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The Influence of Altered Habitat: Landscape Ecology of Freshwater Turtles in Rhode Island

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THE INFLUENCE OF ALTERED HABITAT: LANDSCAPE ECOLOGY OF FRESHWATER TURTLES IN RHODE ISLAND

BY

SCOTT W. BUCHANAN

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OF

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Abstract

Beginning several centuries ago, European settlement brought great change to the landscape of New England. Vast deforestation, subsequent reforestation, and rapid development that continues to this day have had a profound impact on wildlife populations. Elements of this legacy of landscape change have been studied, but the ways in which past and current landscape patterns affect freshwater turtles remains poorly understood. The primary objective of this research was to better understand the influence of the landscape of Rhode Island on populations of freshwater turtles. It is in many ways a work in landscape ecology, but with components of spatial ecology, habitat selection, and population genetics.

Chapter 1 is a stand-alone, opportunistic study of the effects of a small forest clear-cut on a population of spotted turtles (*Clemmys guttata*). We conducted a radio-telemetry study for one year before, and one year after a 3-ha forest clear-cut in close proximity to wetlands known to contain a resident population of the species. The annual home range size of turtles was 18.5% larger post-cut, possibly due to changes in the distribution of resources and suitable habitat after the harvest. However, turtles exhibited fidelity to hibernacula and communal hibernation despite nearby disturbance, and patterns of activity and habitat use were similar in both years and were generally consistent with those of other spotted turtle populations. Our results suggest that timber harvesting of this spatial scale and management approach may not have any short-term effects on the spatial ecology or habitat use of populations of spotted turtles. It is a strong caveat though, that further research is needed to understand longer-term effects.

Chapters 2, 3 and 4 consist of data collected during a state-wide sampling effort of freshwater turtles in small, non-riparian wetlands across a gradient of forest cover. By systematically and intensively sampling 88 randomly selected wetlands across this gradient, we intended to capture the variability in landscape composition and configuration found in Rhode Island and determine how this variability is related to species distribution, abundance, demography, and population genetic structure. In Chapter 2 we report abundances based on capture per unit effort, and use occupancy analysis to determine which among a suite of environmental and within-wetland covariates are driving species occurrence. Eastern painted turtles (*Chrysemys p. picta*) and snapping turtles (*Chelydra serpentina*) were widespread (occurring in 83% and 63% of wetlands, respectively), relatively abundant, and exhibited wide niche breadth. Spotted turtles were far less common, occurring in 8% of wetlands, and exhibited a strong association with forested, shallow, natural (i.e., not manmade or heavily modified) wetlands. Non-native red-eared sliders (*Trachemys scripta*) occurred in 10% of wetlands and exhibited a strong, positive association with road density, likely as a function of human population density. In Chapter 3 we further examine eastern painted turtle abundance, demography, and the relationship between sex ratio and road density. There was no difference in abundance or any demographic trait between natural and manmade wetlands. A negative relationship between abundance and forest cover surrounding wetlands emerged as the best model, but explained very little variation. Contrary to expectations, there was a significant, but weak relationship between increasing road density and the proportion of females in a population. Collectively, these results suggest that eastern painted turtles are exhibiting little to no detectable variation in population demography across the range of landscapes found in Rhode Island and are resilient in the face of human-induced landscape change. Finally, in Chapter 4 we used microsatellite markers to compare the population genetic structure between the common and widespread eastern painted turtle, and the rare and more specialized spotted turtle. Due to their relative rarity and smaller populations, we predicted that spotted turtles were more likely to have experienced the detrimental effects of habitat loss and fragmentation associated with landscape change, and that these effects would manifest in the form of more inbreeding, reduced genetic diversity, and greater population genetic structure. As expected, eastern painted turtles exhibited little population genetic structure, showing no evidence of inbreeding or strong differentiation among sampling sites. For spotted turtles however, results were consistent with certain predictions and inconsistent with others. We found tentative evidence of recent population declines in spotted turtles, as well as a greater degree of inbreeding in the species when compared to eastern painted turtles. Genetic diversity and differentiation among sites were comparable between species, however. As our results do not suggest any major signals of genetic degradation in *C. guttata*, the southern region of Rhode Island may serve as a regional conservation reserve network where the maintenance of population viability and connectivity is prioritized.

Globally, turtles are among the most threatened of vertebrate taxa. Information on how populations respond to human-induced landscape change has important implications for conservation. The work herein will provide wildlife biologists with a better understanding of the current state of populations of freshwater turtles in the state and region, and allow for more informed management decisions.

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Dedication

For Sarah, who was there at the beginning and is here at the end. Thank you for having the patience and optimism to see this thing through. And thanks for being the best part of every day.

Preface

This dissertation has been prepared in manuscript format as specified by the University of Rhode Island Graduate School guidelines. All chapters have been or will be submitted to peer-reviewed journals in a form similar to their current state. Chapter 1 has been through the peer review process and will appear in a forthcoming issue of Herpetological Conservation and Biology. Chapters 2, 3 and 4 have been formatted to Conservation Biology, Journal of Wildlife Management, and Conservation Genetics styles, respectively, and will be submitted at a future date.

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Manuscript I: Responses of a Spotted Turtle (*Clemmys guttata*) Population to Creation of Early-successional Habitat

Accepted in Herpetological Conservation and Biology, August 2017

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Abstract

The maintenance or creation of early successional habitat is commonly employed by natural resource managers, often for the benefit of native wildlife. In southern New England, USA, forest succession has reduced the amount of early successional habitat on the landscape making the creation of such habitat a management priority in the region. However, questions remain regarding the impacts of the creation of early successional habitat on certain species, especially those that are associated with late successional habitats. We conducted a radio-telemetry study of Spotted Turtles (*Clemmys guttata*) in Rhode Island, USA, for one year before, and one year after a 3-ha forest clear-cut in close proximity to wetlands known to contain a resident population of the species. The annual home range size of turtles was 18.5% larger post-cut, possibly due to changes in the distribution of resources and suitable habitat after the harvest. However, turtles exhibited fidelity to hibernacula and communal hibernation, despite nearby disturbance, and patterns of activity and habitat use were similar in both years and were generally consistent with those of other Spotted Turtle populations. Our results suggest that timber harvesting of this spatial scale and management approach may not have any short-term effects on the spatial ecology or habitat use of populations of Spotted Turtles, but further research is needed to understand longer-term effects. We strongly recommend that the timing of clear-cut harvesting be restricted to outside of the region-specific activity season of this species and that land managers avoid significant disturbance to wetlands containing Spotted Turtles, especially those containing hibernacula.

Introduction

Habitat alteration can be an important component of wildlife management (Russell et al. 1999; Degraaf et al. 2006). The maintenance or creation of early successional habitat via mowing, prescribed burns, and clear-cuts is commonly employed by natural resource managers to benefit native wildlife (Greenberg et al. 1994, Van Dyke et al. 2004), including some birds (Degraaf and Yamasaki 2003), mammals (Litvaitis 2001; Fuller and DeStefano 2003), and reptiles (Dovĉiak et al. 2013). In southern New England of the United States, the abandonment of agricultural fields that occurred in the first half of the 20th Century led to an increase in early successional habitat. The gradual process of forest succession that followed however, has greatly reduced the amount of early successional habitat on the landscape (Foster and Aber 2004; Buffum et al. 2011). State wildlife agencies and conservation groups have made the creation of early successional habitat a priority in the region because of its benefits to many species of wildlife including shrubland birds and particularly to the New England Cottontail (*Sylvilagus transitionalis*; Schlossberg and King 2007; Buffum et al. 2014; Fuller and Tur 2015). However, questions remain regarding the effects of early successional habitat creation on certain species, especially those that are associated with mature, forested habitats.

