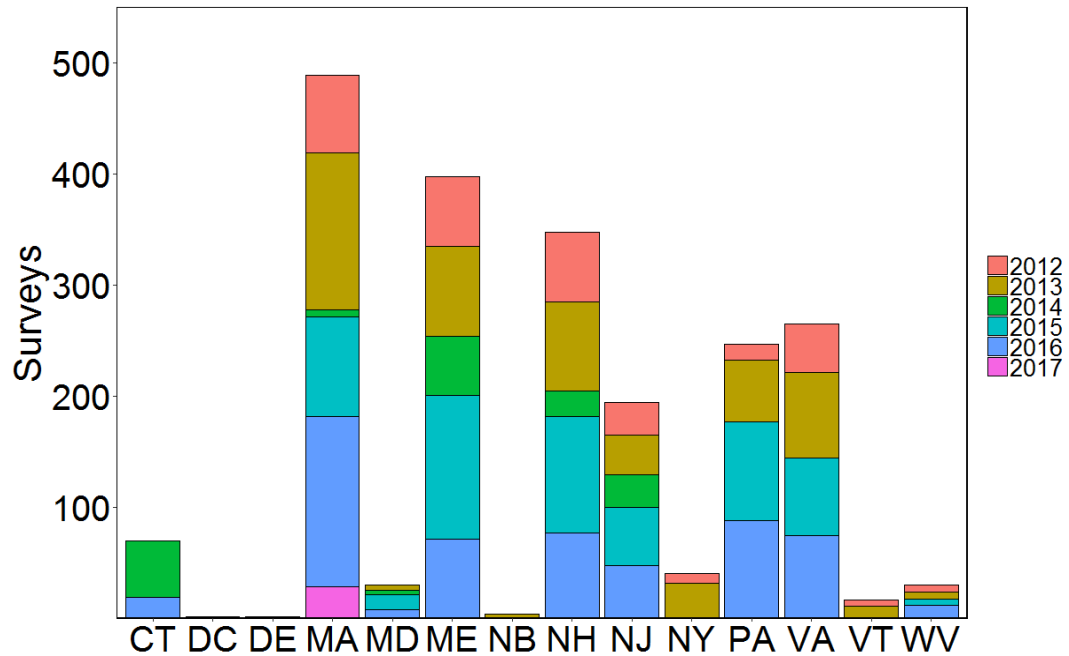


Figure 2.6. Number of standardized Wood Turtle surveys per state/province by year (indicated by colors).

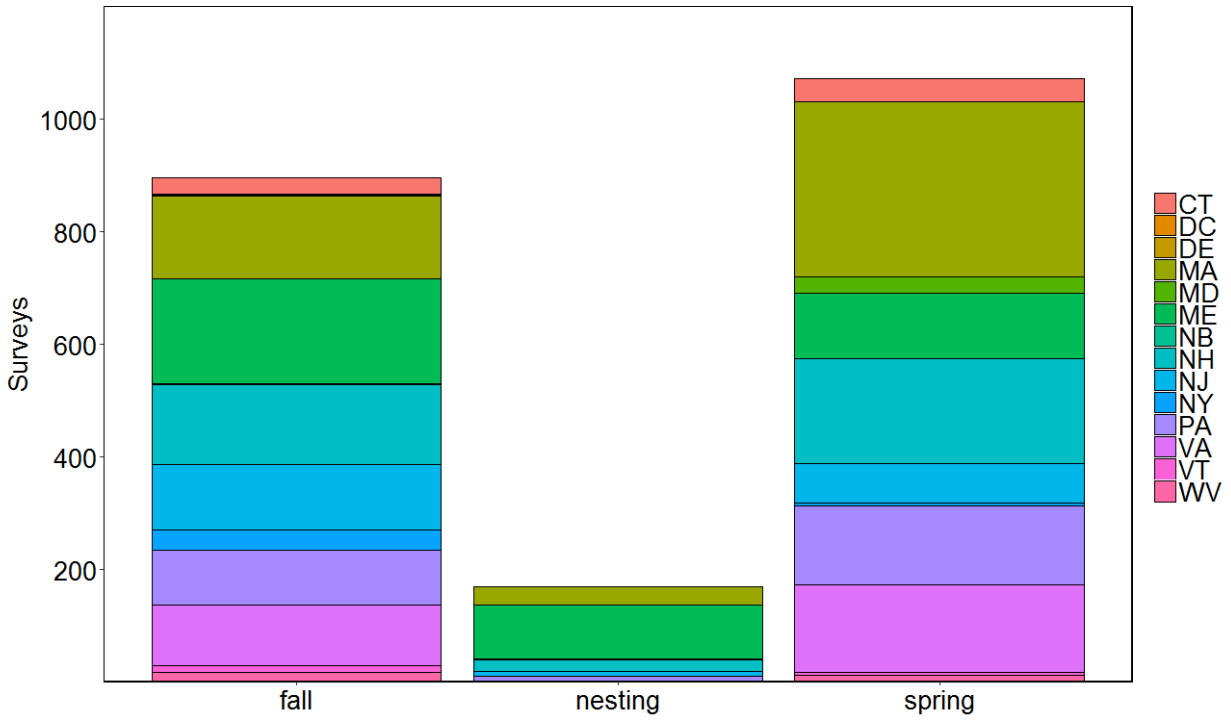


Figure 2.7. Number of surveys conducted during each season across all years (2012–2017). Data were collected during standardized surveys conducted from Virginia to Maine.

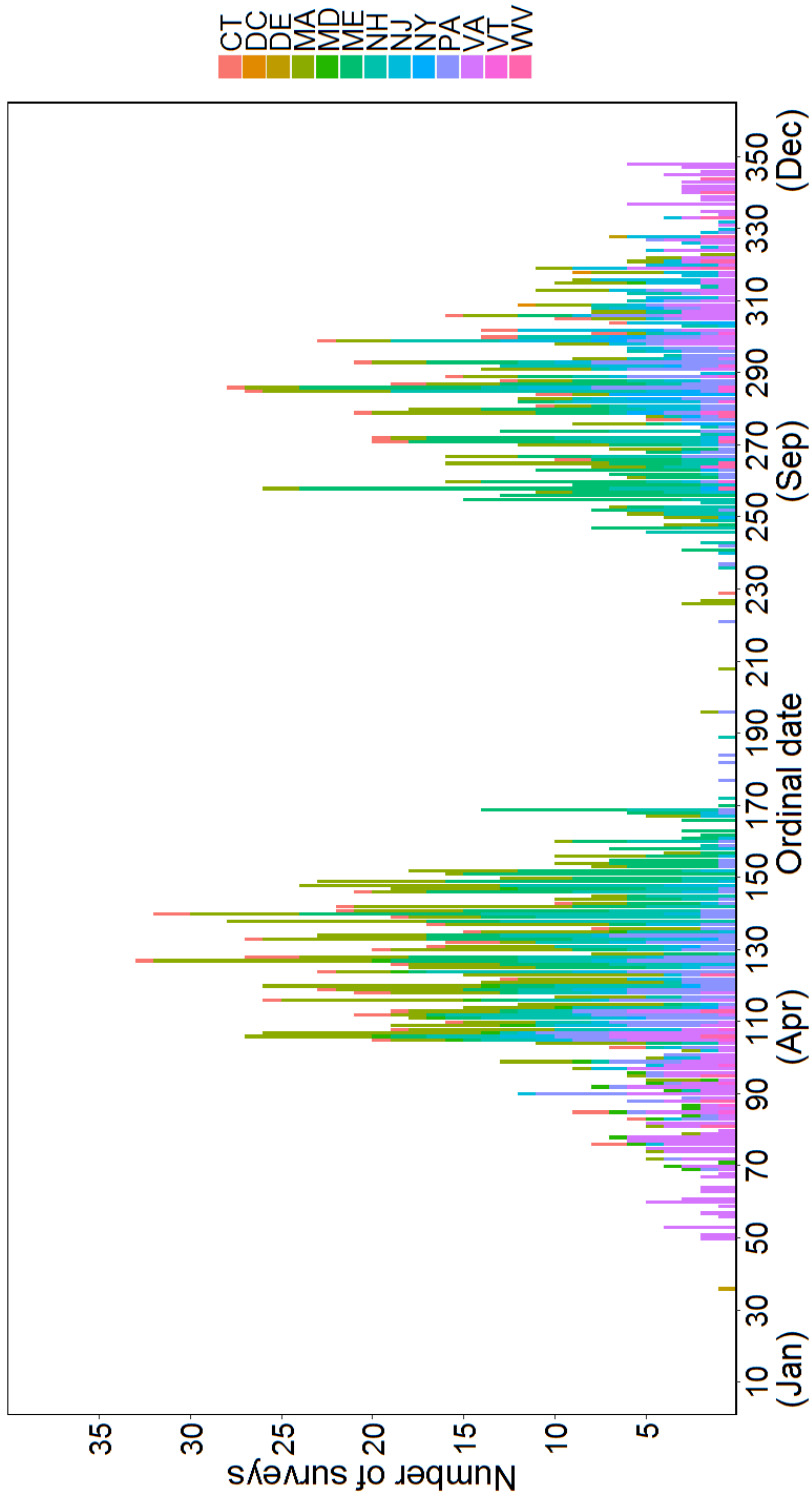


Figure 2.8. Number of standardized Wood Turtle surveys per day of year by state (indicated by colors).

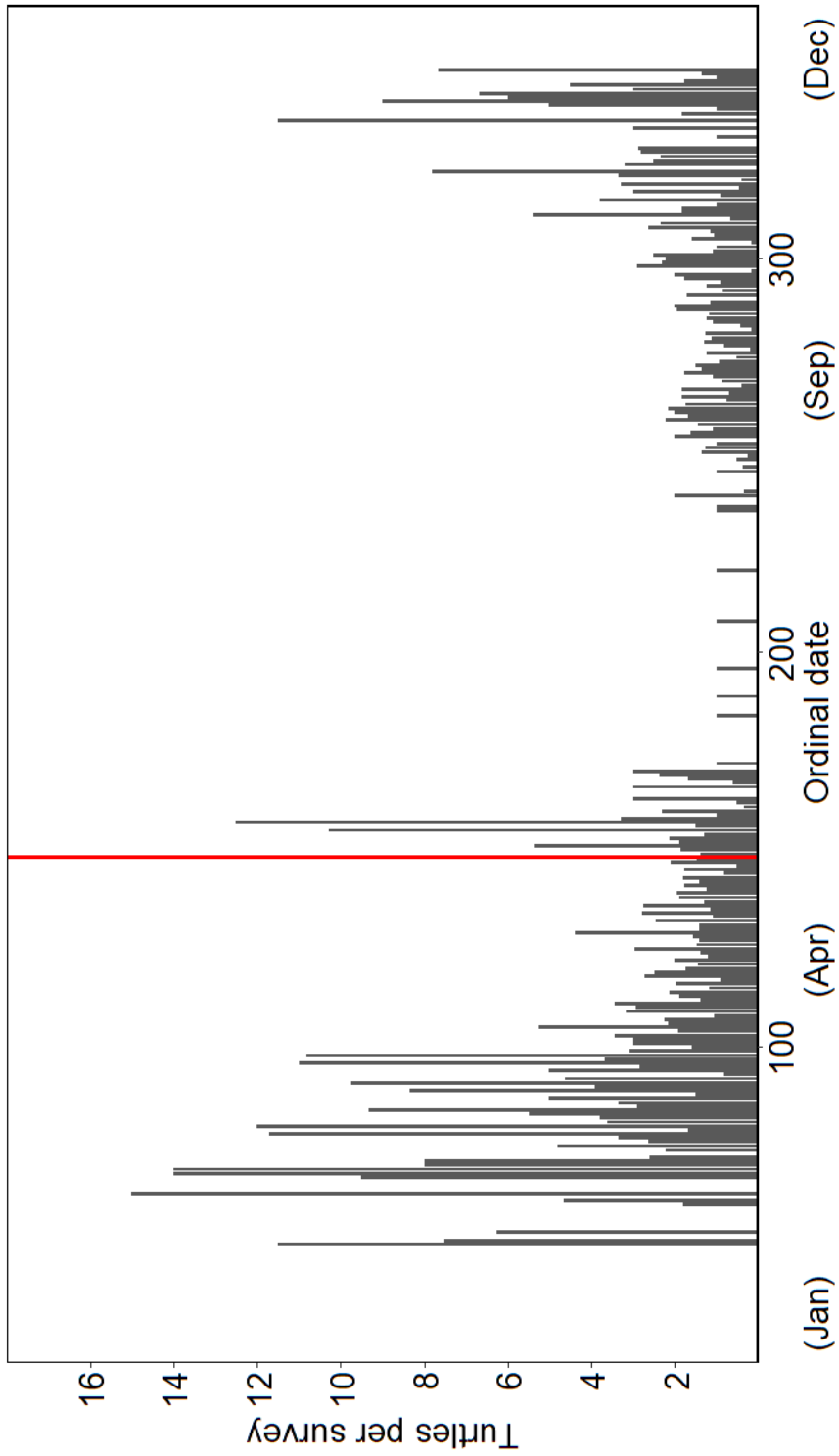


Figure 2.9. Turtle detections per survey by day of the year. Red line indicates the beginning of nesting season. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

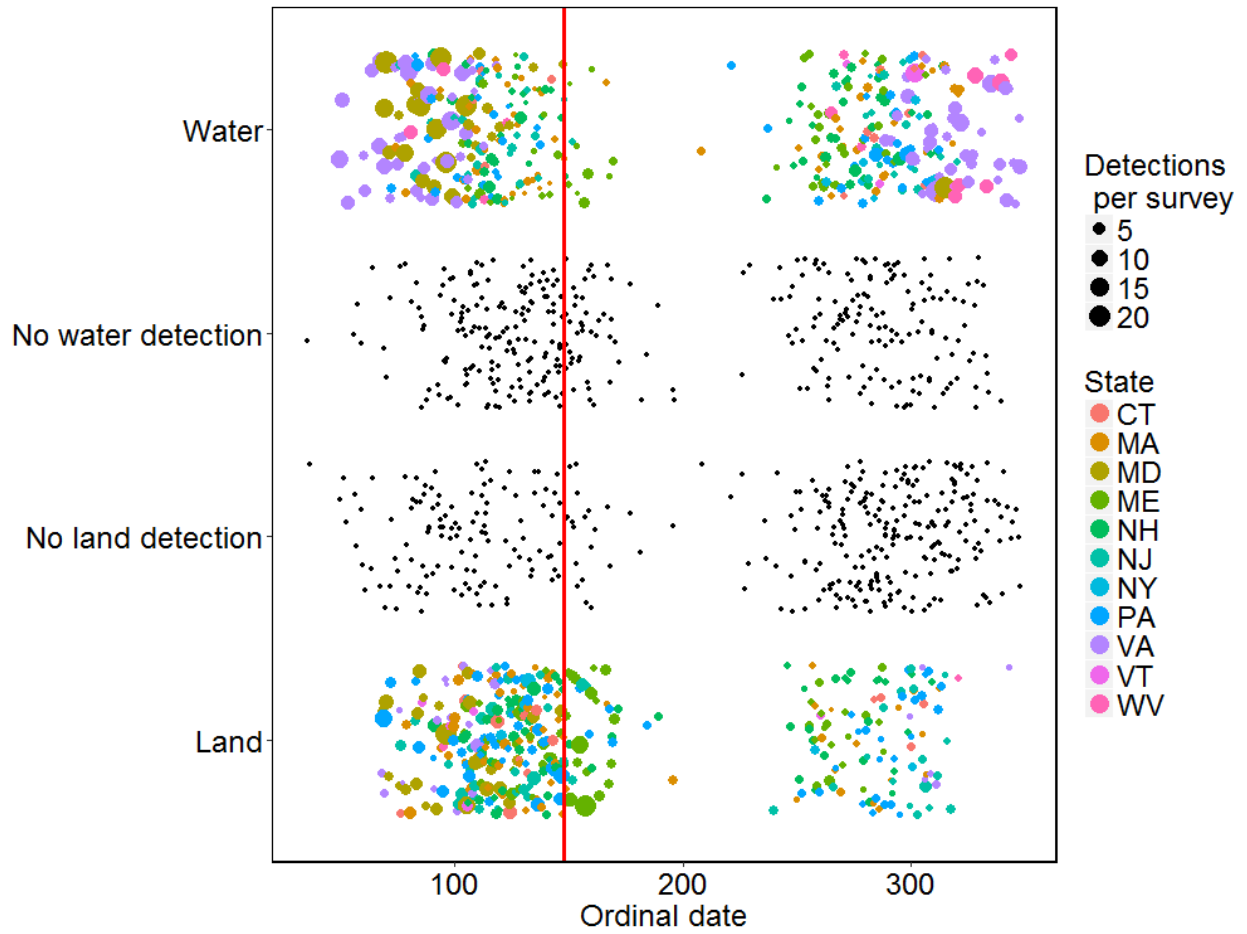


Figure 2.10. Land and water detections per survey for each day of the year for each state. Central rows represented by black points reflect days of year per state that yielded no detections across all surveys. The red line indicates the start of the nesting season (May 28). Data were collected during standardized surveys conducted from 2012–2017 throughout the northeastern United States (Virginia to Maine).

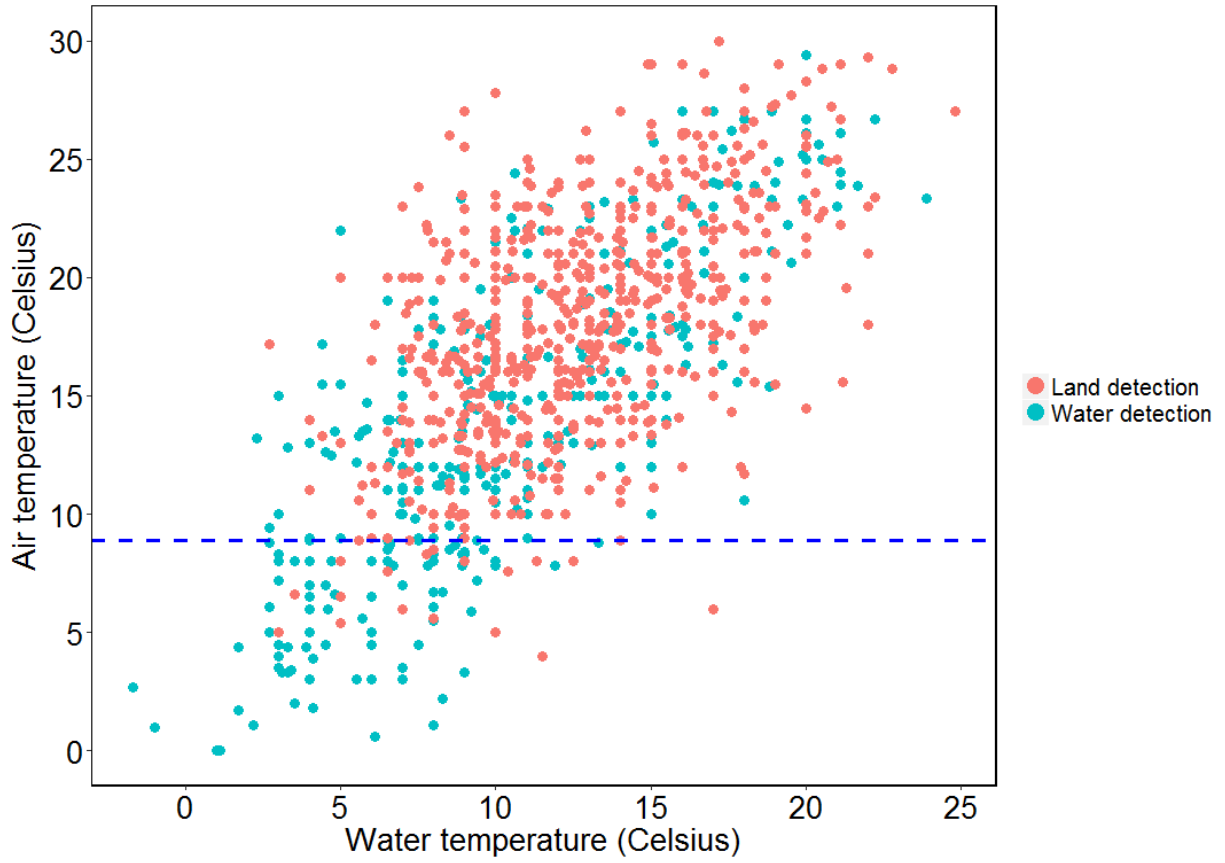


Figure 2.11. Land and water Wood Turtle detections in relation to air and water temperature. Blue dashed line indicates the air temperature (8.9° C) above which 90% of land detections were recorded. Data were collected during standardized surveys conducted from 2012–2017 throughout the northeastern United States (Virginia to Maine).

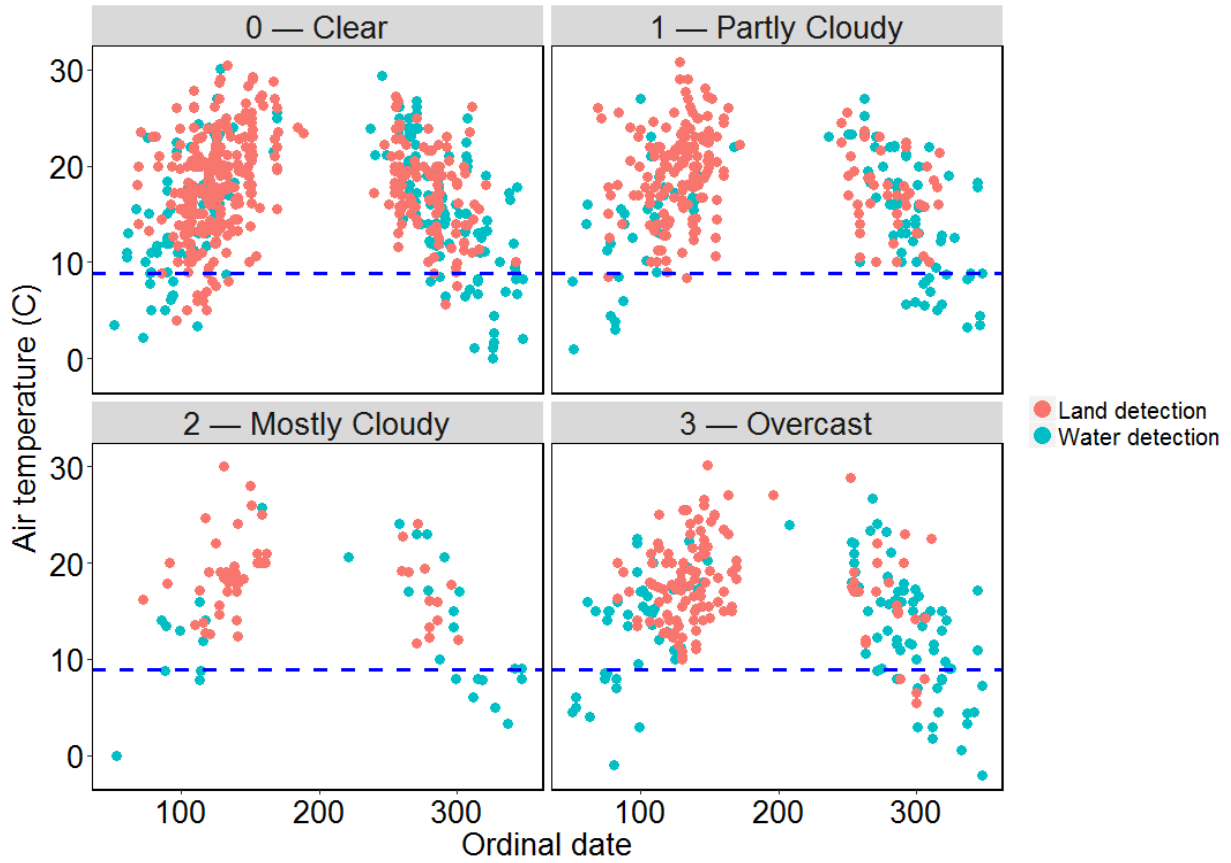


Figure 2.12. Land and water Wood Turtle detections in relation to air temperature and day of year within varying levels of cloud cover. Blue dashed lines indicate the air temperature (8.9° C) above which 90% of land detections were recorded. Data were collected during standardized surveys conducted from 2012–2017 throughout the northeastern United States (Virginia to Maine).

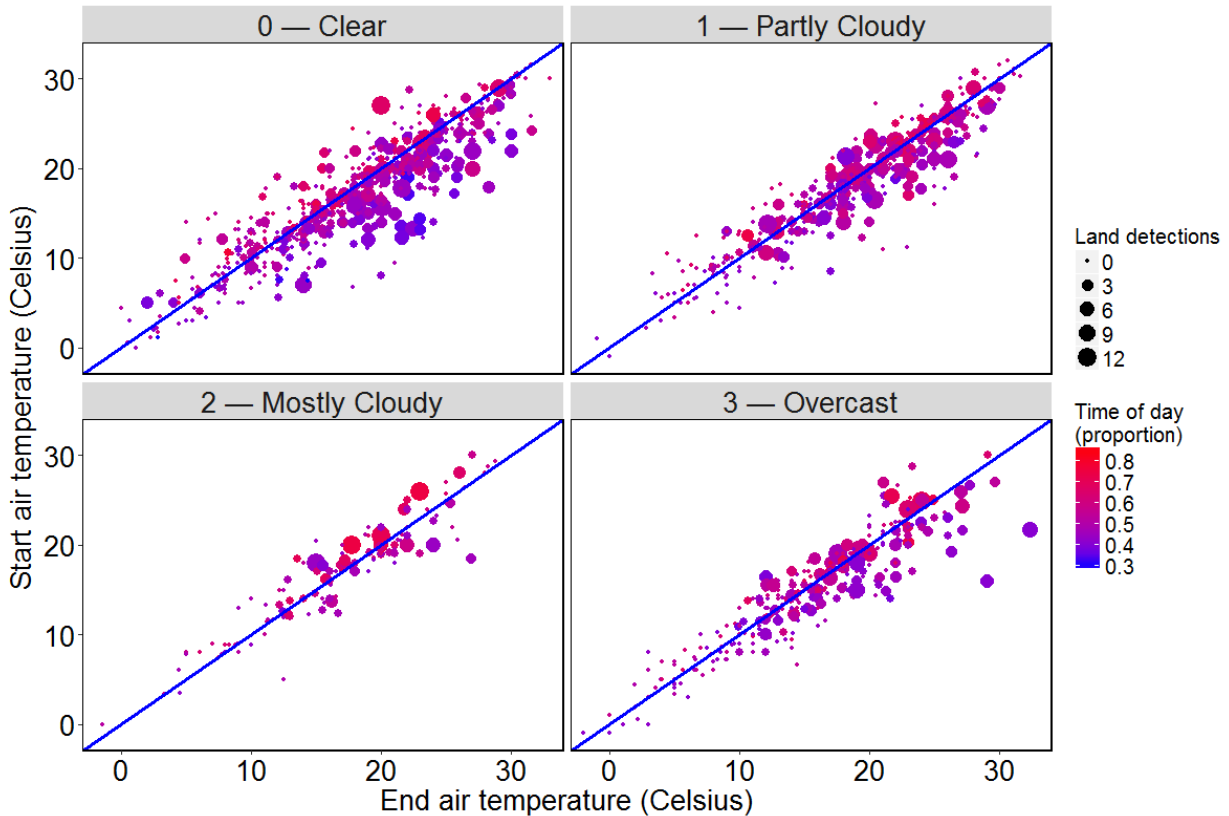


Figure 2.13. Wood Turtle land detections in relation to start and end (of survey) air temperatures by cloud cover. Point color represented the time of day. The largest points represent surveys with 12 or greater land detections. The blue line represents equal start and end temperatures. Data were collected during standardized surveys conducted from 2012–2017 throughout the northeastern United States (Virginia to Maine).

Age distribution.—Juveniles made up 16% of regional detections and state-specific overall juvenile percentages ranged 6–21% for states with >4 surveys (Table 2.4). Regionally, a greater percentage of juveniles were found in the spring (0.67%, excluding nesting season) than fall (Table 2.4); however, seasonal juvenile capture rates varied considerably by state/province (Table 2.4). Juveniles made up a greater proportion of overall within-season detections in spring (18%) than fall (13%; Table 2.5), but the difference between season was smaller (16% and 15% respectively) when only sites with >6 detections were considered. Overall the majority of sites across the Northeast consisted of <25% juveniles (Fig. 2.14, Fig. 2.15). New Hampshire, Virginia, and Connecticut showed the largest overall percentage of detections that were juveniles (19%), while Pennsylvania displayed the lowest (6%; Table 2.4). Overall, the percent of detections that were juvenile did not increase with CPUE and, in fact, appeared to decline with increased CPUE (Fig. 2.16). Overall, because juveniles are harder to detect than adults, we estimated that—on average—juveniles would need to make up 14.3% of survey detections throughout the region in order to represent 25% of populations.

Table 2.4. State-based summary of demographic information collected during standardized Wood Turtle surveys conducted throughout the northeastern United States (Virginia to Maine) and New Brunswick from 2012–2017.

Variable	CT	DC	DE	MA	MD	ME	NB	NH	NJ	NY	PA	VA	VT	WV	Total
Surveys	70	2	2	489	30	400	4	348	196	41	247	265	17	30	2141
Segments (sites)	15	2	2	88	10	115	1	67	51	14	44	48	3	4	464
Juveniles (all seasons)	13	0	0	95	56	128	8	122	25	5	29	206	10	30	727
Proportion of juveniles detected in spring	1	0	0	0.74	1	0.45	0	0.75	0.7	0.2	0.77	0.66	0.1	0.33	0.67
Proportion of juveniles detected in fall	0	0	0	0.26	0	0.55	0	0.25	0.3	0.8	0.23	0.34	0.9	0.67	0.33
Overall proportion of juvenile detections	0.19	0	0	0.18	0.14	0.14	0.4	0.19	0.11	0.12	0.06	0.19	0.4	0.21	0.16
Mean proportion of juvenile detections	0.31	0	0	0.22	0.11	0.16	0.4	0.19	0.06	0.08	0.08	0.14	0.25	0.19	0.16
Mean proportion of juvenile detections across sites with >6 detections	0.18	0	0	0.23	0.14	0.15	0.4	0.18	0.12	0.12	0.09	0.17	0.37	0.19	0.17
Mean juveniles/survey/site	0.25	0	0	0.16	1.4	0.21	2	0.2	0.04	0.11	0.1	0.73	3.03	0.86	0.27
Mean juveniles/survey/site in spring	0.39	0	0	0.2	1.53	0.28	0	0.3	0.16	0.11	0.11	0.98	0.17	0.83	0.36
Mean juveniles/survey/site in fall	0	0	0	0.15	0	0.19	0	0.16	0.02	0.11	0.04	0.79	0.3	0.88	0.23
Males, total	30	0	0	230	154	298	3	296	117	14	261	474	10	71	1958
Mean males/survey/site	0.39	0	0	0.33	4.42	0.36	0.75	0.51	0.3	0.37	0.85	1.37	1.57	2.12	0.62
Males in spring	12	0	0	167	141	73	0	181	66	5	163	269	2	16	1095
Mean males/survey/site in spring	0.24	0	0	0.43	4.41	0.43	0	0.64	0.61	1	0.98	1.69	0.33	1.33	0.84
Males in fall	18	0	0	60	13	173	0	113	49	9	95	205	8	55	798
Mean males/survey/site in fall	0.57	0	0	0.26	13	0.6	0	0.69	0.32	0.25	0.8	1.65	1.59	2.58	0.71
Females, total	25	0	0	168	175	483	7	195	81	18	189	387	5	40	1773
Mean females/survey/site	0.48	0	0	0.25	5.3	0.89	1.75	0.37	0.22	0.37	0.59	1.23	0.44	1.17	0.67
Females in spring	16	0	0	136	167	103	0	145	54	10	148	232	4	11	1026
Mean females/survey/site in spring	0.33	0	0	0.35	5.48	0.77	0	0.54	0.51	1.33	0.82	1.6	0.67	0.92	0.84
Females in fall	9	0	0	23	8	80	0	41	20	8	40	155	1	29	414
Mean females/survey/site in fall	0.63	0	0	0.12	8	0.39	0	0.27	0.16	0.22	0.31	1.4	0.33	1.25	0.44
Overall male:female ratio	1.20	NA	NA	1.37	0.88	0.62	0.43	1.52	1.44	0.78	1.38	1.22	2.00	1.78	1.10
Mean male:female ratio (sites with at least 1 female)	0.71	NA	NA	1.42	1.01	0.86	0.43	1.23	1.48	0.94	1.64	1.33	2.62	2.17	1.25
Sites with 0 females detected	2	0	0	9	0	3	0	8	3	1	6	0	1	0	33
Mean male:female ratio (sites with at least >6 detections)	0.99	NA	NA	1.48	0.73	0.95	0.43	1.59	1.81	0.56	1.84	1.38	2.62	2.17	1.4
Sites with >6 detections and 0 females	0	0	0	2	0	0	0	1	0	0	0	0	0	0	3

Table 2.5. Season-based summary of demographic information collected during standardized Wood Turtle surveys conducted throughout the northeastern United States (Virginia to Maine) and New Brunswick from 2012–2017.

Variable	Fall	Nesting	Spring
Number segments	258	103	312
Mean % of detections that were juveniles	0.13	0.17	0.18
Mean % of detections that were juveniles for sites with >7 turtles	0.15	0.08	0.16
Mean male-female ratio (sites with at least 1 female)	2.26	0.23	1.13
Surveys with zero females	37	6	28
Mean male:female ratio for sites with >7 turtles (sites with at least 1 female)	2.87	0.19	1.31
Surveys with >7 turtles and zero females	2	0	1

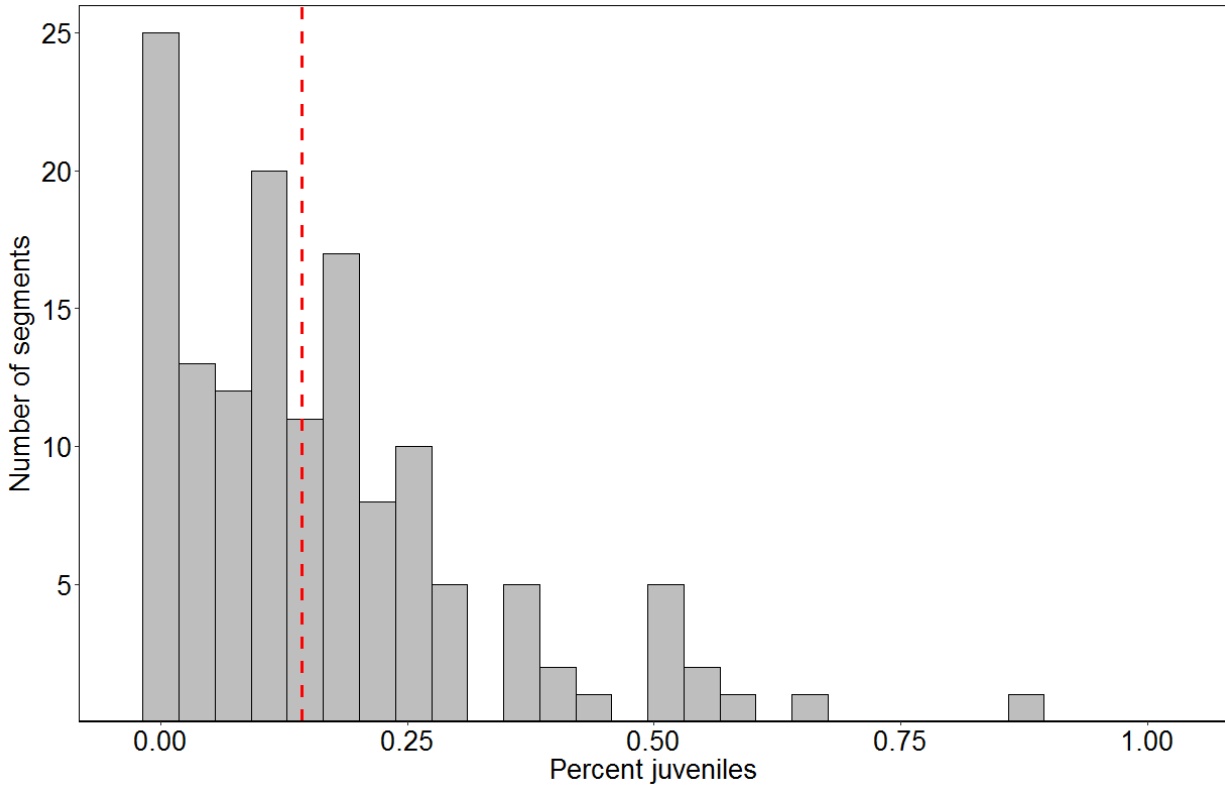


Figure 2.14. Histogram of the percent of juvenile detections per site with >6 total detections. The red dashed line indicates the estimated approximate recapture-rate-corrected juvenile percentage that reflects an average juvenile percentage of 0.25. Data come from standardized Wood Turtle surveys conducted throughout the northeastern United States (Virginia to Maine) and New Brunswick from 2012–2017.

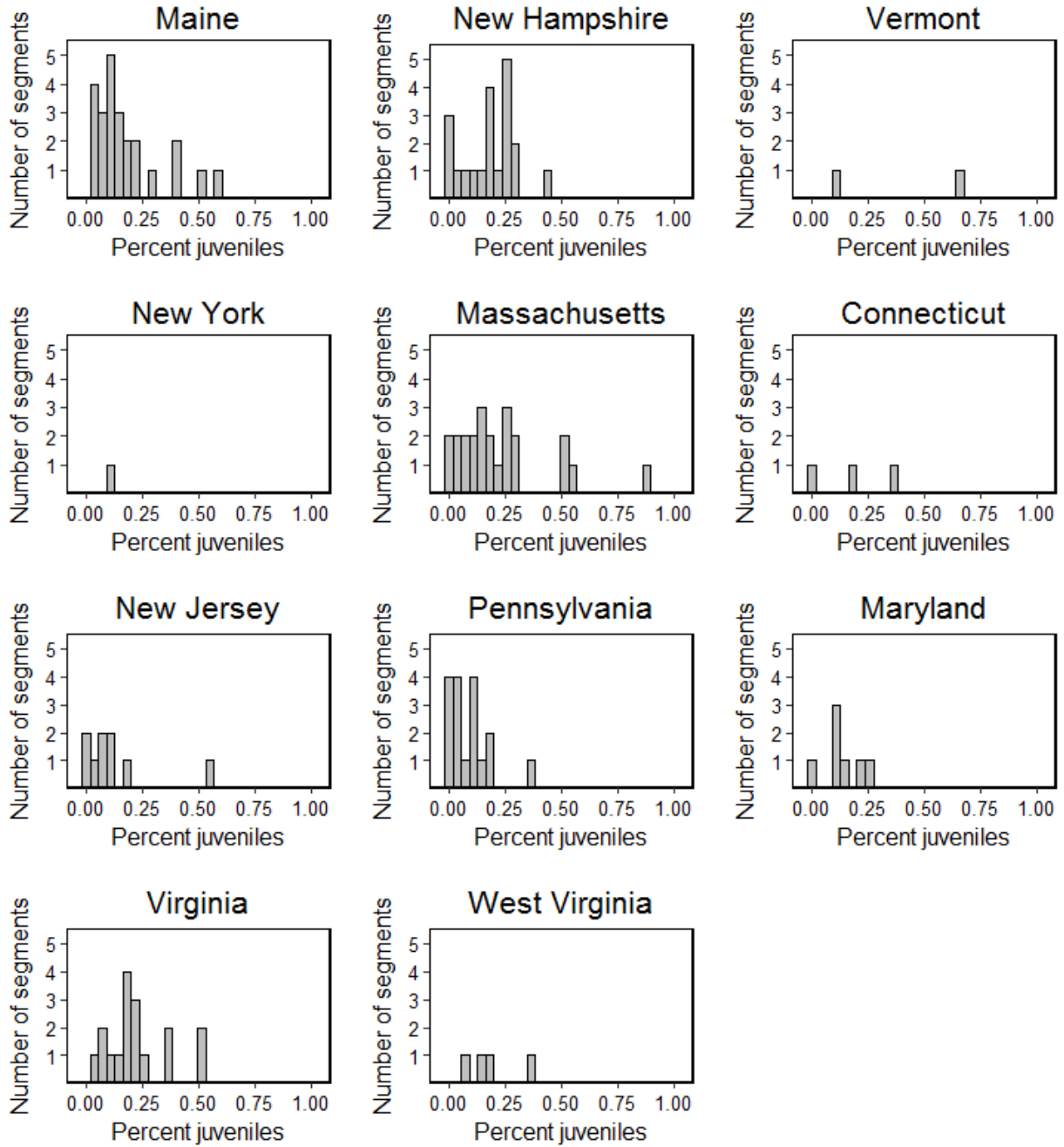


Figure 2.15. Histograms of the percent of detections during standardized Wood Turtle surveys that were juveniles by state in the northeastern United States from 2012–2017. All sites with at least seven detections are included.

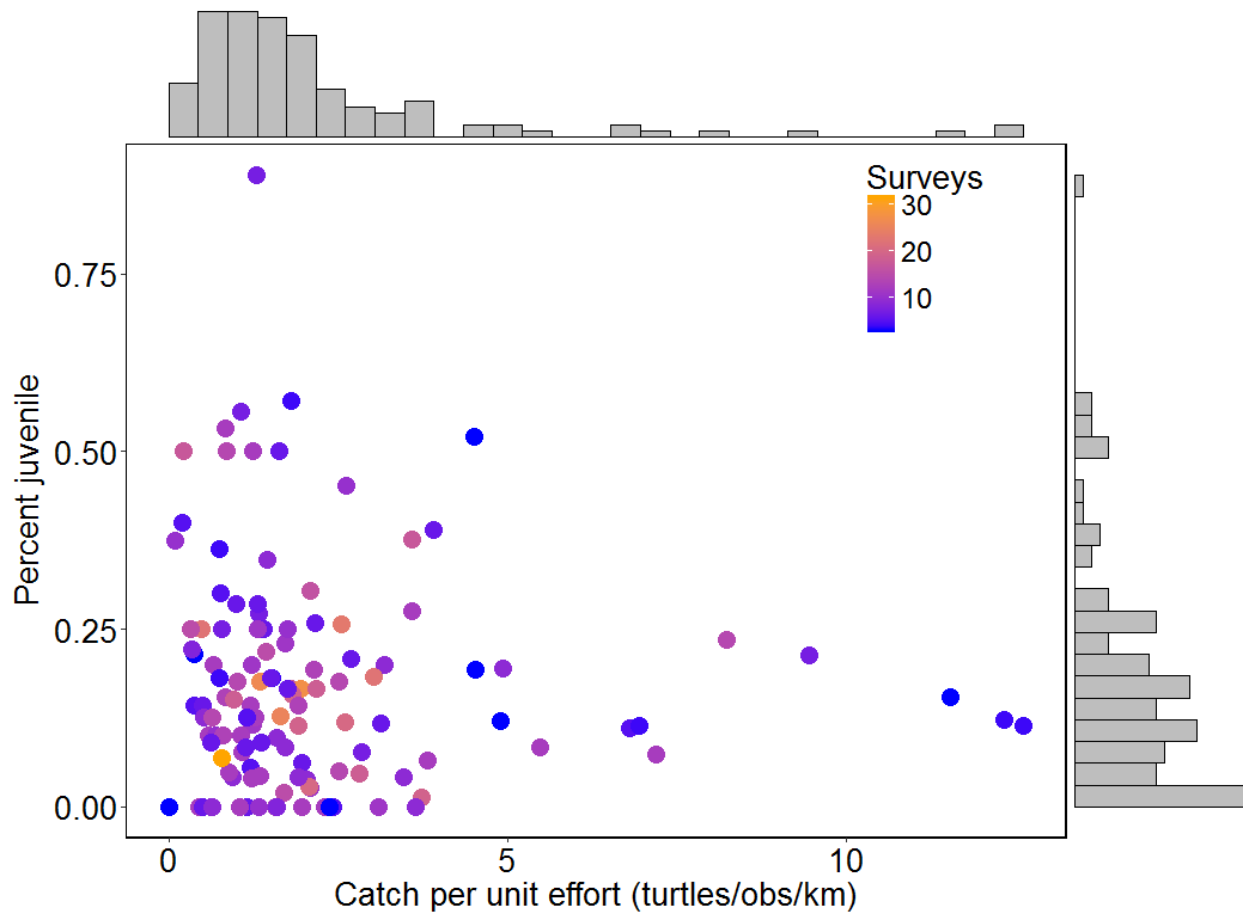


Figure 2.16. Catch per unit effort in relation to the percent of captures that were juveniles (<15 years old). Only sites that were surveyed at least three times and yielded >6 turtles are included. Number of surveys is indicated by color. Gray bars represent histograms of values within each axis.

Sex ratios.—The average male:female ratio was 1.25:1 for all sites with at least one female and 1.4:1 for sites with at least one female and > 6 detections (Table 2.4). Sex ratios varied considerably across states/provinces (Table 2.4). The overall within-season sex ratio in fall was double that of spring (2.26 and 1.13 respectively; Table 2.5, Fig. 2.17) when all surveys with at least one female were considered and this difference was even greater when only sites with >6 detections were considered (2.87 and 1.31 respectively). The male:female ratio was relatively low during the nesting season (0.23 when all sites were considered and 0.19 when all sites with >6 detections were considered; Fig. 2.17).