Although several studies have reported impacts of timber harvesting on reptiles (Enge and Marion 1986; Todd and Andrews 2008; Moorman et al. 2011), including turtles (Currylow et al. 2012), to our knowledge none have focused on how freshwater turtles respond to forest clear-cutting. This may be less important for highly aquatic turtles that make only occasional upland movements, for example, to an open area to nest. However, some freshwater turtle species, including the Spotted Turtle (*Clemmys guttata*) and the

Wood Turtle (*Glyptemys insculpta*), move frequently between ephemeral and permanent wetlands and are known to estivate terrestrially, with some Spotted Turtles spending as much as 30% of their time on land (Milam and Melvin 2001) and Wood Turtles as much as 40% of their time (Arvisais et al. 2004). Use of upland habitats by some forest and wetland-associated turtle species may make them vulnerable to forest alteration if habitat is destroyed or fragmented. Alternatively, the removal of the forest canopy for the creation of early successional habitat may create new microhabitats suitable for thermoregulation and nesting.

The Spotted Turtle is a species of increasing conservation concern. The International Union for the Conservation of Nature (IUCN) reviewed the species in 2013 and upgraded its status from Vulnerable to Endangered (van Dijk 2013). In five of the six New England states where it occurs, the Spotted Turtle has been designated with some type of conservation protection and the status of the species is currently under review by the U.S. Fish and Wildlife Service (USFWS) for federal listing under the U.S. Endangered Species Act (USFWS 2015). Spotted Turtles are relatively small (carapace length up to 142.5 mm) freshwater turtles that are native to the eastern United States and Great Lakes regions of North America. They occur in a variety of wetland types throughout their range and have sometimes been described as habitat generalists (Ernst and Lovich 2009). However, Spotted Turtles have also been shown to exhibit strong habitat selection based on the physical and biological conditions of their environment (Milam and Melvin 2001; Anthonysamy et al. 2014). This selection is detectable at multiple spatial scales and can vary with season and by sex (Litzgus and Mousseau 2004; Rasmussen and Litzgus 2010). Spotted Turtles are often described as semi-aquatic because they use both wetland and upland habitats. They spend the majority of their time in wetlands and depend on these habitats for overwintering, foraging, thermoregulation, and mating (Milam and Melvin 2001; Ernst and Lovitch 2009). Most individuals exhibit high fidelity to wetlands, often overwintering in the same hibernaculum each year (Litzgus et al. 1999; Ernst and Lovich 2009). Spotted Turtles use uplands for nesting and moving between wetlands, and both sexes spend extended periods of time in upland habitat estivating in shallow forms or underneath leaf litter during the warmest periods of the summer (Joyal et al. 2001; Gibbs et al. 2007). Thus, uplands are essential to this species and concern is raised when these habitats are to undergo significant alteration. In Rhode Island, USA, Spotted Turtles are a strongly forest-associated species (Chapter 2), but the implications for the removal of forest surrounding wetlands where they occur is unknown.

We investigated the potential impacts of a clear-cut timber harvest that took place within close proximity to a complex of wetlands in southern Rhode Island that is known to contain a population of Spotted Turtles. We radio-tagged individuals in this population for one year prior to, and one year after, a clear-cut that was implemented to create early successional habitat for wildlife. Our objectives were to examine the effects of forest clear-cutting on Spotted Turtle spatial ecology, activity, and habitat use.

Materials and Methods

Study site.—Our study took place in Washington County, Rhode Island, USA. We have withheld specifics of the location out of concern for making this population of Spotted Turtles vulnerable to collection. Mean annual temperature in the area (Kingston, Rhode Island) is 10.5° C and mean annual precipitation is 134.3 cm (National Oceanic and Atmospheric Administration [NOAA] National Centers for Environmental Information. Available from http://www.ncdc.noaa.gov [Accessed 1 March 2016]). The study area consisted of an arrangement of mowed fields, upland forest, freshwater wetlands, and shrub-dominated habitats along a powerline right-of-way. Management was generally limited to trail maintenance, mowing, and seasonal deer hunting. Soils consist of predominantly fine, sandy loam (U.S. Department of Agriculture. 2016. Web Soil Survey. Available from http://websoilsurvey.sc.egov.usda.gov [Accessed 1 August 2016]). A mosaic of permanent and temporary wetlands were distributed throughout the site consisting of *Sphagnum* Bog, emergent shrub wetlands, and forested vernal pools. Adjacent second-growth forest consisted of an Oak-Maple overstory and a wetland-associated shrub understory. The most common species of understory woody vegetation found throughout the study area in descending order of occurrence were Highbush Blueberry (*Vaccinium corymbosum*), Common Winterberry (*Ilex verticillata*), Sheep Laurel (*Kalmia angustifolia*), Coastal Sweetpepperbush (*Clethra alnifolia*), and Northern Bayberry (*Morela pensylvanica*).

Beginning in December of 2013 and concluding in February of 2014, while turtles were inactive in aquatic hibernacula, approximately 3 ha of mature forest was harvested to create early successional habitat using a Clear-cut with Reserves approach (Miller et al. 2006). The cut retained approximately eight residual trees per hectare to serve as seed trees and sources of food for wildlife. Large amounts of coarse woody debris were left on the ground to reduce deer browse and six large brush piles were created for wildlife habitat. No herbicides were applied after the cut and no rutting or erosion was observed after the cut. The shape of the cut was irregular and a buffer of at least 15.2 m (50 feet) was retained around all wetland habitat (Fig. 1.1 and 1.2).

Radiotelemetry and data collection.—We captured Spotted Turtles using baited hoop traps and by hand. We attached RI-2B 6g radio transmitters (Holohil Systems Ltd., Carp, Ontario, Canada) with waterproof putty epoxy to the right-posterior of the carapace. The combined mass of transmitter and epoxy averaged approximately 6% of body mass and did not exceed 8%. Following transmitter attachment, we released all individuals at their original points of capture. We used an ATS R410 receiver (Advanced Telemetry Systems, Isanti, Minnesota, USA) and a three-point Yagi antenna to track turtles. We recorded geographic coordinates (Universal Transverse Mercator; North American Datum of 1983) for each turtle radio-location using a Garmin Oregon 450 handheld global positioning system receiver (Garmin International Inc., Olathe, Kansas, USA). We conducted radio-telemetry for one season before (2013) and one season after (2014) the implementation of the clear-cut. We radio-tracked turtles approximately once every 5 d (mean = 5.35 ± 0.11 [SE] d, n = 655 intervals) between 1 April and 31 October, and less frequently in the early spring and late fall. We classified radio-locations into one of three categories based on the precision of the detection of the turtle. If we found a turtle and actually saw it, we classified the radio-location as Visual. If we obtained a signal and identified the location to a small area (a few square meters) without use of the telemetry antenna (i.e., using just the receiver), we classified the radio-location as Exact. If we obtained a signal and we estimated the location using the telemetry antenna, we considered the radio-location as Approximate, in which case we used triangulation to confirm that turtles were within wetlands.

We measured midline carapace length (mm) using analog calipers and we measured initial body weight (g) using a digital scale. We obtained daily maximum temperature (° C) and precipitation (mm) data for 1 April to 31 October in both years from a representative weather station (Kingston, Rhode Island; NOAA, National Centers for Environmental Information. *op. cit.*). We used these data to obtain annual means (for temperature) or sums (for precipitation) and we determined averages to compile weekly means over the course of the activity season. We conducted an initial forest inventory of the clear-cut area in October 2013 after the clear-cut area had been delineated but before logging operations began, and a second inventory after the logging was complete in October 2014. In both cases we assessed the vegetation at 56 locations along parallel transects spaced equal distances apart. We used 2 m^2 fixed area plots to record frequency of occurrence of understory vegetation, and variable area plots to measure diameter at breast height and density of overstory vegetation. We measured overstory tree canopy cover at each point using a spherical densiometer.