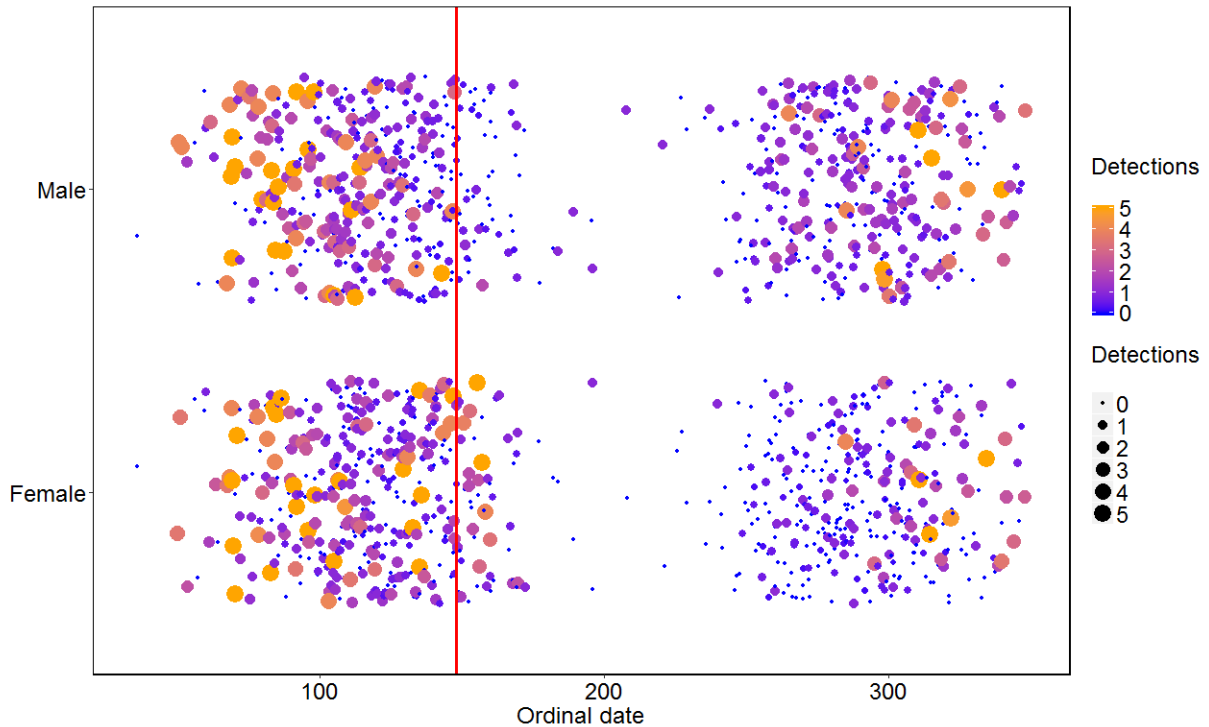


Figure 2.17. Male and female detections per survey by state in relation to ordinal date. The largest orange points represent dates with >5 turtles per survey. Points are randomly distributed along the y-axis for better visualization. The red line represents the beginning of what was considered the nesting season (May 28).

Trends in Relative Abundance Derived from Hierarchical Models

Total Wood Turtle detections in spring with only three surveys.—Best performing N-mixture models ($\Delta AIC < 2$; Fig. 2.4, stage 4) of total Wood Turtle detections in spring using only three randomly selected surveys (within single years)—with a negative binomial error distribution and the common detection covariates of time of day, date, time spent searching, and number of observers—displayed significant relationships with the following state covariates: quadratic terms for agriculture within 5500 m, agriculture within 300, primary habitat within 300 m, and elevation as well as linear terms for elevation, heat index, precipitation, and traffic rate within 5500 m (Table 2.6; Fig. 2.18). Traffic rate within 5500 m, precipitation, heat index, and quadratic agriculture terms at both 300 and 5500 m appeared in the majority of top models. Wood Turtle detection (Fig. 2.19; Fig. 2.4, stage 2) was positively related to time spent searching and number of observers and negatively related to time of day and day of year. A parametric bootstrap of the best performing model (Table 2.8, model 1) produced a $P = 0.56$ suggesting no evidence of lack of fit.

Table 2.6. Coefficients of state covariates within best performing N-mixture models of total Wood Turtle detections during spring when only three surveys were used within a single season. Asterisks indicate coefficients that were statistically significant. Detection covariates for time of day, date, time spent searching, and number of observers were included in all models, but their coefficients are not shown.

Model	State Covariates								Δ AIC	Weight	
	ag300 ^{2a}	ag5500 ^{2b}	elev ^c	elev ^{2c}	for300 ^{2d}	heat ^e	precip ^f	pri300 ^{2g}			traf5500 ^h
1	-0.141*	-0.086*			-0.134	-0.204*	-0.297*	0.076	-0.193*	0	0.127
2	-0.160*	-0.079*				-0.244*	-0.326*	0.045	-0.177*	0.206	0.115
3	-0.161*	-0.078*				-0.264*	-0.337*		-0.167*	0.347	0.107
4	-0.157*	-0.085*		-0.072		-0.220*	-0.304*	0.045	-0.182*	0.553	0.096
5	-0.159*	-0.084*		-0.072		-0.241*	-0.316*		-0.173*	0.712	0.089
6	-0.151*	-0.083*	0.189*	-0.163*		-0.223*	-0.285*			0.875	0.082
7	-0.142*	-0.090*		-0.055	-0.113	-0.192*	-0.284*	0.071*	-0.195*	1.080	0.074
8	-0.149*	-0.084*	0.190*	-0.163*		-0.204*	-0.274*	0.041		1.108	0.073
9	-0.151*	-0.087*	0.121	-0.133		-0.191*	-0.266*	0.044	-0.129	1.177	0.070
10	-0.153*	-0.085*	0.125	-0.134		-0.212*	-0.279*		-0.118	1.263	0.068
11	-0.137*	-0.091*	0.116	-0.113	-0.109	-0.164	-0.249*	0.069	-0.143	1.789	0.052
12	-0.140*	-0.086*	0.016		-0.137	-0.202*	-0.293*	0.076*	-0.186*	1.958	0.048

^aPercent agricultural cover within 300 m

^bPercent agricultural cover within 5500 m

^cElevation

^dPercent forest cover within 300 m

^eHeat index

^fPrecipitation

^gPercent primary habitat cover within 300 m

^hTraffic rate within 5500 m

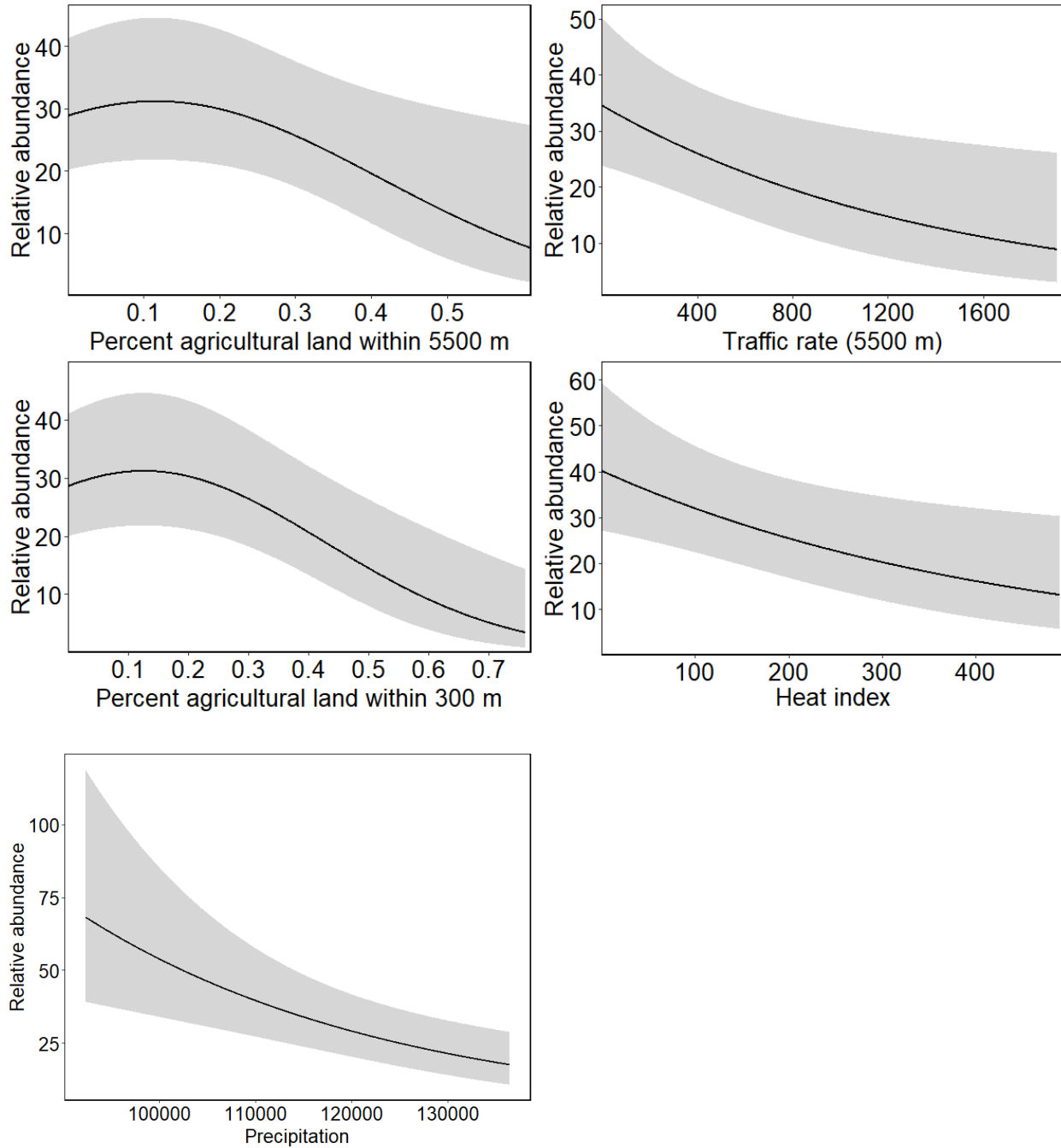


Figure 2.18. Relative Wood Turtle abundance in relation to significant state covariates included in the best performing model of total Wood Turtle detections in spring. Three surveys were selected randomly for each season. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

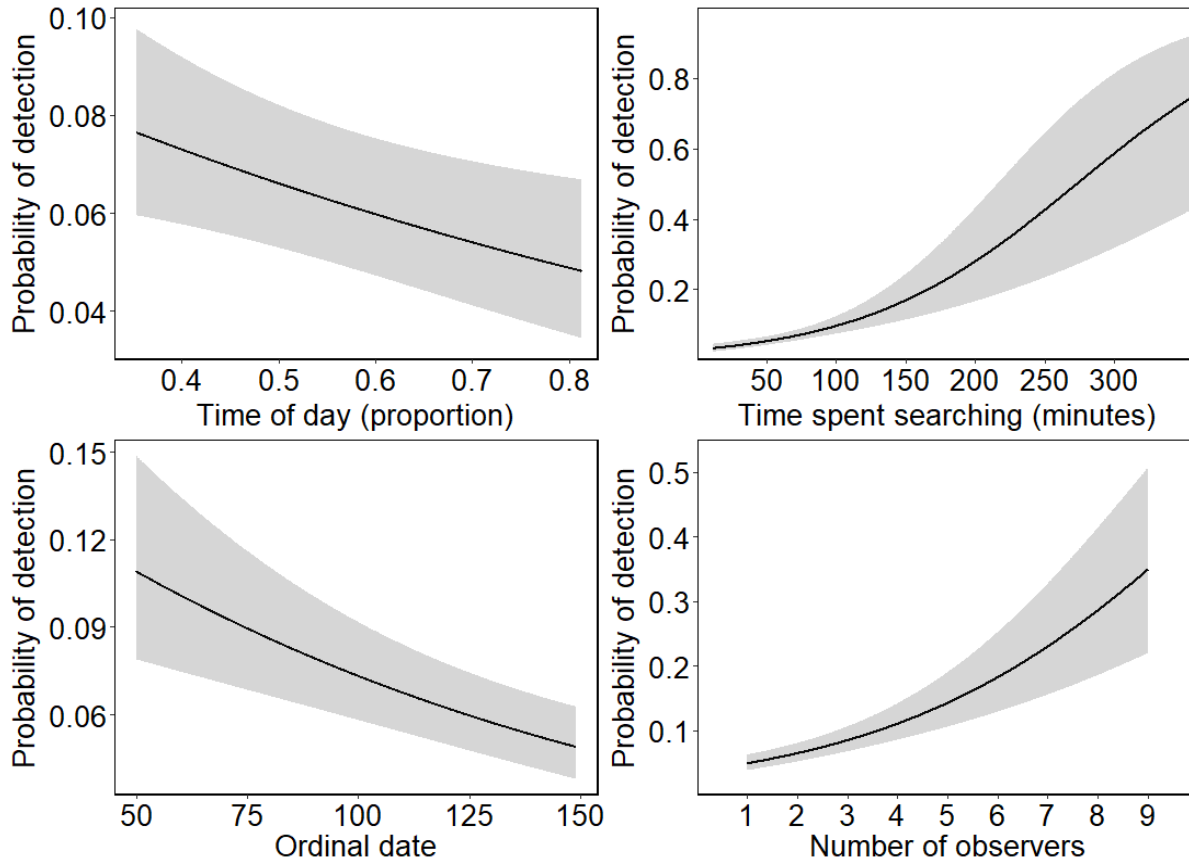


Figure 2.19. Probability of detection in relation to significant covariates included in the best performing model of total Wood Turtle detections in spring. Three surveys were selected randomly for each season. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

Total Wood Turtle detections during spring.—Best performing N-mixture models ($\Delta\text{AIC} < 2$; Fig. 2.4, stage 4) of total Wood Turtle detections during spring—with a negative binomial error distribution and the common detection covariates of air temperature, time spent searching, number of observers, growing-degree-days, ordinal date, and the interaction between growing-degree-day and ordinal date—displayed significant relationships with the following state covariates: quadratic term for agriculture within 300 m, quadratic term for elevation (indicating a unimodal relationship), heat index, primary habitat within 5500 m, precipitation, and undeveloped land within 5500 m (Table 2.7, Fig. 2.20, Fig. 2.21). Wood Turtle relative abundance was only significantly related to heat index and primary habitat within a subset of top models (4 of 6 and 1 of 6 respectively). Wood Turtle detection (Fig. 2.4, stage 2) was positively related to air temperature, number of observers, and search time and negatively related to growing-degree-days, time of day, ordinal date, and the interaction between growing-degree-days and ordinal date (Fig. 2.22). All relationships were significant except for that with growing-degree-days. A parametric bootstrap of the best performing model (Table 2.7, model 1) produced a $P = 0.29$ suggesting no evidence of lack of fit.

Table 2.7. Coefficients of state covariates within best performing N-mixture models of total Wood Turtle detections in spring. Asterisks indicate coefficients that were statistically significant. Detection covariates for air temperature, number of observers, time spent searching, growing-degree-days, time of day, ordinal date, and the interaction between ordinal date and growing-degree-days were included in all models, but their coefficients are not shown.

Model	State Covariates								ΔAIC	Weight
	ag300 ^{2a}	elev ^b	elev ^{2b}	heat ^c	precip ^d	pri300 ^{2e}	pri5500 ^f	undev5500 ^g		
1	-0.148*		-0.134*	-0.198*	-0.286*	0.089	-0.199*	0.270*	0	0.261
2	-0.145*		-0.135*	-0.195*	-0.316*		-0.142	0.295*	0.145	0.243
3	-0.142*	0.140	-0.186*	-0.152	-0.316*			0.282*	1.192	0.144
4	-0.138*		-0.132*	-0.164	-0.353*			0.328*	1.329	0.134
5	-0.146*	0.081	-0.166*	-0.182*	-0.303*		-0.113	0.275*	1.558	0.120
6	-0.148*	0.029	-0.145*	-0.194*	-0.283*	0.083	-0.185	0.264*	1.935	0.099

^aPercent agricultural cover within 5500 m

^bElevation

^cHeat index

^dPrecipitation

^ePercent primary habitat cover within 300 m

^fPercent primary habitat cover within 5500 m

^gUndeveloped land within 5500 m

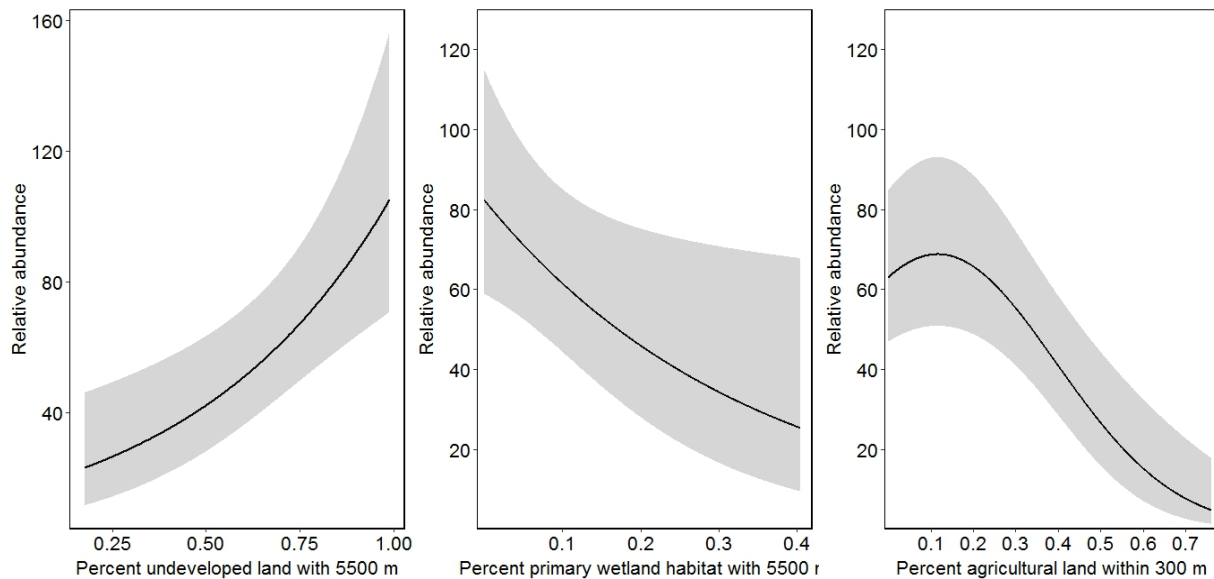


Figure 2.20. Predicted Wood Turtle relative abundance in relation to significant covariates included in the best performing model of total Wood Turtle detections in spring. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

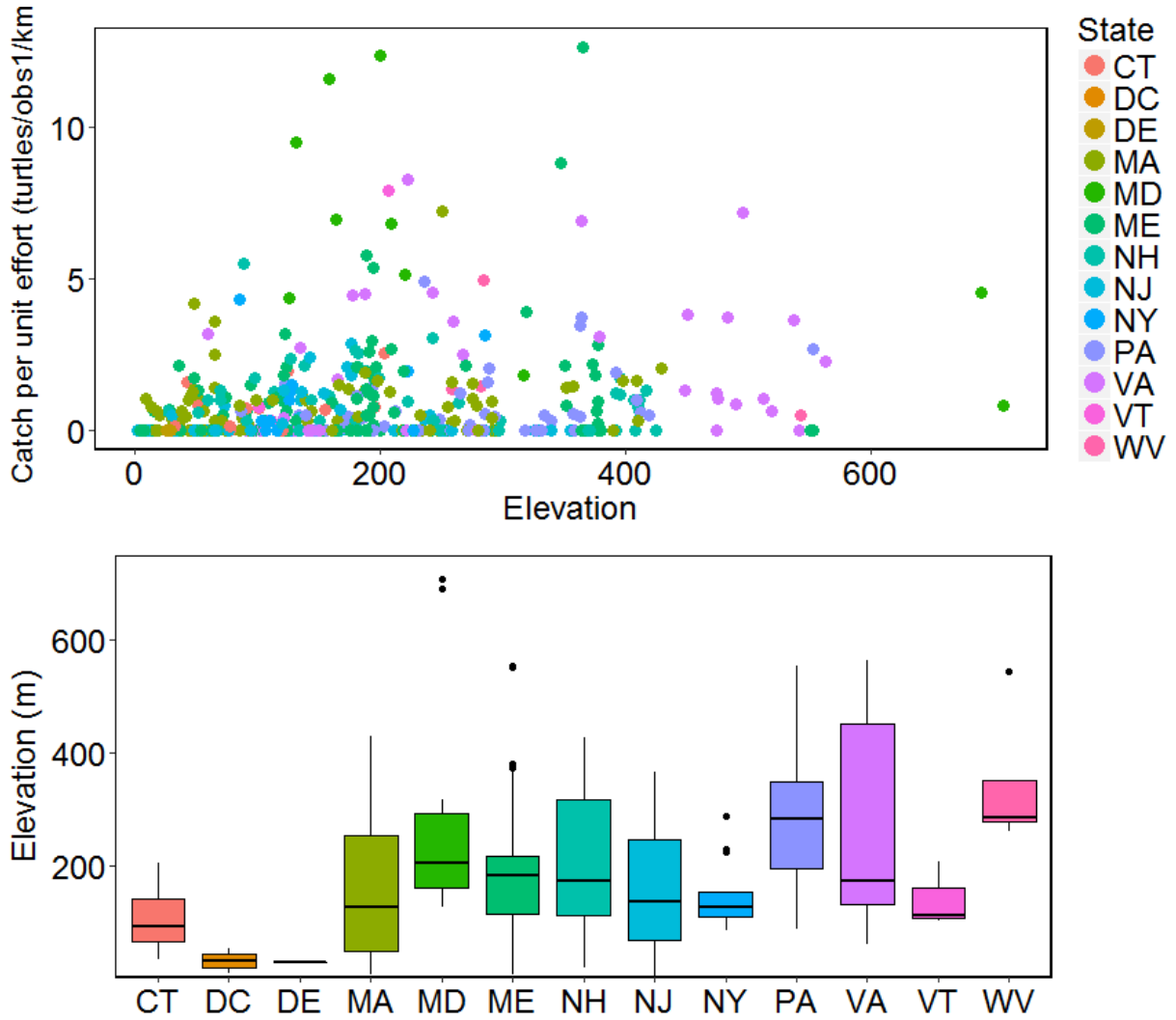


Figure 2.21. Catch per unit effort in relation to elevation (top) and boxplots showing the distribution of sites with respect to elevation within each state (bottom). Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

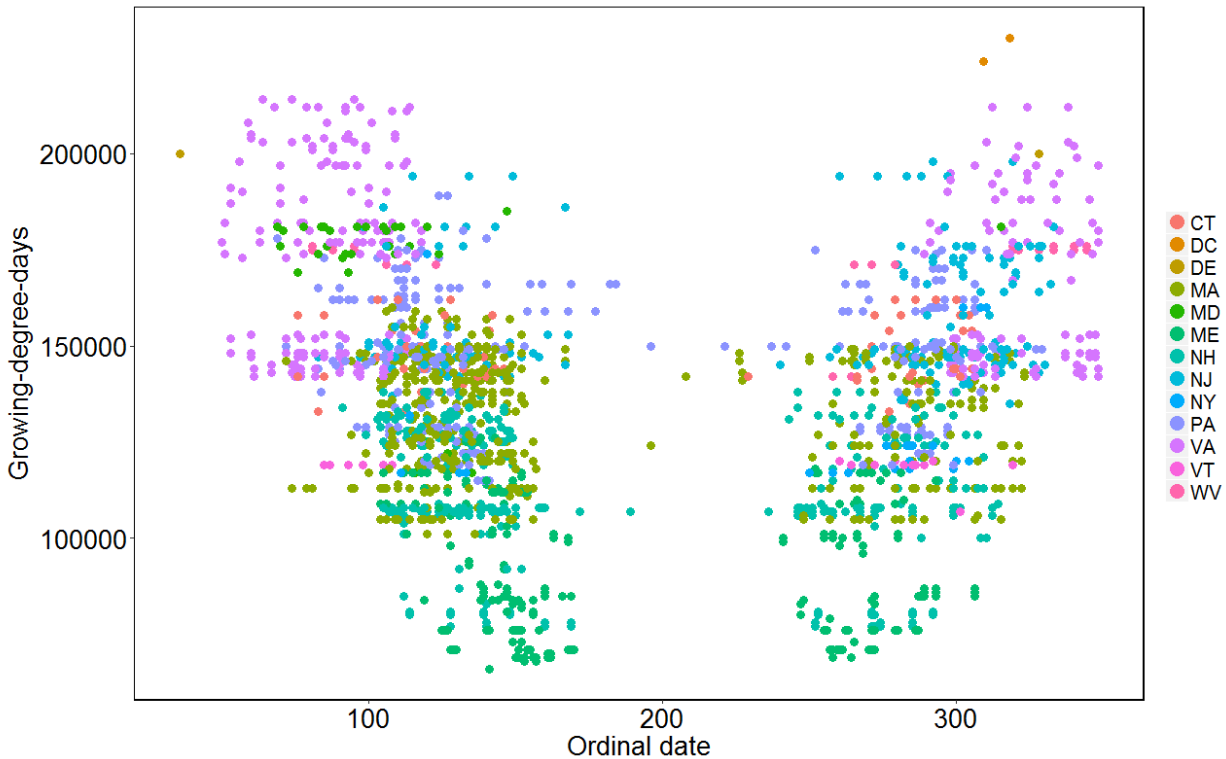


Figure 2.22. Growing degree-days in relation to date of survey for all surveys conducted. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

Lead observer Wood Turtle detections during spring.—All best performing N-mixture models ($\Delta\text{AIC} < 2$; Fig. 2.4, stage 4) of lead observer Wood Turtle detections in spring—with a negative binomial error distribution and the common detection covariates of air temperature, time spent searching, time of day, growing-degree-days, ordinal date, and the interaction between growing-degree-day and ordinal date—were significantly related to the following state covariates: the quadratic term for percent agricultural cover within 300 m, the quadratic elevation term, precipitation, and undeveloped land within 5500 m (Table 2.8). Wood Turtle detection was positively related to air temperature and search time and negatively related to growing-degree-days, time of day, ordinal date, and the interaction between growing degree days and ordinal date (Fig. 2.4, stage 2). These relationships were significant for all covariates except growing-degree-days and the interaction between growing degree days and ordinal date. A parametric bootstrap of the best performing model (Table 2.8, model 1) produced a $P = 0.12$ suggesting no evidence of lack of fit.

Table 2.8. Coefficients of state covariates within best performing N-mixture models of lead observer Wood Turtle detections in spring. Asterisks indicate coefficients that were statistically significant. Detection covariates for air temperature, time spent searching, growing-degree-days, time of day, ordinal date, and the interaction between ordinal date and growing-degree-days were included in all models, but their coefficients are not shown.

Model	State Covariates						Δ AIC	Weight
	ag300 ^{2a}	elev ^b	elev ^{2b}	july_temp ^c	precip ^d	undev5500 ^e		
1	-0.095*		-0.130*		-0.227*	0.329*	0	0.317
2	-0.098*	0.125	-0.176*		-0.196*	0.282*	0.133	0.296
3	-0.095*		-0.138*		-0.253*	0.324*	1.716	0.134
4	-0.098*	0.125	-0.184*		-0.222*	0.277*	1.828	0.127
5	-0.095*		-0.131*	-0.045	-0.231*	0.311*	1.850	0.126

^aPercent agricultural cover within 300 m

^bElevation

^cJuly temperature

^dPrecipitation

^ePercent undeveloped land

Total Wood Turtle detections during entire year.—Best performing N-mixture models (Δ AIC < 2; Fig. 2.4, stage 4) of total Wood Turtle detections throughout the year—with a negative binomial error distribution and the common detection covariates of season, number of observers, cloud cover, time spent searching, growing-degree-days, ordinal date, and the interaction between growing-degree-day and ordinal date—displayed significantly related to the following state covariates: percent agricultural cover within 5500 m, the linear and quadratic terms for elevation, precipitation, traffic rate within 5500 m, the linear term for elevation, and forest cover within 5500 m (Table 2.9; Fig. 2.23). Agriculture within 5500 m, precipitation, and traffic rate within 5500 m occurred within the large majority of top models. Wood Turtle detection (Fig. 2.4, stage 2) was greater in the spring, positively related to growing-degree-days, time spent searching, number of observers and the interaction between growing-degree-days and ordinal date, and negatively related to time of day, ordinal date, and cloud cover. These relationships were significant, except of that with ordinal date. A parametric bootstrap of the best performing model (Table 2.9, model 1) produced a $P = 0.18$ suggesting no evidence of lack of fit.

Table 2.9. Coefficients of state covariates within best performing N-mixture models of total Wood Turtle detections during entire year. Asterisks indicate coefficients that were statistically significant. Detection covariates for season, cloud cover, number of observers, time spent searching, growing-degree-days, time of day, ordinal date, and the interaction between ordinal date and growing-degree-days were included in all models, but their coefficients are not shown.

Model	State Covariates								Δ AIC	Weight	
	ag5500 ^a	elev ^b	elev ^{2b}	es5500 ^c	for5500 ^d	iei300 ^e	precip ^f	pri5500 ^g			traf5500 ^h
1	-0.293*	0.207*	-0.203*	0.148			-0.285*	-0.299*	0.000	0.135	
2	-0.301*	0.228*	-0.151*				-0.343*	-0.305*	0.700	0.095	
3	-0.204*	0.159	-0.187*	0.143	0.156		-0.282*	-0.226*	0.734	0.094	
4	-0.172		-0.131	0.156	0.239		-0.298*	-0.242*	0.980	0.083	
5	-0.311*	0.175	-0.138				-0.324*	-0.104	-0.309*	1.007	0.082
6	-0.322*						-0.347*	-0.146*	-0.379*	1.123	0.077
7	-0.302*	0.172*	-0.185	0.125			-0.280*	-0.076	-0.303*	1.152	0.076
8	-0.207*	0.176	-0.136		0.164		-0.339*		-0.228*	1.318	0.070
9	-0.166				0.246		-0.366*		-0.257*	1.325	0.070
10	-0.277*	0.188	-0.205*	0.152		0.050	-0.281*		-0.286*	1.682	0.058
11	-0.333*		-0.071				-0.340*	-0.155*	-0.374*	1.757	0.056
12	-0.325*		-0.120	0.128			-0.295*	-0.125	-0.367*	1.848	0.054
13			-0.126*	0.151	0.401*		-0.279*		-0.142	1.943	0.051

^aPercent agricultural cover within 5500 m

^bElevation

^cPercent early-successional cover within 5500 m

^dPercent forest cover within 5500 m

^eIndex of ecological integrity

^fPrecipitation

^gPercent primary habitat cover within 5500 m

^hTraffic rate within 5500 m

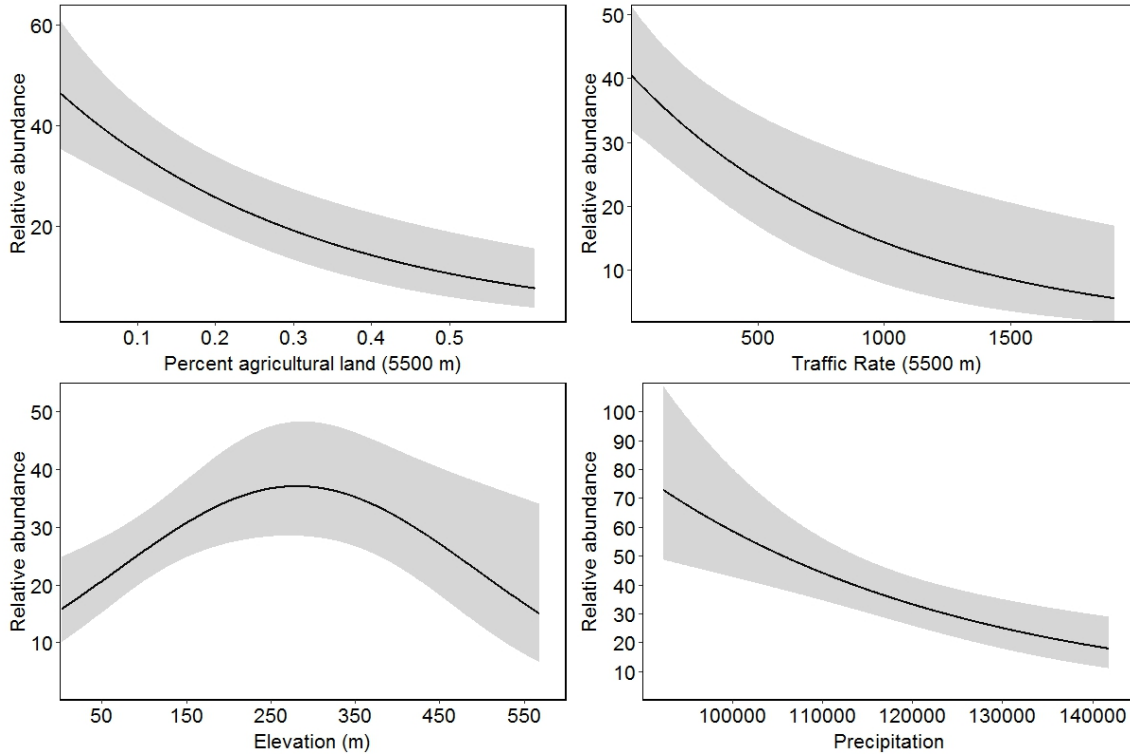


Figure 2.23. Predicted Wood Turtle abundance in relation to significant covariates included in the best performing model of total Wood Turtle detections throughout the year. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

Relative Population Size Estimates

We estimated relative population size for 80 stream segments using closed-population CMR models and 58 stream segments using open-population CMR models across 9 of 13 northeastern states: Maine, New Hampshire, Vermont, Massachusetts, Connecticut, New Jersey, Pennsylvania, Virginia, and West Virginia. We were unable to estimate standard errors for 12 of the 58 open-population estimates. Sites were surveyed between 9 and 31 times across as many as 11 seasons. Twelve streams had more than one segment (ranging 2–10) for which relative population size was estimated (Table 2.10).

Relative population size estimates—which should **not** be interpreted as *absolute* population sizes—ranged dramatically among segments. Relative closed-population estimates, where surveys were not pooled within seasons, ranged 4–140 turtles/km and averaged 41.3 turtles/km (sd = 33.5). Relative pooled closed-population estimates ranged 4–211 turtles/km and averaged 47.5 turtles (sd = 43.5). Relative open-population estimates ranged from 3–282 turtles/km and averaged 41.2 turtles/km (sd = 50.2). Twenty-two of 80 sites (28%) had closed-population estimates (non-pooled) >50 turtles/km and 7 sites (9%) had estimates >100 turtles/km (Fig. 2.24, Fig. 2.25). Twenty-five of 80 sites (32%) had pooled closed-population estimates >50 turtles/km and 8 of 80 sites (10%) had estimates >100 turtles/km. Thirteen of 58

sites (22%) had open-population estimates >50 turtles/km and 4 sites (7%) had estimates of >100. Closed population estimates displayed a positive relationship with the number of turtles detected per lead observer per km, but there was considerable variation associated with this relationship (Fig. 2.26). Segments with greater closed population estimates did not appear to yield greater percentages of juveniles (Fig. 2.27).

Pooling surveys by season increased relative population estimates when compared to non-pooled estimates, most dramatically at larger populations (Fig. 2.28, Fig. 2.29). The mean difference between estimates was 8.2 (median = 3.5) and standard error bars overlapped for all sites except one. Two sites with exceptionally large disparities between estimates (56 and 104 turtles/km) were sites where surveys were conducted in close temporal proximity out of necessity.

Relative population estimates using all surveys across all years (Table 2.10) were generally larger than those following an “ideal” sampling regime (Table 2.11), with sites with more additional surveys over more years typically producing relatively larger estimates (Fig. 2.30, Fig. 2.31, Fig. 2.32). Overall, however, relative population sizes when comparing sites remained similar (i.e., the largest sites were still the largest, etc.).

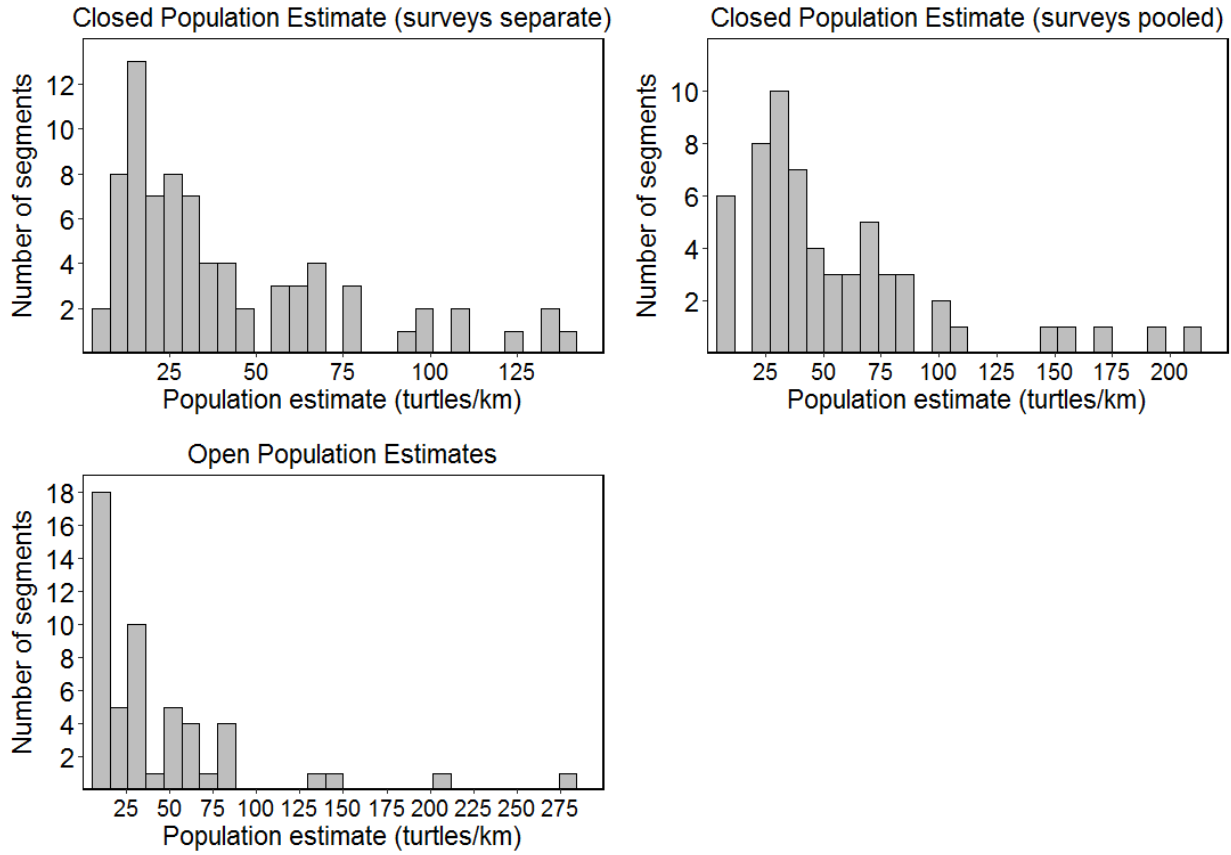


Figure 2.24. Histograms of capture-mark-recapture estimates for the three methods used (closed with surveys not pooled by season, closed with surveys pooled by season, and open) derived from standardized surveys conducted throughout the northeastern United States from 2012–2017.

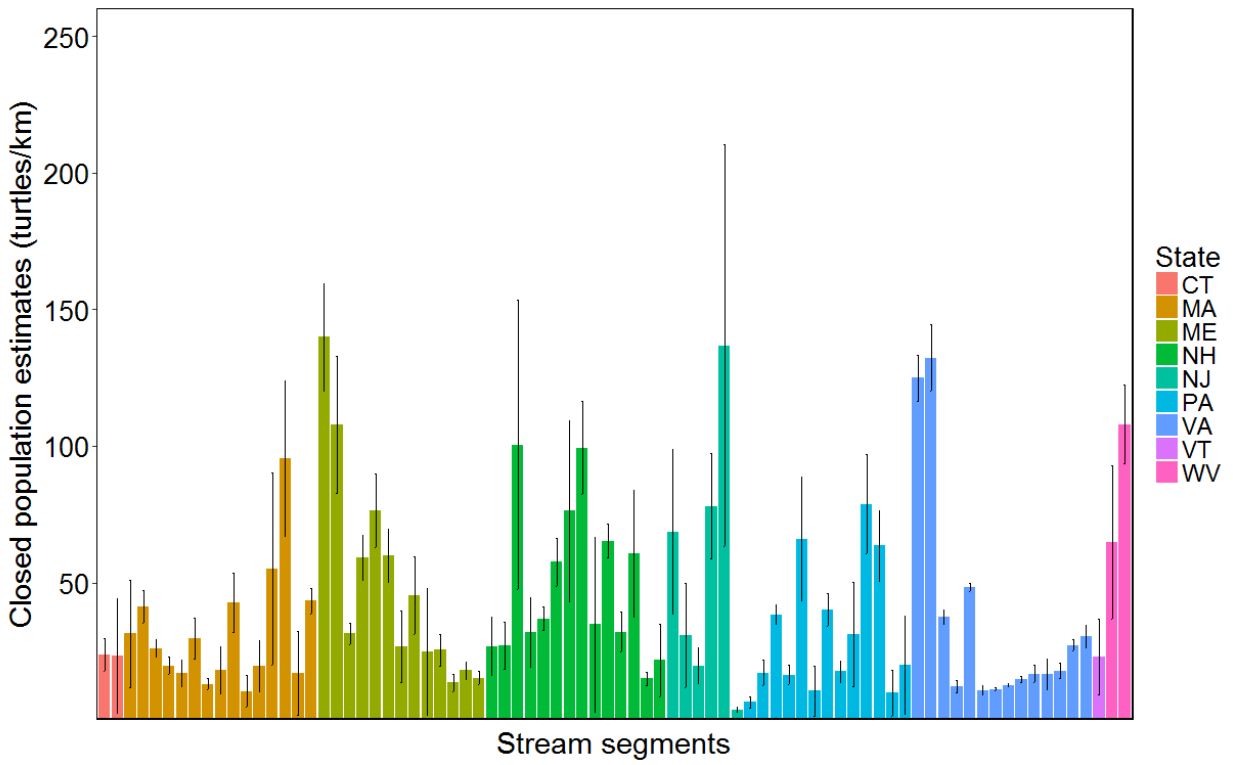


Figure 2.25. Closed population estimates (surveys not pooled) by state (indicated by color). Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

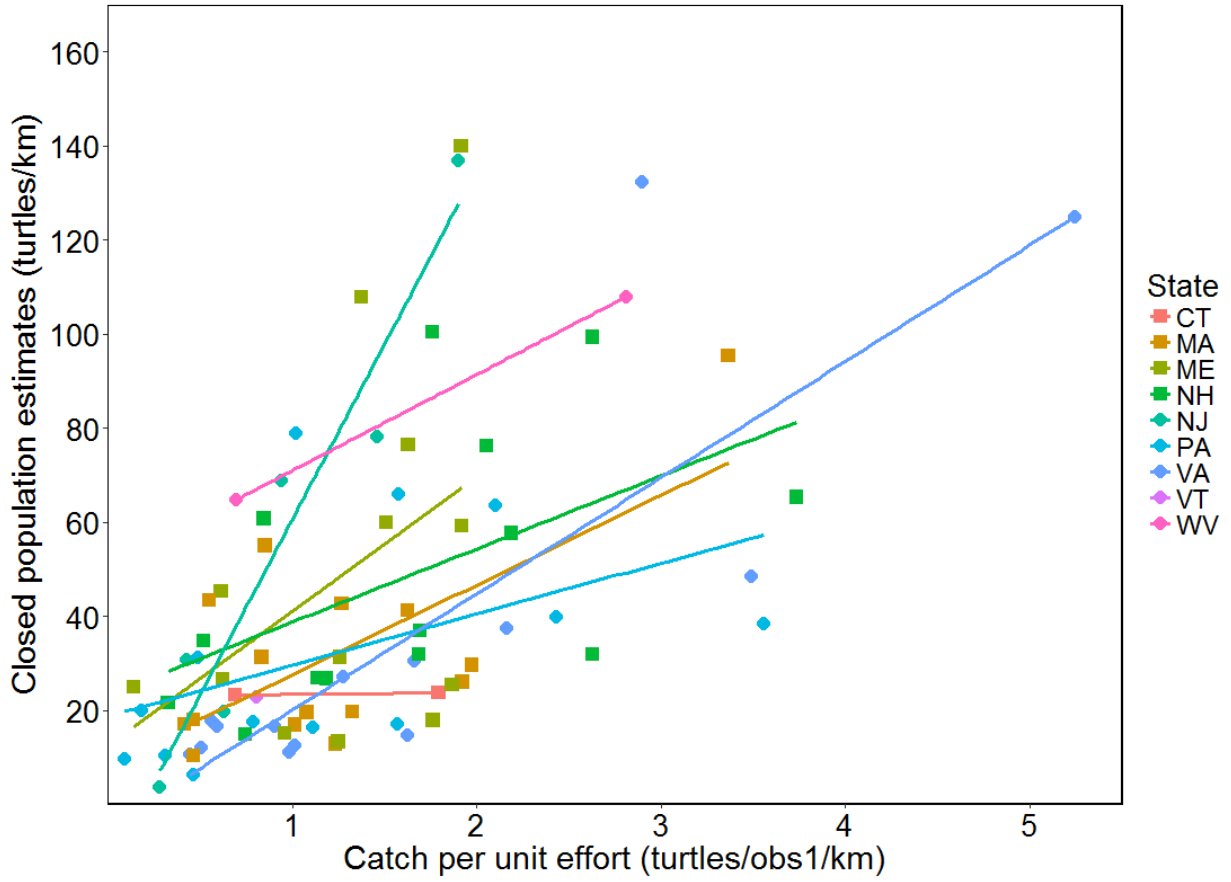


Figure 2.26. Closed population estimates (surveys not pooled) in relation to catch per unit effort (turtles/observer 1/km). Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

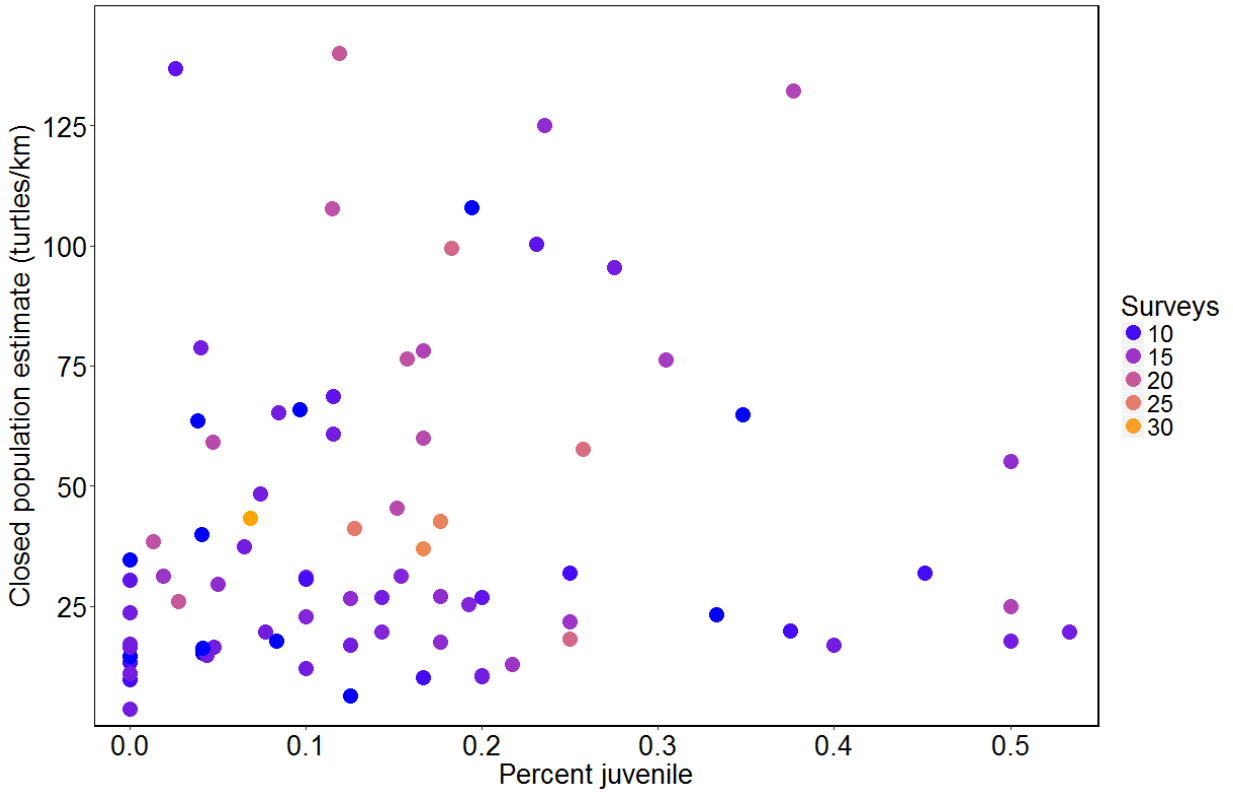


Figure 2.27. Closed population estimates in relation to the percentage of detections that were juveniles (number of surveys indicated by color). Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

Table 2.10. Relative population estimates (turtles/km) and standard error for sites using three methods: closed (surveys separate within season), closed (surveys pooled within season), and open.

Pseudonym	Surveys	Seasons	Closed (separate)		Closed (pooled)		Open	
			Est.	SE	Est.	SE	Est.	SE
arroyo del cuervo 1	11	4	26.8	10.7	27.9	12.7		
arroyo del cuervo 2	12	4	26.9	8.6	23.2	6.6	13.7	
arroyo del cuervo 3	11	4	100.4	52.9	84.3	42.8	34.9	13.2
arroyo los barrancos 1	26	10	36.9	4.4	38.9	4.6	48.5	11.3
arroyo los barrancos 2	23	9	57.6	8.7	61.0	10.4		
arroyo los barrancos 3	17	8	76.3	33.0	71.6	30.4	79.6	
arroyo tierra blanca 1	18	9	59.2	8.1	54.4	10.9	84.4	29.7
arroyo tierra blanca 2	19	9	76.4	13.4	81.0	15.6	86.3	22.5
arroyo tierra blanca 3	18	9	59.9	9.6	60.8	10.3		
arroyo tierra blanca 4	14	9	26.6	13.1	25.6	12.4		
arroyo tierra blanca 5	18	8	45.4	14.3	46.8	16.1	63.4	41.5
arroyo tierra blanca 6	17	8	24.9	23.2	23.3	21.4		
arroyo tio lino 1	20	7	140.0	19.6	195.6	40.0	208.5	
arroyo tio lino 2	19	7	107.8	25.0	211.4	86.6	282.3	253.8
arroyo tio lino 3	15	7	31.4	4.0	32.4	4.6	36.0	19.9
aspen brook	14	5	55.1	35.3	87.5	82.0		
barney creek	11	5	68.7	30.0	77.3	39.2		
big cypress creek	9	5	15.2	2.3	19.2	5.2		
big reedy creek	12	5	19.6	9.3	41.2	37.7		
brattle brook	11	4	10.5	9.2	8.9	7.3	3.6	
bumblebee creek lower	12	3	16.9	4.9	27.2	15.6	13.3	
bumblebee creek upper	14	3	29.6	7.5	37.5	36.1	28.2	12.0
captain jacobson creek	12	4	3.6	0.8	3.9	1.2	3.0	
castle brook lower	22	8	18.1	8.7	22.6	13.8		
castle brook upper	26	8	42.7	10.9	58.5	21.7		
charcoal house creek	31	11	43.4	4.6	45.2	5.6	46.5	8.7
chicken run	14	5	27.1	2.1	33.3	5.4		

Pseudonym	Surveys	Seasons	Closed (separate)		Closed (pooled)		Open	
			Est.	SE	Est.	SE	Est.	SE
coral creek	12	4	78.8	18.3	71.2	16.1	58.1	16.5
cottonwood creek	12	5	17.0	15.3	15.0	13.1	12.2	
crosby river	13	5	31.4	19.8	49.6	46.0	12.2	
cyclone creek	13	5	14.9	2.3	14.3	2.0	13.7	2.0
dude creek	15	6	12.9	2.0	16.1	4.4	14.3	
fish creek	10	4	10.3	5.9	8.2	3.8	6.2	2.1
fortification creek	22	9	99.4	17.1	103.8	19.4		
foxtail creek	11	4	30.5	4.1	33.4	6.0	26.7	5.2
grindstone creek	13	3	22.9	13.9	17.4	9.3	18.0	
happy creek	9	3	6.3	2.0	5.9	1.9	4.6	
hidden wash	14	5	132.3	12.2	157.5	20.2	134.6	19.5
jackie creek	11	5	136.8	73.4	174.3	115.9		
lee creek	10	4	19.9	18.0	16.9	14.7		
little bearskin creek	13	5	19.7	3.1	18.7	2.6	21.4	8.0
lone tule wash 1	9	3	107.9	14.4	108.2	16.2	61.9	23.5
lone tule wash 2	9	3	64.8	27.9	88.4	55.3	24.8	
magazine brook 1	12	4	65.3	6.0	70.0	8.2	67.5	12.1
magazine brook 2	10	4	32.0	7.4	38.4	12.0	33.0	20.4
mastodon creek	12	3	17.1	4.6	30.9	18.3	30.0	
monroe creek 1	10	4	13.4	3.2	13.9	3.9	10.3	1.7
monroe creek 2	9	4	17.9	3.2	21.4	5.8	13.9	
mystery creek	9	3	63.5	12.9	73.6	19.9	47.1	14.4
nancy creek	9	3	66.0	22.7	70.3	29.1		
panther branch c1	12	4	37.5	2.4	39.2	3.4	34.1	0.8
panther branch c2	12	4	12.0	2.2	11.1	1.7	9.4	
panther branch c3	12	4	48.4	1.5	49.7	2.1	47.5	1.3
panther branch c4	12	4	10.6	1.7	10.2	1.5	9.8	1.4
panther branch p1	9	3	11.0	0.6	12.0	1.5	10.7	
panther branch p2	9	3	12.6	0.7	12.6	0.7	12.1	
panther branch p3	12	4	14.6	1.1	15.4	1.8	14.3	0.8

Pseudonym	Surveys	Seasons	Closed (separate)		Closed (pooled)		Open	
			Est.	SE	Est.	SE	Est.	SE
panther branch p4	12	4	16.6	3.2	16.2	3.1	12.7	
panther branch p5	12	4	16.5	5.5	16.3	5.6		
panther branch p6	11	4	17.8	2.8	17.8	3.0	15.6	1.3
pickle creek	15	3	21.7	13.3	28.0	24.3		
potato run	17	8	77.7	19.1	78.1	19.9	71.9	21.1
powderhorn creek	10	4	9.7	8.5	8.3	6.8	3.3	
prairie creek	19	4	38.4	3.4	54.5	10.9	33.6	
roaring lion creek	13	5	25.4	5.8	32.1	10.9		
saint sebastian river	10	4	125.0	8.4	148.8	15.4	140.4	26.4
sheep creek	12	4	23.8	6.0	15.1	6.0	16.9	3.9
snow brook	9	3	40.0	6.0	53.5	13.7	31.6	6.7
sombrero creek	9	3	16.3	3.4	18.2	5.2	12.3	1.5
sourdough creek	9	3	34.8	32.1	26.7	23.2		
strawberry creek 1	14	5	17.5	3.9	21.5	11.5	14.5	2.9
strawberry creek 2	14	5	31.1	19.1	29.5	26.1		
sucker run	12	4	19.7	6.7	19.4	6.9	11.1	
turkey meadow brook	10	5	31.9	12.7	35.3	16.5	48.8	41.1
turpentine still brook	12	4	60.8	23.3	76.3	38.3	78.5	47.3
wheeler fork	9	4	23.3	21.1	20.0	17.4		
wildcat brook lower	25	10	41.2	5.8	42.4	6.5	49.5	15.5
wildcat brook upper	20	8	26.0	3.1	28.3	4.6	31.8	12.0
williamson creek	10	4	30.7	19.0	46.4	42.3	13.3	
worcester	12	3	95.4	28.4	103.8	38.4	67.2	23.7

Table 2.11. Relative closed population size estimates (turtles/km) using an “ideal” sampling regime where three surveys are conducted within each of three seasons across no more than two years.

Pseudonym	Surveys removed	Period	Ideal Sampling Regime Estimates					
			Closed (Separate)		Closed (Pooled)		Open	
			Est.	SE	Est.	SE	Est.	SE
worcester	3	cswg	74.2	23.4	74.8	27	47.8	15.2
castle brook upper	17	rcn	25.4	8.9	30.6	14.2	23.9	20.2
dude creek	6	rcn	14.3	2.8	17.6	5.8	11	
bumblebee lower	creek 3	2016-17	30.9	19.1	24.1	13.6	12.8	
bumblebee upper	creek 5	2016-17	12.9	5.7	10.4	3.8	8	2.1
charcoal house creek	22	rcn	23.3	6.4	22	6	20.8	10.9
wildcat brook lower	16	rcn	33	7.5	30.3	6.5	28.8	6.5
wildcat brook upper	11	rcn	15.7	3	15.3	2.9	19.3	9.7
arroyo tio lino 1	11	rcn	99.3	22.4	150.2	53.2	111.6	56.9
arroyo tio lino 2	10	rcn	81.7	24.2	118.1	51.9	32	
arroyo tio lino 3	6	rcn	16.2	2.9	16	2.7		
arroyo tierra blanca 5	9	rcn	50.6	25.5	54.4	33.1		
arroyo tierra blanca 6	8	rcn	16.5	14.7	12.8	10.5		
turpentine still brook	3	cswg	35.4	17.4	67.9	62.1		
arroyo los barrancos 1	17	rcn	29.8	7.2	29.7	7.7	26.1	9.6
arroyo los barrancos 2	14	rcn	46.8	12.1	47	13.3	53.4	29.5
arroyo del cuervo 1	2	rcn	42.3	26.7	58.7	53.3		
arroyo del cuervo 2	3	rcn	40.2	19.9	31.5	14.1		
arroyo del cuervo 3	2	rcn	104.2	54.8	80.4	39.8	24	2
fortification creek	13	rcn	85.1	22.1	77.7	20.1	107.1	49.4
magazine brook 1	3	cswg	60.5	5.6	64.8	7.6	62.5	11.2
cyclone creek	3	cswg	14.7	2.3	14.1	2	13.5	2
pickle creek	6	cswg	11.5	10	8.9	7.1		
williamson creek	1	cswg	30.9	19.1	42.3	37.7		
captain jacobson creek	3	cswg	4.7	1.1	5.1	1.6	4	
brattle brook	2	cswg	4	3.3	3.1	2.2		

Pseudonym	Surveys removed	Period	Ideal Sampling Regime Estimates					
			Closed (Separate)		Closed (Pooled)		Open	
prairie creek	10	cswg	34.4	5.8	36.9	7.8	24	
coral creek	3	cswg	85.7	19.9	77.4	17.5	63.2	17.9
panther branch c1	3	cswg	36.8	2.4	38.5	3.3	33.5	0.8
panther branch c2	3	cswg	14	2.6	13	2	11	
panther branch c3	3	cswg	53.3	1.7	54.7	2.3	52.3	1.4
panther branch c4	3	cswg	11.9	1.9	11.5	1.7	11	1.6
panther branch p3	3	cswg	11.6	0.9	12.2	1.4	11.3	0.6
panther branch p4	3	cswg	15.7	3	15.3	2.9	12	
panther branch p5	3	cswg	16.9	5.6	16.6	5.7		
panther branch p6	2	cswg	12	2.8	11.7	2.8	9	
chicken run	5	rcn	11.2	1.4	12.5	2.5	8	2.8
foxtail creek	2	rcn	29.5	5	34.7	8.7	18.5	4
grindstone creek	4	rcn	16.5	14.7	12.8	10.5		

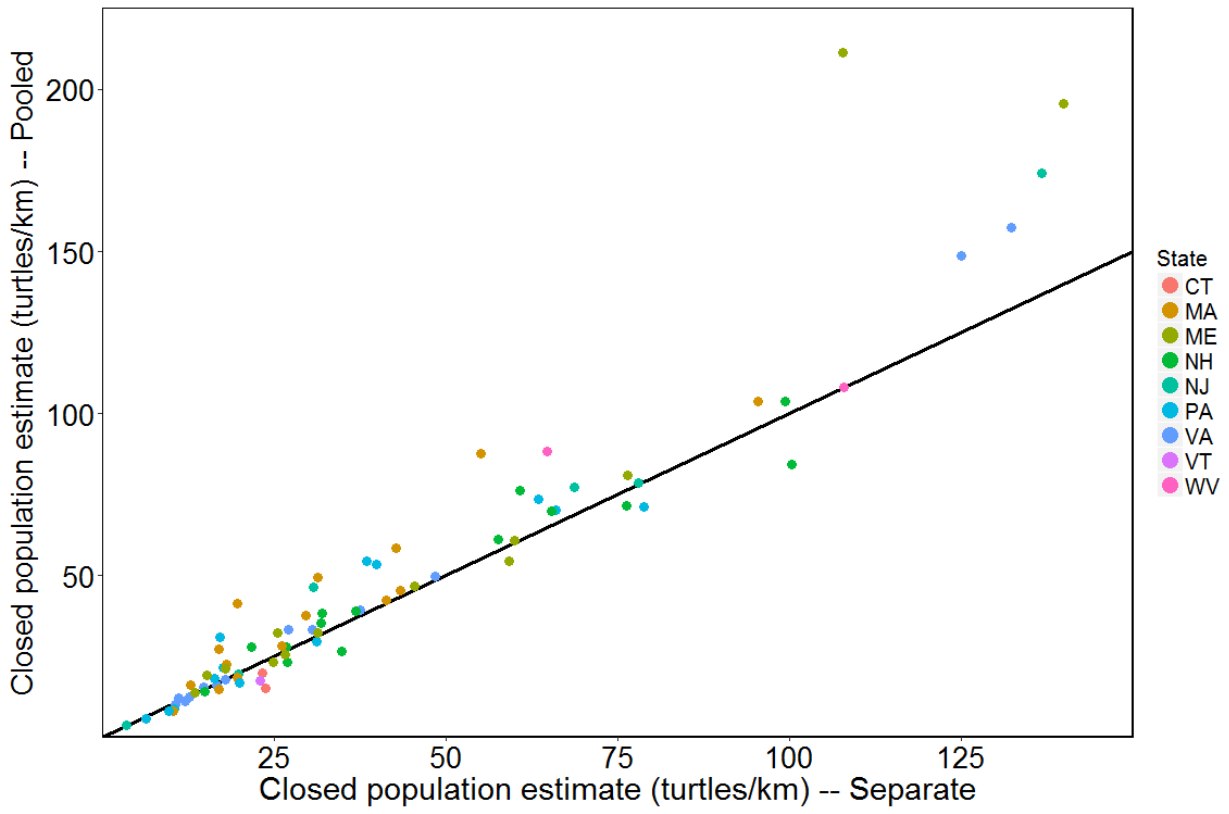


Figure 2.28. Relative closed population estimates where survey returns within seasons are pooled in relation to closed population estimates where surveys are kept separate. The black line reflects a direct relationship between the variables. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

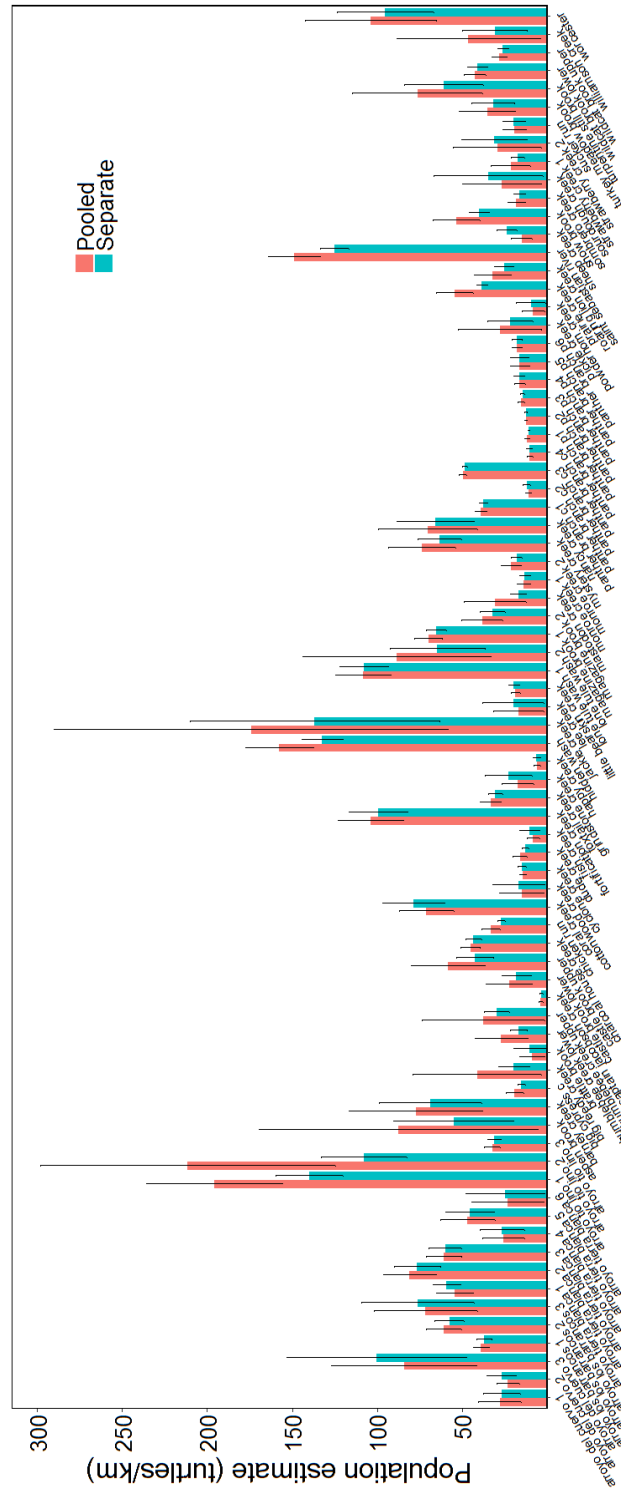


Figure 2.29. Relative closed population estimates when pooling and not pooling turtles captured within season (indicated by color). Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

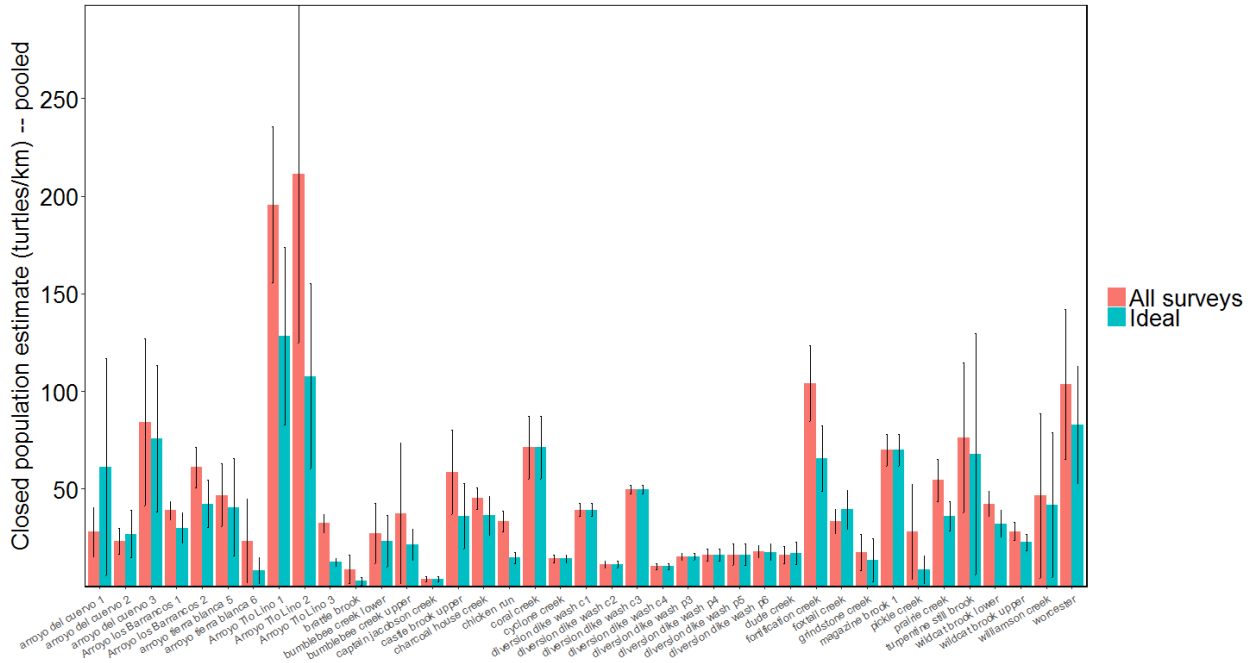


Figure 2.30. Relative closed population estimates (surveys pooled within season) when all surveys are used compared to the “ideal” sampling regime (three surveys in each of three seasons across two years). When >3 surveys were surveyed within a season, surveys were randomly selected. Only sites that could achieve an “ideal” sampling regime are shown. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

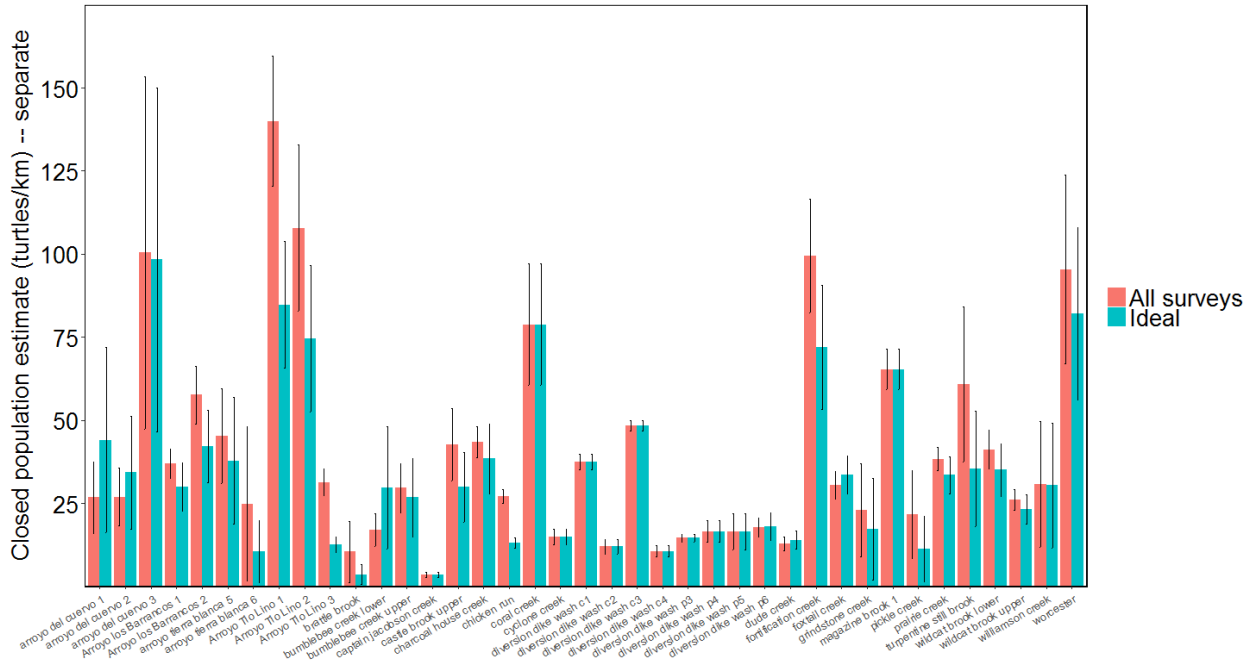


Figure 2.31. Relative closed population estimates (surveys kept separate within seasons) when all surveys are used compared to the “ideal” sampling regime (three surveys in each of three seasons across two years). When >3 surveys were surveyed within a season, surveys were randomly selected. Only sites that could achieve an “ideal” sampling regime are shown. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

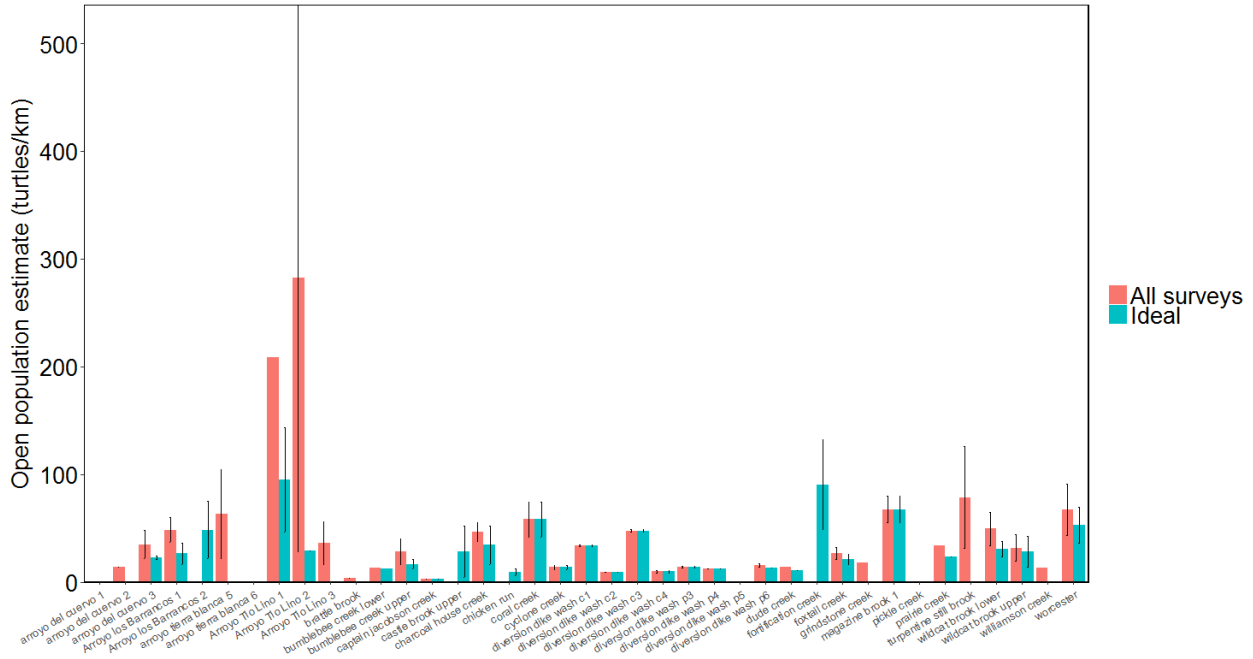


Figure 2.32. Relative open population estimates when all surveys are used compared to the “ideal” sampling regime (three surveys in each of three seasons across two years). When >3 surveys were surveyed within a season, surveys were randomly selected. Only sites that could achieve an “ideal” sampling regime are shown. Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

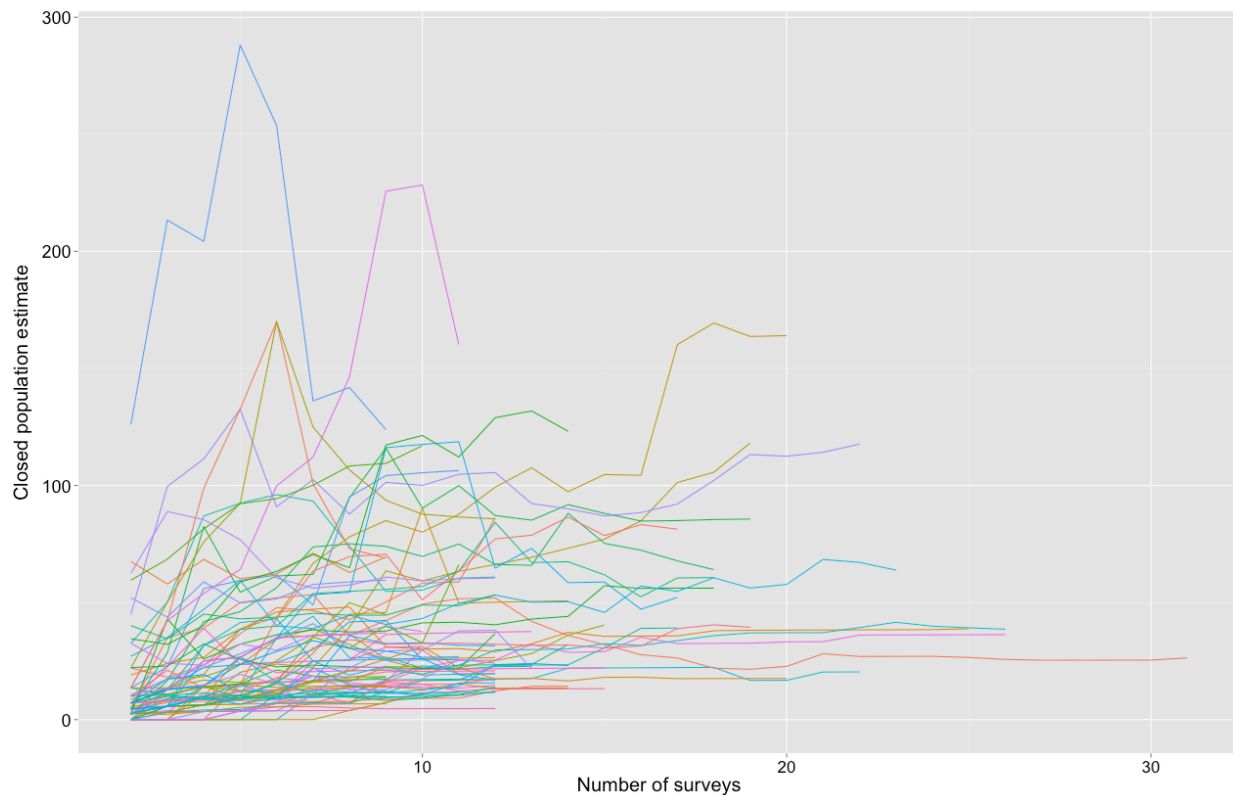


Figure 2.33. Change in closed population estimates (separate surveys within seasons) with increased survey effort for each site (indicated by color). Data were collected during standardized surveys conducted throughout the northeastern United States from 2012–2017.

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Discussion

Regional Trends in Relative Abundance

The findings of this regional population assessment provide further support for the understanding that large-scale landscape-level patterns play an important role in predicting Wood Turtle abundance and occurrence throughout the northeastern United States. Land cover variables at the 5500 m scale appeared in top models across all datasets, with percent undeveloped land (positive relationship), traffic rate (negative relationship), and percent agricultural cover (negative relationship) emerging as three of the more effective predictors of abundance at this scale across the response variables examined. Imperviousness did not appear within top models as it did for analyses completed for the Status Assessment (Jones and Willey 2015). This discrepancy is likely due to the fact that more variables (many

of which were highly correlated with imperviousness) and more complex models were examined during this most recent assessment. Overall, these results reinforce the growing contemporary understanding among conservationists that successful Wood Turtle conservation strategies must extend beyond the streams and riparian areas that encompass the annual activity areas of most individual Wood Turtles in any given subpopulation.

The site-level 300-m scale also played an important role in predicting Wood Turtle abundance even when factors at the landscape-level 5500-m scale were taken into account. Wood Turtle abundance displayed a unimodal relationship with agricultural cover within 300 m, peaking at relatively low levels of agriculture (approximately 15% of the landscape within 300m) and declining thereafter, reaching and maintaining its lowest predicted value at values above ~70% agriculture. While this finding appears to suggest that Wood Turtles may benefit from low levels of agriculture (<15% land cover) within 300 m, this pattern should be interpreted cautiously because Wood Turtles can be attracted to the conditions that agriculture provides despite the negative impacts exerted on the population by machinery. Therefore, it is possible that this finding captures, at least partially, a sink phenomenon. It is also possible that Wood Turtles are simply more detectable within agricultural settings, thus making abundance appear greater than it is. Finally, it is likely that the type of agriculture present when agricultural cover is <15% of the 300 m landscape is of lower intensity—and thus less harmful to Wood Turtles—than when agriculture dominates the landscape. Future, targeted or rigorously designed reassessments of this relationship should provide a chance to elucidate whether abundance at sites with low levels of agriculture are declining or demographically stable.

Contrary to our expectations, Wood Turtle abundance displayed a negative relationship with primary habitat (a variable reflecting the wetland cover types Wood Turtles were found in throughout the region) within 5500 m (albeit only in a small portion of top models). Because this variable represented certain marsh and swamp habitats, this observed relationship may simply reflect the well-documented avoidance of large, slow-moving bodies of water by Wood Turtles.

Our finding that the total number of Wood Turtles detected within the spring sampling period produced the best goodness-of-fit statistic suggests that this may be the most effective method (of the three options examined) for analyzing this type of survey data. Analyzing the total number of Wood Turtles detected while accounting for the total number of observers may have been more effective than analyzing the lead observer's returns because of substantial observer bias that has been shown to be evident (Jones and Willey 2015). Using the total survey returns may have helped to reduce some of the observer-associated bias. This finding may also suggest that the independence of the lead observer's returns—which is an

important assumption underlying the justification of analyzing the lead observer's detections alone—is frequently violated. The improved results from subsetting the response data to the spring season-only may reflect the existence of clear seasonal bias of both overall and sex-based detection that we were unable to fully account for by including a detection covariate for season.

It is important to bear in mind that the results of the N-mixture models represent *relative* abundance. The overall trends and relative comparisons should be emphasized rather than the absolute scale of the response variable (i.e., population size estimate). In addition, because of the high correlation between many potential explanatory land cover variables, we were unable to examine all variables and combinations of variables using this model building process. Therefore, the absence of certain variables within the top models presented does not mean that they do not have the potential be important factors influencing Wood Turtle abundance.

Our observation that stream variables did not appear in best performing models for any subset of response data was unexpected given Wood Turtle's reported strong association with, for example, low gradient streams and high sinuosity (Appendix VII). It is possible that our sampling did not capture a broad enough gradient in these variables to allow for the detection of clear relationships since random surveys only made up a relatively small proportion of all surveys, and these were constrained to suitable stream habitat. Further, most sampling efforts were directed toward known occurrences and "high quality" Wood Turtle habitat. It is also possible that our stream metrics could use improvement. For example, our sinuosity metric (straight-line distance between survey start and end divided by the total length of stream) could produce the same value for a section of stream with a single bend as a section with several changes in direction. The ability to account for important stream variables within future research and regional analyses—through a broader range of sampling or improved metrics—will allow for a more robust understanding of the factors impacting Wood Turtles throughout the region.

Population Estimates

Capture-mark-recapture population estimates from throughout the region show that—even among sites that were selected by experts based on their potential to support Wood Turtles—large populations appear to be rare within the northeastern United States. The observed pattern mirrors that which exists throughout the region and highlights the clear tendency for Wood Turtles to reach high densities only within ideal landscape contexts and where key microhabitat and geomorphological features are juxtaposed. This is exemplified by streams such as Arroyo del Cuervo or Arroyo Tio Lino, for which adjacent segments (i.e., less than 1 km apart) yielded extremely different CMR estimates.

Overall, estimates where surveys were pooled within biological seasons (spring/pre-nesting, nesting, and fall) produced larger estimates than estimates that did not pool surveys, especially for larger populations. Pooling surveys within seasons may represent more accurate relative population estimates by reducing bias associated with temporally autocorrelated capture histories, but it is also possible that useful information is lost in this process.

An apparent lack of strong relationship between relative population size and the percent of captures that were juveniles/subadults could reflect a recruitment deficit even in some of the largest populations. If this is true, it could be explained by one of three major factors: (1) unsustainable depredation rates; (2) suboptimal nesting habitat that cannot produce viable young; (3) high rates of emigration by young turtles. However, juvenile recapture (and detection) rates were highly variable, suggesting observed proportions in many streams are underestimates. Juvenile and subadult percentages should be used in conjunction with closer examination of the nesting features to assess the state of recruitment at sites and the overall of threat of lack of recruitment to the long-term persistence of the subpopulation. Alternatively, low juvenile detections at more dense sites could be related to greater habitat heterogeneity supporting preferred juvenile habitats away from primary sampling areas.

Considerations, Caveats, and Directions for Future Research and Assessments

Although a relatively large proportion of sites sampled (40.3%) yielded zero Wood Turtle detections, the sites that made up this regional dataset were clearly biased toward known populations high Wood Turtle densities. Therefore, results of these analyses should be interpreted accordingly and with caution. When examining the findings of this population assessment it is also important to acknowledge the immense ecological variation that is ignored when combining all data for regional assessments such as these. The regional patterns presented here should be interpreted with the understanding that certain trends may differ by subregion. For example, while regionally Wood Turtles appear to be most abundant at mid-elevations (200-300 m), populations in the southern portion of the region (PA, MD, VA, WV) tend to be found at higher elevations.

Variations in state-specific survey effort represent another potential avenue for the introduction of bias into these analyses. Streams were sampled within all range states in the Northeast, but the vast majority of surveys were concentrated within six states—Massachusetts, Maine, New Hampshire, New Jersey, Pennsylvania, and Virginia—and consequently, analyses are biased toward the ecological settings and environmental conditions present within those states. Fortunately, these states likely represent a large portion of the range of ecological conditions in the northeastern States. Future research and/or regional

assessments should attempt to apply elevated sampling effort toward underrepresented states, especially the largest under sampled state, New York, in order to more accurately reflect the relative proportion of Wood Turtle habitat within respective states, as well as to further clarify regionally significant sites within these areas.

Future research and regional reassessments should consider implementing modeling strategies that provide more robust means for assessing relative abundance and population size and are better suited for the idiosyncrasies associated with broadscale, Wood Turtle data. Spatially-Explicit Capture Recapture models (SECR) may provide an effective means of estimating population size. However, this effort would necessitate the documentation of coordinates for all turtles captured with the established survey segment, which was not consistently reported throughout this five-year study. Some changes to the regional monitoring protocol, data forms, and data management systems may also be needed to better accommodate and efficiently implement SECR models at a regional scale. “Robust” design (Pollock 1982) CMR models offer another potential alternative for analyzing these data. Through this method, populations are assumed to be closed during sampling periods (i.e., biological seasons), but experience deaths, immigration, emigration between periods.

Part III. Population Genetics of the Wood Turtle

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Summary

A major objective of this regional Wood Turtle conservation planning initiative was a regional assessment of Wood Turtle population genetics. Individual goals of the study were to (1) identify genetic diversity across the study area (Maine to Virginia), (2) identify the number of populations in the study area, and (3) determine the success of genetic assignment of individuals to sites of origin. Tissue samples were primarily collected as blood, tail tips, toenails and shell shavings from 1,895 Wood Turtles. Most tissue samples were collected in 2015 and 2016; however, some collectors submitted tissue samples from tissue archived from previous collections with the earliest collection dated 2005. Tissue samples were genotyped at 16 microsatellite markers for 1,244 individuals. Genetic data were analyzed for genetic diversity (using HP-RARE, GENEPOP and GENALEX), allele frequency exact test (using GENEPOP), genetic clustering (using STRUCTURE), full siblings (using COLONY), and genetic assignment (using GENECLASS). Sample sizes ranged from 5 to 50 individuals (average $n = 17.4$) collected from 62 sites. Unbiased allelic richness ranged from 3.4 to 6.2 (average 5.1), private alleles ranged from 0 to 0.3 (average 0.05), unbiased expected heterozygosity ranged from 0.5 to 0.7 (average = 0.6) and F_{IS} ranged from -0.21 to 0.14 (average = 0). F_{ST} ranged from 0 to 0.23 (average = 0.07). Allele frequency exact tests identified significant pairwise differences between 91% of the sites. A Bayesian genetic clustering analysis indicated that there are likely three to five genetic clusters in the northeastern United States, with four clusters providing the most optimal clustering pattern in the data set. These four major population groups identified were northern ME, Potomac, coastal MA, and NJ/NY. Sites in PA and NH showed admixture with the neighboring clusters. The Bayesian clustering analysis indicated that an island stepping-stone model describes the population genetic structure where sites are exchanging individuals

with neighboring sites creating a gradation of genetic structure over the study area. For the purposes of conservation in the Northeast, we recommend that managers consider tailoring management actions to five Evolutionarily Significant Units: the four genetic clusters described above, and the geographic area encompassing the Connecticut, Merrimack, and Kennebec basins. Isolation by distance was statistically significant for two of three clusters tested (Potomac and Maine/NH). The northern Maine cluster showed a similar pattern but was not significant for isolation by distance ($p = 0.17$). The results of this study indicated that clear genetic differences among subpopulations are detectable across the study area. Tests for full sibling families indicated a maximum distance between related turtles of 50 km. Genetic assignments indicated that 52% of individuals in the dataset assigned correctly to the collection site. The genetic assignment was relatively successful for some sites (>75% correct genetic assignment); however, assignment success using these markers varied dramatically across sites/populations and, at some sites, correct assignment was low (<50%), limiting the application of this method for management and enforcement for Wood Turtles confiscated from illegal harvest.

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Introduction

Population genetic analyses can be used to support management assessments and conservation planning. Specifically, these analyses can identify genetic diversity, low population size, fragmentation, population structure or designation, gene flow and migration rates—all of which assist the management of populations and associated habitat (Paetkau et al. 2004; Manel et al. 2005). Management Units are defined as demographically independent units based on genetic divergence (Pasboll et al. 2007). Understanding the genetic and demographic interactions is important for predicting how populations will respond to environmental and anthropogenic disturbances.

In addition to the identifying populations, genetic data can be used to assign individuals (or parts thereof, such as shells, horns or teeth) to populations of origin (Paetkau et al. 2004; Manel et al. 2005). This method is commonly applied in the illegal animal trade and can be useful to identify illegal poaching activity. In some circumstances, genetic assignment may be used to release confiscated animals to their population of origin (such as Gaur et al. 2006). The ability to identify the site of origin of confiscated animals may assist species' conservation efforts as the threat from illegal harvest continues to increase while the population abundances and habitat quality are continuing to decline.

Currently, population genetic information on the Wood Turtle across its range is limited. One study found little genetic variation and structuring across the range of the Wood Turtle examining mitochondrial DNA

(Amato et al. 2008). Several other studies have used nuclear microsatellite DNA markers to examine patterns of population genetic structure at smaller geographic scales, within or across adjacent major basins (Tessier et al. 2005; Castellano et al. 2009; Spradling et al. 2010; Fridgen et al. 2013; Willoughby et al. 2013). While these studies provide some information about the genetic status of the Wood Turtle, the limited geographic scope precludes the identification of species-wide genetic diversity. Therefore, the overarching goal of this component of the Wood Turtle Competitive State Wildlife Grant was region-wide genetic sampling to fully complement the broad scope of concurrent conservation planning efforts.

The specific objectives of this study were to: (1) describe population genetic diversity (heterozygosity, allelic richness, private alleles); (2) identify the most likely number of population groups in the northeastern United States; (3) measure relative isolation by distance comparing genetic and geographic distances; (4) estimate contemporary migration rates; and (5) test population genetic assignment methods to identify the origin of confiscations from the illegal animal trade.

Methods

Tissues were collected from participating states in the Northeast including: Maine, New Hampshire, Massachusetts, Connecticut, New Jersey, Pennsylvania, Maryland, Virginia and West Virginia. Samples were also collected by cooperators in Vermont, New York and Rhode Island. Additional samples were submitted from other studies from the Midwestern U.S. as out-groups for this study.

Tissue was collected as blood, tail tissue, toenail, and shell. Other soft body parts were occasionally collected from recent mortalities (such as toes or foot). Blood was preserved in 95% ethanol, lysis buffer (e.g., Queens lysis) and PBS, depending on the collector. Other tissue types were preserved in 95% ethanol. Samples were stored at -20°C until processed in the lab.

Laboratory Methods

DNA extraction varied for tissue types. DNA was extracted using a MoBio Ultra Clean Tissue and Cells DNA isolation kit™ (MoBio, Inc., Calsbad, CA) (blood, tail) or a Qiagen DNeasy Blood and Tissue Kit™ (Qiagen, Inc., Germantown, MD) (blood, tail, nail, shell) according to manufacturer's protocols. Blood and tail and other soft tissue were incubated overnight at 55°C, and nail and shell samples were incubated for 2 days on a shaking incubator (Henry Troemner LLC, Thorofare, NJ). The concentration of DNA was measured in each sample using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Samples with DNA concentrations greater than 40 ng/ul were diluted with DNA/RNA-free water to 20-25 ng/ul.

Seventeen microsatellite markers were selected based on the performance in other published studies of Wood Turtle. Most markers were described for Bog Turtle (*Glyptemys muhlenbergii*), but were tested on the Wood Turtle (King and Julian 2004). Additional markers were included that were described for Blanding's Turtle (*Emydoidea blandingii*) and Painted Turtle (*Chrysemys picta*) (Table 1). Four multiplexes were performed with 3 to 5 markers each. GmuD51 was isolated for the PCR reaction and then added to multiplex 1 prior to electrophoresis.

Table 3.1. Microsatellite loci, citations and multiplex assignment used for this study.