Home range and habitat use.—We categorized all turtle radio-locations as occurring in either wetland or upland habitat. We calculated percentage wetland use by dividing the number of radio-locations that occurred in a wetland by the total number of radio-locations. For all upland radio-locations, we calculated the distance to the nearest wetland edge. The lack of consistent, precise radio-locations (particularly when turtles were in wetlands) made it impossible to calculate distance between radio-locations throughout the activity season, but did not preclude the calculation of home range size estimates. We estimated home range sizes using minimum convex polygons (MCPs). MCPs are widely used in home range analyses of reptiles and have been used in multiple studies of Spotted Turtles making them the most useful for comparison with other studies (Litzgus and Mousseau 2004; Row and Blouin-Demers 2006). We included Approximate radio-locations in the construction of MCPs, as these were the majority of locations because many turtles were located in the interior of a wetland and their precise location could not be determined. The majority of these points fell within the interiors of constructed polygons and did not influence MCP size. We also inspected all radio-locations for each turtle and manually removed points from the home range analysis that were ambiguous or erroneous due to transcription errors (n = 7 points).

We calculated overall home range size and overall percentage wetland use by combining all available data from both years. In addition, to examine both home range fidelity and potential differences pre- and post-clear-cut, we calculated annual home range size and annual percentage wetland use in 2013 and 2014 and compared these data between years. To maximize the comparability of these metrics between years, we also calculated constrained post-clear-cut values by constricting the radio-locations used to the range of dates when turtles were tracked in both years. We estimated annual home range percentage overlap between years to compare potential changes in resource use overall and between sexes. For all turtles tracked in both years, we divided the common area of both MCP polygons (one for each year) by the total merged area of both polygons. We used all available radio-locations to estimate annual home range overlap. We also determined all instances in which an individual used any of the area inside the delineation of the clear-cut, in a given year, by identifying all the instances in which an annual MCP (constrained MCP for post-cut) overlapped the area of the clear-cut.

Statistical analyses.—We assessed normality using Shapiro-Wilk tests and equality of variances using Levene's tests. All data were normally distributed and homoscedastic. We used paired *t*-tests to compare home range sizes and percentage use of wetlands pre-and post-clear-cut. We used an independent samples *t*-test to determine if home range sizes differed by sex pre-and post-clear-cut, using the difference between pre- and post-clear-cut MCP as the dependent variable. The paired *t*-tests and the independent samples *t*-test used observations only from individuals tracked in both years (n = 9), and the post clear-cut observations were constrained to the dates when turtles were tracked in the previous year. We compared overall home range size and annual home range percentage overlap between males and females using independent samples *t*-tests. We used linear regression to examine the relationships of body size (midline carapace length) and the number of radio-locations, and of body size and overall home range size. We compared overall percentage of wetland use between sexes with an independent samples *t*-test. For descriptive statistics, we report means ± one standard error (SE), and we defined statistical significance as $P \leq 0.05$. We calculated MCPs and distance to nearest wetland using Geospatial Modeling Environment (version 0.7.3.0, www.spatialecology.com/gme, Accessed 15 January 2013) and ArcGIS 10.2. All other statistical analyses were performed using R (R Core Team 2013).

Results

Radiotelemetry and data collection.—We tracked 12 turtles over the 2 y (six females, six males), nine of which (four females, five males) were tracked in both years (Table 1.1). We logged 712 radio-locations with a mean of 59.3 ± 5.1 (SE) radio-locations per individual. Tracking of individuals began in late May or early June in 2013, and March or April in 2014 (Table 1.2). We directly observed turtles in 24% of radio-locations (172/712), identified locations without visual observation (Exact) in 20% (143/712) of radio-locations, and estimated locations using triangulation (Approximate) for 56% of radio-locations. Approximate radio-locations occurred almost exclusively when turtles were in interior sections of a wetland.

Home range and habitat use.—Mean constrained annual home range was 18.5% larger post-cut (mean = 1.41 ± 0.21 ha, n = 12) than pre-cut (mean = 1.19 ± 0.27 ha, n = 9), but the difference was not significant ($t = -2.02$, df = 8, $P = 0.078$). The mean difference between pre- and post-cut constrained annual home range was larger for females (mean = -0.74 ± (SE) 0.31 ha, n = 4) than for males (mean = -0.09 ± (SE) 0.16 ha, n = 5), but this difference was not significant ($t = 1.84$, df = 4.7, $P = 0.128$). Between years, mean annual home range overlapped by 56.6 % (± (SE) 3.2 %, n = 5) for males, 29.9 % (± (SE) 5.8 %, n = 4) for females and 44.8 % (± (SE) 6.1 %, n = 9) for both sexes combined. Overlap between years by males was significantly higher than that of females ($t = -2.86$, df = 3.8, $P = 0.048$). Spotted Turtles exhibited a mean overall home range of 1.95 ha (± (SE) 0.26 ha, n = 12, range = 0.59–4.07 ha), and mean female home range size (2.04 ± (SE) 0.46 ha, n = 6) did not differ from mean male home range size (1.85 ± (SE) 0.30 ha, n = 6; $t = 0.362$, df = 8.7, $P = 0.73$). We found no relationship between overall home range size and number of radio-locations ($r^2 = 0.04$, $t = 0.61$, $P = 0.56$), or between overall home range size and carapace length ($r^2 = 0.01$, $t = 0.37$, $P = 0.72$). One female (turtle C) moved outside of the study site in 2014, yielding an underestimate of home range size for that year as we were not allowed access to the adjacent property.

Mean overall wetland use was 84.6 % (± (SE) 3.4 %, n = 12) and mean wetland use did not differ (*t* = -1.95, df = 9.5, *P* = 0.079) between males (mean = 90.4 ± (SE) 3.7 %, n = 6) and females (mean = 78.7 ± (SE) 4.7 %, n = 6). Three turtles were found exclusively in wetlands. However, each of these individuals were radio-tracked in different, discontinuous wetlands, indicating that they too made terrestrial movements during the activity season. There was no difference (*t* = -0.994, df = 8, *P* = 0.35) in annual wetland use between 2013 (mean = 82.9 ± (SE) 5.8 %, n = 9) and 2014 (mean = 83.2 ± (SE) 4.1 %, n = 12), but persistent use of upland habitat occurred later in 2014 by approximately three weeks (Figure 1.3).

Turtles moved from hibernacula in mid- to late-March and appeared to congregate in nearby vernal pools. Seven of 12 (58 %) tracked turtles were found in the same small vernal pool (~0.05 ha) in the same two-week period of May 2014 and as many as five turtles were found in the vernal pool on the same day. Annual home range overlapped with the clear-cut delineation in 4 of 9 (44.4%) instances in 2013, and with the clear-cut in 8 of 12 (66.7%) instances in 2014 (Figure 1.4). There were only two confirmed observations (i.e., visual or exact) in 2013 (late July, mid-August) of individuals using the area of the clear-cut prior to cutting, and both involved estivation in which turtles were buried below vegetation and leaf litter. There were no confirmed observations of individuals in the clear-cut area in 2014, after the trees were harvested. Spotted Turtles found in uplands occurred a mean distance of 7.56 m (± (SE) 5.42 m, n = 107, range = 0.1–33.4 m) from the nearest wetland. Of these observations, 11 % occurred at a distance greater than 15.2 m (50 feet) from the nearest wetland (the buffer distance mandated by state wetland regulations).

Turtles hibernated exclusively in wetlands. Several individuals exhibited fidelity to hibernacula and we observed the use of communal hibernacula in both years (Table 1.2). Of the six instances in which individuals were tracked to hibernacula in both years, four individuals (67 %) used the same hibernaculum. Another individual hibernated in different locations within the same wetland. All turtles occupied hibernacula by 12 November in 2013, and by 28 October in 2014. Turtles remained in the uplands as late as 31 October in 2013, and as late as 14 October in 2014. Hibernation sites were all associated with *Sphagnum* hummocks and/or the roots of woody shrubs. An untracked Spotted Turtle was found dead in the adjacent mowed field on 28 October 2014, suggesting use of the field at some time of the year. The turtle was decomposed, so it was not clear how long the turtle had been dead, but the shell remnants were found in many pieces suggesting that it had been crushed.

From 1 April to 31 October, mean daily maximum temperature was 21.5° C (range = 5.8–33.4° C) in 2013 and 21.0°C (range = 6.9–29.7° C) in 2014. Total precipitation was 69.3 cm in 2013, and 56.9 cm in 2014 (Figure 1.5). Basal area of trees in the clear-cut was 17.3 m^2/ha in 2013 prior to harvest, and 5.3 m^2/ha in 2014 after harvest (Table 1.3). Average canopy cover of the area was 76 % in 2013, and 35 % in 2014. Clear-cut border trees and a few remaining seed trees contributed to post-clear-cut estimates of canopy cover.

Discussion

Home range and movements.—The duration and timing of the activity season was consistent with other observations of Spotted Turtles at the northern portion of their range (Haxton and Berrill 2001; Beaudry et al. 2009; but see Milam and Melvin 2001). Surface activity began in mid- to late-March and ceased in late October or early November, after which turtles entered wetland hibernacula. Overlap of annual MCPs with the delineation of the clear-cut in both years suggests that turtles used the area both before and after the cut took place. Spotted turtle home range size was nearly 20% larger post-clear-cut, but lack of a statistical difference precludes a clear interpretation of this result, particularly given our relatively small sample size. Habitat alteration can cause wildlife to travel greater distances to locate necessary resources, which for turtles may include food items, mates, thermoregulatory habitat, nesting habitat, and overwintering habitat. However, the creation of early-successional habitat (such as a clear-cut) could also create new opportunities for thermoregulation and nesting, thereby reducing the distance required to locate these habitat types. Open areas including power line rights of way and recent clear-cuts have been used by Spotted Turtles for nesting (Litzgus and Mousseau 2004). Whether a habitat alteration serves to expand or reduce home range size probably depends on the proximity of the alteration to established home ranges as well as the nature of the alteration itself. Spotted Turtle home range size increased after disturbance in the form of flooding by Beaver (*Castor canadensis*) dams, but probably because the turtles were using newly available aquatic habitat (Yagi and Litzgus 2012); the flooding was interpreted as beneficial to this population of Spotted Turtles in Ontario.

We detected a difference in annual home range overlap between sexes. Male turtles exhibited greater overlap between years, suggesting a higher fidelity to specific sites. If males can reliably locate females for mating during early spring congregations, the additional distances a male must travel are potentially limited to those where it can find food, thermoregulatory habitat (e.g., for basking and estivation) and hibernation habitat. In addition to these types of movements, females must also locate nesting habitat. As a proportion of female Spotted Turtles in a population do not breed every year (Litzgus and Brooks 1998; Ernst and Lovich 2009), differences in reproductive condition between years may explain the observed differences in annual home range overlap in females. Alternatively, the clear-cut may have influenced female movements by altering habitat selection. The clear-cut could have created new areas that had potential to serve as nesting and thermoregulatory habitat. Females may have moved greater distances while seeking out these newly available sites. Determining the proximate effects of a given habitat alteration is difficult. Our inference is limited in this case due to insufficient information (e.g., reproductive condition of females), the lack of additional treatment and control sites, and the fact that our data are limited to one year before, and one year after the clear-cut.

Spotted Turtles exhibited smaller home range sizes at our study site in Rhode Island than those from populations of Spotted Turtles in Massachusetts (Milam and Melvin 2001), South Carolina (Litzgus and Mousseau 2004), and Ontario (Rasmussen and Litzgus 2010), but were larger or comparable to those of other studies (Ernst 1970; Wilson 1994; Graham 1995). Differences in home range size among studies are usually attributed to distribution and density of resources (i.e., food items, critical habitat, and mates) on the landscape. Intermediate home range sizes suggest a moderate density of resources at our study site. Males and females exhibited similar overall home range size. In turtles, males generally engage in larger movements during the mating season to locate mates, and females exhibit larger movements during the nesting season to locate nest sites (Morreale et al. 1984; Parker 1984). Movements of Spotted Turtles do not always follow this pattern, though. Early season congregations in Spotted Turtles appear to be common (Ernst 1967; Milam and Melvin 2001) and likely take place for breeding purposes (Litzgus and Mousseau 2004), thus limiting the distance that males must travel to actively search for mates. Larger home range sizes were observed for gravid females in South Carolina (Litzgus and Mousseau 2004), and results of other studies support the idea that gravid females exhibit larger home ranges because they must find appropriate nesting habitat (Haxton and Berrill 1999; Milam and Melvin 2001; but see Rasmussen and Litzgus 2010). The fact that we did not observe a difference in home range size between sexes may be due to an absence of gravid females, or the fact that appropriate nesting habitat existed in close proximity to wetlands used throughout the activity season. We suspect that, among populations, the location and configuration of appropriate nesting habitat plays a large role in the home range sizes of females.

Habitat use.—Turtles used wetlands with much greater frequency than uplands. Most likely, the majority of observations of upland use were associated with summer estivation, possibly influenced by water levels in ephemeral wetlands (Milam and Melvin 2001; Rasmussen and Litzgus 2010). Vernal pools in the area dry in late June through late July, and increased use of upland areas may reflect decreases in available wetland area. Overall wetland use was consistent between years, but the shift from wetland use to persistent use of uplands occurred about three weeks later in 2014. Total precipitation was greater in 2013 though, and data from a different study confirms that 2014 was a drier year in small wetlands state-wide (Scott Buchanan, unpubl. data). Thus, the timing of wetland drying does not explain the difference in timing of upland use between years, which remains unexplained. Future studies should investigate what factors influence the shift between wetland use and upland use for this species.

Upland areas surrounding wetlands, often termed buffer zones or core terrestrial habitat, are important for ensuring the protection of wetland fauna that use both habitat types. Use of upland areas appears to be variable among populations of Spotted Turtles. In 12 instances (approximately 11 % of upland radio-locations), turtles in our study were found in upland areas beyond the protected buffer of 50 ft (15.2 m) required for ‘perimeter wetlands’ (pond area > 0.10 ha [0.25 ac] and standing water for ≥ 6 mo/y) under the Rhode Island Fresh Water Wetlands Act (Rhode Island Department of Environmental Management 1998). In addition, there were many instances in which individuals moved from one wetland to another, and in doing so used upland habitat outside of the regulatory buffer zone. In our study population, current RI regulations would not be adequate to ensure that upland habitat used by Spotted Turtles was protected from development projects or other activities that would result in the destruction or fragmentation of upland habitat. In Massachusetts, > 90% of Spotted Turtles nested or estivated outside the 30 and 60 m upland buffer zones (for palustrine and permanently flowing wetlands, respectively) stipulated by Massachusetts wetlands regulations at the time of study (Milam and Melvin 2001). In Ontario, one population of Spotted Turtles nested between 2–139 m from a wetland (Rasmussen and Litzgus 2010). In contrast, individuals in another population in Ontario were described as rarely observed farther than 2 m from a wetland except in instances of nesting or movements between areas (Haxton and Berrill 1999); the study did not quantify these distances. A review of aquatic turtle nesting data estimated that a core area of 127 m surrounding wetlands would be required to protect 95% of Spotted Turtle nests (Steen et al. 2012). Our results and those of other studies of Spotted Turtle habitat use suggest that protection of upland habitat around wetlands is important to ensure that habitat used for nesting, thermoregulation, and movement between sites is not compromised.