Locus	Citation	GenBank no.	Wood Turtle Genetics Studies by location						CSWG study multiplex
			Castellano et al. 2009	Chinnici and Huffman 2016	Spradling et al. 2010	Willoughby et al. 2013	Fridgen et al. 2013	Tessier et al. 2005	
GmuA19	King and Julian 2004	AF517227					X		4
GmuA32	King and Julian 2004	AF517228					X		2
GmuB21	King and Julian 2004	AF517231		X	X	X	X	X	4
GmuD16	King and Julian 2004	AF517235	X	X	X	X		X	1
GmuD28	King and Julian 2004	AF517237			X	X	X		3
GmuD40	King and Julian 2004	AF517238	X	X	X	X	X	X	4
GmuD51	King and Julian 2004	AF517239	X	X					1
GmuD55	King and Julian 2004	AF517240			X	X			2
GmuD62	King and Julian 2004	AF517241		X					
GmuD70	King and Julian 2004	AF517242							4
GmuD79	King and Julian 2004	AF517243			X				3
GmuD87	King and Julian 2004	AF517244	X	X	X	X	X	X	2
GmuD88	King and Julian 2004	AF517245	X	X	X	X			1
GmuD90	King and Julian 2004	AF517247			X ^a				
GmuD93	King and Julian 2004	AF517248	X ^a	X		X		X	
GmuD95	King and Julian 2004	AF517249	X ^a	X					
GmuD114	King and Julian 2004	AF517251		X	X ^a				
GmuD121	King and Julian 2004	AF517252			X				3
Eb17	Osentoski et al. 2002	AF416295							1
Eb19	Osentoski et al. 2002	AF416296							3
BTCA9	Libants et al. 2004	AY335790							2
Cp2	Pearse et al. 2000								2

^a indicates the marker was dropped from analysis

We conducted 10 µl PCR reactions in a 96-well plate using a thermal cycler (MJ Research, PTC-200). Each reaction consisted of 5 µl of Qiagen Multiplex PCR Master Mix, 1 µl template DNA, 1 µl of primer (6-FAM primers were 0.15 µM, PET and VIC primers were 0.2 µM and NED primers were 0.25 µM), and 3 µl of PCR grade water. The PCR reactions were adjusted for nails to include 1 µl BSA and 2 µl of PCR grade water. Forward primers were fluorescently labeled and acquired from Applied Biosystems (colors

NED and PET, Foster City, CA) and Integrated DNA Technologies (colors 6-FAM and VIC, Coralville, IA). Thermocycling conditions included an initial 15 mins at 95°C followed by 35 cycles of 94°C for 30 sec, 57°C for 90 sec (or 51°C for GmuD51), 72°C for 90 sec and a final cycle of 72°C for 10 mins. One negative control was included on each plate. PCR products were diluted to 1:50 with PCR grade water. The PCR products were run on an Applied Biosystems 3130xl Genetic Analyzer with a LIZ600 ladder for size standard. Peaks were scored using Geneious version 9 (Biomatters Ltd, Auckland, New Zealand). Peaks were visually checked for conformity to expected profiles. Duplicate samples for the quantification of error rates ranged for the multiplex and the locus. The number of duplicate samples ranged from 33 for multiplex 1 to 48 for multiplex 2. The number of re-run samples by locus ranged from 23 for GmuD51 to 48 for GmuD87. The percent error was estimated as the percent of alleles from the total duplicated samples that were not equal. This estimate would include scoring error, binning error, variation in runs and null alleles.

Statistical Analysis

Individuals without location information or with fewer than 8 successfully genotyped loci were removed from the data set prior to statistical analysis. Sites with fewer than 5 individuals were removed prior to statistical analysis. One individual identified from a pair of full siblings with a 95% confidence using 100 randomizations in ML Relate (Kalinowski 2006) was removed from the data set; this was done to avoid bias in site-based population genetic measures.

Exact tests for deviation from Hardy Weinberg proportions and linkage disequilibrium were performed using GENEPOP version 4.5 (Raymond and Rousset 1995). Heterozygosity and unbiased estimates of allelic richness and private alleles were calculated using HP Rare (Kalinowski 2005). F_{IS} was calculated using GENALEX (Peakall and Smouse 2006, 2012). A log likelihood G test from Goudet et al. (1996) in GENEPOP version 4.5 was used to test for genetic differences among sites. A Bonferroni correction was applied to all significance tests with multiple comparisons (Rice 1989).

We used STRUCTURE version 2.3.4 (Pritchard et al. 2000) to estimate the number of populations. STRUCTURE is a Bayesian-based model that clusters individuals according to allelic frequencies while minimizing linkage disequilibrium and deviation from Hardy–Weinberg equilibrium. The model allows for admixture between population groups. The admixture model with correlated allele frequencies in STRUCTURE was run by using 10,000 iterations for burn-in and 100,000 iterations with a Markov-chain Monte Carlo resampling algorithm as described by Pritchard et al. (2000). Ten runs were performed for each K value tested (K=1 to 20). Data from sites with more than 15 genotyped individuals were used in

this analysis except NY where sites had 11 individuals. We conducted an initial analysis on all the genotyped individuals included in the complete data set, hereafter referred to as uneven data set. Due to bias inherent in structure-based analyses (see Kalinowski 2011; Puechmaille 2016), we performed a secondary analysis on a subset of the data by reducing sites to a sample size of 16-18 individuals, hereafter referred to as the even data set. Individuals with incomplete data were removed from the data set first, followed by a random selection as needed. Finally, a last set of runs was performed with the location prior option, which uses the capture location as a prior in the model. STRUCTURE output was compiled and visualized using STRUCTURE HARVESTER (Earl and vonHoldt 2012). After identifying the K value, a final run (hereafter called full data set) with all sites with $n > 6$ and individuals with 14 or more loci were tested using this optimal K value.

A K-means test was performed using the even sample in GENODIVE version 2.0b27 using a Bayesian Information Criterion (BIC) (Meirmans and Van Tienderen 2004) to verify the number of clusters we identified using STRUCTURE. The K-means clustering identifies the optimal clustering as the K value with the smallest amount of variation within clusters, which is calculated using the within-clusters sum of squares. The value of K with the lowest BIC value is identified as the best fit for the data. Finally, a principal components analysis (PCA) was performed in GENODIVE using allele frequency data and covariance matrix, and graphed in R version 3.4.1 (R Corps Team 2013).

Isolation by distance was tested using a Mantel test on pairwise F_{ST} values and geographic distance (Euclidean and stream distance) for sites within the clusters identified using STRUCTURE. The test was performed using IBDWS version 3.23 using 1000 randomizations (Jensen et al. 2005). Several sites are not connected by stream or river corridor in the clusters, such as the Allegheny River and the Potomac River sites. The stream distance tests were done using 10,000,000 km as a pairwise distance value for these unconnected sites. The stream distance analysis was also performed without including the unconnected sites.

Full siblings and parent-offspring pairs were identified among the sites within the clusters identified in the structure analysis. Colony version 1.2 (Wang 2004) was used to identify full sibling groups. Simulations for similar analyses found that full sibling groups with 3 or more individuals was 97% accurate using 16 loci (Whiteley et al. 2014). This method has also been found to out-perform STRUCTURE for identifying recent migrants (Whiteley et al. 2014). Sites were grouped according to the major population groups identified via the Bayesian clustering analysis were used for this test. Population groups in northern Maine, ME/NH, western MA, and Potomac were tested for family groups. The specific sites used for this

analysis are listed in Appendix E, Table E.2. Some overlap was considered between northern ME and ME/NH in order to test movement between sites and across major population groups.

Samples from the known collections (sites) were tested in GENECLASS version 2.0h (Piry et al. 2004) using a leave-one-out method where each sample is sequentially removed from the data set and assigned to a population. This test provides an estimate of accuracy for assignment success. Additionally, unknown samples from captive populations or from the illegal pet trade were assigned to reference populations. The frequentist analysis described in Petkau et al. (1995) was used for this test as it slightly outperformed the other options. Individual samples with more than one missing locus were removed from the data set prior to performing this analysis.

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Results

More than 1,895 tissue samples of various tissue types (Fig. 3.1, 3.2) were collected from across the northeastern United States. Samples were prioritized for genotyping based on site location, sample size and success by tissue type. Blood and soft tissue (toes, tail tips) were selectively chosen for genotyping due to ease of extraction and higher success rates. Toenails were highly successful, but only when an adequate amount of nail and associated soft tissue was sampled (see Lutterschmidt et al. 2010 for details on toenail tissue success). Shell shavings and scutes also had sufficient success rates. Nails and shells were used to increase sample sizes when other tissue types were not available. To estimate the success rates of the tissue types, we examined a sub-set of samples where the collection and treatment of the samples provided a fair estimation of the sample success. A tissue was considered failed if 3 or more loci were missing. Tail tissue was the most successful (97%), followed by blood (87%). We did not examine blood by preservation method, but ethanol and PBS provided higher success rates and more ability to manipulate the amount of tissue used in the extraction. Toenails were successful when the nails provided sufficient soft tissue, and the more successful samples ranged from 70% to 94.5% but certain sites provided high failure rates (90-100%) when the sample collection did not provide adequate tissue. Shell samples were the smallest portion of our data set and were about 60% to 80% successful depending on the collector.

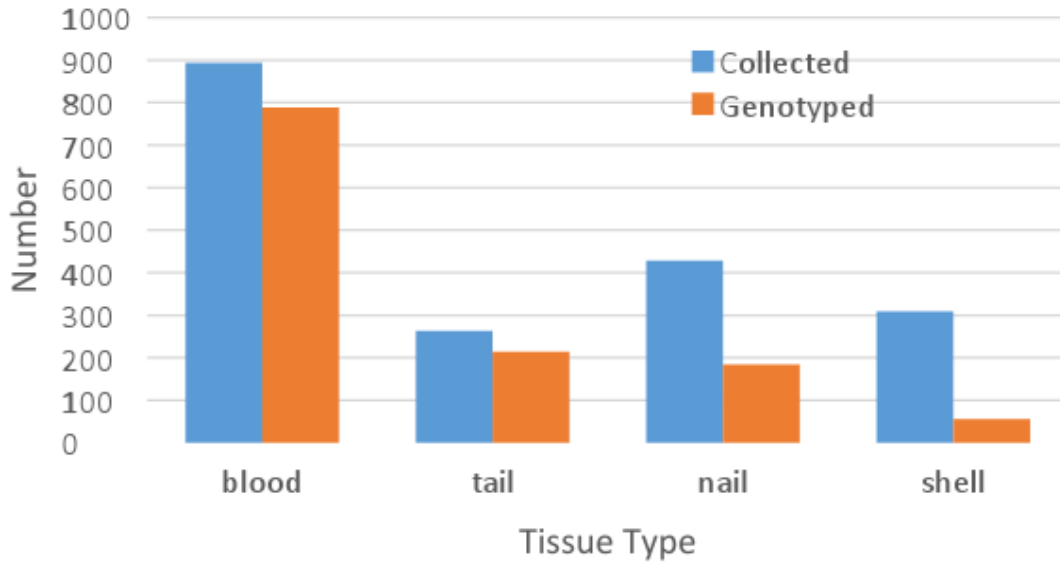


Figure 3.1. Tissue types included in the study (blood, tail or soft tissue, toenail, shell shavings) collected and genotyped for this study.

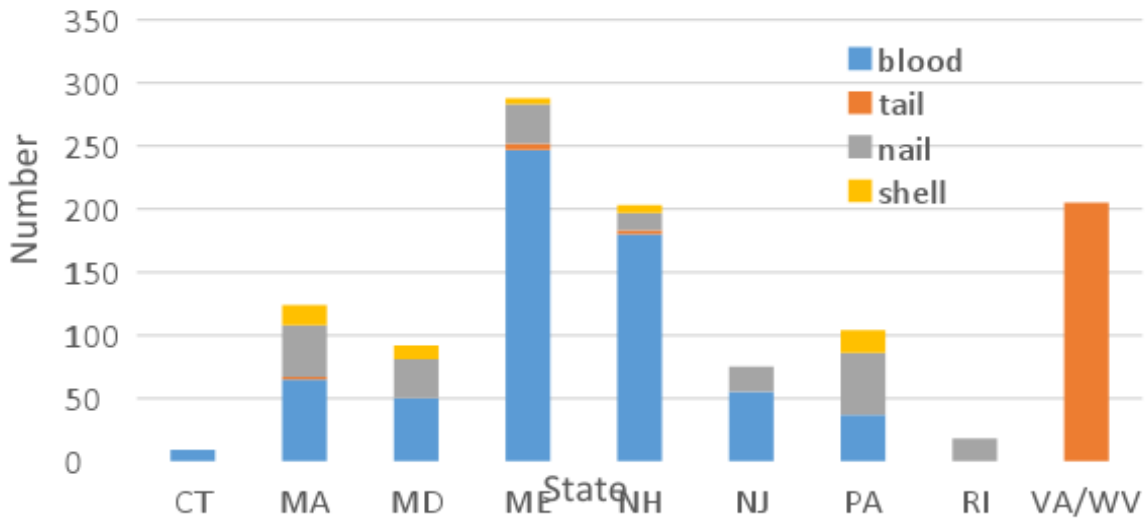


Figure 3.2. Number of samples successfully genotyped by state and tissue type (blood, tail or soft tissue, toenail, shell). The sample must have >7 loci amplified to be considered successful and included in data analysis.

Tests for Assumptions and Genetic Diversity

Samples sizes ranged from 5 to 50 individuals (average $n=17.4$) collected from 62 sites. One locus, GmuA19, was removed due to scoring difficulties. For the remaining 16 loci, genotyping error ranged from 0 to 3.4% (Table 2). Exact tests for deviations from Hardy-Weinberg proportions identified significant deviations for GmuA32 at three populations (MA Worcester, NH Turpentine, NH ArBar), GmuD51 at two populations (MA Wildcat, NJ Potato) and GmuD21 at one population (PA Coral). Significant linkage disequilibrium was detected at 6 pairs of loci, but there was no pattern to the loci or populations. Based on these results, we kept all these loci in the analysis due to potential for a significant test based on random chance, uneven sample sizes (which we address later), and the robustness of many statistical tests to deviations from Hardy-Weinberg proportions.

Unbiased allelic richness ranged from 3.4 to 6.2 (average 5.1), private alleles ranged from 0 to 0.3 (average 0.05), unbiased expected heterozygosity ranged from 0.5 to 0.7 (average = 0.6) and F_{IS} ranged from -0.21 to 0.14 (average =0). F_{ST} ranged from 0 to 0.23 (average 0.07). The overall genetic diversity is within the range documented in other studies of Wood Turtle (Table 3).

Table 3.2. Loci, size ranges (bp), number of alleles, percent failed amplification and genotyping error for this study.

Locus	Size Range min	Size Range max	No. alleles	% fail amplification	Genotype error	Comments
GmuA19						Removed due to difficulties scoring Stutters; high failure in USFS Midwest samples
GmuA32	147	208	29	6.7	0	
GmuB21	193	204	21	3.2	0	
GmuD16	151	237	27	18.6	3.4	
GmuD28	185	258	25	3.6	0	
GmuD40	136	197	24	3.9	0	
GmuD51	220	396	51	8.7	0	
GmuD55	182	204	17	10.6	0	
GmuD70	151	193	9	12.4	0	
GmuD79	149	265	6	10.2	0	
GmuD87	226	303	25	4.5	1.0	
GmuD88	102	185	29	3.2	0	
GmuD121	124	174	12	1.8	2.3	
Eb17	88	104	11	3.0	1.7	
Eb19	92	97	4	1.7	0	
BTCA9	136	152	8	4.0	1.1	
Cp2	188	263	22	8.7	0	

Table 3.3. Summary of genetic diversity in studies of Wood Turtle by citation, location, number of loci, unbiased allelic richness (asterisk indicates number of alleles as only value reported), expected heterozygosity and F_{IS} .

Citation	Location	No. loci	AR	He	F_{IS}
Castellano et al. 2009	Delaware Water Gap	7	10.3-13.8	0.88-0.95	-0.20-0.019
Fridgen et al. 2013	Southern Ontario, Canada	5	3.6-5.4	0.48-0.87	-0.07-0.33
Spradling et al. 2010	Iowa, Minnesota, WV	11	1.0-16.4	0-0.9	0-0.007
Tessier et al. 2005	Quebec, Canada	5	13-36*	0.8-0.89	
Willoughby et al. 2013	Michigan	9	7.11-10.7*	0.37-0.91	-0.10-0.48
This study	NE – mid Atlantic states	16	3.4-6.2	0.53-0.70	-0.21-0.14

Three sites in Maine (Tananger, Arroyo Frijoles, and Arroyo Colorado) were tested for genetic differences by age class. These data do not indicate any differences across ages within a site (Table 4), and all of the pairwise allele frequency exact tests were not significant following correction for multiple tests. The sample sizes for juveniles are low which is likely due to collectors avoiding natal areas and young turtles during sampling. Similarly, Fridgen et al. (2013) did not find statistically significant differences among age classes at a site in Ontario, Canada. Although these results indicate little genetic changes among the broad age classes at these sites, this type of test could be improved with greater sample size targeting as large an age range as possible and implementing this test in areas where populations are fragmented and/or declining. It should also be noted that this test was only possible in these relatively intact sites due to sampling limitations.

Table 3.4. Genetic diversity (observed heterozygosity, unbiased expected heterozygosity, unbiased allelic richness, unbiased private alleles) of age classes at three sites. Age categories are juvenile (J=<15 years old), middle (M=15-25 years old) and oldest (O=>25 years old). Ages were provided by M.T. Jones, unpublished data.

Site	Age	N	Ho	He	AR	PA
Tan	J	4	0.63	0.57	3.3	0.5
	M	10	0.52	0.59	4.7	0.8
	O	4	0.61	0.63	3.9	0.6
ArF	J	4	0.55	0.60	3.4	0.3
	M	9	0.53	0.58	4.9	1.3
	O	13	0.56	0.61	4.7	0.9
ArCol	J	5	0.61	0.57	3.5	0.2
	M	8	0.50	0.60	4.6	0.8
	O	8	0.60	0.60	4.7	1.0

Population Differentiation

Allele frequency (genic) exact tests indicated that 91% of the pairwise comparisons were statistically significant after correcting for multiple tests. F_{ST} ranged from 0.01 to 0.23, and generally lower F_{ST} values will correspond with insignificant allele frequency differences. Both tests indicate the amount of pairwise differences among sites. F_{ST} values among the sites are shown in Appendix C.

Within Maine, Camel Hut and Big Cypress were not significantly different, while all of the other pairwise comparisons were significantly different. F_{ST} values ranged from 0.03 to 0.13. Many of the sites in NH were not significantly different. The NH Fortification site showed the greatest divergence from the other sample sites, but all of the sites in NH had lower F_{ST} values (0.01 to 0.08). In general, most of the pairwise tests across sites within the Merrimack basin were not significant. Dead Lizard and Arroyo del

Cuervo were not significantly different from several of the Merrimack sites. Bullhead was not significantly different from Sourdough and Crow but was significantly different from other sites in the Connecticut basin. Overall, the sites sampled in NH appear to have some migration among the sites and low genetic drift.

Within Massachusetts, Bumblebee, Charcoal House, Wildcat and Little Bearskin were not significantly different and could be considered one subpopulation. All of the MA sites had F_{ST} values ranging from 0.01 to 0.08 with the higher values (0.05-0.08) associated with MA Worcester. The other pairwise F_{ST} values at MA sites ranged from 0 to 0.03. MA Worcester was significantly different from the other Massachusetts sites, but not significantly different from the Rhode Island site. Connecticut Wheeler was not significantly different from NH Pickle and NH Millstone.

Maryland sites (Mary Davis, Wolfpen, Moose Meadow and Tomahawk) were not significantly different from each other, but were significantly different from Pumpkin Field. MD Pumpkin Field also had the highest F_{ST} values within the MD sites with the F_{ST} values ranging from 0.02 to 0.04 among the sites. Among the New Jersey sites, Potato, Barney, and Jackie were not significantly different and are likely one subpopulation. Barney and Sucker, and Barney and Bulldozer were not significantly different. Williamson was the most divergent of the sites sampled in the state. F_{ST} values for NJ sites ranges from 0.02 to 0.11. In New York, Barrel Ranch and Yankee were not significantly different. In Pennsylvania, Snow was not significantly different from Nancy and Nancy was not significantly different from Coral. F_{ST} values in PA ranged from 0.02 to 0.04.

In Virginia/West Virginia, many sites sampled were not significantly different. St. Sebastian, Silvertip, Box Canyon, Waterfall, Hidden, Diversion and Lone Tule were not significantly different from each other and should be considered one subpopulation. Chicken Run was not significantly different from Waterfall Wash indicating some genetic exchange or lack of genetic drift. Chicken Run and August were the most divergent sites included in the state, and Chicken Run was the most divergent site in the complete, northeast sample showing the largest F_{ST} differences from the sites further north. F_{ST} values from the VA/WV sites ranged from 0.03 to 0.06.

STRUCTURE indicated that there are likely 3 to 5 clusters region-wide. The likelihood plot of the number of clusters (K) did not change substantially between the uneven and even runs without location prior and the even with location prior runs (Fig. 3.3). The clusters identified with and without the location prior provided similar clustering results (data not shown). Our presentation is focused on the results without using the location prior.

The most distinct clusters were the differentiation of the northern sites from the southern sites and sites in coastal MA/RI. For example, at $K=4$, the clusters are: coastal MA (MA Wo/RI); Potomac/Allegheny sites (MD, VA and WV); northern ME (Ar. Coyote, Ar. Frijoles, Ar. Tio Lino, Ar. Colorado, Ar. Yupa, Camel Hut, Tanager, Baxter); and NJ/NY sites. The Connecticut (MA Cr, MA Bu, MA LB), Merrimac (NH Tu, NH ABa, NH FI, NH Cy), and Kennebec (ME Sm, ME CaH) basins indicate mixed ancestry between the coastal MA/RI cluster and the northern ME cluster, and should be considered a genetically similar group. Some locations in PA and NY showing mixed ancestry (Fig. 3.4). PA has one site that groups with the Potomac and two sites in the Susquehanna basin that cluster with NJ/NY. The sites in PA show admixture between NJ, MA and VA. The NH and coastal MA/RI sites separate from the ME sites when increasing from $K=3$ to $K=4$, while increasing from $K=4$ to $K=5$ separates the NH cluster from the coastal MA/RI cluster (Fig. 3.4). The K-means test also indicated 4 clusters as the best fit for the data, providing additional support for the STRUCTURE inferences.

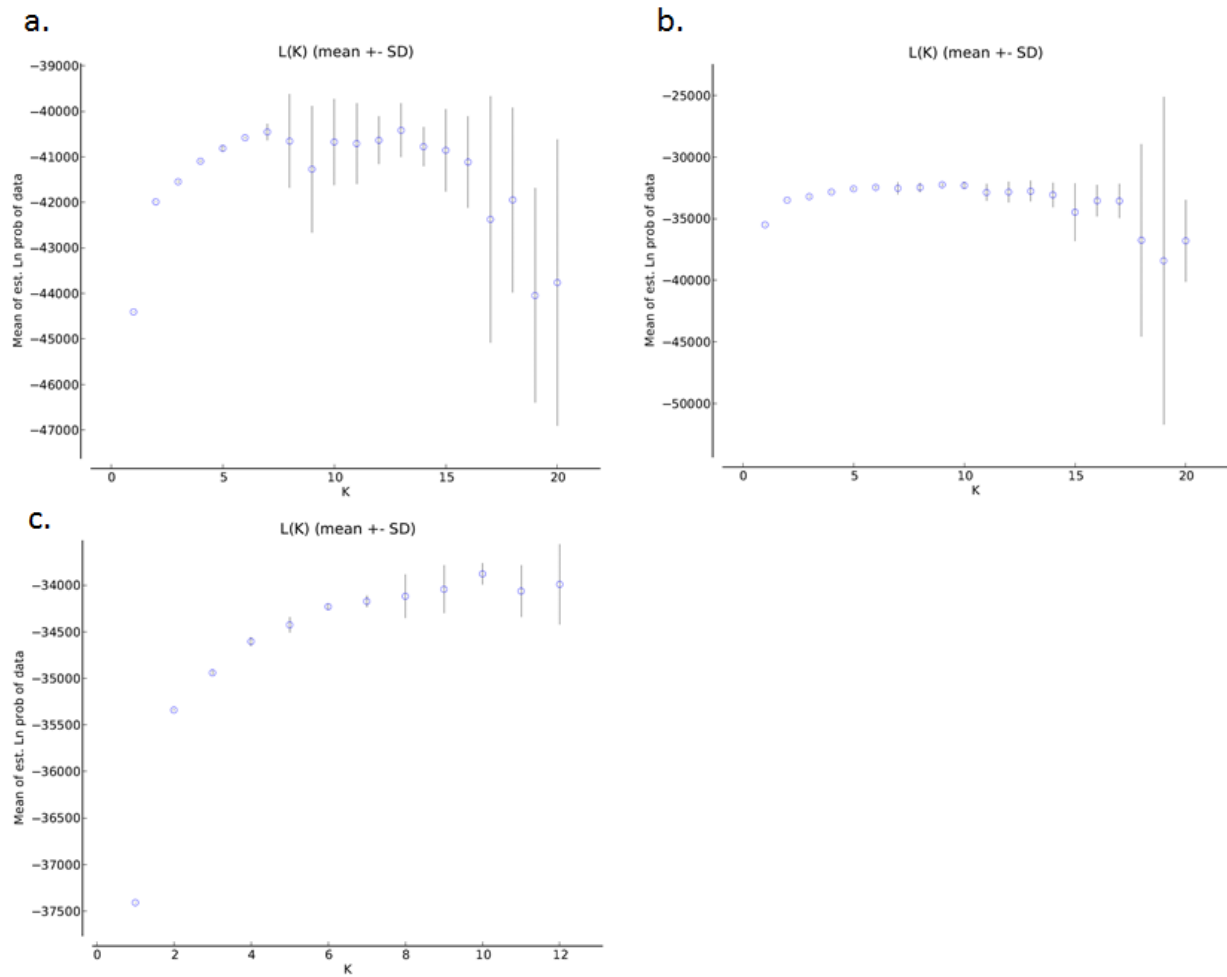


Figure 3.3. Log likelihood for the number of clusters (K) in the data sets with (a) uneven sample sizes without location prior; (b) even sample sizes without location prior and (c) even sample sizes with location prior. Note different scale on x-axis for (c).

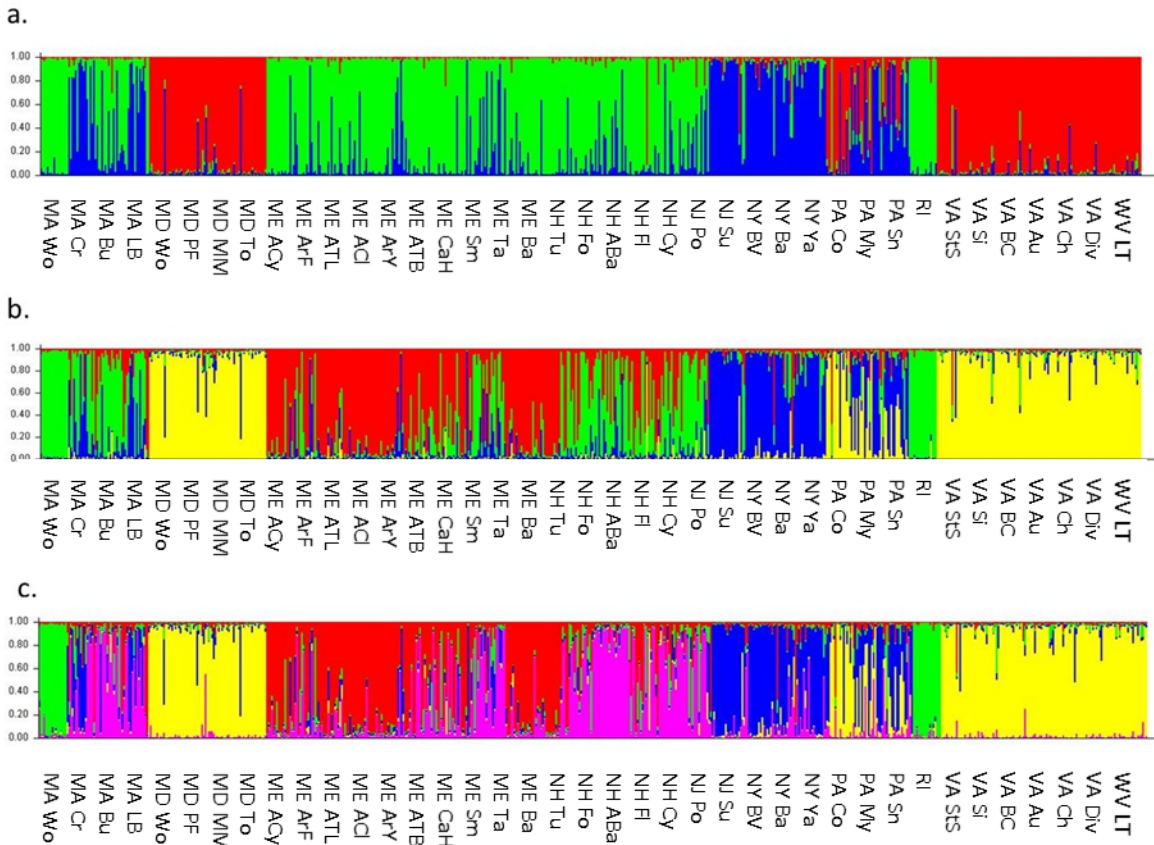


Figure 3.4. STRUCTURE plots for the even sample size without location prior runs for (a) $K=3$, (b) $K=4$, and (c) $K=5$ clusters. The y-axis shows the admixture coefficient (Q -value) and each bar or column in the figure represents one individual Wood Turtle. Site abbreviations are shown below the x-axis.

Principal components analysis revealed similar major groups as those identified with the STRUCTURE and the K-means analyses. The northern and southern sites were the most distinct clusters, the coastal MA/RI cluster was separate from both of these clusters and the sites geographically between these showed a gradation primarily along the cluster with the northern sites. PA, NJ and NY fall in between the northern and southern clusters (Fig. 3.5). One site (MA Crosby) clustered with the NY/NJ group, but locates just to the right of the ME/NH group. The other western MA sites group with the ME/NH group but toward the bottom of the cluster in the direction of the eastern MA/RI group.

We used analyses of isolation by distance within the major STRUCTURE-defined clusters of populations to test for nearest-neighbor patterns of gene flow (over land or via waterways). The isolation by distance

tests for sites in the NH/ME group and the Potomac group were significant for Euclidean distance tests; however, the test in the northern Maine cluster was not significant (Table 3.5, Fig. 3.6). The northern Maine sites were significantly correlated with stream distance, but 13 out of 28 pairwise comparisons were not connected by stream corridor. Eliminating these unconnected data points reduced the correlation among sites. The NH/ME group was significantly correlated with stream and Euclidean distance, but the correlation between Euclidean distance and genetic distance was stronger. These results suggest that gene flow occurs both over land and by waterways. This group had too few sites connected by stream corridor to perform this test excluding the unconnected sites.

Table 3.5. Summary of Mantel test by major population group, pairwise distance calculation, unconnected sites out of total pairwise comparisons (unconnected/total), correlation coefficient (r) and p-value (p) for the isolation by distance tests. Sites that were not connected by a stream corridor were given a maximum value of 10,000,000 km pairwise distance in the stream corridor distance test. NA indicates there were too few populations to perform the analysis.

Population Group	Distance	Unconnected / Total	r	p
North Maine	Euclidean	0/28	0.21	0.170
	Stream	13/28	0.67	0.001
	Stream connected only	0/15	0.47	0.045
NH-ME	Euclidean	0/21	0.74	0.002
	Stream	16/21	0.60	0.011
	Stream connected only	0/5	na	na
Potomac	Euclidean	0/66	0.74	0.001
	Stream	21/66	0.34	0.070
	Stream connected only	0/45	0.15	0.160

The average site Q values from STRUCTURE run on the full data set with K=4 showed similar patterns as Fig. 3.4 (Fig. 3.7). Specifically, the CT and western MA sites showed mixed ancestry. CT has more of the NJ/NY ancestry and western MA sites show more of the coastal MA influence. ME Monroe and ME Smiley show ancestry similar to the NH sites and NY and PA show mixed ancestry with the major influence from the NJ/NY group.

Dispersal and Relatedness Tests

Full siblings were detected among the ME Arroyo Coyote and Tanager sites (individuals 237, 245 and 493) and Camel Hut and Baxter sites (individuals 433, 444, 214). Euclidean distances between these sites are 50.6 km and 30.5 km, respectively. Detection of related individuals is sample size dependent, so these results should be interpreted similarly to presence/absence data, where absence of detection does not indicate a lack of connectivity. Other clusters tested for full sibling groups did not detect full siblings

across sites (Potomac, NH/ME, and western MA). Full siblings detected within sites are listed in Appendix E, Table E.2.

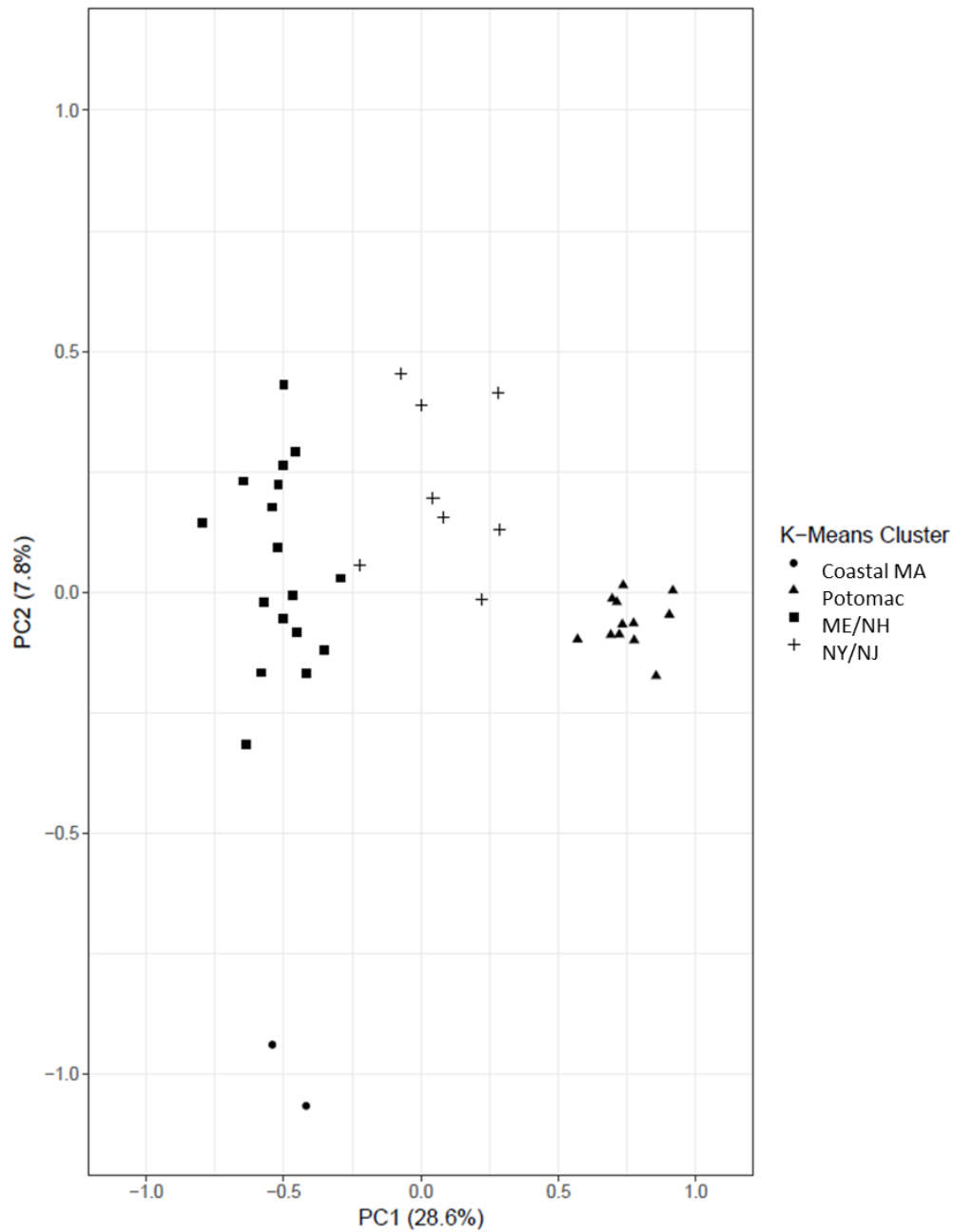


Figure 3.5. Principal components analysis showing average allele frequency by site. Symbols represent the major clusters identified in the K-Means analysis. The values on the axes represent the percent variation explained by PCA 1 and PCA 2.

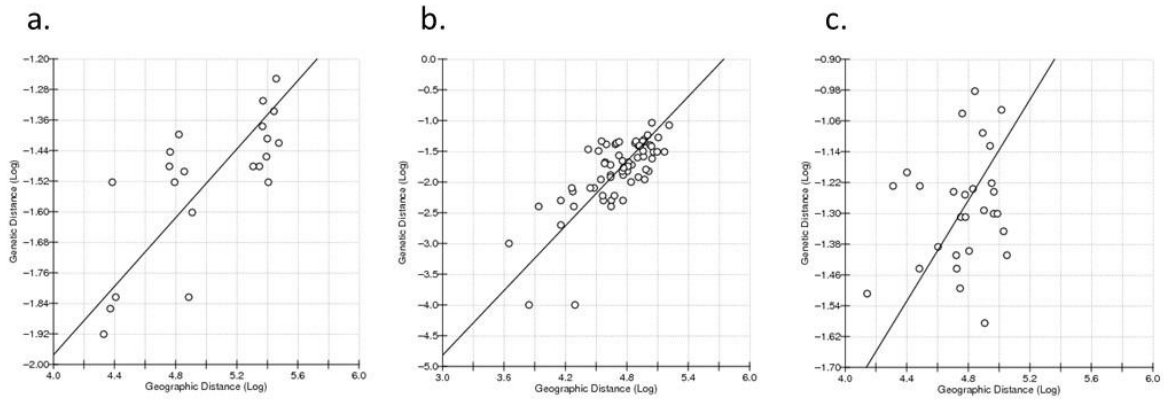


Figure 3.6. Isolation by distance tests for Euclidean geographic distance and pairwise F_{st} among clusters identified by the STRUCTURE analysis (shown in Fig. 3.4b): a. NH/ME (red and green); b. Potomac (yellow); and c. northern Maine (red). The NH/ME and Potomac groups were significant, the northern Maine cluster was not significant (see Table 5).

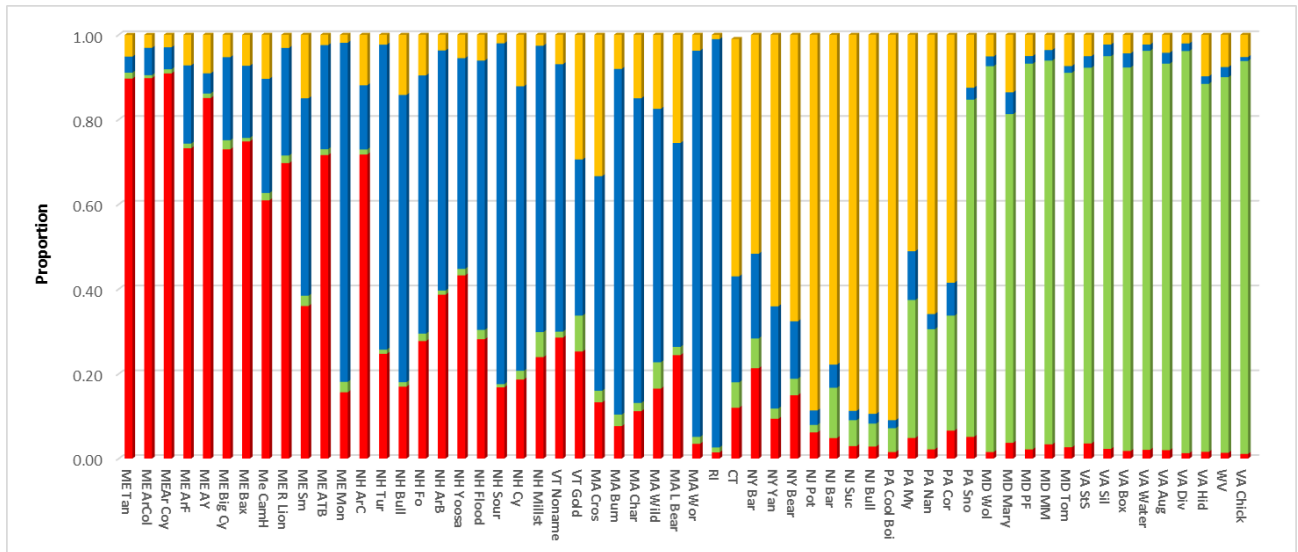


Figure 3.7. The average ancestry value (Q -value) for each major group identified in the Bayesian clustering analysis. Sites are ordered from north (left) to south (right) on the x-axis. This figure is color coded to match Figure 5. Each bar in this figure represents one site.

Individual Genetic Assignments

All sites with $n \geq 7$ were used in the genetic assignment tests. There was a weak relationship between sample size and the proportion of correct assignments (Fig. 3.8); however, genetic distinctness was also related to the proportion of correct assignments. In other words, the more genetic uniqueness from others (such as coastal MA) the greater the success in genetic assignment. Genetic assignment to the correct site was fairly low (51.9%). However, when allowing the individual to assign to any site where pairwise allele frequencies were not significantly different, assignment success increased and ranged from 12 to 100% by site (average = 73%; Fig. 3.9). Overall, genetic assignment varied geographically. Assignment success was generally high in the Maine and Potomac sites and low in the New York and Pennsylvania sites (Fig. 3.10). Therefore, genetic assignment will work better for some locations than others, and in most cases it is unlikely that the exact site of origin can be identified using these markers. However, in many cases, the correct major population group can be identified for more than 70% of the samples. Generally, the sites with higher admixture (such as PA and NY) had lower assignment success.

The unknown samples provided by the U.S. Fish and Wildlife Service from the southern part of the study area mostly assign to a Potomac site (56%). The remainder of the samples assigned to various locations in Maine, Pennsylvania and New Hampshire. These individuals were presumably from a site in the study area. However, these results could arise from samples from the Potomac cluster as some individuals show admixture from these other clusters (Fig. 3.4). One confiscated sample submitted from Massachusetts assigned to the Potomac cluster.

The unknown samples provided from captive populations in New Jersey may or may not originate from samples included in the study area. The NJA samples assigned to all states included in the analysis with the most samples assigning to the Potomac basin sites (33%), and the western MA sites (24%). Other individuals assigned to sites in New York, New Jersey, New Hampshire, Maine and Pennsylvania. One sample did not assign strongly to any sampled site indicating that the site of origin may not be included in the reference sites tested. The NJB samples similarly assigned to many different sites with 33% not assigning strongly to any particular sampled site, followed by 28% assigning to the Potomac cluster. Other sites included in this sample were New Hampshire, Pennsylvania, New Jersey and Maine.

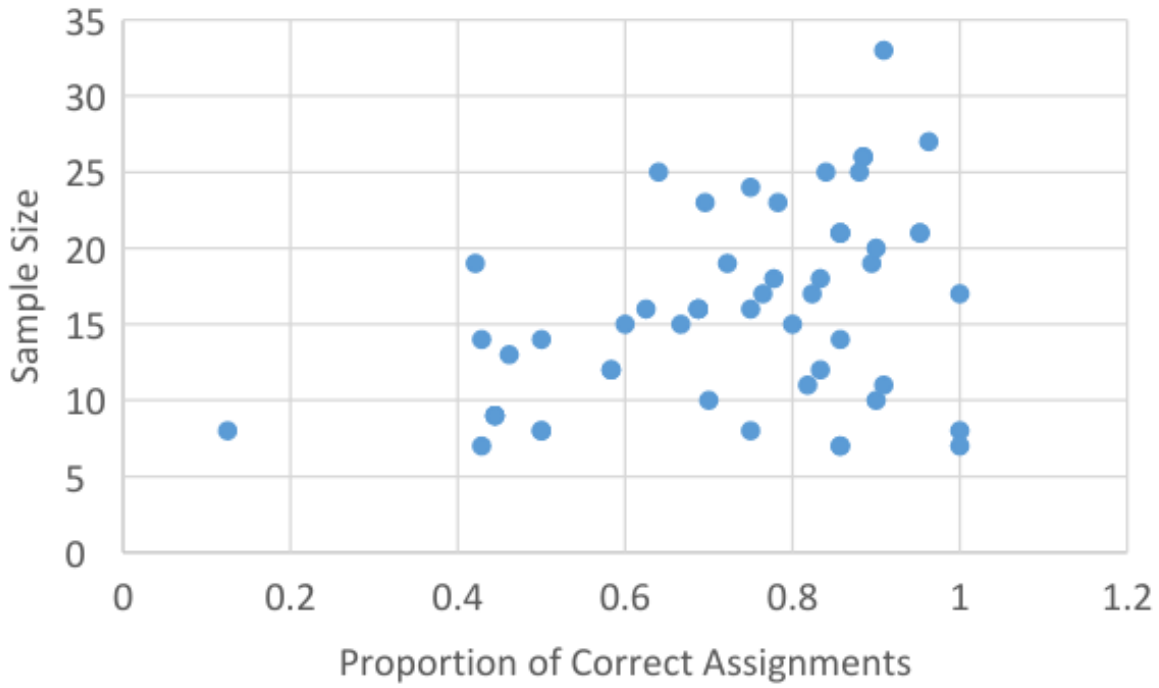


Figure 3.8. Sample size and proportion of correct assignments for sites included in the genetic assignment test.

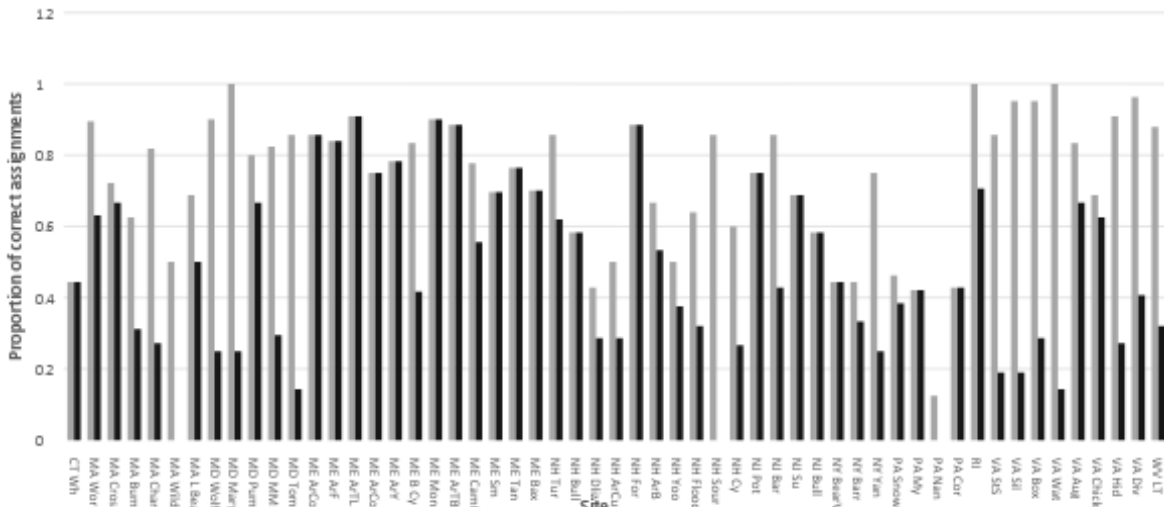


Figure 3.9. Proportion of correct genetic assignment by site. Black bars indicate the proportion of correct assignments to the site where the sample was collected. Gray bars indicate correct assignment to the sample site and any site where no significant allele frequency differences were detected.

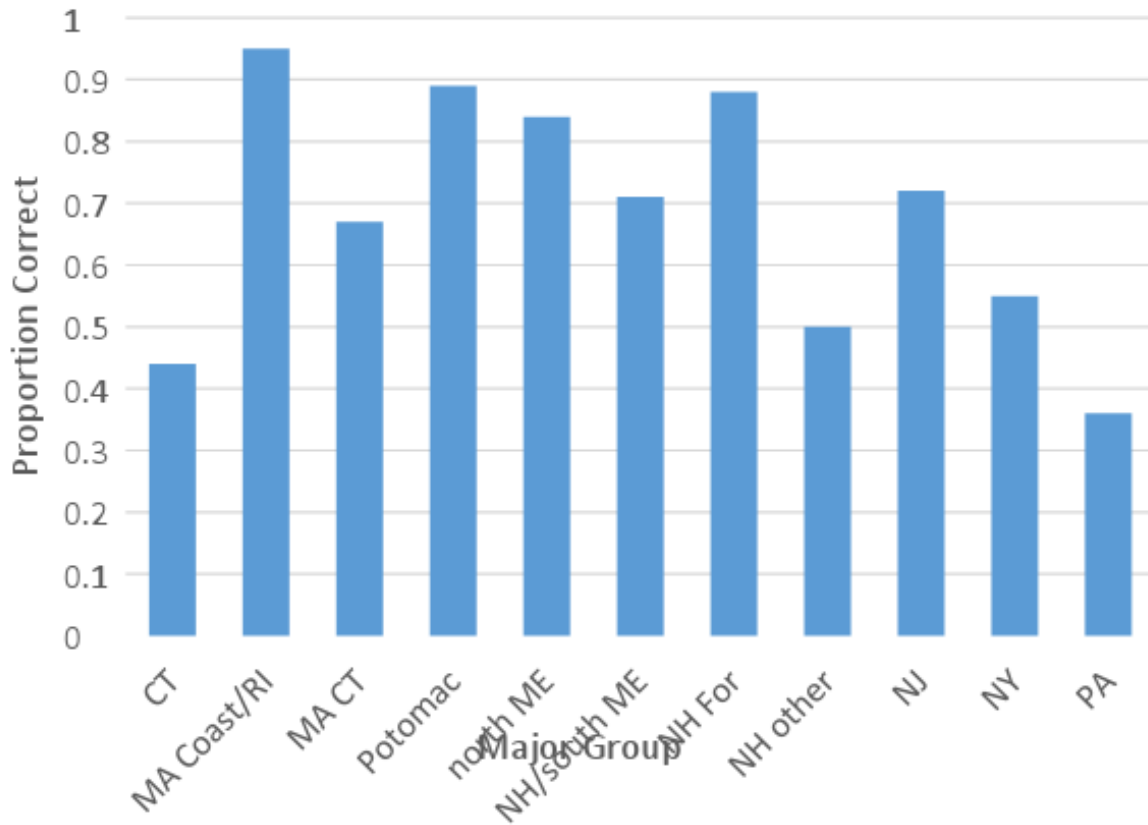


Figure 3.10. Proportion of correct genetic assignments by major group (or cluster).

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Discussion

The objectives of this population genetics study center on conservation planning and particularly the potential identification of Evolutionary Significant Units that could support regional conservation planning initiatives (e.g., the Northeast Wood Turtle Conservation Area Network, Part IV). We identify major population groups throughout the northeastern United States, evaluate genetic diversity throughout the region, assess potential migration of turtles among the sampled sites, and quantify the accuracy of population assignment for the potential application of releasing turtles confiscated from illegal trade. Results from these analyses should be interpreted cautiously as Wood Turtle biology may not indicate current patterns and processes in the landscape. Specifically, given the longevity and generation time (~50 years) of this species, contemporary genetic signals may reflect conditions that

existed approximately 100 years ago. Landscapes have changed substantially in the study area during this period, and processes such as fragmentation may not be detected for more than seven generations depending on the dispersal ability and analytical methods (Blair et al. 2012) and abundances.

The population genetic differences and admixture detected in this analysis may reflect historic genetic-demographic signals, current population interactions, or a combination of historic and current effects. For example, large population genetic differences can arise from a founder effect, population bottleneck, or genetic drift (which is accelerated with smaller population sizes and isolation). Post-glacial colonization will also influence the genetic structure and admixture can result from colonization patterns or inter-population migration patterns. Demographic studies can assist in determining the level of population interaction and dispersal abilities of a species; however, when migration rates are low, but genetically influential—such as one individual per generation—identifying this signal from demographic studies can be challenging (Lowe and Allendorf 2010). Additionally, demographic studies often cannot identify the difference between dispersal (movement) and successful migration or mating in a new population (genetic exchange). Due to the longevity of the Wood Turtle, current population genetic data can reflect conditions as long as 100 years ago. Although this can make determination of current levels of connectivity challenging, it can describe gene flow and movement patterns in less developed and impacted landscapes before modern development and fragmentation, and can be used as a guide for conservation planning.

Genetic Diversity

Genetic diversity measured across the northeastern United States was similar to other studies reported for Wood Turtles in the literature. Heterozygosity and allelic richness did not indicate loss of genetic diversity in the samples. The age-based test also did not indicate any genetic diversity differences across generations, but power to detect this trend is limited. Based on these tests, there is no indication of the detrimental effects of fragmentation or inbreeding in these samples; however, these results should be interpreted cautiously and with consideration of current demographic information as the longevity of the species and other behavioral attributes could potentially mask the genetic effects until the population has reached very low population sizes.

Population Differentiation

Evolutionarily Significant Units (ESU) and Management Units are used in the conservation and management of threatened species. ESUs are populations or groups of populations that merit separate management or priority for conservation because of high genetic or ecological distinctiveness. Management units are generally smaller than ESUs and define demographic units for monitoring and

management (Allendorf and Luikart 2007). Importantly, management units are demographically independent populations or meta-populations. Stepping stone models make the designation of management units ambiguous because admixture between the more divergent groups can impede the identification of clear boundaries (Palsboll et al. 2007). In this model, sites between major population groups are a combination, or rather, gradation of the groups. A trade-off exists between management units that are too large and do not provide adequate protection to the species and associated critical habitats, and those that are too small and may provide over-protection and undue costs of management or associated economic impacts. Genetic data are useful to quantify genetic distinctness among major population groups and subsequent management units, but should be considered a guide in the identification of distinct groups that are demographically independent.

Our results revealed a hierarchical genetic structure, with larger cohesive assemblages that exhibit stronger genetic differentiation. Within these cohesive assemblages, genetic differentiation was weaker, suggesting that there is more gene flow and possible metapopulation structure. The pattern in the clustering data generally indicated that the most genetically unique clusters in the study area were northern ME, coastal MA, Potomac, and NJ/NY. Areas of admixture were located between these major groups, such as the Merrimack, Connecticut and Kennebec basins and areas in PA and NY. Although the genetic data indicate four major clusters, we have recommended five major population groups to guide management planning. It might be warranted to consider these larger assemblages ESUs. Definition of ESUs for a species generally draws from genetic, life history, ecological, geological, and socioeconomic sources of information (Allendorf et al. 2013). We provide one of these sources of information here. Genetic differentiation among the major assemblages of populations is on the scale observed with ESUs of other species, such as Pacific Salmonids (NMFS 2018, WDFW 2018).

These major groups (ESUs) could be further divided into management units based on demographic independence. Most (91%) of the sites were significantly genetically differentiated from each other, indicating that the Wood Turtle is finely genetic structured across the study area. Whether subsets of populations within the major clusters should be considered management units depends on determining the degree of demographic independence among the populations under consideration. Demographic independence will rely on the maximum dispersal ability of the Wood Turtle. Other studies of Wood Turtle did not detect significant genetic differences among sites <50 km unless there was a barrier to movement such as a large water body (Tessier et al. 2005; Castellano et al. 2009; Spradling et al. 2010; Fridgen et al. 2013; Willoughby et al. 2013). Therefore, sites less than 50 km apart with functional pathways for connectivity are probably not demographically independent. The pairwise F_{ST} and allele frequency tests indicated that the Wood Turtle is maintaining gene flow across drainage boundaries,

emphasizing the importance of considering terrestrial connectivity within conservation efforts for the species.

The cluster pattern we observed indicates that, although grouping by major basin will capture much of the genetic diversity, there is some indication that gene flow or colonization has occurred across the headwaters of adjacent basins, such as the Potomac and the Allegheny, and the Delaware and Susquehanna. Therefore, an island stepping stone model describes the patterns of genetic structure and connectivity among clusters may be important for maintaining genetic diversity and exchange.

Migration and Gene Flow

Significant isolation by distance was detected in all the population groups tested. Isolation by distance in freshwater turtles has been detected in other studies, but appears to be spatially scale dependent, with a lack of evidence at smaller geographic distances (Castellano et al. 2009; Howeth et al. 2008), but clear evidence at larger distances (Howeth et al. 2008; Shoemaker and Gibbs 2013). Sethuraman et al. (2014) found a positive, but non-significant correlation for isolation by distance in the Blanding's Turtle from sites located across Iowa, southern Minnesota and northern Illinois. Isolation by distance indicates a stepping stone model where neighboring subpopulations have a higher probability of sharing migrants. In the case of a freshwater turtle, the stepping stone model would be a two dimensional network of sites with the neighboring sites surrounding a site sharing individuals (Kimura and Weiss 1964). With this movement model, the sites most distant will show greater genetic divergence. Collectively, these studies indicate that population or group boundaries are fairly large (~100 km) for freshwater turtles. As populations decline, it will be increasingly more important to maintain connectivity among adjacent sites, and ideally this connectivity would be maintained across the entire study area to support the movement of turtles from one site or population to the next.

Euclidean distance provided a stronger correlation with F_{ST} than stream distances for the Potomac and the NH/ME groups, but provided a weaker correlation for the northern Maine group. These findings indicate that overland corridors are more likely connecting sites than pathways along the stream corridor—particularly for the Potomac sites. It is also possible that the turtles are utilizing both types of corridors and perhaps for different purposes. For example, turtles may make local movements along the stream corridor while making less frequent and longer distance migrations overland. The genetic data suggest that overland movements happen across basins as well as within basins and most likely in an overland pathway that is closer by Euclidean distance than travel restricted within the stream corridor.

Little is known about longer dispersal distances for Wood Turtles. Only a few observations of longer range movements exist for Wood Turtles, and these movements were observed overland and along stream corridors. Individual turtles moving among sites are documented based on individual identification or notch codes, and movements up to 50 km are known to occur (T. Akre, personal communication). An individual male turtle equipped with a GPS tag moved at least 16 km overland and over basin divides in 6 months (T. Akre, personal communication). Turtle migrations may be necessary to reach critical habitats for feeding and reproduction, and could also be made by individuals emigrating from sites. Longer distance movements may be infrequent or sporadic based on alterations in habitat or high water events. Jones and Sievert (2009) documented Wood Turtles in Massachusetts displaced up to 16.8 km after flood events. Turtles tracked by Jones and Sievert (2009) confirm that Wood Turtles can move between populations overland or in the stream corridor. Additionally, it appears that males may be more likely to disperse longer distances than females (Jones and Sievert 2009). Long-term studies are needed to accumulate observations to understand these movements and connectivity among sites.

Relatedness tests of full sibling groups may provide some indication of dispersal abilities. Based on this test, dispersal distances for Wood Turtles were a maximum of 50 km. Although, we cannot rule out human transport as a possible mode of movement, this 50 km distance is similar to the scale of genetic relatedness reported by several previous studies (Tessier et al. 2005; Castellano et al. 2009; Spradling et al. 2010; Fridgen et al. 2013; Willoughby et al. 2013). Increasing sample sizes would improve the conclusions from these analyses, particularly when considering maximum migration distances. Certainly, more information about the dispersal of the species, including the landscape attributes and habitats where the turtles travel would provide valuable information about corridors for managing connectivity between sites.

Genetic Assignment

Genetic assignment was only moderately successful for Wood Turtles in the study area and the level of success varied across sites. Certain sites in the study area have high site-level success rates where as other sites only can identify individuals to a major population group. Some sites had low genetic assignment success, particularly those with admixture from neighboring populations. Our study found that only 52% of individual turtles assigned correctly to the sample site. This low success rate can be due to closely located sites (<40 km) and a lack of genetic distinctness. A study of a freshwater turtle from South America found similar genetic assignment results where 59% of individuals correctly assigned to their sample site (Escalona et al. 2009). Tessier et al. (2005) found assignment success ranged from 84 to 98% when assigning individuals to population groups; however, this study was limited in geographic scope

and examined populations divided by the St. Lawrence River, which showed high genetic divergence between the north and south shore.

Based on our results, genetic assignment using the microsatellite markers we used would have limited application for enforcement in the illegal animal trade, and results may not be reliable if desiring to identify the exact site of origin for unknown samples. Newer population genomic methods that use large numbers of single nucleotide polymorphisms (SNPs) should be investigated for the potential for finer-scale differentiation among sites or smaller groups of sites due to the potential to obtain and efficiently genotype high numbers of loci (>100 – 1000's). SNP data generated by this method are also more easily compared across different laboratories and may provide finer genetic differentiation than microsatellites (see Malenfort et al. 2015). Alternate methods, such as permanent tagging methods like passive integrated transponder tags, may provide more certainty in the identifications and also allow more detailed demographic data to accumulate over the life span of the turtles, while also providing site of origin for enforcement and repatriation.

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Recommendations and Data Gaps

Major Population Groups and Genetic Assignment

This study identified significant isolation by distance and a stepping stone pattern of admixture. The study identified four major population groups or clusters: northern ME, Potomac, coastal MA/RI, and NJ/NY. The Connecticut, Merrimack and Kennebec basins showed admixture between the coastal MA and the northern ME group and could be managed as an additional group based on similar genetic attributes. The sites included from PA showed admixture among the NJ, coastal MA and Potomac groups and we recommend should be managed according to the genetic admixture reflected in the data. For example, the Susquehanna basin should be managed with the NJ/NY group where it predominantly clusters, whereas the site in the Potomac basin in PA should be managed with the other Potomac sites.

Updating to genomic sequencing methods can provide many loci that improves the resolution for analysis of population differentiation. Additionally, these techniques have numerous applications in evolution and ecology that can assist conservation planning (see Andrews et al. 2016).

Migration and Connectivity

The site-based genetic differentiation combined with the estimate of contemporary migration rates and relatedness indicates that Wood Turtles are capable of migrating 50 km and perhaps greater distances. Therefore, sites less than 50 km apart should be managed to maintain connectivity to support adjacent populations. More information on maximum dispersal distances and habitat attributes associated with the movement corridors is greatly needed to identify the preferred migration habitats and target them for habitat restoration and conservation. Acquiring these data and associated GIS based analyses should be a high priority to inform conservation planning efforts.

Landscape and Conservation Planning

Landscape and conservation planning should strive to maintain long-term genetic diversity and stable, or increasing, population growth. Therefore, the genetic data and population designations need to be considered in terms of demographic data (abundances, age class diversity, reproduction, sex ratios, and dispersal). All of these factors will directly influence genetic diversity and the resilience of individual populations. Data analyses in this report considered collectively with other genetic studies in Wood Turtles indicate that migration distances are more than 50 km. Additionally, considering the stepping-stone model of migration, connectivity among sites less than 100 km apart should be a high priority. Connectivity among sites across basins and also across the major population groups should be maintained in any planning and restoration efforts. This will allow populations to exchange individuals in source-sink dynamics, reduce the risk of extinction, and promote the conservation of genetic diversity.

Genetic Assignment

The success of the genetic assignments indicate that the population (site) where an individual was sampled could be correctly identified for 52% of the individuals in the sample, only slightly higher than random chance. When considering assignment to major population groups within which we detected no significant allele frequency differences, correct assignment ranged from 12 to 100%. High assignment success (>75% correct) could be identified for several population sub-groups: coastal MA, northern ME, Potomac and NH Fortification. These groups are genetically distinct from other groups. The success of assignment to the exact site where an individual was captured was relatively low, but identifying the subpopulation or cluster from where an individual originated may be possible with these markers depending on where the individual originated. Assignment success was low (less than 50% correct) for CT Wheeler, NH (except NH Fortification) and PA sites, which limits the application of these markers in the enforcement of the illegal harvest of Wood Turtles across the broad geographic area.

A transition to next generation genomics could also improve population genetic assignments. SNPs have a lower error rate than microsatellites and the data are comparable across labs without requiring a standardization process needed with microsatellites. Therefore, the application of genomics and identification of SNP panels for Wood Turtles could improve the genetic assignment success for forensic applications. If this route is pursued, expanding the reference collections to the entire range of the Wood Turtle would increase the assignment success and consider all potential sources for release of confiscated turtles.

Tissue Sampling Strategies for Future Genetic Collections and Monitoring

Tissue sampling for turtles is challenging and should consider the intrusion and stress to the turtle, genotyping success of the tissue type, experience of the collector and logistic difficulty in collecting the samples. Specifically, tissues that require the least handling with the highest success rates are desired when the study requires high numbers of samples with rapid processing in the lab (i.e. > 500 samples and < 12 months). Minimizing the different tissue types within a large study allows more streamlining in the lab for faster processing. Tail tips and toes were the most successful tissue type, followed by blood. Shell samples were reasonably successful and seemed to have higher consistency across samplers than toenails. In other words, shell samples seemed to require less information or experience by the collector whereas toenails had considerable variation across collectors. Specifically, some collectors provided multiple nails for small turtles which increased the successful extraction, some collectors cut nails deeper than others or the nails at certain sites were larger and provided more soft tissue. Overall, there are multiple tissue types that genotype successfully, and the selection of the tissue type for any future studies should consider these various factors. If a study desires high success rates in the lab and uses experienced collectors, then tail tips or blood would be preferred. However, if the study can tolerate some failed samples in the lab and/or uses inexperienced collectors then shell or toenail might be preferred. Sampling should be coordinated among collectors and the lab, and designed to best fit the questions and goals of the study.

Part IV. A Conservation Area Network for the Wood Turtle in the Northeastern United States

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Summary

In this section, we (1) provide an overview of fundamental concepts and theory underpinning many contemporary conservation planning efforts; (2) highlight several existing single-species Conservation Area Networks; and (3) describe the Conservation Area Network (CAN) that we have developed for Wood Turtles (*Glyptemys insculpta*) in the northeastern United States.

The Northeast Wood Turtle CAN is the core concept underpinning this Conservation Plan. Sites included in the CAN represent Wood Turtle subpopulations that have been determined, through an empirically driven process, to be regional priorities for conservation actions, particularly land protection. Sites were scored and ranked using an expert-weighted composite metric reflecting an array of multi-scale factors including observed abundance, demographic structure, and genetic traits as well as measures of habitat quality and landscape integrity. Sites selection was stratified across ecoregion, watershed, and state boundaries to ensure adequate representation of ecological, geographic, and political settings. CAN sites fall under two major tiers: high priority Focal Core Areas and lower priority Management Opportunity Sites. Focal Core Areas primarily represent highly sensitive, robust Wood Turtle subpopulations that, when considered together, are critical to the long-term persistence and evolutionary potential of the species in the northeastern United States. Thus, Focal Core Areas are intended to be the primary focus of conservation efforts and especially land protection resources. Management Opportunity Sites represent

several classes of Wood Turtle streams but include some areas that, with concentrated management and mitigation efforts, have a high likelihood of supporting a functioning Wood Turtle subpopulation. Some Management Opportunity Sites are ideal targets for agricultural mitigation programs (e.g., Natural Resources Conservation Service Working Lands for Wildlife), federal engagement (i.e., located within National Wildlife Refuges), and/or international collaboration.

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Key Concepts in Conservation Planning

Over the last several decades, a wealth of research has accumulated around the challenges associated with conservation planning. While the field continues to rapidly advance, there are several fundamental concepts that have emerged and remained relevant and stable, and ultimately become core components of most contemporary conservation plans. In this section, we provide a brief overview of the concepts and theory that have influenced the conception and development of the Northeast Wood Turtle Conservation Area Network.

Margules and Pressey (2000) argued that there are two major goals of conservation planning: representation and persistence. In this context, representation refers to the need for all essential components of what is targeted for conservation—whether it be a single species or biodiversity in general—to be fully accounted for within a conservation or management plan. This concept is relatively intuitive, however, the challenge surrounding representation is the need to attain spatial economy, i.e., the achievement of conservation goals with minimal spatial cost (Sarkar et al. 2006). **Persistence** reflects the understanding that organisms and populations as well as the systems they exist within, are enormously complex and refers to the need for conservation efforts to do more than simply preserve areas with the highest diversity or abundance—ecological principles and evolutionary processes such as metapopulation dynamics, source-sink population structures, dispersal, broad-scale connectivity, climate change, and genetic lineages must be acknowledged and planned for as well (Margules and Pressey 2000).

Shaffer and Stein (2000) established the conservation principles termed the “three Rs”: representation, resiliency, and redundancy. **Representation**, in this context (and in contrast to Margules and Pressey 2000), refers to the importance of geographic representation for protecting the variations in ecological roles, behavioral traits, and genetic distinctiveness and diversity that occur across a species’ range. **Resiliency** regards the presence of population-specific attributes that allow for long-term persistence despite stochastic disturbance, dynamism in the availability of habitat patches, and projected environmental change. **Redundancy** refers to the need for replicated representation of populations within

each ecological setting that a species occurs, in order to reduce extinction risk and preserve the evolutionarily potential of the lineage. Shaffer and Stein (2000) stated that conservation efforts that follow the three Rs should ultimately contain several large populations that are resistant to disturbances and distributed across the full array of ecological settings within a species' range. These basic guidelines have gained traction in recent years (Redford et al. 2011) and have even been argued for incorporation into the Endangered Species Act (Wolf et al. 2015).

Redford et al. (2011) attempted to build upon of the three Rs, suggesting six attributes that should be present in plans that successfully conserve species. Those attributes include:

Demographic and ecological self-sustainability.—Populations should not only possess the demographic parameters needed for persistence (e.g., population viability analyses), but also be capable of sustaining ecological roles (e.g., species interactions) for the sake of the species and the broader ecosystem. Redford et al. (2011) stress that ecologically functional populations are often considerably larger than demographically functional populations.

Genetic robustness.—Defined as “the genetic capacity to survive and respond to environmental changes within populations, among population, and across the range.” Small populations that result from the fragmentation of a larger population, typically experience diminished genetic variation, which threatens the ability of the population as a whole to adapt to environmental changes.

Population health.—The health and physical condition of the individuals that make up a population can play a large role in determining its overall vulnerability.

Representative populations.—Species exist within a gradient of ecological contexts. Representation of populations throughout the range of current and historical ecological settings plays a vital role in effective conservation efforts by protecting local adaptations and therefore, the evolutionary potential of the species as a whole.

Replicate populations.—A reiteration of the previously outlined concept, replication provides a safeguard for protection against factors that could cause local population extinctions such as disease and catastrophic natural or anthropogenic disturbances.

Range-wide resilience.—The ability to sustain healthy, functioning, robust populations in the face of environmental change and disturbances such as climate change.

Decision-making represents one of the major challenges with regard to conservation planning and design. Sarkar et al. (2006) emphasized three concepts important for the decision-making process of conservation design: complementarity, irreplaceability, and vulnerability. Although, there is considerable overlap with concepts previously highlighted, these three can be subtly distinguished by their slightly more site-specific focus and application to the site comparison and selection. The first, **complementarity** (Kirkpatrick 1983; Justus and Sarkar 2002), centers on the idea that sites within a network of conservation areas should maximize their differences with regard to factors that vary throughout a population, such as genetics and ecological settings. The second concept, **irreplaceability**, refers to the reality that hierarchy often exists with respect to the site-specific conservation value. Certain sites, because of their uniqueness—biologically, ecologically, genetically, or otherwise—are irreplaceable. The quantification of irreplaceability has been the focus of considerable research (Rebelo and Siegfried 1992; Pressey et al. 1994; Csuti et al. 1997; Ferrier et al. 2000; Leslie et al. 2003; Tsuji and Tsubaki 2004), but remains difficult to realistically implement in many scenarios. The third concept is site **vulnerability**. Because the persistence of a target species (or some measure of biodiversity) is typically the primary goal of any conservation plan, conservationists should avoid focusing their sole effort upon sites that are especially vulnerable to extirpation or loss, such as those that have low abundance estimates, are unlikely to remain suitable or sustain necessary dynamic processes, or may be adversely affected in the future by development or climate change (i.e., have low predicted viability or resilience). We used these core concepts, in combination with a review of relevant conservation planning case studies, outlined below, to develop of a Conservation Area Network for Wood Turtles in the northeastern United States.

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Examination of Relevant Conservation Area Network Case Studies

The general concept of the Conservation Area Network (CAN)—a collection of protected areas that function to protect natural resources from anthropogenic pressures—has been integral to conservation biology and conservation planning for several decades (Sarkar et al. 2006). Initial attempts to design CANs were driven by the theory of Island Biogeography (MacArthur and Wilson 1967; Diamond et al. 1976)—or relatively straightforward species-area relationships—but fell short of expectations because they ignored the range of additional factors that can influence wildlife populations outside of a true island-based context (Sarkar et al. 2006). Steady acknowledgment of the immensely complex array of factors influencing the effectiveness of the conservation planning process—such as landscape structure, landscape ecology, socioeconomics, and population dynamics—has shifted the field of CAN design toward computerized approaches that often rely heavily on statistical modeling (Ochoa-Ochoa et al. 2016)

and automated software (Ciarleglio et al. 2009; Ciarleglio et al. 2010; Lehtomaki and Moilanen 2013). In recent years, there have also been numerous efforts to establish systematic CAN design frameworks that can be applied broadly across different planning scenarios (Faith 1995; Shafer 1999; Margules and Pressey 2000; Groves et al. 2002; Cowling and Pressey 2003; Sarkar 2004a; Gagne et al. 2015). Notably, however, much of the published literature focuses on the design of CANs that aim to maximize biodiversity (or other similar goals) rather than ensure the persistence of a single threatened species. This distinction is important because while broad biodiversity-focused conservation efforts can afford to utilize generalized spatial modeling approaches to identify likely biodiversity hotspots, single-species CANs for imperiled species often require a population-level degree of specificity. A detailed understanding of population demographics, dynamics, connectivity, and genetics become critical when developing a conservation plan for a single imperiled species and this presents many challenges during the conservation planning process.

There are numerous CANs that have been developed for individual species, but many remain unpublished or are available only as gray literature. Here, we provide brief overviews of four large-scale, single-species conservation plans that have recently been developed for different taxa.

Blanding's Turtle (*Emydoidea blandingii*)

In 2014, the Northeast Blanding's Turtle Working Group finalized a conservation area network to sustain functioning and viable populations of Blanding's Turtles in the northeastern United States (Willey and Jones 2014). Sites representing relatively closed populations were first delineated using a standardized methodology and subsequently ranked using a combination of two methods: an expert-based metric and an empirically-derived metric. The expert-based metric was calculated using a poll in which Blanding's Turtle experts were asked to rank various metrics relative to one another. These metrics fell under six broad categories: (1) site size; (2) site fragmentation; (3) habitat abundance and quality; (4) landscape context; (5) population size; and (6) conservation measures already underway. Metrics were calculated for each site, weighted according to the expert-based rank, and combined to produce a single, final expert-based metric score. Empirically-derived metric scores were created by extrapolating results from models based on standardized sampling efforts.

Once sites were scored relative to one another, they were assigned into one of four tiers (high priority sites, mid-tier sites, supporting landscape, or non-priority) using a series of assessment criteria. This systematic placement process used the site ranking metrics described above, but also allowed for sites to be elevated in status by taking into account sites that were: (1) exceptionally large or well protected; (2)

strategic corridors between priority sites; (3) within an underrepresented watershed or ecoregion; (4) genetically distinct; (5) exceptionally genetically diverse; (6) exceptional with respect to population density.

New England Cottontail (*Sylvilagus transitionalis*)

A conservation strategy was developed in 2012 (and subsequently ratified) in an effort to protect and promote the New England Cottontail (NEC; Fuller and Tur 2012). Overarching goals, which incorporated the “three Rs” (Shaffer et al. 2012) were to ensure (1) representation of populations across the species’ historic range; (2) overall resiliency by increasing population sizes enough to buffer environmental and genetic uncertainties; and (3) redundancy of populations to bolster resistance to catastrophes (Fuller and Tur 2012). A key distinguishing component of the NEC conservation plan was the extension of conservation efforts beyond the estimated NEC range and an emphasis on *creating* additional NEC habitat. This was deemed to be reasonable in light of the evidence of historic range contraction, unfragmented habitat within the historic range, and the biology of the NEC.

First, a combination of both habitat suitability models and carrying capacity estimates was used to identify landscapes that possess the potential to support persistent NEC populations. Next, general areas where conservation efforts would be focused were delineated. Because the large majority of the southern New England landscape is privately owned and most parcels are relatively small in size (<9 acres), considerable care was given to identifying areas where large privately owned parcels or secured lands (e.g., state forests, wildlife management areas, National Wildlife Refuges) corresponded with optimal habitat model results. Parcels were ranked according to size, distance to the nearest recent NEC occurrence, habitat capability score, habitat capability index score, maximum and mean predicted suitability, and distance to nearest conservation land. Parcels in the 94th percentile were considered “high-value” and were the subsequent focus of site-specific assessments to gauge landowner willingness to manage land and feasibility of habitat management.

Site-specific population goals were informed by computer simulations and carrying-capacity extrapolations, but final management goals were established using local judgments that considered the feasibility of management at each site, the possibility of competition by eastern cottontails, and local habitat assessments.

Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*)

With the objective of reaching a specified population size within a 10-year period, the Lesser Prairie-Chicken Range-wide Conservation Plan (2013) identified conservation areas and goals that would achieve representation, resiliency, and redundancy as well as connectivity throughout each of four ecoregions within the Lesser Prairie-Chicken (LPC) range (Van Pelt et al. 2013). This plan's central method for achieving conservation goals was the identification and establishment of focal areas that were most important for the species.

General guidelines for focal areas were first developed according to expert opinion, which reflected the best available science on the species. Focal area requirements included a goal for overall average size of areas, a minimum percentage of focal areas that needed to be high quality habitat, and a maximum distance that focal areas could be from one another in order to facilitate connectivity. Focal areas were selected based on (1) existing population sizes; (2) habitat quality; (3) fragmentation; (4) presence of preferred ecological sites; (5) presence of public or other conservation lands; (6) the degree of preexisting demands for land use, and (7) receptivity of the landowners to incentive programs. Connectivity zones between focal areas were also established based on expert opinion and specific criteria including a minimum percentage of high quality habitat, a maximum distance between focal areas, a minimum width, and rules regarding barriers to movement.

One noteworthy aspect of this plan was the establishment of "strongholds" within core areas that were considerably smaller, but served as more permanent conservation areas that have the capability of sustaining a population of LPC. This was considered particularly important for conserving this species because 95% of its range fell within private lands at the time of this plan's development. These strongholds served as a way to focus limited resources on the most critical areas as well as identify areas where development will have the most impact on the species.

Mojave Desert Tortoise (*Gopherus agassizii*)

A recovery strategy was proposed for the Mojave Desert Tortoise in 2011 (U.S. Fish and Wildlife Service 2011) with the primary goal of the recovery and delisting of the species. This conservation plan does not focus on the creation of a CAN, but its overarching goals and proposed methods provide some insight and guidance for large-scale single-species conservation efforts for a long-lived turtle species. Overall objectives included (1) achieve self-sustaining populations in all recovery areas; (2) achieve geographic representation of self-sustaining populations throughout recovery areas; and (3) protect and maintain habitat that would support the long-term persistence of the species. Individual recovery actions included:

(1) develop, support, and build partnerships to facilitate recovery; (2) protect existing populations and habitat; (3) augment depleted populations through a strategic program; (4) monitor progress toward recovery; (5) conduct applied research and modeling in support of recovery efforts within a strategic framework; and (6) implement an adaptive management program.

Bog Turtle (*Glyptemys muhlenbergii*) - Northern Population

A Recovery Plan for the northern population of the Bog Turtle (*Glyptemys muhlenbergii*) was developed by Michael Klemens (USWFS 2001) under contract with the U.S. Fish and Wildlife Service, Pennsylvania Field Office. Five recovery units were identified: Prairie Peninsula / Lake Plain; Outer Coastal Plain; Hudson/Housatonic; Susquehanna/Potomac; and Delaware. The plan deemed that 185 stable or increasing populations defined recovery. Recovery would be met when long-range protection was secured for 185 populations distributed across the five recovery units, and monitoring at five-year intervals over a twenty-five year period indicated that these populations were not declining. Further, collection and trade must no longer threaten the survival of the species, and habitat dynamics must be sufficiently understood to manage successional threats to the species.

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A Conservation Area Network for the Wood Turtle in the northeastern U.S.

Overview

Expanding upon the relevant and key concepts from the theoretical and applied frameworks described above, we utilized Wood Turtle occurrence and empirical population data, Species Distribution Models, habitat suitability models, landscape characteristics, and genetic information to design a Conservation Area Network (CAN) for Wood Turtles in the northeastern United States (Maine to Virginia). This design process primarily followed an automated and repeatable, quantitative selection process that was informed by Wood Turtle experts and was tailored to reflect the unique natural history and ecology of the species as well as accommodate the challenges and idiosyncrasies associated with the available, region-wide occurrence and landscape data. Automation of the ranking and stratified selection process was important in providing a high degree of objectivity, while expert opinion ensured the incorporation of human judgment and personal experience in the final site selection process (Sarkar et al. 2006).

We utilized spatial, political, ecological, and hydrographic stratification as the primary means of guaranteeing adequate geographic, ecological, political, and genetic **representation**. Minimum site

requirements within stratification levels as well as a multi-tiered framework or site classifications ensured further **redundancy**. Prioritization metrics were utilized in an attempt to account for vulnerability of sites to both anticipated change in climate as well as threats associated with future land development. This allowed for the identification of high-risk sites of high conservation value while otherwise maximizing the **resiliency** of sites within in the Conservation Area Network.

Here, we detail our methodology for standardized site delineation and site ranking for the Wood Turtle in the northeastern United States and describe the framework and site selection process of the Northeast Wood Turtle CAN. We also outline the steps of further fine-scale delineation of CAN sites.

Conceptual Scales and Framework

The Northeast Wood Turtle CAN reflects two fundamental scales: (1) the site-scale (as defined by the mapping procedure and site delineation process described below) and (2) the basin-scale, meant to identify connected landscapes at the 8-digit Hydrologic Unit Code (HUC8; Fig. 4.1). Sites are the primary unit of the CAN and represent the units of prioritization as well as the basic scale at which implementation of the associated Conservation Action Plan (CAP, Part V) will ultimately manifest. Based on the best available information, sites reflect areas along suitable rivers where demes or subpopulations of Wood Turtles will regularly interact and mate in a given year. Analyses by Jones and Willey (2015) provided evidence that broader landscape scales of several kilometers from the stream are more predictive of Wood Turtle abundance, strongly suggesting that adequate conservation at a broader, watershed scale is important to the persistence of local populations. For this reason, the Northeast Wood Turtle CAN also focuses on the identification of the HUC8 watersheds (“Connectivity Landscapes”) that possess features that will promote the persistence of Wood Turtles at timescales of evolutionary consequence with minimal management (i.e., multiple generations). This “Basin” scale reflects aggregations of potentially, but not necessarily regularly, interacting demes or subpopulations. The HUC8 watershed was chosen because it most closely corresponds to the scale at which subpopulations or demes appear to be related genetically (~100 km, see Part III; Tessier et al. 2005; Castellano et al. 2009; Spradling et al. 2010; Fridgen et al. 2013; Willoughby et al. 2013).

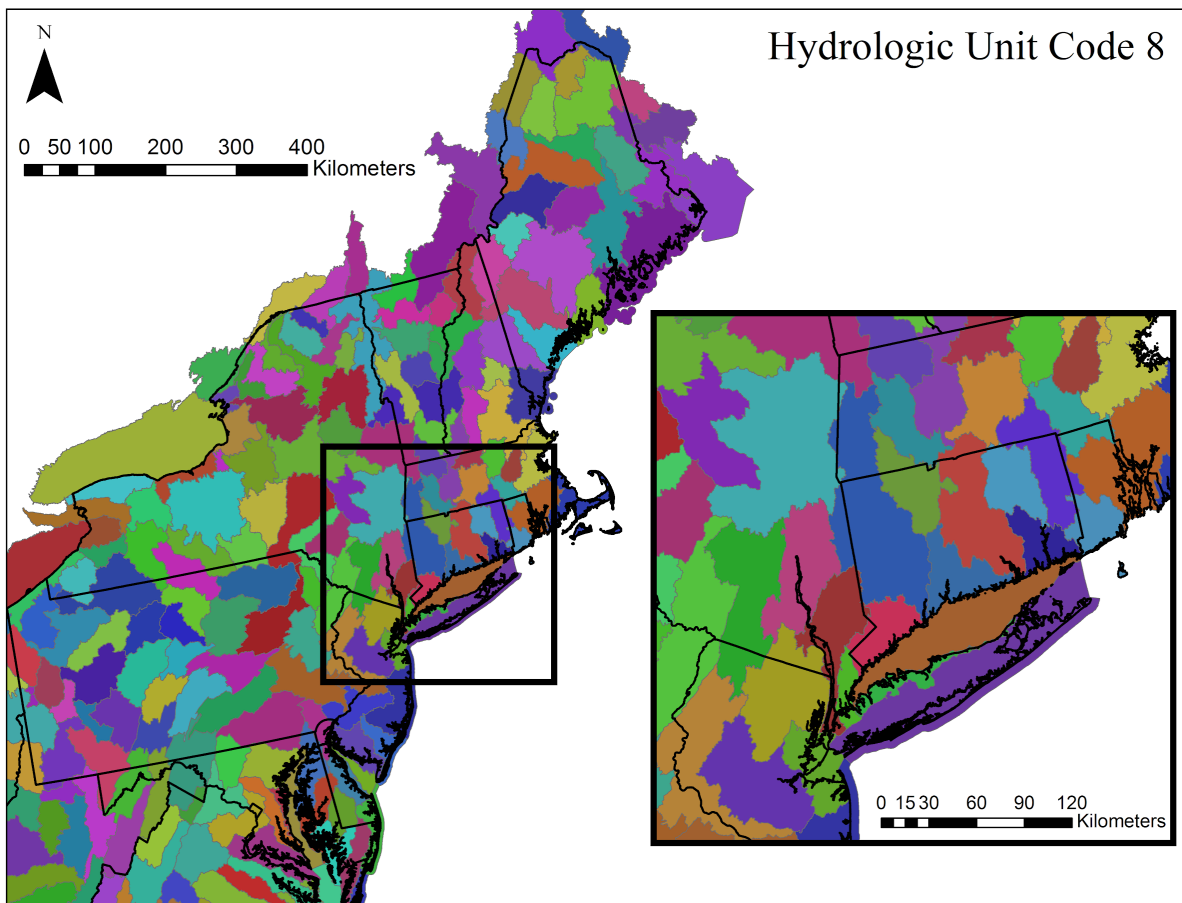


Figure 4.1. 8-digit Hydrologic Unit Code (HUC8) in the northeastern United States. Colors distinguish HUC8 watersheds.

Site Delineation

In this subsection we describe the identification and delineation process to produce the units (i.e., sites) that would eventually make up the Wood Turtle CAN. We established standardized and biologically meaningful mapping criteria by consulting NatureServe (2016) criteria, reviewing the existing Wood Turtle movement literature (Compton et al. 1999; Saumure 2004; Jones 2009), and examining state mapping strategies (Appendix X).

Compilation and Treatment of Occurrence Data

We compiled Wood Turtle occurrence information from three general sources: (1) an updated corroborated occurrences database that was originally developed as a part of the 2015 Status Assessment and constitutes a wide array of data sources from state and federal agencies, literature, and experts (Jones and Willey 2015); (2) updated state-level occurrence data, including Element Occurrence source features;

and (3) standardized surveys that detected Wood Turtles as a part of this regional effort (Status Assessment and Competitive State Wildlife Grant [CSWG], see Part II). Following the treatment of data that were used in the development of distribution models for the regional Status Assessment (Jones and Willey 2015), we only considered occurrences reported or updated from 30 years prior to 2011 (i.e., 1981) to present.

Defining Subpopulations for the Purposes of Prioritization

Sites delineated in the Wood Turtle CAN are intended to reflect relatively closed subpopulations in accordance with the definition of Element Occurrences provided by NatureServe (2016). While animals likely interact across the boundaries of the delineated sites within an average lifetime, and in some cases with regularity, the sites are meant to reflect discrete areas where most individuals within the local population are expected to reside within any given year.

NatureServe Element Occurrence definition:

“An Element Occurrence (EO) is an area of land and/or water in which a species or natural community is, or was, present. An EO should have practical conservation value for the Element as evidenced by potential continued (or historical) presence and/or regular recurrence at a given location. For species Elements, the EO often corresponds with the local population, but when appropriate may be a portion of a population (e.g., long distance dispersers) or a group of nearby populations (e.g., metapopulation).”

NatureServe refers to two key concepts when mapping EOs: barriers to movement and separation distances. Barriers to movement are features on the landscape that completely (or almost completely) restrict dispersal or movement of the focal species, thus having a limiting effect on gene flow. Separation distances are described by NatureServe as “distances of intervening area that restrict movement” and represent habitat-specific reaches beyond which gene flow is significantly diminished. Because patterns of gene flow are unknown for most species, NatureServe recommends that separation distances be based on the best available information. Below are NatureServe’s recommended barriers to movement and habitat-specific separation distances for Wood Turtles.

Barriers to movement:

- Busy highways or roads with obstructive structures
- Impassable topography
- Urbanized areas without aquatic/wetland habitat
- Large impoundments or lakes

Separation distances:

Continuous upland habitat: 1 km

Along riverine corridors: 5 km

Intermediate situations (e.g. mixed upland-riverine habitat): 3 km

We employed a mapping strategy that centered on stream locations because, while all Wood Turtles use both stream and terrestrial habitat, streams are required for overwintering and entirely determine species distribution throughout the landscape within the species range.

Site Delineation

Distinguishing sites.—We considered all occurrences that were connected by ≤ 5 km of stream (the NatureServe riverine corridor separation distance, also used by the Massachusetts Division of Fisheries and Wildlife to define Wood Turtle Species Habitat) to be of the same subpopulation (i.e., the same site). We viewed all spatial data in Google Earth. We used NHD Flowlines to assist the visualization of stream networks and measurement of stream distances between occurrences. If occurrences were not directly located within stream channels, we measured distances based on their approximated snapping point to streams. For state agencies that represented occurrences using circular polygons, we treated the centroid of the polygon as the occurrence point. We drew polygons around each cluster of occurrences that met the separation distance, and assigned a unique identification code to each polygon.

Assigning identification codes.—We converted polygons denoting subpopulations, as well as all of the supporting occurrence data, to shapefiles in ArcGIS. We converted state agency occurrence polygons and Wood Turtle-occupied survey segment polylines into points. We replaced circular polygons with a point at the centroid of the polygons. We represented complex polygons (non-circular) by placing points where NHD flowlines intersected the polygon boundary. We represented survey segments by converting all polyline vertices into points. We then used the Spatial Join Analysis Tool in ArcGIS to assign the unique identifier of each population polygon to each newly defined observation point within the subpopulation polygon.

Mapping sites for CAN ranking.—While Wood Turtles require certain stream habitat characteristics and environmental conditions (see Part I and Appendix VII), they are also highly mobile and, as a result, are often discovered by humans far from ideal stream habitats. Therefore, assigning Wood Turtles to the closest stream could sometimes lead to inaccurate representation of Wood Turtle stream habitats. For this reason, we employed a mapping strategy that deliberately distinguished between suitable stream habitats

using Species Distribution Models (SDM) developed as part of the Status Assessment (Jones and Willey 2015).

To begin the mapping process, we first snapped all points to the nearest NHD flowline. We then buffered these points by 1735 m, which was the euclidean distance between 20 randomly selected 2.5-km meandering stream segments throughout the Northeast. We used this buffer in order to approximate the 5 km distance used to separate populations. We selected stream habitat within this buffer that was identified as suitable by at least two of three species distribution models (SDM) built as a part of the 2015 Status Assessment (Jones and Willey 2015). We removed all suitable stream segments that were not directly connected to occurrence points by continuous stream habitat (suitable or non-suitable) within the buffer. This entailed removing streams within adjacent watersheds as well as segments of stream that originated within the buffer, but flowed out of the buffer boundary and then back in. We removed stream segments < 1/3 of a km in length. We included all occupied standardized stream survey segments (the exact segment only) that were not considered suitable Wood Turtle habitat by the SDM. We assigned unique identifiers to all flowline segments using the Spatial Join Analysis Tool in ArcGIS. In some cases, this created duplicate stream segments when a single segment crossed the boundary of more than one buffer polygon. We removed these duplicate segments individually by hand. Occasionally, adjacent sites shared small amounts of stream habitat because the respective 1735-m buffers overlapped while still capturing >5 km of stream between occurrences—these sites were kept separate.

Because the stream-based SDM was intended to capture suitable stream habitat, and not optimal habitat, it tended to overpredict suitable Wood Turtle habitat within certain areas, such as New Jersey. As a result, most sites within New Jersey contained more stream habitat relative to comparable sites in adjacent states, which could potentially introduce bias into the ranking process. This discrepancy in the SDM appeared to reflect a lack of emphasis on stream gradient within the state-based SDM for New Jersey. Therefore, we applied a modified mapping strategy to sites in New Jersey that removed high gradient streams. To do this, we used Northeast Aquatic Habitat Classification System (NEAHCS) spatial stream gradient flowlines (“Simplified Gradient Classes - 5 Classes” datalayer; rcngrants.org/content/northeastern-aquatic-habitat-classification-project) and removed the highest gradient stream classification (>5%). We then clipped the resulting flowlines using the original 300-m site buffer. We included both original and “reduced” sites in the CAN ranking process to determine the effect of this change on the ranking process.

We initially mapped candidate CAN sites on a state-by-state basis, which meant that there was the potential for single, trans-state populations to be considered as two separate populations within each state

and lead to a lower overall ranking of the population as a whole. To address this, we examined all sites in close proximity of the all state borders and combined sites from different states if any of their respective occurrences were within 5 km.

Streams selected through this mapping procedure—and the surrounding terrestrial habitat—served as our representation of functionally distinct populations that would be ranked based on their relative conservation value for the species.

Existing Strategies for Prioritizing Conservation Areas

To inform our methodology for ranking Wood Turtle sites (described below), we reviewed several existing site-ranking strategies including NatureServe ranking guidelines, existing state agency guidelines in the Northeast, and a recently developed process used for the Northeast Blanding's Turtle Conservation Plan (Willey and Jones 2014). We provide an abbreviated overview of these ranking strategies here:

NatureServe ranking criteria.—NatureServe (2008) provides a generic approach to assign the following ranks to Element Occurrences. These are provided below:

A: Excellent viability.—Occurrence exhibits optimal or at least exceptionally favorable characteristics with respect to population size and/or quality and quantity of occupied habitat; and, if current conditions prevail, the occurrence is very likely to persist for the foreseeable future (i.e., at least 20-30 years) in its current condition or better.