Spotted turtles hibernate in wetlands, hibernate communally, and show fidelity to overwintering sites (Litzgus et al. 1999; Ernst and Lovich 2009). Most (66%) of the individuals tracked to hibernacula in both years exhibited fidelity to hibernacula. This level of fidelity is comparable to other studies of Spotted Turtles at undisturbed sites in Ontario (Haxton and Berrill 1999; Litzgus et al. 1999; Rasmussen and Litzgus 2010), and suggests that turtles were able to navigate to and from specific wetlands, even after the dramatic alterations to our study site associated with the clear-cut. Wetland habitat is critical to this species and, from the perspective of conservation, the protection of wetlands containing Spotted Turtle hibernacula is of preeminent importance.

Management implications.—Overall, our observations should be considered descriptive. Our data are limited to one year before, and one year after the clear-cut at only one study site. Multiple years of data collection, both before and after the cut, would have improved our ability to gauge the direct influence of the clear-cut by establishing interannual variation for the ecological parameters of interest under both conditions. In addition, monitoring populations at control sites that did not undergo a clear-cut would have been helpful in establishing inter-population variation in home range size and timing of upland use (Currylow et al. 2012). We did however document potential effects of the clear-cut; marginally larger home range sizes in 2014 could have come as a result of the clear-cut. But whether home range sizes were larger for positive (e.g., new opportunities for nesting or thermoregulation) or negative (e.g., more area needed to obtain resources) for this population of Spotted Turtles remains an open question. Nonetheless, our data suggest that timber harvesting of this intensity (i.e., percent of forest removal and management practices carried out) and spatial scale may be compatible with maintaining populations of Spotted Turtles, even when the harvest takes place in close proximity to wetlands where the species occurs. However, the spatial configuration of the clear-cut relative to wetland habitat is probably an important factor to consider. Although the clear-cut did come very close to several wetlands containing Spotted Turtles, the continuity of forest north of the rights of way (where turtles spent the majority of their time) remained largely intact (see Figure 1.1) and no wetlands were completely fragmented. A larger cut or a cut that completely fragmented individual wetlands may have had a more dramatic effect on turtle movements. In addition, the availability of longer-hydroperiod wetlands at our study site may have ameliorated some of the effects of the clear-cut. The study site contains several vernal pools, which dry nearly every year, and one permanent wetland on the site and another just off-site. Permanent wetlands in the area of the study provide refugia for turtles as vernal pools dry, probably reducing the need for long-term estivation in upland sites, as has been documented in other populations (Litzgus and Brooks 2000; Beaudry et al. 2009). Thus, a clear-cut similar to this one is probably less likely to impact Spotted Turtle populations where turtles are able to move from ephemeral into permanent wetlands during the hottest and driest parts of the activity season.

To our knowledge, this is the first study to investigate responses of Spotted Turtles to creation of early successional habitat. Given that more than 3,300 ha of early successional habitat was created for New England Cottontail in six northeast states in 2013 and 2014 (Fuller and Tur 2015), we are encouraged that we did not detect major impacts of this activity on the turtle population in our study. However, we strongly recommend that the spatial arrangement and hydroperiods of wetlands near a proposed clear-cut area be investigated prior to commencement of operations and that the entire harvesting process take place during months when turtles remain in or near wetland hibernacula. In the Northeast, this would generally be between mid-November and early March, but may vary depending on weather conditions in a given year. Additionally, care must be given to avoid any significant disturbance to wetlands that contain Spotted Turtles at any point in the year, especially those containing hibernacula.

Spotted Turtles are a species of increasing conservation concern. Habitat destruction and modification, vehicular mortality (i.e., automobiles and agricultural equipment), and personal and commercial collection are considered the greatest threats to the species (Ernst and Lovich 2009; van Dijk 2013). An improved understanding of how early successional habitat creation affects populations of Spotted Turtles will allow resource managers to identify instances in which the implementation of the practice is consistent with the site-specific conservation goals for the species.

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Tables

Table 1.1. Summary of all Spotted Turtle (*Clemmys guttata*) individuals tracked Washington County, RI 2013-2014.

Individual	Sex	Initial weight (g)	Midline carapace length (mm)	Dates tracked	Number of relocations	Fate	MCP (ha)	Percent wetland use (%)
A	M	195	123.7	10 JUN 2013 - 11 NOV 2014	71	Tracked to hibernaculum	0.59	80.3
B	M	175	117.7	10 JUN 2013 - 11 NOV 2014	65	Tracked to hibernaculum	2.29	89.1
C	F	210	115.2	25 May 2013 - 23 SEP 2014	41	Moved outside study area	2.35	100
H	F	165	98.7	10 JUN 2013 - 11 NOV 2014	76	Tracked to hibernaculum	1.95	81.6
I	M	155	108.5	25 May 2013 - 11 NOV 2014	68	Tracked to hibernaculum	2.78	100
K	F	235	118.6	10 JUN 2013 - 11 NOV 2014	75	Transmitter failure	4.07	70.8
M	M	175	113.6	10 JUN 2013 - 30 SEP 2014	67	Died (unknown causes)	1.73	100
N	M	165	109.3	10 JUN 2013 - 11 NOV 2014	66	Tracked to hibernaculum	2.07	93.8
O	F	180	104.7	10 JUN 2013 - 11 NOV 2014	79	Tracked to hibernaculum	1.08	68.4
AC	F	183	102.9	1 APR 2014 - 11 NOV 2014	34	Tracked to hibernaculum	1.84	73.5
AH	M	227	122.2	11 APR 2014 - 11 NOV 2014	34	Tracked to hibernaculum	1.64	79.4
AN	F	203	104.2	18 APR 2014 - 11 NOV 2014	36	Tracked to hibernaculum	1.00	77.8
Sum	-	-	-	-	712	-	-	-
Mean (SE)	-	189 (7.3)	111.6 (2.3)	-	59.3 (5.1)	-	1.95 (0.26)	84.6 (3.4)
Female mean (SE)	-	196 (10.2)	107.4 (3.1)	-	56.8 (8.9)	-	2.04 (0.46)	78.7 (4.7)
Male mean (SE)	-	182 (10.5)	115.8 (2.6)	-	61.8 (5.6)	-	1.85 (0.30)	90.4 (3.7)

Table 1.2. Summary of Spotted Turtle (*Clemmys guttata)* annual home range and wetland use Washington County, RI 2013-2014. An asterisk indicates an individual that exhibited hibernaculum site fidelity in consecutive years. Individuals sharing superscript numbers indicates communal hibernation in the winter beginning that year.

Individual	2013 dates tracked (number of relocations)	2014 dates tracked (number of relocations)	2013 MCP (ha)	2014 MCP (ha)	2014 constrained MCP (ha)	Annual home range overlap (%)	2013 wetland use (%)	2014 wetland use (%)	2014 constrained wetland use (%)
A*	10 JUN - 12 NOV (34)	21 MAR - 19 OCT (37)	0.37	0.45	0.29	47.8	85.3	78.4	68
B	10 JUN - 31 OCT (29)	6 APR - 11 NOV (36)^1	1.77	1.71	1.66	57.2	78.6	97.2	96.3
C	25 MAY - 31 OCT (23)	25 APR - 23 SEP (18)	1.05	1.47	1.47	27.4	100	100	100
H*	10 JUN - 20 NOV (36)^1	21 MAR - 11 NOV (40)^2	0.20	1.95	1.86	10.6	80.5	82.5	74.0
I	10 JUN - 20 NOV (31)	21 MAR - 11 NOV (37)	1.77	2.34	1.93	52.4	100	100	100
K	10 JUN - 11 NOV (37)^1	21 MAR - 11 NOV (38)	2.67	3.55	3.06	53.0	55.5	86.1	82.6
M	10 JUN - 20 NOV (34)	21 MAR - 30 SEP (33)	0.99	1.70	1.70	58.4	100	100	100
N*	10 JUN - 7 NOV (32)	21 MAR - 11 NOV (34)^1	1.51	1.88	1.32	67.2	90.3	97.1	95.2
O*	10 JUN - 20 NOV (39)	21 MAR - 11 NOV (40)	0.37	1.01	0.86	28.9	56.4	80	70.4
AC	-	1 APR - 11 NOV (34)^2	-	1.84	1.04	-	-	73.5	65.4
AH	-	11 APR - 11 NOV (34)^2	-	1.64	0.68	-	-	79.4	73.1
AN	-	18 APR - 11 NOV (37)	-	1.00	1.00	-	-	77.8	73.1
Mean (SE)	-	-	1.19 (0.27)	1.71 (0.26)	1.41 (0.21)	44.8 (6.1)	82.9 (5.8)	87.7 (3.0)	83.2 (4.1)
Female mean (SE)	-	-	1.07 (0.56)	1.80 (0.38)	1.55 (0.34)	29.9 (8.7)	73.1 (10.7)	83.3 (3.8)	77.6 (5.0)
Male mean (SE)	-	-	1.28 (0.27)	1.62 (0.26)	1.26 (0.26)	56.6 (3.2)	90.8 (4.2)	92.0 (4.2)	88.8 (5.9)

Table 1.3. Basal area estimates for all tree species before and after clearcut, Washington County, RI 2013-2014. Estimates for 2014 include the trees on the perimeter of the clear-cut.

Species	2013 basal area (m^2 / hectare)	2014 basal area (m^2 / hectare)
Black Oak (*Quercus velutina*)	6.76	0.98
Red Maple (*Acer rubrum*)	4.14	1.15
White Oak (*Quercus alba*)	1.89	1.44
Northern Red Oak (*Quercus rubra*)	2.00	0.74
Eastern White Pine (*Pinus strobus*)	1.27	0.25
Scarlet Oak (*Quercus coccinea*)	0.04	0.25
Eastern Redcedar (*Juniperus virginiana*)	0.41	0.37
Bigtooth Aspen (*Populus grandidentata*)	0.25	0
Gray Birch (*Betula populifolia*)	0.12	0
Black Cherry (*Prunus serotina*)	0.12	0
Pitch Pine (*Pinus rigida*)	0.12	0.04
Swamp White Oak (*Quercus bicolor*)	0.08	0
Paper Birch (*Betula papyrifera*)	0.04	0
Sassafras (*Sassafras albidum*)	0	0.04
Sum	17.26	5.25
Mean (SE)	1.23 (0.53)	0.37 (0.13)

Figures

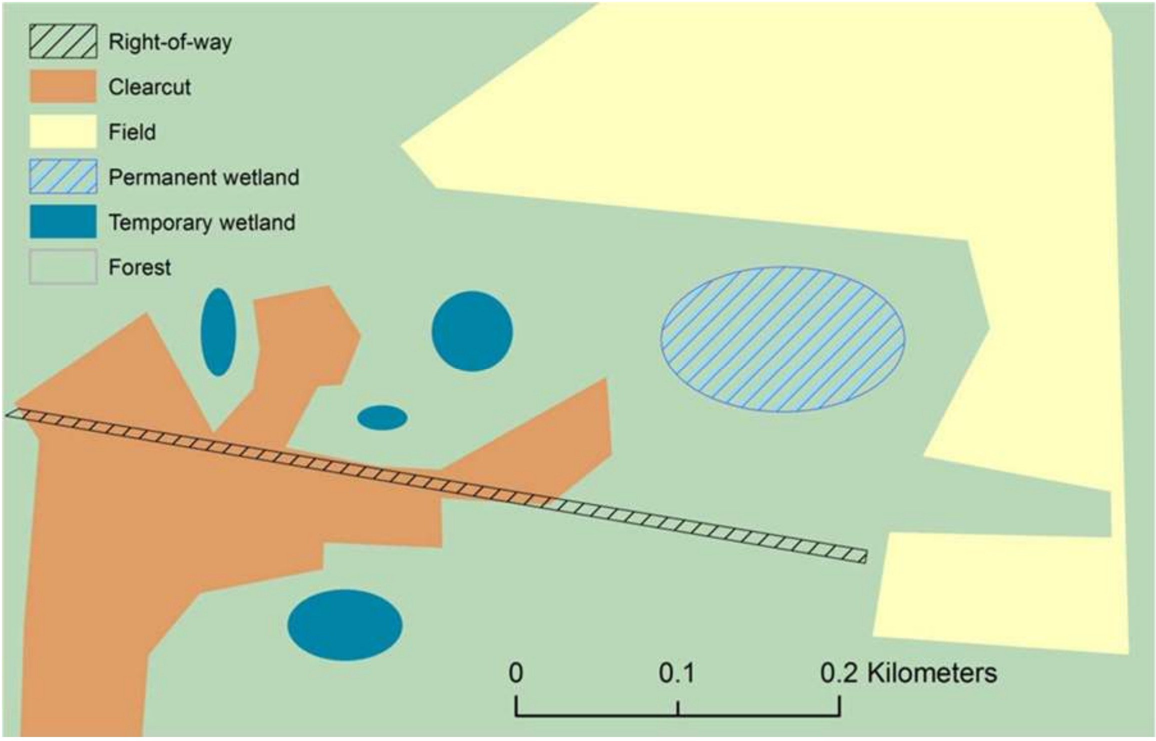

Figure 1.1 A simplified map of the study area showing the configuration of important features. All shapes are approximate.

Figure 1.2 Representative photograph of the clear-cut area. Seed trees and coarse woody debris were purposefully left behind by the logger.