B: Good viability.—Occurrence exhibits favorable characteristics with respect to population size and/or quality and quantity of occupied habitat; and if current conditions prevail, the occurrence is likely to persist for the foreseeable future (i.e., at least 20–30 years) in its current condition or better.

C: Fair viability.—Occurrence characteristics (size, condition, and landscape context) are non-optimal such that occurrence persistence is uncertain under current conditions, or the occurrence does not meet A or B criteria but may persist for the foreseeable future with appropriate protection or management, or the occurrence is likely to persist but not necessarily maintain current or historical levels of population size or genetic variability.

D: Poor viability.—If current conditions prevail, occurrence has a high risk of extirpation (because of small population size or area of occupancy, deteriorated habitat, poor conditions for reproduction, ongoing inappropriate management that is unlikely to change, or other factors).

E: Verified extant.—Occurrence recently has been verified as still existing, but sufficient information on the factors used to estimate viability of the occurrence has not yet been obtained.

H: Historical.—Recent field information verifying the continued existence of the occurrence is lacking.

F: Failed to find.—Occurrence has not been found despite a search by an experienced observer at a time and under conditions appropriate for the Element at a location where it was previously reported, but the occurrence still might be confirmed to exist at that location with additional field survey efforts.

X: Extirpated.—Adequate surveys by one or more experienced observers at times and under conditions appropriate for the species at the occurrence location, or other persuasive evidence, indicate that the species no longer exists there or that the habitat or environment of the occurrence has been destroyed to such an extent that it can no longer support the species.

State agency EO ranking strategies in the Northeast.—Of the eight CSWG-participating states, only Massachusetts uses a ranking strategy that expands upon the generic NatureServe guidelines. Massachusetts' ranking approach provides numeric criteria that reflect the species' habitat needs. The Massachusetts guidelines are as follows:

A-Rank.—

Size: Large amount of suitable habitat (200+ ha; 500+ acres)

Landscape Condition:

Habitat available beyond mapped polygon and/or corridors to other wood turtle polygons

Development is <25% of surrounding landscape at 1:50,000 scale

Largely unfragmented habitat

No or few roads adjacent to the polygon

Population Condition: 10+ individuals observed

B-Rank.—

Size: Medium amount of suitable habitat (101-200 ha; 250-499 acres)

Landscape Condition:

Habitat is available beyond mapped polygon with corridors to other stream systems

Development is 25-50% of surrounding landscape at 1:50,000 scale

Largely unfragmented habitat

No or few roads adjacent to the polygon

Population Condition: Observation of 2-9 individuals and/or multi-year observations and/or evidence of breeding/recruitment or one gravid female

C-rank.—Minimum rank for a site that can support a viable population.

Size: Small amount of suitable habitat (50-100 ha; 125-249 acres)

Landscape Condition:

Habitat is available beyond mapped polygon, but can only be accessed by crossing roads

Development is 50-75% of surrounding landscape at 1:50,000 scale

Largely unfragmented habitat

Has roads running within 100 m of it for at least 1/3 of the polygon

Population Condition: Single observation of non-gravid turtle

D-rank.—Sites cannot support viable populations.

Size: Minimal amount of suitable habitat (<50 ha; <124 acres)

Landscape Condition:

No suitable habitat adjacent to polygon or isolated due to roads

Development is 75-100% of surrounding landscape at 1:50,000 scale

Habitat is fragmented by roads and/or development

Polygon has road running within 100 m of it for 1/3 or more of the polygon or road length to stream length ratio is greater than 1

Population Condition: Single observation of a turtle or shell

Blanding's Turtle site ranking procedure.—In Conservation Plan for the Blanding's Turtle and Associated Species of Conservation Need in the Northeastern United States (Willey and Jones 2014), northeastern partners expanded upon NatureServe guidelines and existing ranking frameworks for Blanding's Turtle by ranking sites according to important characteristics affecting the species such as site size, landscape context, and population information. They expanded the approach by using expert opinion to weight each characteristic according to their perceived relative importance. This expert-based metric was combined with an objective, empirically-derived metric to produce an overall score that was used to compare sites.

Site Prioritization and Characterization

Of the basic approaches outlined above, we chose to employ a ranking strategy that was based on the Blanding's Turtle methodology (Willey and Jones 2014). Following Willey and Jones (2014), we assigned prioritization scores to Wood Turtle sites using a composite metric based on empirical data and

expert opinion that reflected the site-, landscape-, and population-level factors contributing to the relative conservation value of a given site. Our definition of “conservation value” refers to the conditions necessary to sustain demographically functional and ecologically viable Wood Turtle populations, within the context of the overarching goal of this Conservation Plan—to protect the evolutionary potential of the Wood Turtle across ecological, watershed, and political units.

Site Attribution

We attributed sites with site-specific information that collectively reflected the overall conservation value of each site for supporting Wood Turtles based on expert opinion. This information was categorized into eight broad metric classes: (1) site size; (2) site fragmentation, (3) habitat abundance and quality; (4) landscape integrity; (5) site-level population information, (6) landscape-level population information; (7) vulnerability to development; (8) climate change vulnerability. Each class contained 1–7 individual submetrics (detailed below). We calculated each submetric at one of three scales, specified below: the 300-m, 5500-m, or Hydrologic Unit Code 12 (HUC12; Fig. 4.2) scale. We used 300 meters to represent the “site” boundaries because this distance has been shown to encompass the 95th percentile of daily Wood Turtle locations relative to their stream origin (see reviews in Jones and Willey 2015). We used 5500 meters to characterize the surrounding landscape because the Status Assessment (Jones and Willey 2015) identified this scale as an effective predictor of population status. Based on expert opinion, we chose HUC12 as a functionally relevant (with respect to the natural history and ecology of the species) scale by which to characterize the landscape at a multi-population scale. Metric classes and respective submetrics (Fig. 4.3) are detailed below (respective scales are provided in parentheses). We used Classes VII and VIII to assess site vulnerability, but did not include these classes in metric ranks. We attributed CAN sites with these variables using the same methodology used to attribute survey segments in Part II.

Class I. Site Size (300 m)

- 1.) Total area of the site.

Class II. Site Fragmentation (300 m)

- 1.) Percent undeveloped land.
- 2.) Impervious surface cover.—Average impervious surface score.
- 3.) Road density.—Total length of all roads divided by the total area of the site. We used all roads provided in the U.S. Census Bureau TIGER/Line® online database (2017).

- 4.) Traffic rate.—Estimate of relative average annual daily traffic rate (data source: NALCC DSL).

Class III. Habitat Abundance and Quality (300 m)

- 1.) Habitat Suitability Model.— Predicted relative Wood Turtle abundance from the hierarchical N-mixture model developed from 2012–2017 survey data.
- 2.) NALCC Designing Sustainable Landscapes (DSL) Wood Turtle Landscape Capability model.—The results of this model reflect both the modeled habitat capability and climate niche of the species (McGarigal et al. 2016). Factors contributing to the habitat capability component include habitat classification, stream size, stream gradient, extent of riparian forest on the landscape, and development. Factors contributing to the estimation of climate niche included growing degree days, precipitation, growing season precipitation, annual temperature, and maximum summer temperature.

Class IV. Landscape Integrity (5500 m and HUC12)

- 1.) Percent undeveloped land (5500 m).
- 2.) Road density (5500 m).
- 3.) Impervious surface cover (5500 m).—Average impervious surface score.
- 4.) Percent forest cover (5500 m).
- 5.) Traffic rate (5500 m).—Estimate of relative average annual daily traffic rate (data source: NALCC DSL).
- 6.) Percent agricultural cover (5500 m).
- 7.) Amount of suitable habitat (HUC12).—Total length of suitable stream habitat according to Species Distribution Model.

Class V. Site-level Population Information

- 1.) Relative abundance.—Estimated using N-mixture models based on 1-km survey data.
- 2.) Average 1-km population estimate.—The average relative population size estimate per segment (of three methods: two closed population estimates using different methods and an open

population estimate). If there were more than one survey segments within a site, the average estimate was calculated.

3.) Maximum 1-km population estimate.—The maximum average relative population estimate for a single segment within each site.

4.) Average number of turtles detected by observer 1 per survey.

5.) Maximum number of turtles detected by observer 1 in a single survey within the site.

6.) Age structure.—Percent of Wood Turtles caught that were subadults (<14 years old). Only sites for which >6 turtles were captured (this number reflects the average number of turtles needed to capture a juvenile).

7.) Total occupied habitat.—Total area of occupied habitat within a site when a 300-m buffer is applied to each occurrence.

Class VI. Landscape-level Population Information (HUC12)

1.) Total occupied stream.—Total area of occupied habitat within the respective HUC12 when a 300-m buffer is applied to each occurrence.

2.) Density.—Calculated as the total area of known occupied habitat within the encompassing HUC12 when a 300-m buffer is applied to each occurrence divided by the total length of suitable stream habitat within the HUC12. This metric underestimates the actual occupied stream area and is intended to be a relative proxy for distributional density.

Class VII. Vulnerability to Development (300 m, 5500 m, HUC12)

1.) Conservation protections in place (300 m).—Percent of the site that is protected from development (data source: USGS Protected Areas Database).

2.) Probability of development (5500 m).—Average probability of development by 2080 (data source: NALCC DSL).

3.) Probability of development (HUC12).—Average probability of development by 2080 (data source: NALCC DSL).

Class VIII. Climate Change Vulnerability

Description: projected to change of site-level climate variables.

- 1.) Heat index.— Estimated change by 2080 (datasource: NALCC DSL).
- 2.) January temperature.— Estimated change by 2080 (datasource: NALCC DSL).
- 3.) Precipitation.— Estimated change by 2080 (datasource: NALCC DSL).
- 4.) Growing-degree-days.— Estimated change by 2080 (datasource: NALCC DSL).

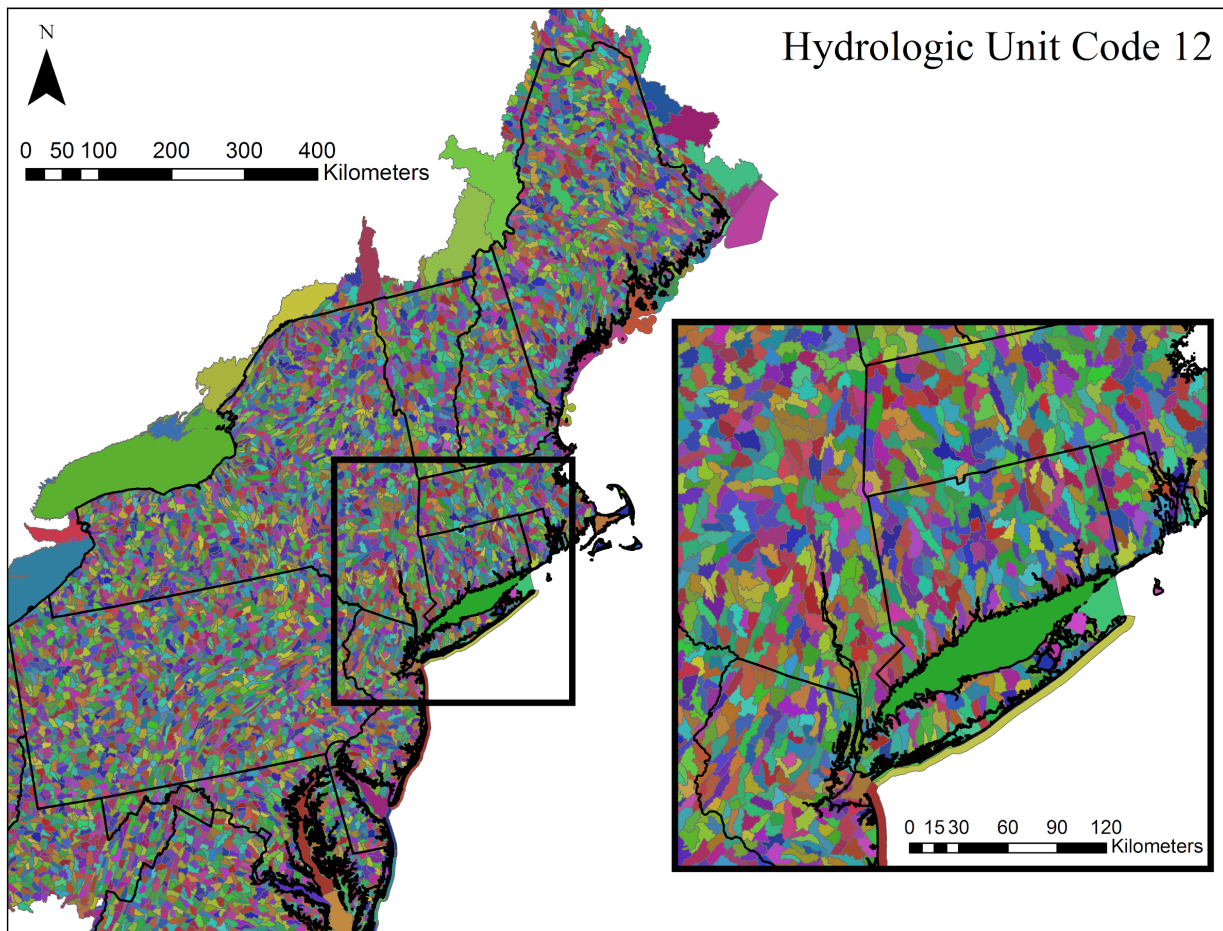


Figure 4.2. Map of the 12-digit Hydrologic Unit Code (HUC12) watersheds in the northeastern United States.

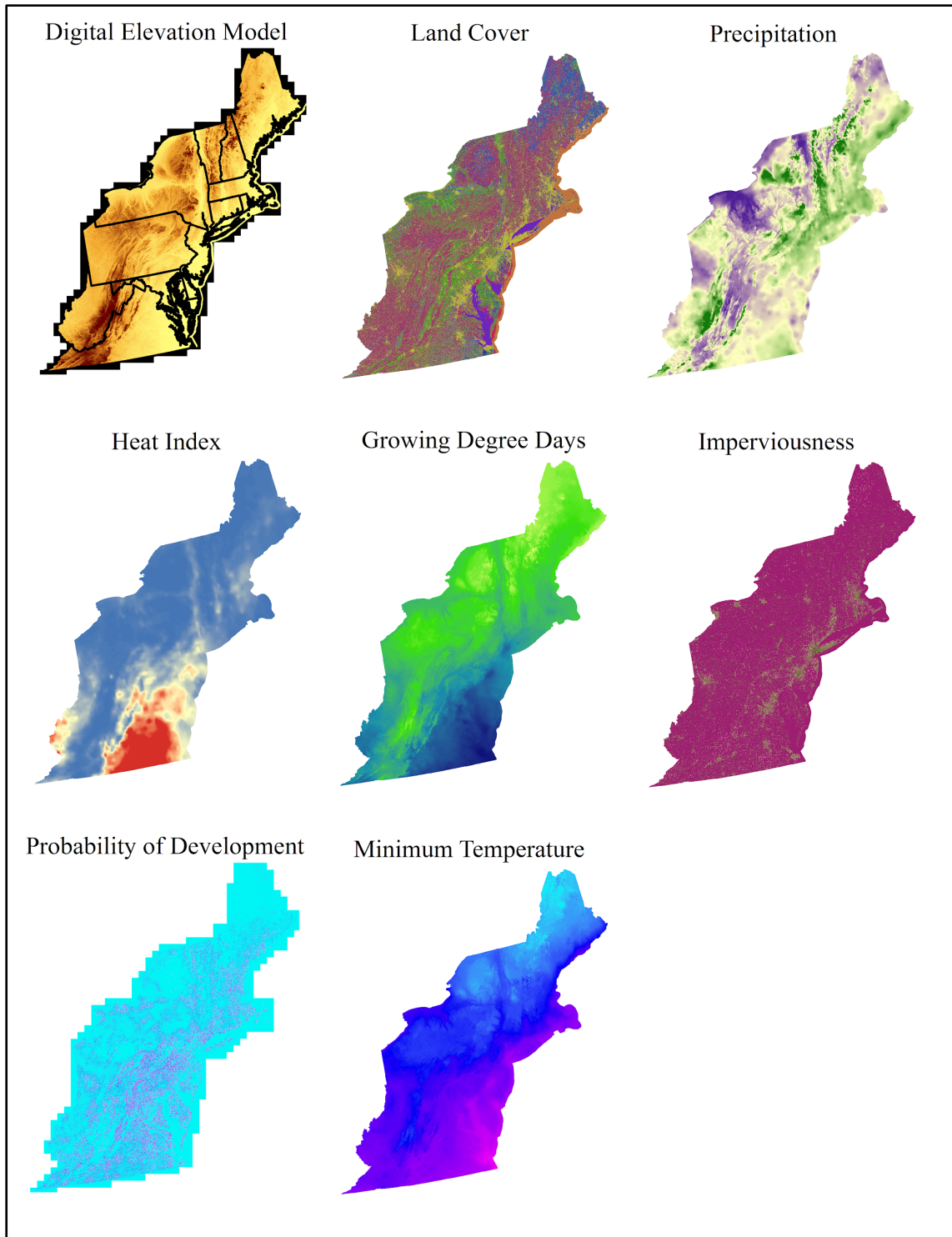


Figure 4.3. A subset of the regionwide spatial data provided by the North Atlantic Landscape Conservation Cooperative Designing Sustainable Landscapes project that was used to attribute Wood Turtle sites.

Expert Opinion Survey

We used the results of an expert opinion survey to score the relative importance of each class and submetric described above. This information was used to determine the contribution of each submetric to each class and each class to the overall ranking metric.

We distributed an electronic expert opinion survey in July of 2017 to state agency biologists throughout the Northeast as well as key CSWG partners and biologists in order to gauge the relative importance of site-ranking criteria. We asked recipients to distribute the survey to other Wood Turtle experts in the Northeast Region. In total, the survey was completed by 20 Wood Turtle experts with experience in all 12 northeastern states where Wood Turtles are known to occur. Respondents averaged 12.6 years of experience working with Wood Turtles.

The expert opinion survey consisted of three major components. First, we asked respondents to score the relative importance of each overarching classes of variables that would be used to assess sites. Categories were given a score of 1–5, where 1 = “not very important” and 5 = “very important.” Next, respondents applied the same scoring criteria to sets of submetrics within each of the respective classes. We asked participants to score each submetric with respect to the other submetrics within same class.

We calculated weights by dividing the average score for each class or submetric by the sum of all average values within the same category (Table 4.1). Class I contained only one submetric, therefore its submetric was not scored. We gave six new submetrics, which were incorporated after the expert survey was distributed, a weight equal to that of the most similar submetric included in the survey or derived based on the editors’ expert opinion.

Table 4.1. Classes and submetrics that contributed to the overall rank of each Wood Turtle site within the Conservation Area Network. The number of votes for each of the five (1–5) possible importance values, the average importance value, and weight (average score divided by the sum) of the class or submetric are provided. Scales are provided in parentheses. Submetrics with NA values were included after the expert survey was distributed. Their weights were assigned according to the most similar variable that was included in the survey or expert opinion. Only classes (and respective submetrics) that contributed to the ranking metric are included.

Classes							
	1	2	3	4	5	Average	Weight
Class I. Site Size (300 m)	0	1	0	9	10	4.4	17.1
Class II. Site Fragmentation (300 m)	0	0	0	8	12	4.6	17.9
Class III. Habitat Abundance and Quality	0	0	7	5	8	4.05	15.7
Class IV. Landscape Integrity (5.5 km or HUC12)	0	0	4	7	9	4.25	16.5
Class V. Site-level Population Information (300 m)	0	0	0	8	12	4.6	17.9
Class VI. Landscape-level Population Information (HUC12)	0	0	7	9	4	3.85	15.0
Submetrics							
Site Fragmentation (Class II)							
	1	2	3	4	5	Average	Weight
1. Percent undeveloped land (300 m)	0	0	2	7	11	4.45	26.6
2. Impervious surface cover (300 m)	0	2	3	9	6	3.95	23.6
3. Road density (300 m)	0	0	2	5	13	4.55	27.2
4. Traffic rate (300 m)	0	1	8	5	6	3.8	22.7
Habitat Abundance and Quality (Class III)							
	1	2	3	4	5	Average	Weight
1. Habitat suitability model	NA	NA	NA	NA	NA		0.5
2. Landcape capability model	NA	NA	NA	NA	NA		0.5
Landscape Integrity (Class IV)							
	1	2	3	4	5	Average	Weight
1. Percent undeveloped land (5.5 km)	0	0	5	7	8	4.15	15.2
2. Road density (5.5 km)	0	0	2	12	6	4.2	15.4
3. Impervious surface cover (5.5 km)	0	1	7	8	4	3.75	13.7
4. Percent forest cover (5.5 km)	0	0	6	8	6	4	14.6
5. Traffic rate (5.5 km)	0	3	8	5	4	3.5	12.8
6. Percent agricultural land (5.5 km)	0	1	7	7	5	3.8	13.9
7. Length of suitable habitat (HUC12)	0	0	4	13	3	3.95	14.4
Site-level Population Information (Class V)							
	1	2	3	4	5	Average	Weight
1. Relative abundance	0	0	4	9	7	4.15	32.7
2. Average 1-km population size estimate	0	0	2	6	12	4.5	35.4
3. Maximum 1-km population size estimate	NA	NA	NA	NA	NA		35.4
4. Average number of turtles per obs. 1 per survey	NA	NA	NA	NA	NA		32.7
5. Max number of turtles caught by obs. 1 for single survey	NA	NA	NA	NA	NA		32.7
6. Age structure	0	0	6	7	7	4.05	31.9
7. Occupied habitat	NA	NA	NA	NA	NA		32.7
Landscape-level Population Information (Class VI)							
	1	2	3	4	5	Average	Weight
1. Total occupied stream (HUC12)	0	0	4	12	4	4	53.0
2. Density (HUC12)	1	1	7	8	3	3.55	47.0

Site-Ranking Metric Derivation

While we included vulnerability to development (Class VII) and climate change vulnerability (Class VIII) and their respective submetrics in the expert opinion survey, we did not use these factors to create the overall ranking metric. We excluded these variables because there was considerable disagreement among experts as to whether vulnerable sites should be prioritized for conservation or down-ranked.

We derived the overall site ranking metric using the following process:

1. All submetrics were scaled 0–1, with 0 representing the smallest value and 1 representing the largest
2. Metrics that are considered to negatively impact Wood Turtles were subtracted from 1, such that large values for all submetrics indicated beneficial conditions for Wood Turtles with respect to that variable
3. Each submetric was multiplied by its respective weight
4. Submetrics were summed to produce an overall score for each class
5. Classes were rescaled 0–1
6. Each class was multiplied by its respective weight
7. Class values were summed to produce the overall metric

Sensitivity Assessment

We assessed the overall influence of each class and submetric by examining the relative change in rank of each site when an entire class or individual submetric was removed. To quantify the influence of each class and submetric, we calculated the correlation coefficient for the original rank and new rank without the class or submetric. We calculated correlation coefficients for the top 50 sites, top 100 sites, and all sites.

To provide an individual measure of the relative importance of each variable for each site, we calculated percentile with respect to rank change when each variable was removed. We differentiated between sites that positively or negatively impacted each variable.

Prioritization of Agricultural Mitigation Opportunities

We defined ideal areas for agricultural mitigation as large sites for which the single act of removing of the negative impacts of agricultural practices would greatly improve the likelihood of the existing Wood

Turtle population persisting into the future. Therefore, we scored sites based on their capacity for agricultural mitigation by multiplying site size, overall site rank, road density, and percent agricultural cover values by each other. These scores served as ranks of relative opportunity for agricultural mitigation.

Vulnerability Characterization

We assessed the relative vulnerability of each site with respect to development and climate change. We did this by first multiplying each site's submetric score by the respective expert-based weight and summing all submetric scores within each class. We then assigned each site its respective percentile score with respect to each variable. We considered sites within higher percentiles to be more vulnerable to development (i.e., less protected land and greater probability of development within 300 m and 5500 m) and climate change (i.e., greater projected overall change in the climate variables outlined above).

Site Tiers

CAN sites exist within two tiers that reflect their relative importance and function within the perspective of the overarching goal of the CAN. These tiers include **Focal Core Areas** and **Management Opportunity Sites**.

Focal Core Areas (FCA).—FCAs represent the highest priority sites that, when considered together, are critical to the long-term persistence and evolutionary potential of the species in the northeastern United States. FCAs represent not only the most robust Wood Turtle populations in the region, but also sites that represent geographic, ecological, and genetic variation throughout the species range.

Management Opportunity Sites.—Management Opportunity Sites represent lower priority areas/subpopulations that are ideal opportunities for agricultural mitigation, federal engagement, or international collaboration.

Site Selection within Tiers

We designed the CAN site selection process (Fig. 4.7) to incorporate the Wood Turtle sites with the greatest conservation value to the species while simultaneously ensuring representation of important geographic, ecological, genetic, and political boundaries. We selected sites using a step-by-step procedure where sites were sequentially selected within defined categories and according to specified criteria. Here we describe these site selection processes.

Focal Core Areas

Primary FCA selection criteria.—We used the selection criteria below, and the order in which they are listed, to stratify the selection of FCAs for inclusion in the CAN. A single site was selected for each component of categories 2–4.

1. Top 15 sites regionwide (~1% of mapped sites)
2. Top site in each state (that was not already included)
3. Top site in each ecoregion (EPA Level II; Fig. 4.4) (that was not already included)
4. Top site in each HUC4 (Fig. 4.5) (that was not already included)

Secondary selection criteria.—We used the following selection criteria to incorporate additional sites that were regionally important for achieving representativeness (Redford et al. 2011). We selected these sites after the primary selection criteria were already applied.

Genetic diversity.—The three most genetically diverse sites within each of three measures of genetic diversity: allelic richness, heterozygosity, and private alleles.

Genetic uniqueness.—The most genetically unique site within the Northeast Region based on F_{st} values (see Part III) as well as four additional sites identified as unique through expert opinion (Weigel and Whiteley, pers. comm.).

Exceptional population density.—All sites within the top 20 of relative (CMR) population estimates for a single km.

Exceptional survey returns.—All of the top 20 sites with regard to maximum survey return for observer 1 for a single survey.

Previous selection rule.—If the best site within any selection category was already included via a previous selection criteria, the next best ranked site within that category was taken (e.g., if a state had two sites that were initially selected because they were in the top 15, the third highest ranked site within the state would be selected during the state-based selection). This rule played a key role in providing redundancy within the CAN.

Linking rule.—During the selection process, each newly selected site was examined to assess its likelihood of regularly sharing the same individuals with a previously selected site (e.g., because the intervening stream habitat is highly suitable and without clear obstructions to movement). If expert

opinion dictated that the sites were very likely “linked,” we included that site in the CAN as linked to its counterpart, but selected an additional site within the category it had been identified for. This procedure provided a means for effectively connecting sites that were mapped separately due to insufficient data.

Vulnerability rule.—For sites that were within the 75th percentile of the previously described vulnerability metric (i.e., high probability of future development based on NALCC models [McGarigal et al. 2017] and very little protected land), we selected an additional site, using the same criteria, that was not vulnerable to development. This served as one measure to ensure resiliency of the CAN to potential future threats. We did not include a similar rule for climate vulnerability because climate vulnerable sites were primarily located in the same areas, and thus additional selections yielded more climate vulnerable sites.

Data deficiency rule.—Population information related to standardized surveys played an important role in the identification of sites. However, survey effort was not distributed evenly throughout the region and certain states had considerably more suitable Wood Turtle habitat than others, making it difficult for certain states to be represented in the CAN proportionally to the amount of suitable habitat. In an attempt to correct for any potential disparities, we first used a linear regression (Quinn and Keough 2002) to relate the amount of suitable stream habitat included in the CAN for each state to the total amount of mapped stream habitat for each state. We included additional sites for each state that was below the regression line by more than the mean distance to the line for all states (Fig. 4.6). One new site was added for every multiple of the mean a state was below the regression line. This led to the inclusion of one additional site for Vermont and two additional sites for Pennsylvania.

Supporting sites.—Those sites that were identified by Wood Turtle experts during meetings and conference calls with state agency personnel and project partners as functionally connected and critical to the persistence of a previously identified FCA were also designated FCAs because of their key supporting role.

Management Opportunity Sites

To complement the FCAs, we identified four types of management opportunities:

Agricultural mitigation sites.—We designated the four sites that ranked the highest with regard to the agricultural mitigation metric (see Prioritization of Agricultural Mitigation Opportunities subsection above) as Management Opportunity Sites that would be prioritized for USDA Natural Resources Conservation Service (NRCS) Working Lands For Wildlife programs and other agricultural mitigation initiatives. We made appropriate adjustments to these designations if expert opinion indicated alternative

sites were better candidates. Agricultural mitigation Management Opportunity Sites were designed with the understanding that a “scattershot” approach—where small-scale mitigation actions are applied across broad geographic areas—is unlikely to result observable changes in subpopulation trends. Agricultural mitigation Management Opportunity Sites are meant to concentrate mitigation resources within defined areas with known Wood Turtle subpopulations that are most likely to display substantial, observable changes in population parameters with extensive and effective management.

National Wildlife Refuges.—We selected the five highest ranked sites irrespective of state, ecoregion, or HUC4 boundary that were located within National Wildlife Refuges and not already included in the CAN as opportunities for conservation and management.

International coordination sites.—Based on the expert opinion of regional and border-state experts, we selected Wood Turtle occupied streams that span the United States-Canada border that represent an opportunity to collaborate with Canadian partners and may support regionally significant Wood Turtle subpopulations if a comprehensive assessment within both countries is conducted.

Supporting management opportunities.—We included as Management Opportunity Sites, those that are functionally connected and critical to the persistence of already designated Management Opportunity Sites, using the opinion of regional and state Wood Turtle experts. We made these designations during meetings and conference calls with state agency personal and project partners.

Conservation Area Network Area Summary

The Conservation Area Network comprises a total of 145 sites, encompassing 151,675 ha. CAN sites averaged 31% protected land, ranging from 14% in West Virginia to 65% in Rhode Island.

Connectivity Basins

As a guiding concept for future site evaluation and surveys, we identified those basins that contain regionally significant sites as well as existing landscape structure that is highly conducive for connectivity among Wood Turtle populations and that potentially supports metapopulation dynamics (which are poorly understood for the species). To quantify these criteria we created a metric (similar to that used to identify Management Sites) that aimed to identify HUC8 watersheds that maximized site rank, landscape integrity at the 5500-m scale (see metric Class IV above), and site size while minimizing road density within 300 m. To do this, we first scaled each variable from 0–1, where values favorable for Wood Turtle (i.e., high rank and low road density) were closer to 1. We then calculated the average of

these variables within each HUC8 and multiplied the resulting values by each other to produce a single metric for which, overall, higher values indicated HUC8 basins with large, high ranked sites within a high integrity landscape with minimal road density within 300 m. We ranked basins by the sites within the portion of each HUC8 within each respective state and suggest that these areas be considered for future sampling effort.

State-Level Review Meetings

Upon final selection of CAN sites and connectivity basins, we held individual meetings or webinars with most of the northeastern States to review the prioritization criteria, site ranks, and site actions.

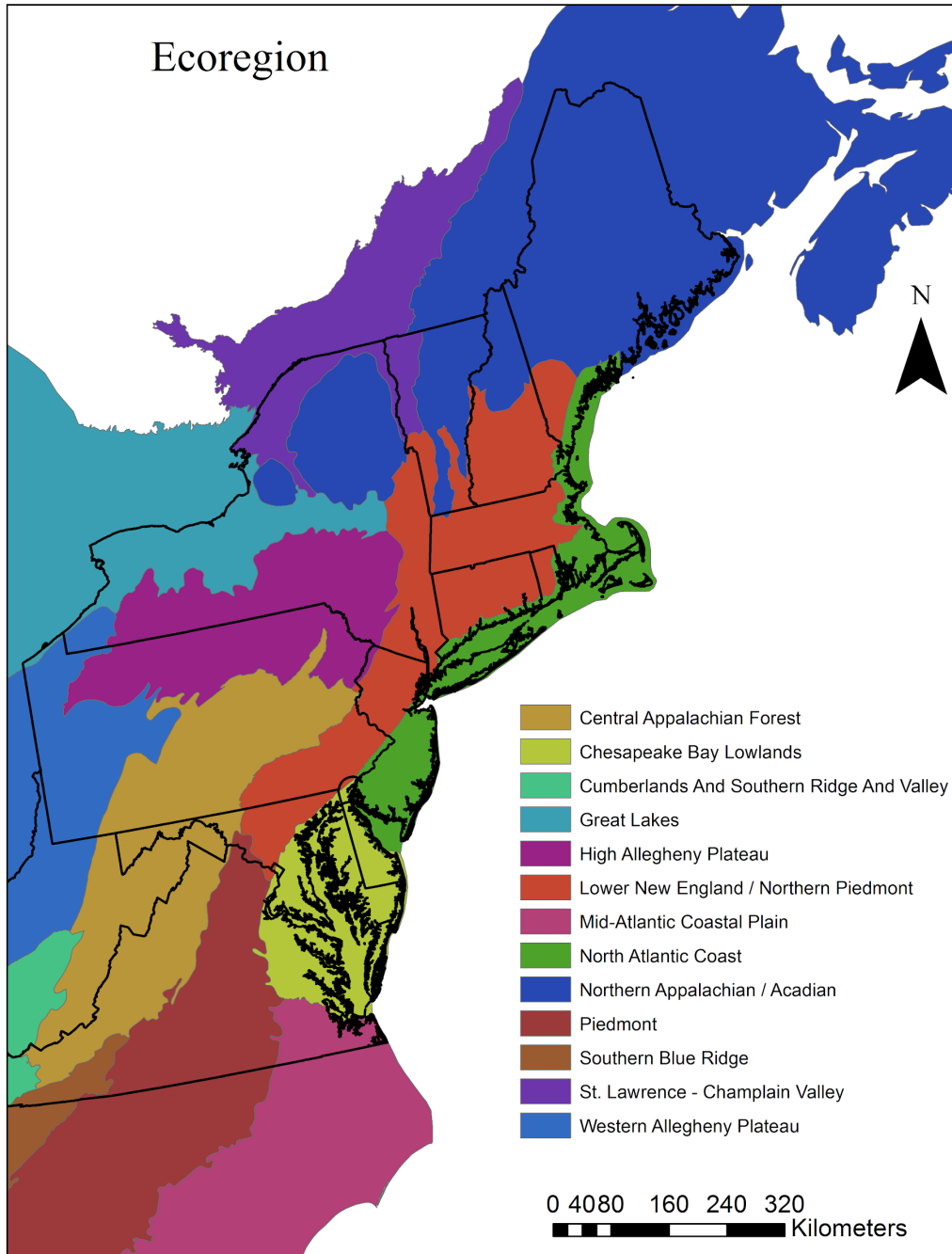


Figure 4.4. Environmental Protection Agency (EPA) Level III Ecoregions.

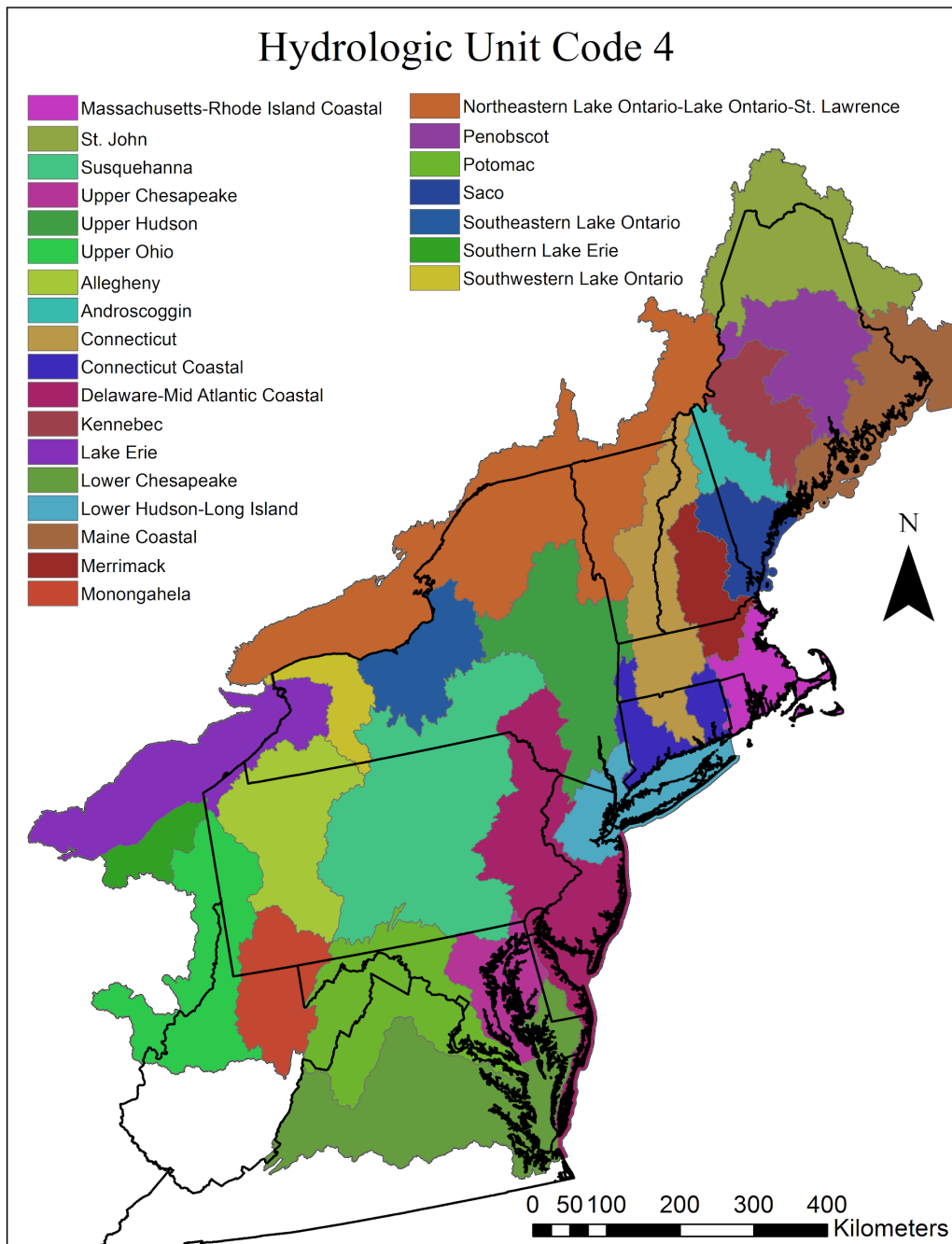


Figure 4.5. Map of 4-digit Hydrologic Unit Code basins (HUC4) in the Northeast region of the United States.

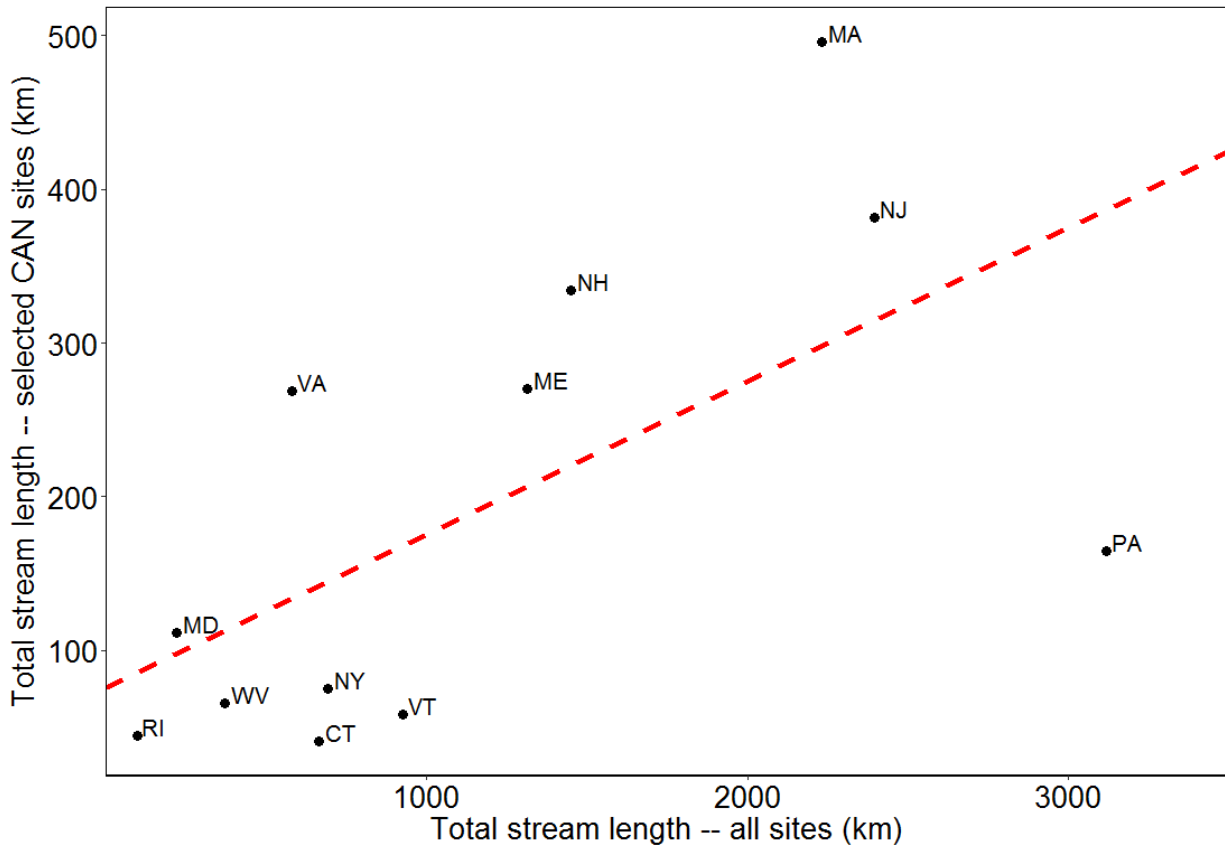


Figure 4.6. The total amount of suitable stream included in the Conservation Area Network for each state in relation (prior to the inclusion of the implementation of the data deficiency rule) to the total length of suitable stream in each state. The line represents the predicted values based on a linear regression.

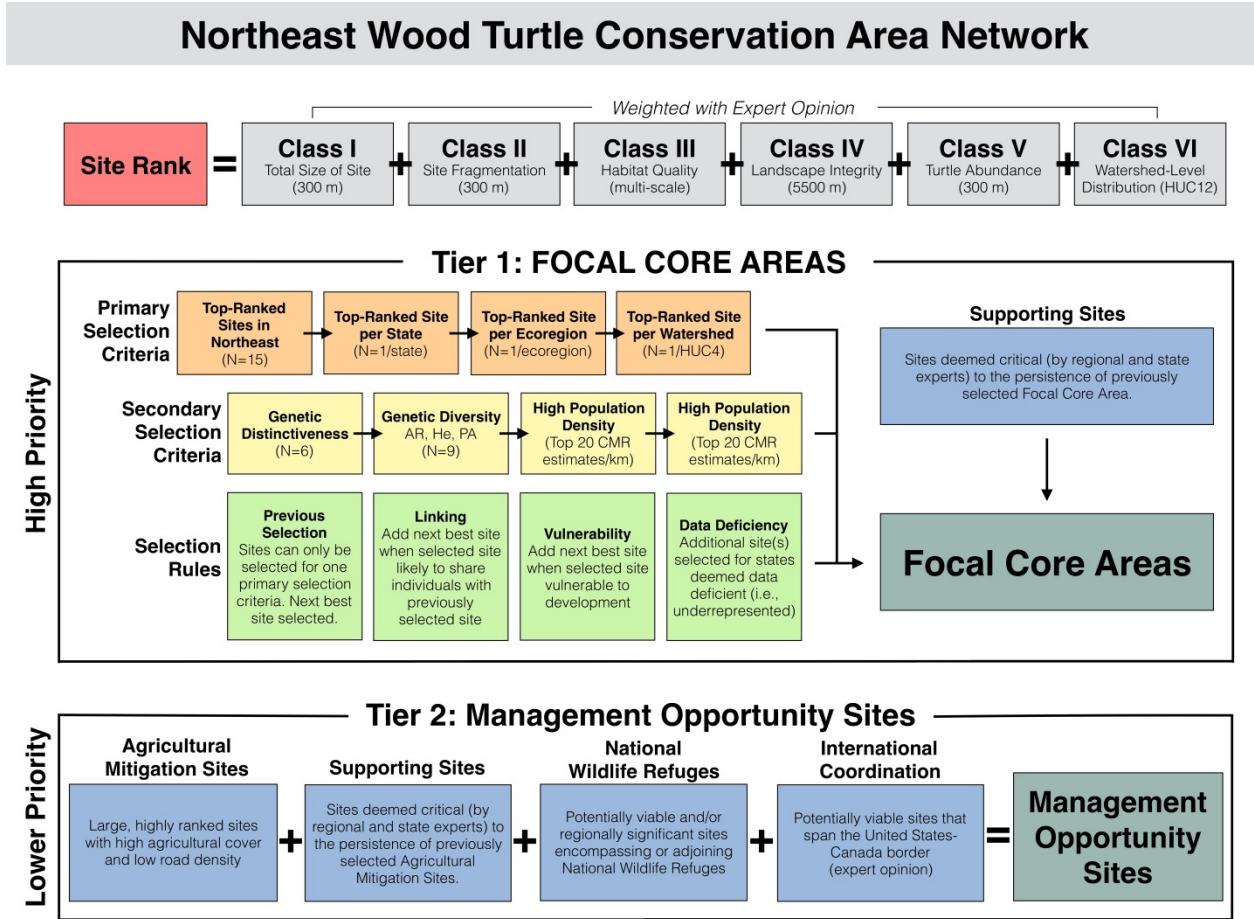


Figure 4.7. Conceptual model of Northeast Wood Turtle Conservation Area Network site selection.

Part V. Conservation Action Plan & Implementation Framework

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Summary

The Conservation Action Plan (CAP) serves as the primary means of achieving the overarching goal of this Conservation Plan: to protect and maintain the evolutionary potential of the Wood Turtle by facilitating the persistence of functional, demographically stable, ecologically viable, and representative populations of Wood Turtles throughout the northeastern United States. The core objective of the Wood Turtle CAP is to stabilize and reverse population declines within the Northeast Wood Turtle Conservation Area Network (CAN; Part IV). The CAP will be overseen and coordinated by the Wood Turtle Council (WTC), a formal Working Group made up of state, federal, and academic biologists throughout the species range. Conservation targets within CAN sites intended to help achieve the core CAP objective include (1) a net gain of protected habitat and secure nesting areas, (2) improved or stable juvenile recruitment without the need for active population management, (3) a measurable improvement in landowner partnerships, and (4) measurable, permanent mitigation of documented or anticipated threats to population persistence.