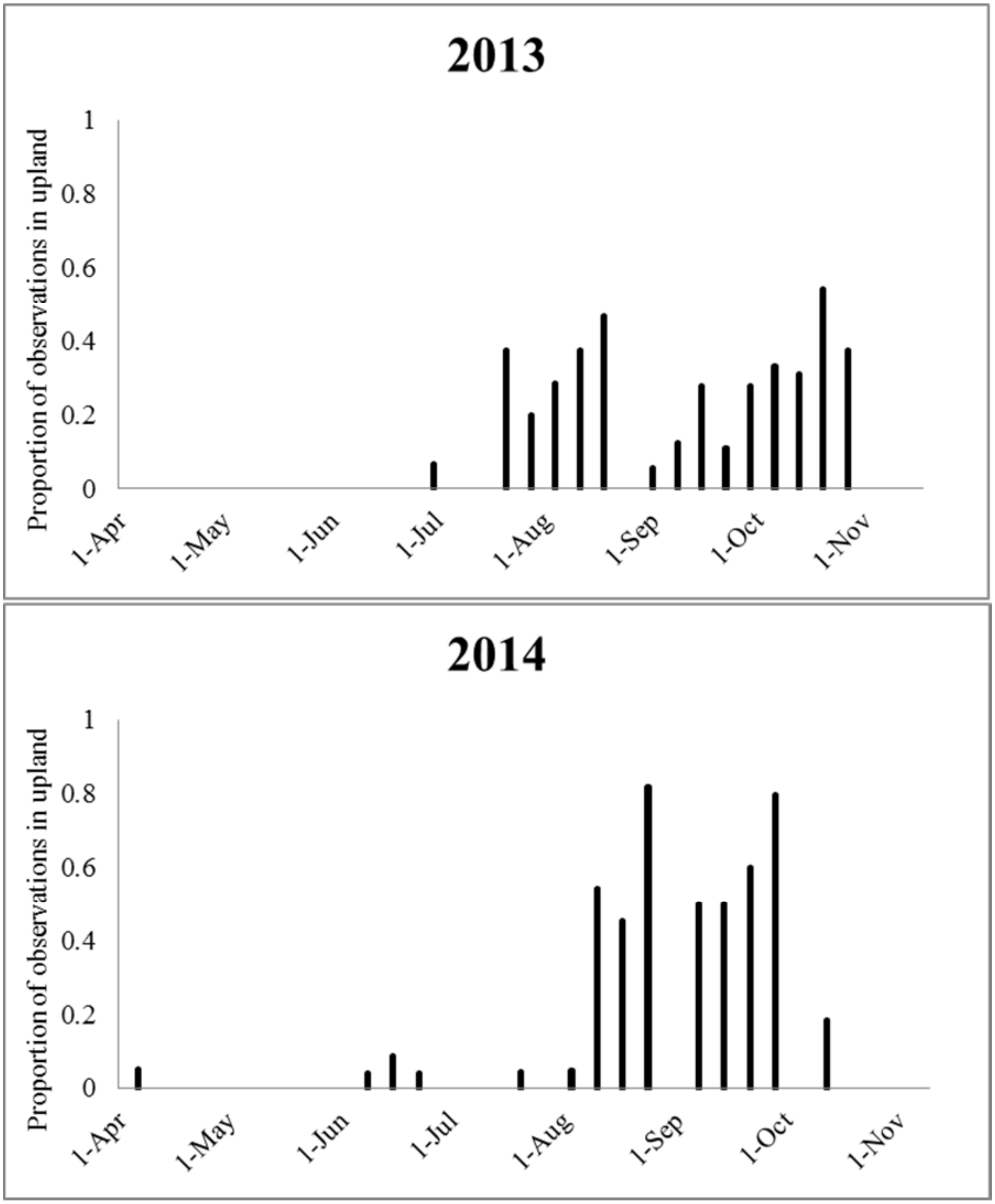

Figure 1.3 Proportion of radio-locations in upland habitat, calculated weekly, for Spotted Turtles (*Clemmys guttata*) tracked in Washington County, RI 2013-2104.

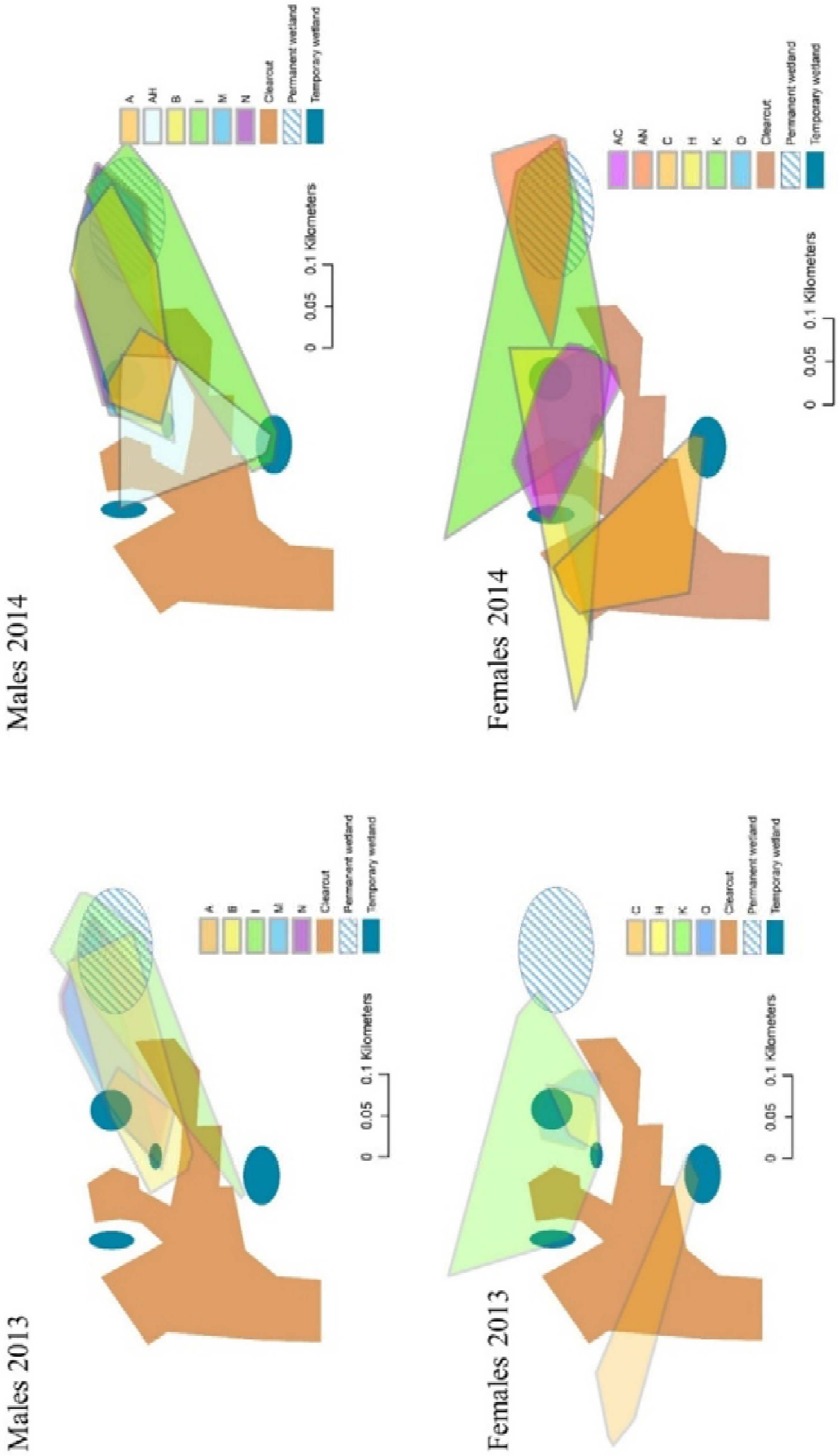

Figure 1.4 Annual home range size estimates (MCPs derived from all available data) for each sex before (2013) and after (2014) the clear-cut. The area of the clear-cut and wetlands occupied by Spotted Turtles (*Clemmys guttata*) are represented by simplified polygons.