The WTC and state agencies will track the condition and status of CAN sites using the Site Action Tracking Database made up of 64 variables within 17 broad categories. This database is intended to facilitate the identification of site-specific conservation priorities and serve as a framework from which

more detailed management plans and spatially-explicit geodatabases can be developed. This CAP provides strategic guidelines for site-level actions such as population monitoring, geodatabase development, targeted land protection, application of Best Management Practices, and others. Conservation actions and recommendations are also provided for Connectivity Basin, state, and regional scales. Regional conservation priorities for the Wood Turtle include the prioritization of land protection of Wood Turtle habitat throughout the range, the reduction of poaching (e.g., through permitting standards and increased penalties), federal conservation opportunities, further genetic assessments, and targeted research. The final component of this section, the Implementation Framework, provides a step-by-step path forward for the implementation of this Conservation Plan, including opportunities of reassessment and refinement of baseline standards and objectives.

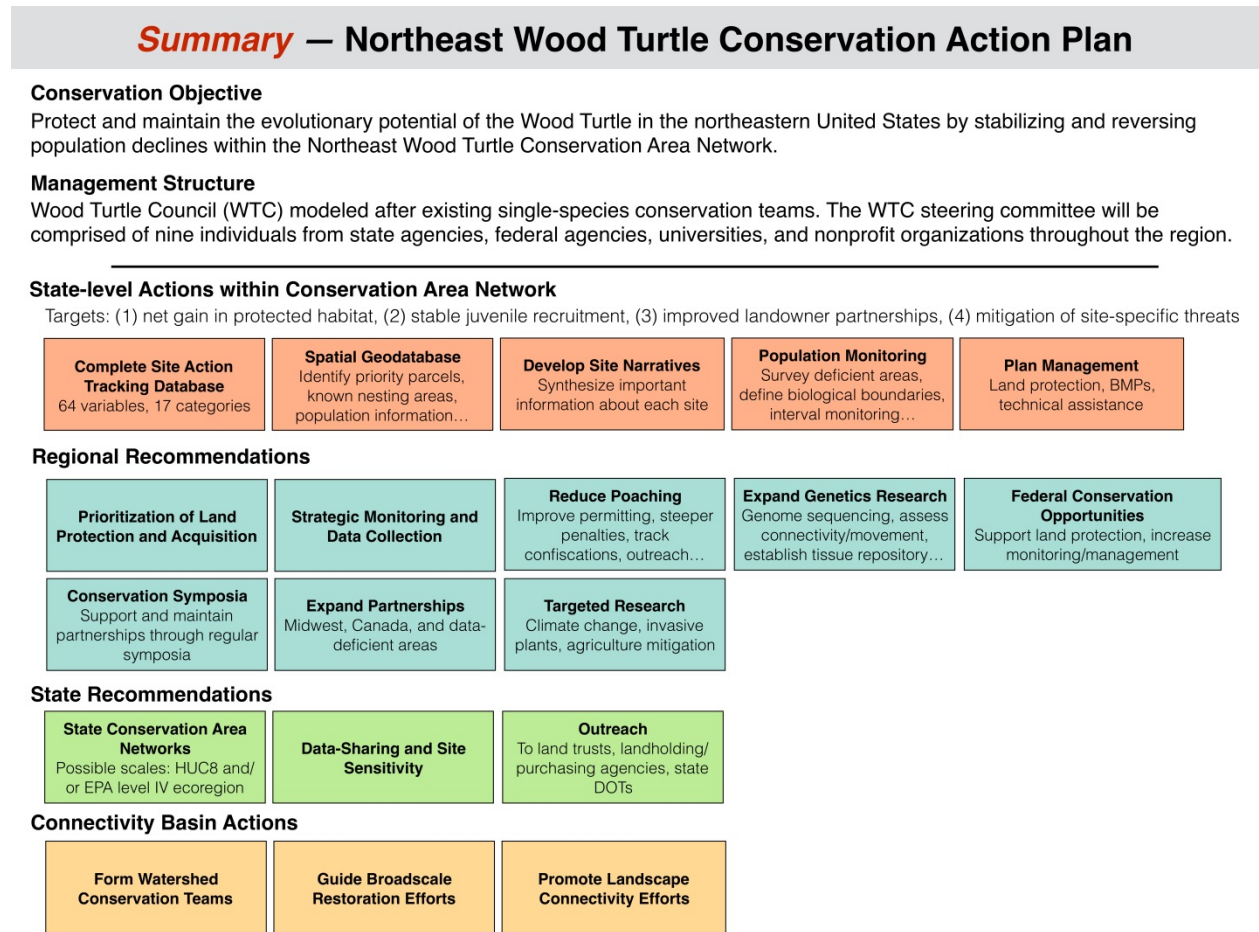


Figure 5.1. Summary of the Northeast Wood Turtle Conservation Action Plan.

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Conservation Objectives

The objective of *Conservation Plan for the Wood Turtle in the Northeastern United States* is to protect and maintain the evolutionary potential of the Wood Turtle by facilitating the persistence of functional, demographically stable, ecologically viable, and representative populations throughout the northeastern United States. To accomplish this, we aim to stabilize populations and reverse population declines within a formal Wood Turtle Conservation Area Network throughout the Northeast through targeted site-specific actions, and to minimize further declines outside of the Conservation Area Network through the application of general practices. The spatially-explicit, multi-tiered Wood Turtle Conservation Area Network (CAN; Part IV) derived from standardized population assessments (Part II), broad-scale genetic analysis (Part III), and landscape analyses (Part II and IV; Jones and Willey 2015) is intended to provide a framework through which to articulate and achieve these goals and track progress and effectiveness. Here, we outline: (1) our proposed personnel management structure for overseeing future Wood Turtle conservation efforts; (2) critical conservation actions needed to address the complex array of threats facing Wood Turtle population throughout the species range at multiple scales; and (3) our proposed strategy for the implementation of these actions.

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Management Structure

We propose that the range of conservation actions outlined below—from those specified at the site scale to those specified for basins and the entire region—be managed through a formal governing body known as the Wood Turtle Council (WTC). The WTC is modeled on elements from other successful conservation teams for turtles, such as the Northeast Blanding’s Turtle Working Group (<http://blandingsturtles.org>) and the Desert Tortoise Council (<http://deserttortoise.org>). Our primary objective in the establishment of the WTC is to facilitate the effective, efficient, and energetic implementation and periodic revision of the Conservation Plan and to supervise subsequent updates, revisions, and reassessments. The WTC is intended to facilitate conservation efforts for Wood Turtles at both the regional scale and site-specific levels, and consists of a Steering Committee, Site Leaders, and other standing or ad hoc committees appointed by the Steering Committee. A full organizational outline of the WTC is provided in Appendix XIII.

The conservation work of the WTC at the regional level will be overseen by a **Steering Committee**, comprised of nine individuals: one representative from each of at least five northeastern wildlife agencies, one representative from the U.S. Fish and Wildlife Service, and three at-large members representing other

partner universities, NGOs, or private entities, who are approved by the agency representatives. The Steering Committee is responsible for furthering the mission of the WTC, specifically by: (1) ensuring that the goals and actions outlined in the regional Conservation Plan are furthered at the regional level; (2) facilitating the revision and updating of the Conservation Plan at appropriate intervals; (3) developing other committees, as necessary, to further the mission of the WTC; (4) supporting conservation at the site level by tracking site-specific actions and appointing **Site Leaders** where appropriate); and (5) ensuring that Wood Turtles are appropriately and adequately represented in regional multi-species planning efforts such as Priority Reptile and Amphibian Conservation Areas (PARCAs) and the North Atlantic Landscape Conservation Cooperative (NALCC). The Steering Committee will be led by two co-chairs.

The entire WTC (the Steering Committee, and other standing or ad hoc committees) should meet at least annually, in conjunction with the Northeast Partners for Amphibian and Reptile Conservation (NEPARC) meeting, during a regional Wood Turtle conservation symposium, or as otherwise necessary. The entire WTC, the Steering Committee, advisory committee, and/or other committees may call meetings and/or conference calls quarterly or frequently enough to ensure that the objectives of the Conservation Plan are met, as well as to initiate new applications for regional funding. Further, the co-chairs will manage a list-serv for Wood Turtle partners to facilitate communication between coordinated efforts and maintain communication with key partners.

With this formal transition, the WTC will cease to function as a Working Group within NEPARC, where it has operated since 2009. The WTC will consider, as an early item of business, to what extent to combine efforts with regional working groups for Spotted Turtles and Blanding’s Turtles.

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Site-Level Actions within the Conservation Area Network

Conservation Targets

By designing a Conservation Area Network and developing a spatially-explicit Conservation Action Plan, we have identified the river segments, basins, sites, landscapes, subpopulations, and metapopulations that are most critical to adequately conserve and appropriately manage in order to protect and conserve representative populations of Wood Turtles from Maine to Virginia. Because the Wood Turtle has already sustained large declines and localized population impairment caused by urbanization and land fragmentation (as much as 58% of the Wood Turtle’s modeled range and suitable habitat in the Northeast is potentially impaired by urbanization [Jones and Willey 2015]), and because the Conservation Area

Network comprises roughly 10% of known occupied sites, our primary target is to prevent further population decline or landscape degradation within the sites comprising the Conservation Area Network, and to minimize declines throughout the range. Thus, our targets for the CAN sites specify (1) a net gain of protected habitat and secure nesting areas; (2) improved or stable juvenile recruitment without the need for active population management; (3) an increase in measurable, quantified landowner partnerships; and (4) measurable, permanent mitigation of documented or anticipated threats to population persistence, such as habitat degradation and illegal collection.

Site Action Tracking Database

We have developed a detailed Site Action Tracking Database for all sites included within the CAN (Focal Core Areas [FCA] and Management Opportunities). This database facilitates the assessment and management of CAN sites by tracking 64 site-specific variables that fall within 17 broad categories such as Nesting Habitat Quality and Status, Site Protected Status, and Technical Assistance Needs (see Table 5.1 below). The database, in its current form, is meant to serve as a basic framework from which more detailed site-specific management plans and spatially-explicit geodatabases can eventually be developed, but also as a guiding document for prioritization and implementation of near-term (<5 years) actions. Further, this database provides a quantitative method by which to assess the status of CAN sites, measure the relative effectiveness of conservation actions that have been implemented, and ultimately inform subsequent actions. Thus, this method of tracking the status and condition of important Wood Turtle sites provides a detailed basis for the iterative assessment of the species' status.

Table 5.1. Site Action Tracking Database for sites identified within the Conservation Area Network. The database is being populated with information from three sources: Expert Opinion, GIS, and standardized population assessments.

Tracking Item	Description
Mapping Considerations	
Full extent of subpopulation known?	Does the site, as mapped, appear to encompass the full range of continuous habitats available to this subpopulation of Wood Turtles?
Adjacent sites to be linked?	Are there sites/populations nearby that should be mapped and managed in conjunction with this site? If, so which?
Truncation needed?	Should this site be made truncated ? Indicate where.
Protected Status	
% protected	Percent of the Focal Core Area (300 m buffer) that is protected. (initial calculation from USGS layer)
% supporting landscape protected	Percent of the surrounding landscape (5.5 km buffer) that is protected. (initial calculation from USGS layer)
Core protected	Based on your knowledge, is the riparian area (to 300 m) sufficiently

Tracking Item	Description
	protected at this site?
Number of high-use areas within site	How many discontinuous high-use (by wood turtles) areas are known in this FCA?
High-use areas protected?	Based on your knowledge, are the critical high-use areas sufficiently protected at this site?
Federal Involvement (USFWS, NPS, NRCS, USFS)	
Percent	Percent of the site that is owned or managed by each respective agency
Acreage	Number of acres owned or managed
Name	Name of the property
Vulnerability	
Development vulnerability	Vulnerability to future development (75th and 90th percentiles indicated)
Climate vulnerability	Vulnerability to climate change (75th and 90th percentiles indicated)
Roads	
Road threat?	0 or 1; Is the site in the top 3rd percentile of a metric reflecting road density and traffic rate?
Road mitigation needed?	Is road mitigation of some form needed for this site?
Agriculture	
Percent agriculture	Percent of the site that is in active agriculture
Total agriculture (acres)	Approximate number of acres of agriculture within the site (300 m)
Mowing threat?	Is mowing (e.g., of fields, powerlines, etc) a major threat?
Livestock threat?	Are factors associated with livestock a threat to the population? (E.g., habitat degradation, water quality, etc)
Agriculture mitigation needed?	Is agriculture mitigation of some sort needed at this site?
Working Lands Site?	Should this be a target site for programs dedicated to mitigating detrimental land management?
Nesting	
Instream nesting available?	Are natural instream nesting features available?
Instream nesting protected?	Are the available natural instream nesting features protected?
Anthropogenic nesting available?	Are anthropogenic nesting features available?
Anthropogenic nesting protected?	Are the available anthropogenic nesting features protected?
Nest depredation threat?	Is nest depredation a major concern?
Nesting management needed?	Is nesting habitat management needed to create new nesting habitat, augment existing habitat, or protect existing habitat?
Invasive Plant Species	
Invasive management needed?	Is invasive plant management needed?
Demographics	
Juvenile percentage	Percent of turtles captured that were juveniles
Demographic stability (% > 0.25 = stable)	If % juveniles estimated to be present is >0.25 the site is given a 1

Tracking Item	Description
Nest protection needed?	Is active nest protection needed to recover the population age structure?
Headstarting urgently needed?	Is the demographic condition and population status such that headstarting is the only means for persistence?
Data Quality	
CMR estimate?	Was a capture-mark-recapture population estimate obtained for the site?
CPUE	Was any catch per unit effort (CPUE) information obtained from this site?
Standardized segment density	How many survey segments per km ²
Year last surveyed	
Year Wood Turtles last observed	
More surveys needed within site?	Based on expert opinion, does this site need more survey segments to evaluate the population status?
More surveys needed outside of site?	Are surveys needed outside of the site's boundaries in order to assess connectivity between sites or establish the biological boundaries of the CAN site?
Data deficient?	Based on expert opinion, is this site data deficient?
Data sensitivity	
High density?	Based on expert opinion, would you consider this a high-density site within the state?
CMR_rank	Did this site have a regionally high density estimate? (Top 20 sites in the region are indicated)
CPUE_rank	Did this site have a regionally high CPUE estimate? (Top 20 sites in the region are indicated)
Withhold?	Should this site be withheld from from any distribution because the potential threats of circulation outweigh the potential benefits?
Poaching risk?	Is this site a poaching risk?
Technical Assistance	
Key landowners identified	Have any key landowners been identified?
Key landowner partnerships	Are partnerships for habitat protection in place?
State agency target for land acquisition?	Is this FCA a target for state agency land acquisition? Indicate which agency.
Land trusts and NGOs updated on FCA	Have Land Trusts and NGOs been contacted and updated about the conservation needs of this FCA?
Species of Greatest Conservation Need	
SGCN expected to occur?	Do any Species of Greatest Conservation Needs (SGCN) occur at this site? Indicate species.
SGCN management incorporated?	Are these SGCN incorporated into the management of these sites?
SGCN conflict?	Does management of an SGCN conflict with the management of Wood Turtles? (e.g., mowing for grassland birds)
Game species conflict?	Does management of a game species conflict with Wood Turtle management at this site? (e.g., Pheasant?)

Tracking Item	Description
Recreation	
ORV threat?	Does ORV activity pose a threat to the population? (e.g., extensive trails near stream or evidence of ORV on nesting areas)
Hunting threat?	
Hiking trails?	Does this site host hiking trails that pose a threat via exposure to humans?
Fishing/canoe/kayak?	Is this site frequented by anglers and other recreationists?
Hydrology	
Stream straightening evident?	From field surveys or aerial photo interpretation, have significant sections of the stream been anthropogenically straightened?
Bank hardening present?	Has the stream bank been hardened (e.g., with cement or riprap) within or adjacent (2 km upstream) to the site?
Major flood risk?	Is this site likely to experience extreme floods with the potential to displace overwintering turtles?
Water quality mitigation needed?	Does water quality threaten the persistence of the site's population?
Other management	
Other management needs	Are there other management consideration for the site?
Telemetry priority?	
Site Leaders	
Site leaders?	Are there any individuals that could lead oversight and management of this site?

Spatial Geodatabase(s)

For each site designated as a Focal Core Area or Management Opportunity, a spatially explicit geodatabase should be developed that corresponds to the actions and conditions in the Site Action Tracking Database. Site geodatabases should identify priority parcels for acquisition or conservation easement; the location, condition, and management needs of known or potential nesting areas; roadway mitigation locations; available population assessment data; and habitat management opportunities and completed actions.

Population Monitoring

Population Monitoring and Standardized Population Assessments

We recognize that the level of population sampling that informed this Conservation Plan—funded by three regional or Competitive State Wildlife Grants (CSWG) and a variety of state- and private funds—will be difficult to undertake again in the near future. For this reason, it is critical to strategically prioritize population assessment efforts within the coming five years (to 2023). Available resources for population

assessment should be directed toward the areas outlined below. In all cases, whenever possible, surveys should be conducted using the regional, standardized protocol (Appendix V), with minimal changes or deviations, and data should be pooled with other regional data on an annual basis.

Opportunistic Collection of Standardized Survey Data

Encourage partners across the Northeast Region to conduct surveys for Wood Turtles using the standard protocol, wherever feasible. As new partners are identified, encourage the use of the regional protocol and continue to amalgamate the new data into the existing databases. Our target upon reassessment is for all state and federal agencies with jurisdiction over all or part of a regionally significant CAN site to be actively engaged in population and site monitoring, and to have identified private partners willing and able to conduct surveys.

Standardized Surveys to Refine the Biological Boundaries of CAN Sites

Within data-deficient CAN sites, establish new standardized survey segments to adequately assess the condition of each regionally significant population. Conduct standardized surveys at strategic intervals up- and downstream to document and establish the biological boundaries of the site. Surveys within and adjacent to CAN sites, especially Focal Core Areas, should be conducted opportunistically as resources allow.

Standardized Surveys in Data-Deficient Areas

Undertake targeted, widespread, and systematic population assessments across data deficient areas of regional significance. These areas have been highlighted throughout this Conservation Plan where known occurrences or prioritized CAN sites do not appear to reflect the estimated extent of suitable habitat. Although numerous data deficient areas remain, generally, areas of regional significance occur in relatively large states with large forested areas and unfragmented river systems. Notable examples include northern New York (Adirondack and Tug Hill Region); western New York (Allegheny Plateau); and northwestern Pennsylvania (Allegheny Plateau).

Interval Monitoring of Established Segments

Undertake reassessment of established segments at 5- to 10- year intervals, as resources allow. Prioritize sites within Focal Core Areas and other CAN designations.

General CAN Site-Level Management Recommendations

The following conservation and management actions are generally prescribed for all of the sites in the Conservation Area Network. Special actions particular only to one class of site are noted.

Targeted Land Protection

It is well established that Wood Turtles persist in remote, mostly forested, unfragmented, and difficult-to-access stream systems. With increasing development pressure expected, it is also anticipated that currently large or healthy populations will continue to decline (Jones and Willey 2015), isolating larger subpopulations in the more isolated mountainous wilderness areas. Further, it is clear that Wood Turtle populations are most stable (i.e., can persist in one area without constant management) in relatively narrowly defined conditions (see Part I). For these and many other specific biological reasons, and as highlighted throughout this conservation plan, **land protection in perpetuity is the primary conservation need of Wood Turtles throughout the region.** At each site within the Conservation Area Network, land conservation should remain a priority until expert opinion deems the site fully protected, with all high-use areas (nesting areas, etc.) fully protected in perpetuity. Further, it is critical that land protection not further facilitate or encourage public access (Garber and Burger 1995).

Deterrence of Illegal Trade

Substantial deterrence of illegal collection/trade is another primary conservation need (in addition to land protection) for the Wood Turtle throughout the species range. Site-level monitoring and deterrence of poaching should be considered in areas where commercial collection is suspected. Active site monitoring via deployment of time-lapse cameras at key Wood Turtle features will allow state biologists and Site Leaders to identify potential poachers and at the very least, understand human activity levels. Widespread public notifications that streams are monitored for turtle poaching may provide some measure of deterrence. Cameras are currently in use at several undisclosed locations in New England and Virginia. Increased use of Passive Integrated Transponders (PIT) at Focal Core Area sites, with updated reporting during regional analyses of monitoring data, would improve the ability of law enforcement to positively determine the site of origin.

Application of Best Management Practices

We have provided template Best Management Practices for Wood Turtles within numerous land-use scenarios in Appendix I. Below, we provide an overview of key guidelines within common management settings.

Agricultural settings.—For a CAN site to be considered secure, land managers should only mow fields and operate heavy machinery during the colder months of the year. In CAN sites where mowing during the active season is unavoidable, unmanaged buffers of 30–100 m should be established adjacent to the stream. When prioritizing expensive or controversial management actions within CAN sites, site

managers should take into account any empirical movement or habitat use information from the site in question.

Nest area restoration and management.—Nesting areas are often a limited and limiting resource for Wood Turtles in the northeastern United States. The influence of the position, location, and configuration of nesting areas can be complex and counterintuitive. For example, females may venture relatively far in order to access suitable nesting areas (Compton 1999), exposing them to high risk of mortality from roadkill or collection. At the regional scale, the highest priority related to nest protection is to identify and protect suitable nesting sites in close proximity to suitable overwintering and foraging areas that are unlikely to require intensive management for years to come (e.g., nesting areas naturally rejuvenated at regular intervals by fluvial processes). Within Focal Core Areas and CAN sites, as well as sites identified within State Conservation Plans (see below), conduct targeted surveys to identify nesting areas and document their condition (invasive plants, predators, public access, security). Update the Site Action Tracking Database and individual site geodatabases as new information is obtained. Nesting areas should be identified from aerial photographs, expert opinion, telemetry, and visual reconnaissance. They may be monitored using the Population Assessment Protocol (Appendix V) in sites where nesting habitat occurs along the stream corridor in the form of beaches and point bars, or by conducting evening surveys of the potential features during the nesting season (primarily the late-May to early July; see Part II), or by using remote-sensing timelapse cameras such as the PlotWatcher Pro (Day 6 Outdoors; day6outdoors.com).

Forest management.—Timber harvests can pose a significant threat to Wood Turtle populations via direct mortality from machinery if conducted during the Wood Turtle active period. Forest management involving heavy machinery should **not** be conducted within 300 m of Wood Turtle CAN sites during the Wood Turtle active period (i.e., forest management should be conducted during the cold months of the year). Under ideal circumstances timber harvests will be limited to the greatest extent possible within 300 m of FCAs and especially within 90 m, unless the creation of early-successional habitat via forest management has otherwise been identified as a management priority. Following major logging operation with new road construction, roads should be permanently retired with berms, or gated to minimize recreational access to priority sites.

Road Mortality Hotspots and Mitigation Opportunities

Road construction.—All new road construction should be avoided within 300 m of CAN-designated streams. New road crossings should be avoided within all Wood Turtle CAN sites. Through land acquisition and regulation, where feasible, restrict the development of new roads within CAN sites.

Pursue conservation partnerships to minimize further fragmentation of the 5.5 km buffer area (Jones and Willey 2015).

Culvert upgrades.—Existing culverts—both aquatic and semi-aquatic—within CAN sites should be upgraded where feasible and appropriate to allow passage of all aquatic organisms (i.e., should not be perched or undersized to constrict flows). Optimally, culverts that are judged to restrict or limit the under-road passage of Wood Turtles should be upgraded to full-span bridges. Further, the construction of additional dry passage culverts and fencing to facilitate movement of Wood Turtles under roads, particularly when roads are running parallel to a stream, may facilitate safe travel of Wood Turtles into surrounding upland areas.

Seasonal road closures.—Wherever feasible, roads should be closed to vehicle traffic within Focal Core Areas. Where roads within CAN sites are judged to pose a risk of roadkill or collection and are within 300 m of the stream, seasonal or permanent closures and/or restricted access should be implemented when possible (e.g., roads on federal lands, some private logging roads).

Identify problematic road crossing areas.—Within documented CAN sites, identify potential road crossing hotspots using aerial photo interpretation, expert opinion, and field surveys. Conduct targeted assessments of potential problem areas to evaluate optimal and feasible mitigation solutions ranging from habitat improvements (replicated nesting areas to minimize road crossings), fencing, signage, traffic controls. Update the Site Action Tracking Database and individual site geodatabases as new information is obtained.

Roadsides.—When roadsides are left bare and consist of sand, gravel, or other suitable nesting substrates they can become attractive to nesting female Wood Turtles, especially when alternative nesting areas are lacking. Efforts should be made to periodically assess roadsides within Wood Turtle sites to determine their potential to attract nesting females. If a roadside is identified as a clear attractant, Sites Leaders and State Wildlife officials should consider working with state Departments of Transportation (DOT) to make the area unsuitable for nesting (e.g., soil compression or large rock deposition).

Additional road mitigation actions.—Signs strategically placed in turtle crossing areas may initially reduce road mortality to some degree, but the influence of signs likely declines with time and the long-term effectiveness of this action is uncertain. It is possible that seasonal sign placement may be more effective than year-round. With the approval of state and local agencies, speed bumps strategically placed within road-crossing hotspots may also lead to a reduction in road mortality. But because of the

uncertainty associated with most post-hoc road mitigation efforts, the primary emphasis and energy should be directed toward preventing new roads and gating existing roads where feasible.

Riparian and Riverine Restoration Management

Due to a legacy of intensive manipulation (straightening, hardening, mill industry) over several centuries, even several of the least degraded and fragmented Focal Core Areas would benefit from strategic riparian and riverine restoration efforts. Measures that restore the natural hydrology of streams are beneficial for Wood Turtles if they can be executed in such a way as to avoid adult and juvenile mortality. Large scale river restoration affecting >1 km of stream within all CAN sites, such as dam removals or channel restoration, should be critically reviewed for (A) review the potential impact (negative and/or positive) on the local Wood Turtle population and (B) assess the appropriateness of proposed restoration actions given the preexisting condition of the site. A concerted effort should be made to thoroughly monitor sites before (at least three surveys during spring) and after restoration efforts take place.

Targeted Management and Early Detection of Invasive Plants

Within documented CAN sites, conduct annual or biennial visits to identify emerging invasive plant species. Update the Site Action Tracking Database and individual site geodatabases as new information is obtained. If the invasive plant is identified early in its establishment, and the species is likely to compromise key nesting areas or early successional habitats, undertake necessary actions to eradicate the species. A wide range of invasive plant taxa occur in northeastern Wood Turtle fluvial and riparian habitats, as catalogued by Jones and Willey (2015). Many species do not appear to structurally influence habitat quality for Wood Turtles (although this should be specifically tested by future studies). However, in several known instances, invasive plants (e.g., Japanese Knotweed [*Fallopia japonica*]) have colonized key features, such as nesting areas. In cases where invasive plants are clearly impacting the quality of major or important nesting areas, they should be controlled at regular intervals (5–10 years). Further, in areas where invasive species are not present and nesting areas are not compromised by invasive plant species, monitoring programs should maintain a status of high alert in order to document the early presence of rare species, and mobilize to eradicate the colonizing plants as they appear, if they might be detrimental to key Wood Turtle habitat features, especially nesting areas.

Promote Local Landscape Connectivity Initiatives

Local landscape connectivity for Wood Turtles should be promoted when possible, where identified as a priority. Stream-oriented efforts centered on CAN sites, such as the the restoration of stream connectivity and practices that promote natural stream hydrology should be encouraged within and among state agencies, land trusts, NGOs, and landowners for all sites identified within the CAN.

Provide Technical Assistance to Key Landowners

“Key landowners” are those that may own or control significant features (nesting areas, overwintering areas, early successional sites) or a significant portion of a CAN site, or own land with habitat features that are critical to the local Wood Turtle population. State agencies and site leaders, as appropriate, should approach landowners about the possibility of implementing land protection and/or management practices beneficial for Wood Turtles. Technical assistance materials (e.g., Appendices I, II, and IX) should be updated as appropriate and distributed to key landowners.

Minimize Impacts of Recreation

Even modest levels of recreation (of all kinds) within Wood Turtle streams and the adjacent landscapes (300 m) poses a threat to Wood Turtle populations (Garber and Burger 1995; Jones and Willey 2015) due to collection, direct mortality, and/or habitat degradation. There are three primary strategies for reducing and preventing recreation within Wood Turtle habitats:

Trail relocation, removal, and prevention.—All new trails (hiking, biking, ATV/ORV, etc.) should ideally be prohibited within Wood Turtle CAN sites (<300 m) region-wide. While this may not be feasible in all sites, it should be heavily emphasized within all FCAs throughout the region. Existing trails should be evaluated for relocation or removal. If outright removal is not feasible, managers should consider redirecting trails >90 m from streams.

Restricted seasonal road access.—Closing or seasonally gating logging roads within CAN sites will help deter recreation. Gates should be located at distances >1 km (preferably several km) to minimize easy access by foot.

Relocation of stream access points.—Stream access points for fishing and boating should be relocated >300 m *downstream* of key Wood Turtle features such as log jams, overwintering areas, and particularly nesting areas within Wood Turtle CAN sites.

Population Management: Nest Protection and Headstarting

The primary objective underpinning the development of the Wood Turtle CAN and conservation plan was the identification of subpopulations and landscapes capable of supporting Wood Turtles for the foreseeable future within minimal or no population management. Thus, population management with techniques such as nest protection or headstarting—which is typically only prescribed when population extirpation is imminent and landscape-level threats have been identified—is only recommended in very rare instances (e.g., for the only known population within a basin or ecoregion, when long-term conservation measures are feasible). The presumption of this conservation effort (based on the best

available evidence [Jones and Willey 2015]) is that areas still exist in the Northeast that can support Wood Turtle persistent populations through natural, dynamic processes, and to focus on population management when landscape conservation opportunities still exist can divert scarce resources away from solutions that are ecologically and evolutionarily viable. However, as already noted, rare instances appear to warrant exploratory population management. These sites are generally one of few remaining populations in an ecoregion, state, or basin; have partners interested in attempting population management; and stand a reasonable chance to resuming landscape-level function within one Wood Turtle generation.

Population Restoration

Considering threats facing existing priority populations throughout each state known to have naturally occurring Wood Turtles, and the potential to adequately conserve and manage representative populations throughout the region, we recommend that regional (e.g., RCN) or federal funds (State Wildlife Grant, CSWG) should not be directed toward restoring extirpated Wood Turtle populations. In rare scenarios—where the threats that caused the extirpation of a known Wood Turtle population are no longer present or have been sufficiently mitigated—the restoration of extirpated populations by non-profit organizations that are willing to cover the expenses may be warranted. However, to maximize the value of the exploratory new population and minimize the detriment to the overall conservation effort, (1) no resources should be used that would otherwise support the conservation of functional, extant populations; (2) no adults or juveniles should be moved from extant populations; (3) hatchlings should be obtained from within the same hydrologic unit (HUC8 if extant; HUC4 if no HUC8 populations exist; restoration efforts should be accompanied by iterative demographic modeling.

Site Narratives

Using the Site Action Tracking Database, Site Narratives should be developed for all sites within the CAN. Site Narratives will provide a synthesis of the actions implemented, subpopulation status, habitat condition, relevant threats, and/or other important information deemed necessary by Site Leaders and state biologists for the management of each site as well as a basic understanding of its overall status. In addition to guiding action implementation, narratives are also intended to serve the important function of maintaining consistency and momentum in the face of inevitable changes in personnel involved with this regional Wood Turtle conservation effort. We provide example narratives in Table 5.2.

Table 5.2. Site management narratives for a subset of Wood Turtle Conservation Area Network sites.

Pseudonym	Management Narrative
Charcoal House Creek	<p>Charcoal House Creek Macrosite has been identified as both a Focal Core Area and a Management Opportunity for agricultural mitigation. This site is now the focus of state-level NRCS outreach. Several distinct but connected Wood Turtle demes occur in the headwaters, two tributaries (“S” and “G”), and mainstem of a named HUC8 basin. A Supporting Site (“B”) was identified in an adjacent tributary to the mainstem. Most of the known Wood Turtle demes within this macrosite were studied intensively by Jones (2009) and Jones and Sievert (2009). The demes along Tributary S appear to be connected by natural movements as well as periodic flooding, which also negatively influences this population (Jones and Sievert 2009). Because of the large contiguous stream area occupied by Wood Turtles, the primary management actions vary throughout the system. One small deme in Tributary S occurs partially on a protected state Wildlife Management Area where Wood Turtle management is considered a high priority. Another, smaller deme downstream in the same tributary occurs on protected State Forest land encompassing one communal nesting area highly impaired by Japanese Knotweed. Between these two subpopulations are multiple problematic areas of moderate intensity agriculture (hayfields, light row crops, pasture). Tributary G is mostly privately owned, with agriculture and roadkill identified as the primary threats. The mainstem is negatively influenced by intensive agriculture and mortality from agricultural machinery is a major threat (Jones 2009). The mainstem deme should not be considered secure until a substantial buffer has been established along the river in the most intensively farmed areas.</p>
Wildcat Brook	<p>Wildcat Brook supports a single, continuously distributed subpopulation in high-elevation spruce-fir forest and light agriculture. The site is entirely privately owned. The threat from agriculture is minimal to moderate. Multiple nesting areas on the west side of the river, documented between 2004 and 2008 (Jones 2009), are now thickly vegetated with alder and Balsam Fir.</p>

Current nesting areas are not known, but it is likely that females are traveling farther than they were historically to access nesting sites. High-use areas, including overwintering sites, are well known and documented. This site is now a Focal Area for land acquisition for the state wildlife agency. There are no known threats from invasive species. A lightly traveled road parallels the river on the east side, where Wood Turtle roadkill has been documented (Jones 2009). Further development on this road should be minimized through land acquisition and regulation under the state Endangered Species Act. This site most urgently needs land protection and nest area rejuvenation.

Bumblebee Creek

Bumble Creek has been identified as both a Focal Core Area and a Management Opportunity for agricultural mitigation. This macrosite is now the focus of state-level NRCS outreach. The site is regionally considered to be an important waterway for freshwater mussels and other SGCN. Several high-use areas are known. All of the high-use areas and most of the site is privately owned. Documented nesting is dispersed in suboptimal conditions (roadsides, yards). Occasional Wood Turtle roadkill associated with nesting forays has been documented on a state highway that traverses the north end of the site; this threat could be mitigated by acquiring the northernmost high-use area and creating a major nesting site. A small portion of the stream is state-owned as part of a Wildlife Management Area; though not optimal, this could be the site of a new nesting area.

Little Bearskin Creek

Little Bearskin Creek is entirely state-owned as part of a State Forest and State Wildlife Management Area. A portion of the lower site is an Army Corps flood control storage facility. The effect on Wood Turtles of periodic flooding (associated with impoundment of the flood control reservoir) is not known, and should be evaluated. A single communal nesting area on an island in the river is of natural origin, but is highly impacted by Japanese Knotweed. To improve recruitment, the nesting area should be restored using herbicide treatments to control knotweed and manual removal of knotweed roots. Opportunities to restore other potential nesting beaches should be evaluated. The long-term influence of knotweed should be evaluated. At present, the

high use areas receive little recreational traffic. A gated forest road traverses the west side of the river, sometimes within a few meters of the stream channel. A ACOE-operated campground on a gated forest road provides access to one high-use Wood Turtle area. Public access to the core areas of the site should be minimized by directing use farther downriver. This site appears to be secure provided that public access does not increase and the nesting areas can be maintained in suitable condition through regular management.

Crosby River

Known high-use areas exist within undeveloped land with few roads. Agricultural/mowing mitigation should be prioritized in areas where fields are mowed to the edge of the stream. Roads may pose a threat in certain areas, particularly in the southern portion of the site. Additional survey segments are needed throughout the site to assess the population within data deficient areas. Instream nesting resources are abundant and should be monitored annually for invasive plant establishment.

Electric Creek
Macrosite

Electric Creek Macrosite encompasses six tributaries and the mainstem. Several of the tributaries are largely state-owned as State Forest and Wildlife Management Area, and Wood Turtles are a management priority for the state wildlife agency.

Worcester River

The Worcester River site is a Focal Core Area that provides important genetic representation (as the most robust known population in the MA-RI genetic cluster). The site is privately owned, though several putative (but not confirmed) nesting areas were created ca. 2011 as part of state-mandated mitigation for a solar field development.* This site appears to have very high rates of juvenile and subadult recruitment. Future efforts should confirm the location, condition, and management needs of suitable nesting areas. Remaining unprotected land should be conserved. Public access to the site—which borders a public high school—should be restricted. Opportunities to expand the distribution of this deme into an adjacent mainstem river should be evaluated. Opportunities to restore the water quality and floodplain habitats of

the adjacent mainstem river should be evaluated. Wood Turtle population status in the adjacent mainstem river should be evaluated through the establishment of one or more standardized survey segments.

Aspen Brook

Aspen Brook is noteworthy as a Focal Core Area because it supports a Wood Turtle population that is probably continuous with the area and subpopulation sampled by Louis Agassiz in the 1850s. Regrettably, the entire site is bisected by an interstate highway that was constructed in the 1970s. The two halves of the site are connected by a cement box culvert that may not permit upstream passage by Wood Turtles. Jones (unpublished data) observed two of ten radio-tagged adults (both nest-searching females) killed on this Interstate in 2009, and the highway is clearly the dominant threat to this important subpopulation. Further, the only known nesting areas are west of the Interstate, and they are at risk from proposed development. This site warrants an aggressive partnership between the state Department of Transportation and the state wildlife agency to evaluate opportunities to reduce roadkill and improve connectivity between the two functional halves of this site. This site also supports Eastern Pearlshell (*Margaritifera margaritifera*) and Spotted Turtle (*Clemmys guttata*).

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Connectivity Basin Actions

The primary conservation unit of this conservation plan, the Focal Core Area, represents known subpopulations of high regional conservation value, which are intended to be prioritized for limited conservation resources. However, connectivity among and between CAN sites is necessary to maintain subpopulation health (i.e., health of individuals within the subpopulation) and metapopulation function, sustain genetic diversity, and ensure the species has the ability to adapt to future shifts in climate and environment. Here, we frame broad, landscape-level actions within the context of basins because, while Wood Turtles readily utilize terrestrial habitats and occasionally disperse between watersheds overland (see Part II), the majority of large movements by Wood Turtles occur along waterways (Compton et al. 2002; Saumure 2004; Jones 2009). There are a multitude of challenges associated maintaining

connectivity along waterways at large scales and within human dominated landscapes, many of which are complex and intractable, but actions aimed at promoting landscape connectivity for Wood Turtles—and other stream-dwelling organisms—even as at low levels, is likely to benefit the species and should be focused within these landscapes deemed most likely to support maximum connectivity.

Conservation Teams for Connectivity Watersheds

The formation of “Watershed Conservation Teams” will encourage progress toward the goal of maintaining and restoring broadscale connectivity within regional Connectivity Watersheds. Ideally Watershed Conservation Teams will represent a diverse collective of conservation-oriented individuals from state agencies, local government, local land trusts, conservation NGOs, and local universities. The WTC will work directly with Watershed Conservation Teams to provide general guidance, identify management and land protection priorities, and ensure appropriate conservation measures are implemented.

Guidance of Broadscale Restoration Efforts

Several regionally significant CAN sites would benefit from broadscale, landscape-scale restoration efforts. Properly directed within Connectivity Basins, these efforts have the potential to improve habitat quality and connectivity and extend the suitable boundaries of Focal Core Areas. The large-scale efforts include dam removal and the restoration of natural stream hydrology. Although, watershed-scale eradication is unfeasible in most case, in some areas it may be possible and appropriate to conduct targeted broadscale eradication of invasive plant species beyond the scale of CAN sites, especially where plant populations have colonized and degraded nesting features.

Promotion of Landscape Connectivity Initiatives

Efforts to preserve land and promote reforestation of the watershed are valuable, if difficult to justify solely for Wood Turtles.

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State Recommendations

State Conservation Area Networks

The goal of the Northeast Wood Turtle Conservation Area Network is to identify *regionally significant* Wood Turtle conservation priorities following an established and peer-reviewed protocol; therefore, the regional CAN is not expected to adequately address each state’s conservation goals for the conservation

of species. However, we highly recommend that state agencies utilize any outputs of the regional CAN development process (i.e., mapped sites, population information, ranking metrics, population assessment data, and/or genetic data, etc.) to build stratified state-specific CANs to address their conservation objectives. HUC8 and/or EPA level IV ecoregion boundaries represent potential scales at which to stratify sites within states (the HUC8 level is the approach undertaken in Massachusetts).

Data-Sharing and Site-Sensitivity

Public Disclosure of Site Information

It is a well-established concern that publicly revealing Wood Turtle sites—especially sites of regional significance or of unusually high density—exposes populations to increased collection activities. For this reason, we recommend that Wood Turtle site locations continue to be managed at the individual state level and provided on a “need to know” basis with key partners under data-sharing agreements (see Basic Data-Sharing Agreements, below).

Data-Sharing Agreements and Permits

To protect sensitive site locations and the locations of viable populations, we recommend that all states within the range of the Wood Turtle require data-sharing agreements to obtain and work with all spatial Wood Turtle data, but especially the prioritized sites within the regional CAN. State Wildlife Agencies should administer data-sharing agreements for information related to CAN sites: Site Actions, site geodatabases, population assessment data, etc. Anyone working within a CAN site must have state-level scientific collecting permits.

Spatial Representation of Critically Sensitive Sites

Due to the risks associated with the distribution of spatial information, there may be few benefits—if any—to sharing coordinates (or general spatial information) for certain extremely high-value sites, such as those with large, stable populations within optimal landscape contexts. Therefore, we recommend that states consider a “Withhold” designation for sites that currently would not benefit from any form of representation or sharing of spatial information—even for conservation planning purposes. In addition, states should assess the relative value of sharing site coordinates for all Wood Turtle sites and particularly those identified within the CAN.

Outreach

Outreach efforts to land trusts, landholding and purchasing agencies, and state DOTs were identified by experts (Appendix IV) as important actions within the context of turtle conservation education. State agencies should consider outreach initiatives that target these entities, with the goal of educating representatives about the basic biology and ecology of the species, general threats to the species, and the role they can play in improving conditions for the species and/or mitigating threats to the species.

Environmental Review Recommendations

The thirteen northeastern states vary considerably in their statutory authority to regulate Wood Turtle habitat through environmental review. The tiered prioritization system provided in this plan could allow more stringent environmental review of Focal Core Areas and Management Opportunities than other sites at the state level. The following environmental review recommendations are adapted from Jones and Willey (2015).

Road Construction

Roads are a clear and persistent threat to wood turtles by facilitating roadkill of all age classes. In many cases, roads reduce the probability of persistence of Wood Turtle populations. Roads that parallel wood turtle streams, especially within 90 m high-use areas, present major conservation challenges. Perpendicular road crossings may exert proportionately similar effects on adult survival in the areas where they cross streams if there are attractive early successional or nesting features near the road, or if the culvert is undersized or perched. To effectively conserve wood turtles, it is important that new roads be prohibited near important wood turtle streams. All road construction should be prohibited within 90 m of Focal Core Area and Management Opportunity streams. New road construction should be prohibited, where feasible, within 300 m of Focal Core Area and Management Opportunity streams. New roads are not only a potential threat to population viability in and of themselves, but they facilitate additional risks such as new development, recreational use, subsidized predation, and mowing along roadsides. Further, to minimize the necessity of long-term population management, roads should be minimized in the land buffer to 5.5 kilometers from Focal Core Area streams. State officials or site managers should take advantage of opportunities to close or seasonally gate existing roads within 300 m of Focal Core Areas. Numerous roads on federal properties, comprising Management Opportunity sites, that serve hunters during the cold season could potentially be closed to protect wood turtles at all other times.

Culverts and crossings—New stream crossings can exert stress or negative influence for decades after installation on the local population, and should be avoided in all possible cases near regionally significant

streams. When it is necessary for roads to cross wood turtle streams, it is critical (A) that the culvert or bridge allow turtles to pass underneath (i.e., it is not perched) and (B) the road surface and side slopes not become an attractive nuisance to nesting females, unless the road will be gated. However, designing road crossing structures for wood turtles has not been experimentally tested, and many examples exist of repeat roadkills at perpendicular crossings, especially in the Northeast.

Agriculture and Mowing

Wood turtles are negatively affected by intensive agriculture because adults may be placed at higher risk of crushing injuries from mowers, combines, tractors, plows, harrows, and other farm machinery (Saumure and Bider 1998; Saumure 2004; Saumure et al. 2007; Jones 2009; Tingley et al. 2009; Erb and Jones 2011). Under certain landscape configurations and timing, mass mortality events or repeated mortalities in the same field occur (Tingley et al. 2009; Jones 2009). Certain landscape configurations probably result in higher mortality rates, although these have not been well-studied. Saumure (2004) noted that mortality rates in fields have probably increased since the 1970s because of the advent of disc and rotary mowers, which are more efficient than sickle-bar cutters but inflict greater damage to turtles. Although Saumure et al. (2007) and Tingley et al. (2009) suggested raising mower heads above 100 mm, Erb and Jones (2011) tested this hypothesis and found that sickle-bar mowers do result in significantly lower mortality rates. Further, they tested whether raising the mower head of disc and rotary mowers reduced mortality rates, but found no significant reduction in mortality by raising the blades. Raising mower blades saves some turtles and is certainly worth the effort where no other option exists, but it is important to note that even with blades set high, both blades and tires kill Wood Turtles at relatively high rates. This suggests that other, more effective alternatives to raising mower heads should be considered whenever possible, and these are discussed below.

Several authors have proposed riparian buffers as a strong mechanism to reduce agricultural mortality (Tingley et al. 2009). Jones (2009) noted the tendency for Wood Turtles to congregate in certain shrub habitats along the edges of fields. These typically had good solar exposure (facing south) and were often close to ditches or damp areas or the river itself. In some cases, it may be possible to delineate high-activity areas through standardized surveys or radiotelemetry. However, at any given site, absence of sightings in fields should not be construed to reflect low use if densities are otherwise high in the river. Wood Turtles are well-documented to heavily use both forb and graminoid-dominated meadows and hayfields, so their presence should be assumed wherever hayfields, pastures, or abandoned farmland comes in close proximity to a high-density overwintering stream.

Other authors have proposed other means of land-clearing, such as grazing, off-season burning, or off-season mowing (Erb and Jones 2011). These seem to be the most compatible with Wood Turtles. Where the primary risk to turtles comes from row-crop agriculture such as corn, Castellano et al. (2008) suggested using late-season varieties that require harvest in October rather than August or September.

All available data support the following Best Management Practices for agriculture in Wood Turtle habitat:

Establish unfragmented riparian buffers of ≥ 90 m at supporting sites;

Establish unfragmented riparian and upland buffers ≥ 300 m at regionally significant sites with no mowed or mechanically cleared areas, where feasible, provided that early successional habitats are available along the river;

Mow or clear existing fields, if necessary, during the cold months of 15 November to 15 March (south) or 15 October to 15 April (north). If warm season mowing or management is necessary leave a buffer at the edge of fields that are only maintained in winter;

Implement off-season burning or year-round grazing if areas must be kept open for other competing interests;

Use late-season crop varieties that require harvest in October rather than August;

Use radiotelemetry on a large sample of adults (>10), or systematic surveys, to identify heavily used areas within the fields and avoid these areas at a bare minimum.

Forestry

Forestry is likely to negatively affect Wood Turtles if adults are crushed by tractors, skidders, or other heavy equipment. Some forms of broadscale, intensive forestry such as clearcutting likely degrades habitat quality by facilitating numerous long-term management concerns. Removal of large wood from the system also decreases the availability of logjams and other overwintering structure in the streams. There are several types of forestry including clear cuts, shelterwood cuts, group selection, patch cuts, and salvage (Sweeten 2008; Martin 2010) and some of these may provide an opportunity to enhance Wood Turtle habitat if conducted when turtles are overwintering. For instance, most northern studies indicate that open, patch cuts near the river (in an otherwise forested landscape) may be beneficial (Compton 1999; Saumure 2004; Tingley and Herman 2008), but it is possible or likely that the relationship to landuse varies with latitude and elevation.

Several authors from disparate regions have proposed best management practices for forestry, and they are in general agreement (Compton 1999; Bol 2005; Tingley and Herman 2008). Harvesting within 300 m of high-quality riparian areas known to be occupied by Wood Turtles should occur only in the cold season when Wood Turtles are inactive (variable by region, but safely late October–late February).

Our recommendations for forestry are:

Minimize or prohibit forestry activities during the active season within 90 m of Wood Turtle streams.

Minimize forest manipulations within ≥ 90 m at Management Opportunity sites;

Minimize forest manipulations within ≥ 300 m at Focal Core Areas;

If early successional habitats or nesting habitats near the stream are lacking, small group selection cuts may enhance riparian habitat quality if conducted during the inactive season;

Logging roads should be discontinued after logging operations are complete so they do not provide multiple new access points to the river or provide for driving access parallel to streams.

Development

Development affects Wood Turtles in a variety of ways ranging from habitat and stream degradation to the facilitation of mortality due to roadkill, collection, and other sources. Parren (2013) noted the tendency of land developers to suggest recreational trails as a component of mitigation; this is counterproductive and probably worsens the outcome for Wood Turtles because of increased collection.

We recommend:

Prohibit all development activities within 90 m of Wood Turtle streams;

Prohibit all development within 300 m of Focal Core Areas and Management Opportunity sites;

Use strategic partnerships and landscape-scale planning to minimize future development within 5.5 km of priority Wood Turtle streams.

Nesting Area Management

Where possible, nesting area management should focus on instream features generated by the stream itself, such as point bars, sand and gravel bars, beaches, and cutbanks. In most cases where they are available, these instream features are probably preferable to anthropogenic nest sites away from the stream. These areas appear to be more abundant in eastern Canada, Maine, and New Hampshire than at the southern edge of the range. At significant sites where instream nesting is not available, management

should focus on maintaining and monitoring existing nest sites, expanding and augmenting existing nest sites, and creating new nesting areas, as appropriate during the off-season from 1 November to 31 March.

New, anthropogenic nesting areas should avoid creating landscape configurations that result in attractive nuisances or ecological traps, in which females are attracted to nesting areas that either result in decreased adult survival rates, decreased nest success, or decreased hatchling survivorship. For example, it is not ideal to have suitable or attractive nesting habitat located across a road from the overwintering stream, even if the road is infrequently traveled. Further, it is not ideal for nesting to be heavily concentrated at a single location because this may result in elevated nest depredation rates (Buhlmann and Osborn 2011; Buhlmann, pers. comm.).

Researchers and managers have successfully created Wood Turtle nesting habitat by constructing piles of sand in open fields (Buhlmann and Osborn 2011). At one site in Morris County, New Jersey, the nesting mound was 18 m long, 8 m wide, and 1.5 m tall (see Part 1, Plate 9).

A summary of considerations for managing nesting habitats follows:

Survey and map potential nesting areas within the stream segment of interest using aerial photographs and ground surveys;

Secure and manage natural occurrences of instream nesting habitat by clearing vegetation (during the inactive season) as necessary;

If instream nesting habitat is not available, evaluate the availability and condition of anthropogenic nesting habitat, and protect, manage or augment it as necessary and as resources allow;

If no nesting habitat is available but the population is otherwise assessed to be a potentially significant population without need of intensive management, construct new nesting areas by clearing land to expose mixed poorly-graded sand and gravel, or build mound(s) of sand in an open field near (≤ 50 m) the stream.

Dam Management

Dams influence Wood Turtles in two major ways, by flooding upstream areas and turning low-gradient stream habitat into deep reservoirs, and by altering the downstream flow regime, which degrades nesting habitat or and/or flood nests near the river.

Compton (1999) provided the most detailed recommendations for dam management in Wood Turtle habitat, focusing mostly on the suitability of nesting habitat. These recommendations probably apply throughout the range, especially within Focal Core Areas and Management Opportunities: 1) minimize large water releases between late May and the estimated date of nest emergence (generally in August) on rivers with Wood Turtles and known or suspected low-lying nesting areas; 2) allow high flows during early spring, before nesting, to encourage natural scouring of vegetation and redistribution of sand and gravel sediments. We recommend adhering to these recommendations throughout the range.

During dam re-permitting near regionally significant and supporting populations, managers should go so far as to map essential resource areas and key features and determine whether nest-site creation or management is necessary as a result of the dam-induced flow regime.

Recreational Access

Wood turtles co-occur with brook trout and are often found on high-quality coldwater trout streams, which may be frequently traveled by fishermen. Furthermore, Wood Turtles often occur on scenic waterways with high value to canoeists and boaters. Even infrequently collection poses a long-term conservation challenge, and so it is critical to re-site recreational access points away from Focal Core Areas and Management Opportunities (preferably downstream, so that boaters aren't carried into priority areas). Where possible, recreational access points for fishing and boating should be installed >300 m downstream of the lower reach of a regionally significant occurrence and >300 m from key features such as nesting areas, logjams, and potential or documented overwintering areas.

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Regional Actions and Recommendations

Prioritization of Land Protection and Acquisition Efforts

Land protection, particularly within FCAs, represents the most important (see Appendix IV), effective, and urgently needed conservation action for Wood Turtles throughout the northeastern United States. While site-specific conservation needs are varied throughout the region and land protection may not present a solution to all problems facing Wood Turtles (e.g., lack of nesting habitat and poaching), it is the single most effective tool for combatting the two greatest threats to Wood Turtles (according to experts throughout North America [Appendix IV]): habitat loss/degradation and elevated adult mortality. However, the conservation value of land protection for Wood Turtles ranges in effectiveness and practicality depending upon both landscape context (i.e., ability to support Wood Turtles) and socio-

economics in the area (i.e., projected price of land and attitude toward land conservation, etc). Priorities for land protection and acquisition that account for population importance (e.g., CAN rank), landscape integrity, and economics, should be developed at both the state and regional level to ensure that limited resources are allotted/available for protection are utilized in the most effective manner possible.

Strategic Monitoring and Data Collection

Strategic population monitoring and improved data collection represent essential components of the Conservation Action Plan. Through the actions outlined below, we hope to develop a robust understanding of Wood Turtle populations and subpopulations within data-deficient and/or under sampled areas, detect meaningful population shifts in abundance and demographics within FCAs, observe regional trends in occupancy and abundance, and identify previously unknown populations of regional significance.

Reassessment of Study Sites from RCN and CSWG, 2012–2017

The network of Rapid Assessment (RA) and Long-Term (LT) sites that were assessed during the course of this conservation plan should be reassessed at 7- to 10-year intervals to evaluate regional trends in occupancy as well as site-level population trend within FCAs. The standardized population assessments reported in this conservation plan occurred between 2012 and 2017. Sites that were first sampled in 2012 may be reassessed as early as 2019. Reassessments should be undertaken using the same protocol as the one presented in Part II, or one that is sufficiently complementary or encompassing to be directly comparable, even if the original results are reanalyzed with other methods.

Standardized Population Assessments in Data-Deficient Focal Core Areas

The Wood Turtle populations within certain FCAs remain poorly understood. FCAs that are considered “Data-Deficient,” either because they have no population estimate for any reference stream segment, or have a sufficiently low density of survey segments per kilometer of suitable stream, should be targets for increased or reallocated survey effort prior to, or during subsequent reassessments.

Basic Data Collection and Standardized Population Assessments in Data-Deficient Areas

Large regions or basins with relatively little occurrence and/or population-level information should be targeted for both basic data collection (i.e., incidental occurrence observations) as well as broadscale standardized sampling. It was clear from the analyses in Part II and Part IV that New York State remains significantly underrepresented compared to other northeastern states with regard to the broadscale collection of element occurrence (Fig. 5.1A) and standardized surveys (Fig. 5.1B). Pennsylvania was well

represented within the CAN site selection process, relative to sheer number of sites (Fig. 5.1A), which was aided by a sizeable element occurrence database; however, given both the size of the state and extent of suitable stream habitat, it appears more population-level information is needed (via standardized surveys) to adequately represent the state relative to other states (Fig. 5.1A, B). In addition, because of the general dispersion of Pennsylvania occurrences (that most likely resulted from random incidental observations), mapped sites were often considered separate (based on our mapping criteria) when in reality they are likely connected. Attention should be directed toward linking separate sites where connectivity between nearby sites is unknown in Pennsylvania and elsewhere. Vermont also stands out as a state with relatively widespread occurrence information that remains somewhat underrepresented with regard to population-level information (Fig. 5.1C). This lack of information about population size and demographics makes it difficult to assess the relative conservation value of sites relative to others throughout the state and region.

Non-political boundaries, such as basins, should also be considered when targeting data-deficient areas for surveys. For example, only one FCA was designated within the Allegheny (HUC4) drainage, in part because of a paucity of sampling in that watershed. States should also consider assessing data-deficiencies at the HUC6 and/or HUC8 scale.

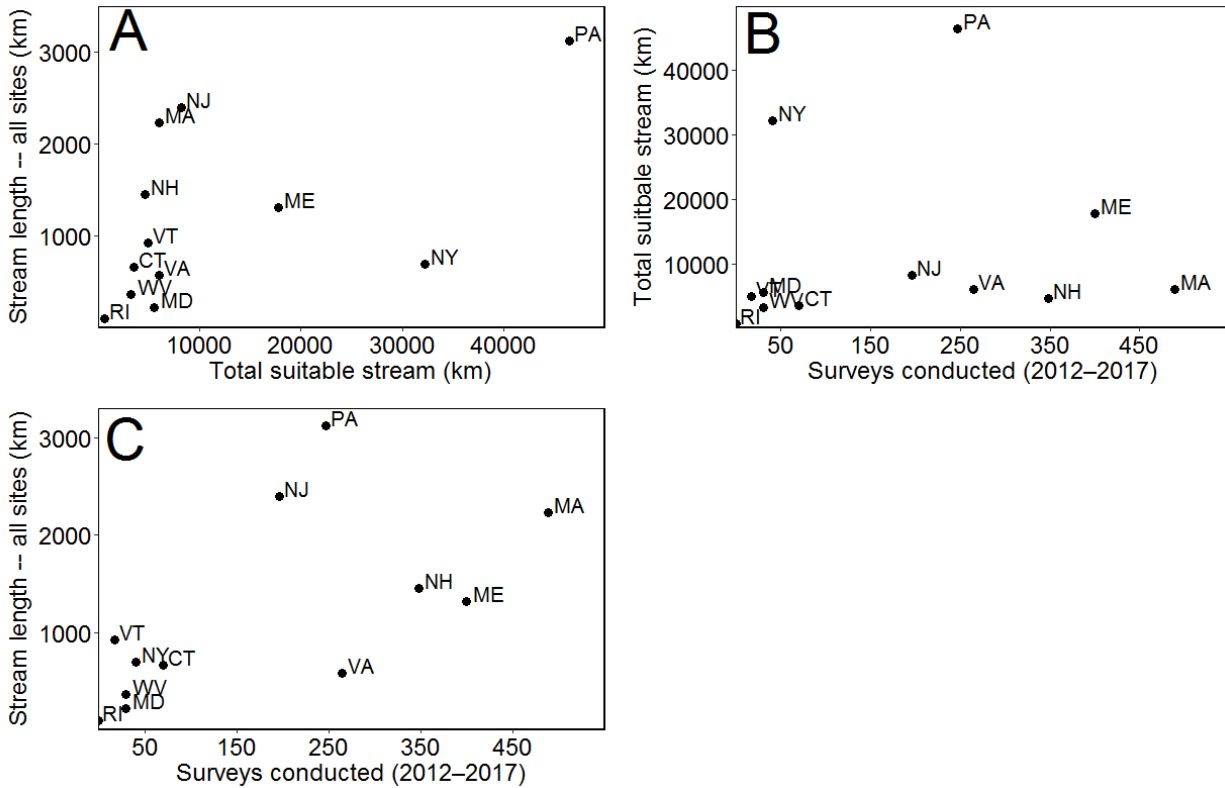


Figure 5.2. Length of mapped Wood Turtle habitat (areas considered for inclusion in the Conservation Area Network in relation to the total modeled suitable stream habitat within each state (A), total modeled suitable stream habitat within each state in relation to the number of surveys conducted (B), and length of mapped Wood Turtle habitat in relation to the number of surveys conducted (C).

Formalize Data Management and Protection

As described above, we recommend that all states within the geographic range of the Wood Turtle track all known occurrences of Wood Turtles. Further, we suggest that standardized data collated by States and combined database managed by WTC (CSWG PIs).

Standardized Sampling within Management Opportunities

Without an understanding of the baseline status of the population, it is difficult to determine whether management is effective or appropriate (e.g., turtles no longer present in sufficient numbers). Therefore, sites that have been identified as Management Opportunities because of emerging partnership—such as highly ranked National Wildlife Refuges and suitable sites for U.S. Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) easements and long-term management agreements—should undergo both initial (before the management action has occurred) and regular standardized survey assessments. While Wood Turtle-oriented management efforts are always intended to improve conditions for Wood Turtles, there is the potential for management efforts to negatively affect a population. For example, nesting areas may be created in locations that expose nesting females to unforeseen threats.

Standardized sampling (in the form of standardized stream and/or nesting area surveys) will provide an opportunity to assess the impact (positive or negative) of management actions on the target population.

Document Key Features within Focal Core Areas

Identification of key features within site can be critical to understanding the resident population and conducting effective habitat management and conservation. Wood Turtle behavior and resource selection is not always predictable among subpopulations—thus key features must often be determined in person rather than from aerial imagery. When possible standardized surveys should be used to identify key site features.

Telemetry

Radio telemetry can serve as a valuable tool for identifying key features within a site (especially within particularly large sites), such as nesting areas, high-use terrestrial habitats, and particularly overwintering locations. Because telemetry is time-consuming and cannot feasibly be applied to all sites, state and site experts should consider the available information, management needs, and threats facing each site within their state and develop telemetry priorities, if financial resources allow. Radio transmitters should ideally be dispersed to individuals throughout sites in order to obtain a more comprehensive picture of habitat use, especially within large sites.

Passive Integrated Transponders

Passive Integrated Transponders (PIT) offer a durable, long-lasting, and relatively painless method for identification of individual Wood Turtles that eliminates the possibility of assigning the same identifier to multiple animals throughout the species range. We recommend that, in conjunction with a future regional assessment effort, a region-wide transition should be made to PIT-tags using a standardized protocol. As a part of this effort, the WTC would oversee and coordinate the database of PIT numbers.

Establish “Natural Condition” Baseline Studies

Even the most remote Wood Turtle populations in the northeastern United States have experienced some degree of anthropogenic habitat alteration over the last several centuries; however, certain subpopulations are still situated within landscapes with relatively minimal anthropogenic influence. These subpopulations offer an opportunity to comprehensively document Wood Turtle population dynamics, spatial ecology, and general natural history within a relatively “natural” landscape context and thus shed light on baseline conditions that managers of FCAs throughout the region may strive to achieve.

Expand and Refine Monitoring Protocols

We recommend that the WTC continue to make adjustments to the regional population assessment protocol. The effectiveness of future population monitoring efforts will benefit from the judicious removal of superfluous and/or cumbersome data collection requirements, further clarification and emphasis of essential components of the protocol, and incorporation of details that will facilitate new modeling strategies (e.g., spatially-explicit capture recapture [SECR]). In addition to refining the existing population assessment protocol, an effort should be made to develop additional protocols/guidelines for monitoring habitat changes (e.g., flow regimes, sediment deposition, erosion, beaver settlement, invasive species), nesting areas, and the overall predator community.

Reduce Collection of Wild Wood Turtles

Over the past several decades, many Wood Turtle populations have been targeted for commercial collection (as opposed to incidental collection for noncommercial purposes, which is likely common and widespread throughout the range). Frequent seizure of adult Wood Turtles overseas and in the United States—some with shell notches (indicating a wild-caught individual from a studied population)—indicates that commercial poaching is an ongoing and persistent threat. Several recent confiscations of several dozen adult Wood Turtles (Part III; Jones and Willey 2015; Kurt Buhlmann, SREL, pers. comm.; Tom Akre, SCBI, pers. Comm.; J.D. Kleopfer, VDGIF, pers. comm.; Ed Thompson, MD DNR, pers. comm.) indicate that **illegal trade is occurring at a scale that is very likely to negatively affect the persistence and viability of regionally significant sites**. The reality that conservation priorities—regionally significant streams, nesting areas, parcels, access points, etc.—cannot be openly discussed with a diverse audience of partners further exacerbates the challenges associated with combatting Wood Turtle poaching. There is strong evidence, based on decades-long trends in market prices, that demand for Wood Turtles has likely increased substantially since the mid-20th century (Jones and Willey 2015). In a 2016 survey of 82 biologists with an average of 10.5 years of experience working with Wood Turtles, poaching was identified as the third greatest threat to Wood Turtles rangewide, behind only elevated adult mortality (2nd) and habitat loss and degradation (Appendix IV). While certain site-level actions may help deter poaching, such as targeted use of cameras at nesting sites and along streams, it is clear that the most meaningful anti-poaching measures will occur at the state and, particularly, federal agency level. Effective anti-poaching actions exist along a spectrum of complexity and sophistication, from the improved communication among entities (i.e., law enforcement, federal agencies, state agencies, and the regional Wood Turtle Council) to the development of more effective genetic assignment techniques and passing of more effective anti-poaching regulations. **Regionally, federal and state agencies must work**

collaboratively and creatively toward the overarching objective of substantially minimizing the risk of illegal trade and collection of adult wild-caught Wood Turtles.

Regional Anti-Poaching/Illegal-Trade Group

A **region-wide anti-poaching/illegal-trade group** that incorporates federal and state agency biologists, law enforcement, and other conservation partners must be developed and maintained. This group should develop a range-wide strategy to combat illegal collection of this species and facilitate the development and maintenance of a confiscation database (see “Coordinated Approach to Tracking Confiscations” below). This group should coordinate with the WTC and Site Leaders throughout the region to implement poaching-deterrence actions.

Federal Trust Permitting Standards for Possession

At present, the Wood Turtle is protected from commercial trade only by state-level regulations and the federal Lacey Act, which prohibits the interstate transportation of wildlife in violation of state law (Jones and Willey 2015). As a result, wild-collected Wood Turtles may be openly traded—and wild specimens laundered—with impunity in any of the ±32 states that do not regulate their possession unless federal authorities have evidence of collection within a native range state. Because the Wood Turtle is not currently a Federal Trust species, the U.S. Fish and Wildlife Service is constrained in the degree to which they can regulate or prohibit the trade in Wood Turtles. This is a substantially different situation than for the Wood Turtle’s congener, the Bog Turtle (*Glyptemys muhlenbergii*), which has been listed federally as Threatened since 1997, for which all specimens in all 50 states require a federal permit or evidence of procurement prior to federal listing. For the Wood Turtle, the burden of proof is on federal wildlife authorities to demonstrate that the animals encountered at point of sale, export, or import, were collected illegally. If the Wood Turtle is listed federally under the Endangered Species Act, it appears likely that the ability of the USFWS to regulate trade would improve, as the same protections in place for the Bog Turtle would be conferred to the Wood Turtle, making it more difficult to collect, trade, and transport wild-caught specimens openly without documentation. If the Wood Turtle is not federally listed, all available regulatory options to require federal permits for the commercial trade and transportation of Wood Turtles should be evaluated. Until new rules or permitting requirements are in place, collecting and poaching and smuggling Wood Turtles from priority sites will remain a major obstacle to successful implementation of the regional Conservation Plan.

Steeper Penalties and Stricter Enforcement of State Regulations

All states and Canadian provinces within the native range of the Wood Turtle, including Ohio, now prohibit the commercial collection of Wood Turtles. However, the penalties for violations can be

relatively minor in states that do not list the Wood Turtle under the authority of their state-level Endangered Species Act. Individual states should consider whether their existing regulations prohibiting the collection, possession, trade, and take of Wood Turtles are sufficient to act as a deterrent. Further, stricter enforcement of existing wildlife regulations was identified by Wood Turtle experts in a survey as the most important action needed to combat poaching and trafficking of Wood Turtles (Appendix IV).

Coordinated Approach to Tracking Confiscations

As already noted above, illegal collection of Wood Turtles for commercial purposes appears to be an existential threat to the persistence of regionally significant populations, but is poorly understood. Understanding the point of origin of confiscated Wood Turtles helps regional partners understand the extent and breadth of the problem, refine methods of reducing collection, and consider repatriation or other conservation actions, but at present, there is no coordinated approach to track confiscations, conduct genetic (and other) assignment tests, or consult with experts who have marked populations of Wood Turtles. Rather, outside of federal investigations, the approach is currently ad hoc. To our knowledge, this regional conservation planning effort (see Part III) is the most thorough attempt to amalgamate and track information about Wood Turtle confiscations and to attempt population assignment using available tools ranging from genetic markers to expert opinion. We recommend a centralized and consistent workflow for Wood Turtle confiscations, as follows. When Wood Turtles are confiscated or suspected to be of illegal origin: (a) repatriate the turtles to a single, designated zoological or research facility in the United States (when detected overseas); (b) assign unique identifying codes to confiscated turtles using passive integrated transponders or plastron photographs (they should not be notched to avoid confusion with marked study animals); (c) obtain blood or (large) toenails from confiscated turtles following regional tissue collection protocols (Appendix VI) and share the tissues with the Wood Turtle Council; (d) engage the Wood Turtle Council by sending photographs and an excel spreadsheet in an attempt to identify the population or origin. Further, we recommend that a centralized database of confiscated Wood Turtles and other threatened turtle species in the Northeast (such as Blanding's and Spotted Turtles), managed by a USFWS field office, will encourage a more organized, effective, and efficient front against poaching. In particular, a centralized confiscation database will assist regional efforts to improve genetic techniques for establishing geographic origin. Moreover, a comprehensive database of confiscations will provide a considerably more accurate estimate of the true demand for Wood Turtle within illegal trading markets. Given that most confiscations are typically made by federal authorities, the U.S. Fish and Wildlife Agency is likely the most appropriate entity for establishing this initiative.

Increase Outreach to Public

Although illegal commercial collection probably results in the most significant and concerning population-level effects (especially within regionally significant populations), incidental collection can also result in Wood Turtle population declines under certain circumstances, such as when the incidental collection is sustained at low levels over long periods, or if it occurs at sensitive natural features such as communal nesting sites, disproportionately affecting nesting females (Garber and Burger 1995; Compton 1999; Jones and Willey 2015). Several FCAs and other sites within the Northeast Wood Turtle Conservation Area Network appear to be susceptible to both types of incidental collection along with sites outside of the CAN and within likely state-level focal areas. For these reasons, the Wood Turtle Council should continue to develop, distribute, and refine a public outreach campaign to improve general awareness about the protected status and regional decline of Wood Turtles. Preliminary materials have been developed and distributed by the Northeast Wood Turtle Working Group (see outreach card, Appendix IX). Another deterrent includes the increased use of cameras within CAN sites and other areas at high risk of illegal collection. These are already in use in New England and Virginia, but have not been publicized. Strategic press releases to highlight the increased use of cameras in regionally significant streams. The USFWS has the geographic scope and capacity to launch a nationwide general campaign to reduce Wood Turtle collection, using public-service announcements as the primary mode of communication.

Improve Assignment Rates

Genomics.—Building upon the microsatellite analysis conducted as a part of this conservation planning effort (Part III), existing samples should be reanalyzed using genomic techniques (SNP) for improved genetic assignment.

Stable isotopes.—Evaluate the feasibility and effectiveness of using stable isotopes to determine population or basin of origin.

Permanent Tissue Repository and Genetics Database

The WTC should finalize and seek funding for a permanent repository for blood and tissues as well as a permanent database of analyzed tissues.

Passive Integrated Transponders

Wood Turtle confiscations occasionally yield individuals that possess marginal scute notches, but conflicting notch systems used by different projects and throughout the years, and a lack of digital photo documentation from early studies can make assigning the origin of these individuals difficult. Increased,

widespread use of PIT-tags will provide a reliable method for identifying confiscated turtles that have been previously captured. Standard use of PIT within FCAs and throughout the range would improve the understanding of where commercial poaching is taking place and provide means for identifying potential “hotspots”. PIT use is underway in New York, Maine, and Massachusetts, and will be expanded in future years of coordination.

Federal Conservation Opportunities

As noted in earlier sections of this Conservation Plan (see Part IV) and in the Site Action Tracking Database (Table 5.1), **relatively few regionally significant Wood Turtle sites and subpopulations occur primarily on federal land**. These federal sites (identified through the CAN site selection process described in Part IV) provide some encouraging long-term conservation opportunities for significant Wood Turtle populations, where other management priorities do not conflict with necessary conservation actions to restore or preserve the natural function of Wood Turtle populations (including isolation of high-use areas from recreation and regular human activity). At these sites, all feasible actions should be undertaken to promote the long-term persistence of Wood Turtles without need for population management (such as headstarting), including: (1) reducing human recreation and regular activity near high-use areas; (2) minimizing collection risk through increased outreach and enforcement; (3) minimizing the risk of machinery and roadkill. In addition to such actions at these sites, several other federal conservation programs can be incorporated into this regional effort to minimize further population declines. These include, but are not limited to, (1) **federal support for a comprehensive anti-poaching strategy**; (2) **prioritized land acquisition and protection**, including targeted USDA/NRCS easements; (3) standardized monitoring on and adjacent to federal lands; (4) prioritized management actions on federal lands and with federal funding.

Federal Support for Land Acquisition

The USFWS should work with state agency leads to guide state-level land protection committees toward federal funding opportunities available for Wood Turtle land acquisition throughout the Northeast. Extreme caution with regard to site-sensitivity should be exercised when communicating and coordinating these activities. When it is determined through standardized monitoring on National Wildlife Refuges (NWR) and federal properties that the most significant or functional Wood Turtle habitat in the vicinity does not occur on the federal property, efforts should be made to acquire the high-use areas.

Standardized Monitoring on and Adjacent to Federal Lands

Through the Conservation Area Network site selection process (Part IV), we have identified FCAs with significant federal land components. We have also identified lower-priority Management Opportunities that encompass NWRs. At NWRs, and on other federal lands where feasible, managers should implement standardized monitoring program using the regional protocol outlined in Appendix V. Standardized monitoring should extend to areas adjacent to federal lands if the most suitable Wood Turtle habitat in the local area does not occur on the federal property. Federal lands managers should consult with state wildlife agency biologists to coordinate notch codes if PIT-tags are not used. Data collected through standardized surveys on federal lands should be shared with the state wildlife agency species lead (usually the state herpetologist or nongame biologist), to be shared with the project managers of the WTC for analysis at periodic intervals, such as for a five-year reassessment of the Conservation Area Network. Standardized monitoring and centralized analysis at intervals provides three basic types of information: (1) contextual population information necessary to identify regionally significant sites in the regional Conservation Area Network; (2) precise information about high-use activity areas such as overwintering sites, nesting areas, early successional aggregation sites, and basking areas; (3) population trend data (if sampled intensively). As new information becomes available for NWRs and other federal properties not sampled during the first phase (RCN, 2012–2013) or second phase (CSWG, 2015–2017) phases of this project, it should be incorporated into management-related decisions including mowing schedules, desired future condition of cover types, public access decisions, outreach materials, logging activity plans, restoration programs, and law enforcement strategies. If higher-resolution information is needed, radio telemetry should be employed.

Natural Resources Conservation Service Programs

In 2017, the NRCS determined that the Working Lands for Wildlife (WLFW) program would extend its purview to encompass the Wood Turtle (as well as Spotted Turtle and Blanding's Turtle) in New England and New York, opening a potential opportunity to facilitate the implementation of Wood Turtle-supportive land management practices, including easements, large riparian buffers, and delayed mowing, at a broad geographic scale. Here, we provide recommendations regarding the prioritization of specific management practices promoted by NRCS, within-state prioritization, and overall geographic scope.

Management agreements.—The delayed sexual maturity (>14 years) and long generation time (35–40 years) of the Wood Turtle preclude the use of short-term management agreements for achieving meaningful conservation outcomes with respect to the long term persistence of the species in a given area. Therefore, we recommend that individual states and respective NRCS offices heavily prioritize the use of

permanent Wetland Research Easements (WRE) and long-term management agreements >30 years wherever possible.

Concentration of efforts.—Given the large homerange, delayed sexual maturity, and long generation time of the Wood Turtle, it is unlikely that a broad “scattershot” approach—where WLFW actions are applied sparsely throughout each state to relatively small parcels of land—would lead to measurable benefits for the subpopulations they are intended to benefit. We recommend that states concentrate WLFW efforts within specified CAN Management Opportunity sites in order to increase the potential for this program to improve the conservation outlook for Wood Turtles within these sites as well as increase the likelihood that measurable population change can be detected in subsequent reassessments.

Broaden geographic scope of WLFW.—Provided that the initial WLFW implementation in New England and New York are judged to be successful, with measurable, stabilizing improvements in population assessments within designated CAN sites, we recommend that NRCS extend the “Northeast Turtles” initiative beyond New York and New England to all Wood Turtle range states (south to Virginia/West Virginia and west to Minnesota and Iowa).

Prioritize Wood Turtle Management on National Wildlife Refuges

Only two Focal Core Areas within the current iteration of the Wood Turtle CAN fall within the boundaries of NWRs. This limited representation of priority sites within NWRs highlights the lack of protection afforded to Wood Turtles by existing federal lands and contrasts with other threatened turtle species in the Northeast such as the Blanding’s Turtle, for which the NWRs demonstrably support a larger proportion of priority habitat (Willey and Jones 2014). However, several NWRs in the northeastern United States are data-deficient due to a lack of detailed population information. In addition to increased monitoring within NWRs, we recommend that NWR-specific management actions be implemented if clear needs (e.g., public access control; law enforcement to minimize collection; mowing mitigation) exist.

Genetics

Additional investigations using samples already collected can improve our understanding of dispersal and connectivity, landscape connectivity priorities, and management units, and improve the accuracy of population assignment following confiscations. Specific research questions are outlined below.

Connectivity and Movement

The extent and mechanisms related to connectivity among Wood Turtle populations and associations with landscape (habitat) attributes needs more investigation to assist conservation planning. Demographic and movement studies should begin long-term efforts to identify individual, longer-range movements. Additionally, the genetic data can be further investigated with a landscape genetics approach to examine correlations among the genetic data, landscape attributes and population demographics. This approach could explore possible habitat or population-related correlates that may be associated with turtle movement among sites that could be important to identify areas where movement may be more critical to population dynamics. For example, the Potomac sites appear to support high movement among sites, whereas the northern Maine sites suggest less movement.

Evolution and Selection

Genomic studies to identify locations on the genome where selection or variation is occurring could inform conservation of the species as well as identify potential threats to Wood Turtles and other freshwater turtles (see Andrews et al. (2016) for conservation applications).

Genomic Sequencing

Single nucleotide polymorphism (SNP) methods should be investigated for potential to identify finer-scale population structure. Panels of approximately 300–500 loci could be developed. Use of these panels would increase the ability to differentiate population groups and would likely increase the success of genetic assignment.

Tissue Repository

The nearly 2,000 blood and tissue samples collected during this project, as well as others collected in the Great Lakes Region and Canada, should be housed permanently at a partner university, such as the University of Montana (where they are stored currently) or the University of Massachusetts Amherst. Regional partners should identify long-term (≥ 10 years) funding to house these materials pending further analysis. Further, partners should establish a permanent database of analyzed tissues to be managed by the WTC.

Emydine Turtle Conservation Symposia

As a component of this CSWG-funded effort, a Wood (and Blanding's) Turtle Conservation Symposium (Appendices III, IV) was held on October 3–4, 2016 at the Massachusetts Division of Fisheries and Wildlife Headquarters in Westborough, Massachusetts. A total of 101 biologists and conservationists

from 58 institutions throughout the eastern United States and Canada attended the symposium. This event provided an opportunity for attendees to share their conservation efforts, participate in discussions about current challenges facing turtle conservation, and initiate new partnerships and collaborations with fellow colleagues. In a post-symposium survey (Appendix IV), a large majority of respondents were in favor of another symposium to continue to improve communication, boost awareness of successful (and unsuccessful) management approaches, and reassess the broad range of threats to these species. We recommend that conservation symposia specifically directed at the conservation needs of the Wood Turtle be held at least every three years. A CSWG-funded Spotted Turtle conservation symposium, geared toward the conservation needs of that related emydine species, is planned to occur in 2019, offering a logical and practical setting under which to hold another Wood Turtle symposium.

Expanded Partnerships

The Midwest Region (USFWS Region 3) and eastern Canadian provinces contain substantial portions of the Wood Turtle species range (see Part I, Fig. 3), and some areas within these regions evidently harbor globally significant subpopulations (i.e., high Wood Turtle densities and diverse age-class structure within high integrity, functioning riparian corridors). The WTC should expand partnerships in these areas with non-profit organizations, universities, and governmental agencies with the overarching goals of improving the flow of information, promoting novel conservation practices, and improving the conservation community's understanding of the global status of the species as a whole. In particular, the 2016 Wood and Blanding's Turtle Conservation Symposium survey (Appendix IV) identified a range-wide email list-serv and a range-wide species status assessment as top priorities for inter-regional coordination. Among other action items, future monitoring work in the Northeast should establish some reference study sites that utilize the primary protocols in use in the Great Lakes region (e.g., OMNRF 2015; Brown et al. 2017)

Several Wood Turtle subpopulations span the international border between the United States and Canada; these may be at greater risk of neglect with respect to conservation efforts because both countries consider the population marginal or peripheral and neither is capable of assessing the cross-border sites entirely. The WTC, and particularly border states in northern New England, should emphasize partnerships with Québec and New Brunswick in priority border regions (see International Coordination Sites, Part IV), and coordinate cooperative monitoring efforts to more effectively assess these subpopulations.

Targeted Research

Climate Change, Stream Temperature, and Dissolved Oxygen

Our assessment revealed that most of the CAN sites near the southern margin of the Wood Turtle range are vulnerable to hypothesized threats associated with rapidly changing climatic conditions. These areas are projected to experience the largest shifts in key climatic variables, including temperature and precipitation, which in turn are expected affect Wood Turtles directly by influencing their seasonal ecology (mating, emergence, nesting), reproductive success, overwintering physiology, foraging efficiency, and trophic relationships. Further, increasing winter and summer temperatures and a changing precipitation regime will exert influence on habitat quality by elevating stream temperatures, which, in turn, can reduce the dissolved oxygen content of streams and influence water chemistry. Conservation Area Network sites in Virginia, West Virginia, and Maryland, including extirpated and degraded sites in the Piedmont, provide a natural laboratory to evaluate the influence of these processes on Wood Turtles *in situ* without conducting a manipulative experiment. However, animals confiscated from illegal markets that cannot be repatriated to their state of origin could also form the basis of experimental tests of the effect of climate change on regionally significant Wood Turtle populations.

Invasive Vascular Plants

Of the dozens of invasive and exotic vascular plants known to occur within regionally significant CAN sites, Japanese Knotweed appears to exert the largest direct, negative influence on Wood Turtle habitat quality. Anecdotal evidence in New England suggests that *F. japonica* expanded its range and distribution following major storm events in 2011 and 2012 (Irene and Sandy), compromising known or expected Wood Turtle nesting areas in Massachusetts and Vermont. While habitat restoration efforts are underway in some areas, including Massachusetts, there is not a targeted research program in place to evaluate whether invasive plant management will be necessary in perpetuity, or whether sites can be restored to the original level of function (in the case of nesting beaches).

Major Stream Restoration Projects

Although large-scale stream and riparian restoration efforts are underway across the northeastern United States, no systematic study has been completed of the response of Wood Turtle populations to such actions as bank restoration, channel restoration, or dam removal. Large-scale projects throughout the Northeast should be cross-referenced early in the design phase to identify feasible and appropriate long-term, standardized, Before-After-Control-Impact studies of individual- and population-level response to large-scale stream restoration. These sites, once identified, should become long-term monitoring sites.

Agricultural Machinery

Agricultural machinery is known to be a primary source of mortality in Wood Turtle populations from Nova Scotia and Québec throughout New England. Most studies of this phenomenon have been purely descriptive (e.g., Saumure 2004; Jones 2009), and the few experimental studies have either not looked specifically at Wood Turtle behavior (Erb and Jones 2011) or did not consider individual response to machinery (Saumure 2004). Wood Turtle populations within CAN sites should be evaluated for potential intensive research on methods and techniques to minimize or reduce mortality during regular agricultural activities. Findings from such a study should be incorporated into Best Management Practices and used to inform NRCS programs.

Semi-Natural Laboratory for Confiscated Animals

The process and procedure for placing animals seized during confiscations has been improvised, haphazard, and ad hoc. One or more facilities should be designated, or constructed, to house confiscated Wood Turtles under standardized guidelines pending their population assignment. Once assigned to a state or basin, the animals should be repatriated to the jurisdictional state agency or housed permanently in the approved facility. As already noted, direct releases of confiscated turtles should only occur following peer review by the WTC, at the discretion of the state wildlife agency. Ultimately, nonreleasable turtles should be housed in a semi-natural enclosure that allows for experimental tests of physiological and behavioral responses to documented and expected threats, ranging from agricultural machinery to climate change.

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Implementation

Site Action Implementation

Conservation action priorities vary dramatically from site to site, with certain sites needing little more than periodic population and invasive species monitoring and other sites requiring extensive human intervention with respect to habitat management, poaching mitigation, and/or other areas highlighted above. To address the logistical challenges associated with the implementation of actions and tracking of progress across this collection of sites, we have developed step-by-step implementation guidelines intended to help streamline CAN site action implementation. All sites identified within the CAN should undergo the following process (Fig. 5.2):

Finalize CAN Site Boundaries

The standardized site mapping procedure was developed (Part IV) based upon existing mapping methodologies in order to objectively delineate the rough site boundaries that would be used to compare and rank >1200 extant Wood Turtle sites throughout the northeastern United States. However, in most cases, further refinement of site boundaries is needed in order to (1) expand boundaries where necessary based on existing data, (2) identify additional terrestrial activity zones, (3) remove unsuitable habitat, and (4) exclude anthropogenic and natural barriers. Final site delineation should at minimum follow the Regional Wood Turtle Mapping Guidelines (Appendix X). Subsequent site delineation will be considered during regional reassessments (see below).

Designate Site Leaders

At every feasible CAN site, a Site Leader will serve as the conservation point person for their respective site and work to (1) identify priority habitat features within sites, (2) finalize site-specific Site Action Plans, (3) guide and oversee the implementation of Site Action Plans, (4) ensure necessary population and habitat monitoring is undertaken in a timely manner, (5) conduct landowner outreach when appropriate, (6) submit progress reports, and (7) assist the WTC in reassessing site populations and adjusting Site Action Plans when necessary. A Site Leader will be designated for each FCA within the CAN as well as high-priority Management Opportunity sites, and other CAN sites where expert opinion deems necessary. Site Leaders will be identified by respective state agency representatives within the WTC. State agency representatives to the WTC will serve as a *de facto* Site Leader when no individual has been proposed.

Identify Key Wood Turtle Habitat Features and High-Use Areas within CAN Sites

Wood Turtle populations rely heavily on combinations of specific habitat and structural features, the availability of which may influence the probability of persistence of a subpopulation. These features include, but are not limited to: (1) hibernacula, (2) nesting areas, (3) early-successional foraging and basking areas, (4) woody material such as logs and rootballs, as well as (5) permanent geologic and hydrographic features that support the occurrence of the preceding features, such as extended areas of moderate stream gradient and glaciofluvial deposits. Site Leaders should attempt to identify and document such features within sites, map them within site geodatabases, rank their condition and availability within the Site Action Tracking Database, and outline specific management needs in the narrative for the Site Action Plan (where feasible). Finally, known or expected high-use areas (aggregations of Wood Turtles exceeding 20 adults) should be identified and mapped.

Complete Site Action Tracking Database for all CAN Sites

State agency biologists and partners should complete as many of the variables within the Site Action Tracking Database as possible. Providing answers for unknown variables should become a priority for Site Leaders.

Develop Spatially-Explicit Site Geodatabases to Track Features and Actions

Site-specific spatially-explicit geodatabases should be developed in ArcMap or Google Earth and housed in a secure location (i.e., password-protected computers and encrypted hard drives). These geodatabases should track all features and actions deemed important by respective Site Leaders and state biologists. Important features may include, but are not limited to, overwintering sites, high-use aquatic and terrestrial areas, ephemeral early-successional habitats, nesting areas, and hiking paths. Actions may include Wood Turtle-oriented management such as nesting area creation and invasive plant treatment, but may also include other activities such as logging and various agricultural practices

Parcel Analysis

Parcel analyses should be conducted for all sites within the CAN in order to identify and prioritize parcels for protection, estimate the potential cost of land acquisition throughout the region, and identify priorities for partnerships with landowners, land managers, non-profit organizations, land trusts and other entities. Parcel analysis should first incorporate the best available spatially-explicit information about key features and high-use areas.

Develop and Finalize Site Action Plan for all CAN Sites

WTC members and Site Leaders will use “Site Action Narratives” and additional information to develop and finalize a 5–7 year Site Action Plan. Site Action Plans will serve as the primary guiding document for achieving respective conservation objectives for each site. Thus, Site Action Plans will be the framework through which the overarching goal of this Conservation Plan will be achieved. Key actions identified within Site Action Plans will be clearly defined using result chain diagrams (Reynolds et al. 2016) when necessary and prioritized with respect to both conservation importance for the Wood Turtle population at hand as well as feasibility.

Site Action Plan Implementation and Annual Progress Reports

Site Leaders will guide the implementation of objectives outlined within Site Action Plans. Annually, Site Leaders will provide progress updates to the WTC. Site Leaders will also convene targeted meetings with key landowners, non-governmental organizations, and interest groups to design site-specific conservation or preservation outcome, as feasible.

Reassessment

FCAs should be reassessed at 7–10 year intervals within the context of regional reassessments. Reassessments will involve renewed sampling efforts to evaluate population statuses, site-specific and overall trends, as well as existing and emerging threats to the species (and sites). Upon reassessment, Site Action Plans will be adjusted accordingly.

Conservation Area Network Site-Level Planning & Implementation

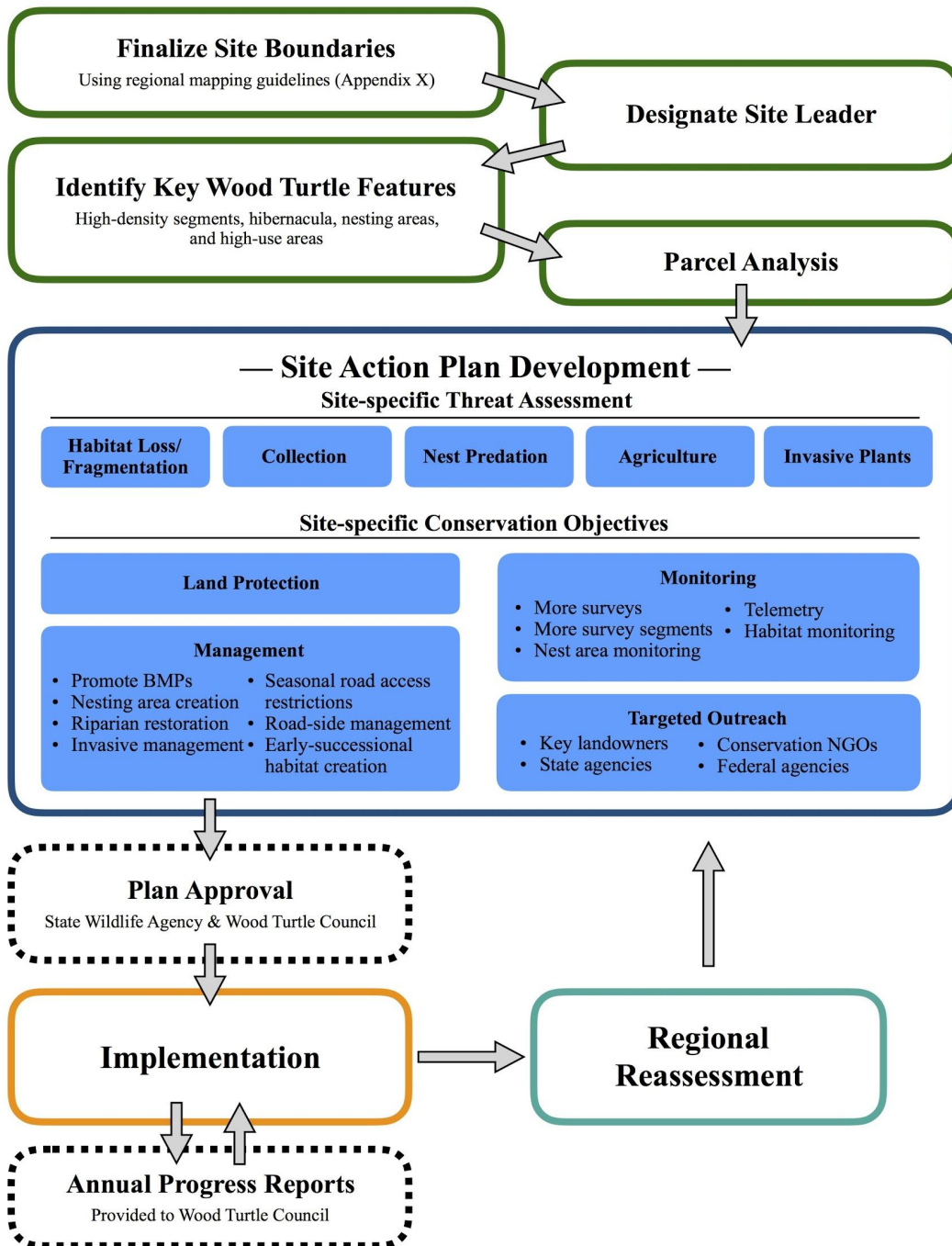


Figure 5.3. Generalized flowchart of the proposed Conservation Area Network site-level implementation process.

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