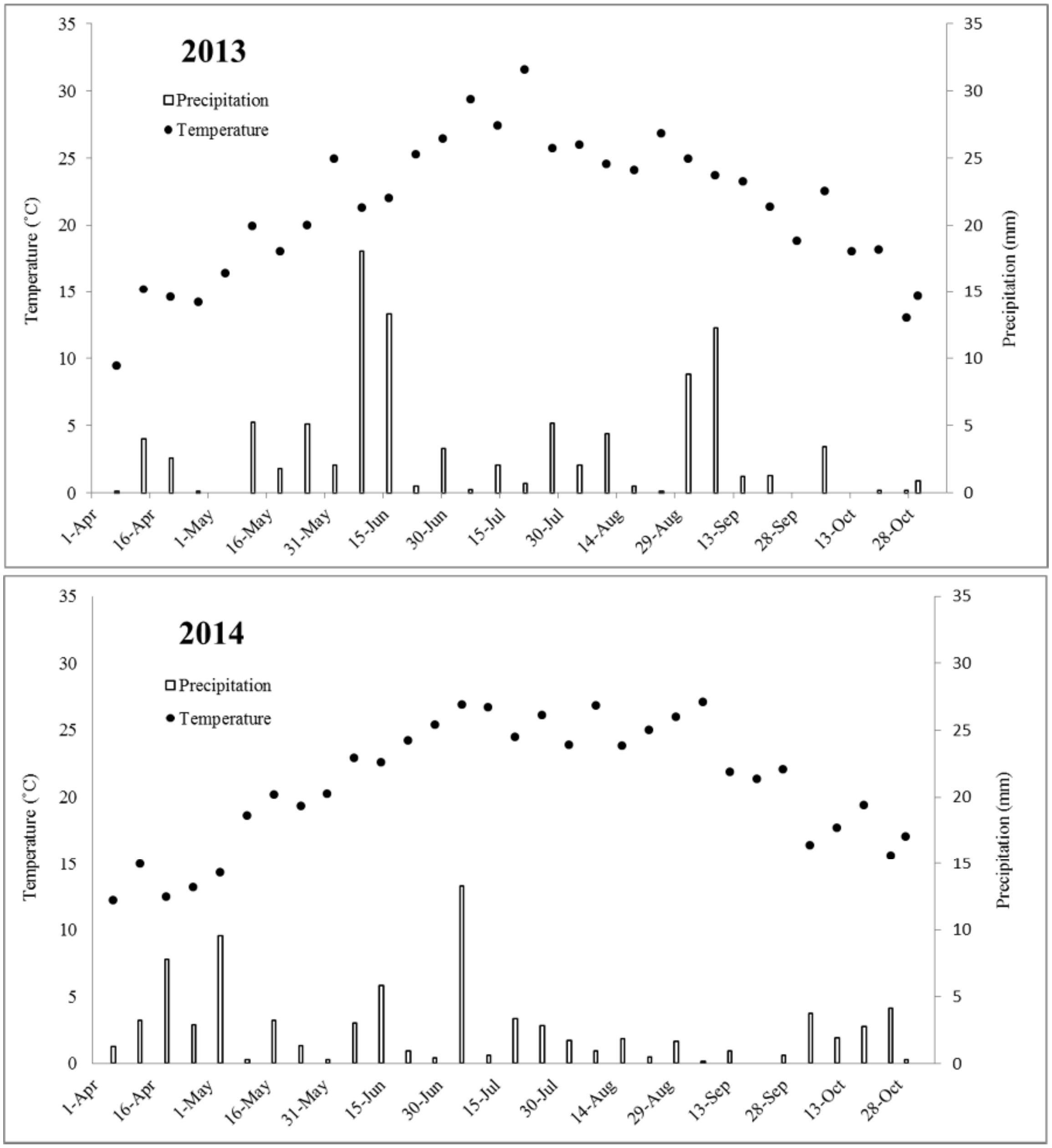

Figure 1.5 Weekly means for maximum temperature (°C) and precipitation (mm) Washington County, RI 2013-2014.

Manuscript II: Occupancy and Abundance of Freshwater Turtles in Rhode Island Across a Gradient of Altered Landscapes

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Abstract

Turtles are one of the most threatened groups of vertebrates worldwide. In the northeastern United States, a legacy of centuries of dramatic landscape alteration has impacted freshwater turtle populations, but the relationships between the current landscape and distributions and abundances of freshwater turtles remain poorly understood. We used a stratified random approach to select small, isolated wetlands across a gradient of forest cover throughout Rhode Island and systematically sampled freshwater turtles in these wetlands. We performed occupancy analysis to determine which environmental variables drive the occurrence and probability of detection of different species. We report naïve estimates of abundance and generated estimates of niche breadth for each species and partitioning among species. Eastern painted turtles (*Chrysemys p. picta*) and snapping turtles (*Chelydra serpentina*) were widespread (occurring in 83% and 63% of wetlands, respectively), relatively abundant, and exhibited wide niche breadth. Spotted turtles (*Clemmys guttata*) were far less common, occurring in 8% of wetlands, and exhibited a strong association with forested, shallow, natural (i.e., not manmade or heavily modified) wetlands. Non-native red-eared sliders occurred in 10% of wetlands and exhibited a strong, positive association with road density, likely as a function of human population density. Identifying landscape-scale habitat features that are associated with the occurrence of sensitive species can improve the ability of biologists to identify and protect populations of that species.

Introduction

Human-induced landscape alteration is often implicated in compromising vertebrate biodiversity, with habitat loss and degradation widely recognized as the leading contributor to a loss of population stability across taxa (Gibbons et al. 2000; Brooks et al. 2002; Johnson et al. 2011). New England, in the northeastern United States, has experienced dramatic shifts in landscape composition since the time of European settlement. Deforestation associated with agriculture and logging peaked in the mid-nineteenth century when as much as 80% of the landscape had been cleared. Beginning around 1850 agriculture shifted to states farther west, ushering in a period of reforestation lasting approximately 100 years (Foster and Aber 2004). In Rhode Island, a small state in southern New England, this period was followed by another phase of deforestation for urban and suburban development. Total forested land area in Rhode Island has been decreasing since at least 1953 (RIDEM 2010; Butler and Payton 2011; but see Butler 2013) and was recently estimated as 147,000 ha, approximately 54% of the total land area of the state (Butler 2013). This extreme landscape alteration in a relatively short period of time has certainly led to changes in the distribution and abundance of wildlife, but the legacy of this change is poorly understood for many species, including freshwater turtles.

As a vertebrate group, turtles have an extremely high risk of population extirpation and extinction (Bohm et al. 2013). In the United States, freshwater turtles are of particular conservation concern largely due to pervasive wetland draining and filling that has resulted in a significant loss in wetland area beginning in the eighteenth century (Dahl 1990, 2000). Additional factors putting freshwater turtle populations at risk include the loss of meta-population structure associated with terrestrial habitat loss and degradation (Dodd 1990; Gibbs 2000), collection for pet, food, and medicine trades (Shiping et al. 2006; Luiselli et al. 2016), and life history characteristics that include delayed sexual maturity and low recruitment (Congdon et al. 1993; Congdon et al. 1994; Heppell 1998). In Rhode Island, native freshwater turtles include the common snapping turtle (*Chelydra serpentina*), eastern painted turtle (*Chrysemys picta picta*), spotted turtle (*Clemmys guttata*), wood turtle (*Glyptemys insculpta*), and musk turtle (*Sternotherus odoratus*). An additional species, the red-eared slider (*Trachemys scripta elegans*), has been introduced to Rhode Island from the southern United States. The spotted turtle and wood turtle have been identified as Species of Greatest Conservation Need in the Rhode Island State Wildlife Action Plan (Rhode Island Department of Environmental Management 2015) and as Endangered by the International Union for the Conservation of Nature (IUCN; van Dijk 2011; van Dijk and Harding 2011), and both are currently Candidate Species under review by the U.S. Fish and Wildlife Service (USFWS) for listing under the U.S. Endangered Species Act (USFWS 2015).

All freshwater turtle species use terrestrial habitats to some extent, but the proportion of time spent on land varies. Freshwater turtles use uplands to nest and to move between wetlands, and some species spend substantial periods of time estivating in uplands (Ernst and Lovich 2009). Spotted turtles are known to move frequently between temporary and permanent wetlands and to estivate terrestrially, spending as much as 30% of their time on land (Milam and Melvin 2001). The landscape adjacent to and between wetlands is directly linked to many ecological processes of freshwater turtles (Bodie and Semlitsch 2000; Joyal et al. 2001).

Landscape gradient analyses have been used for decades to investigate how changes in composition and configuration of the landscape affect wildlife (Limburg and Schmidt 1990; Gibbs 1998; Riem et al. 2012). The approach has its roots in earlier studies that characterized biological change, often involving plants, along physical, chemical, and ecological gradients (Waring and Major 1964; Whittaker 1967; Peet and Loucks 1977). Driven by a desire to better understand how human activity affects species, communities, and ecosystem change, many landscape gradient studies have taken place along urban gradients, sometimes referred to as urban-rural, land-use, or fragmentation gradients. Typically, data are collected along some direct or indirect measure of varying anthropogenic intensity. For certain taxa, these studies have led to broad generalizations about the relationships between urbanization and patterns of distribution, abundance, and diversity (McDonnell and Hahs 2008). For example, it is widely held that bird diversity decreases and population density increases with increasing urbanization (Marzluff 2001; Chace and Walsh 2006; but see Saari et al. 2016). The increased prevalence of non-native species with increasing urbanization is another pattern revealed by these types of studies (Hansen et al. 2005; Schochat et al. 2010). Very few studies however, have examined patterns in reptile distributions across urban gradients. A major review (McDonnell and Hahs 2008) of 201 studies investigating organismal distributions along urbanization gradients published between 1990-2007 included only one study of reptiles. By comparison, there were 49 studies of birds and 13 of mammals during that same time period. If we are to advance our understanding of how human activity affects reptile ecology and susceptibility to population loss, data on species distribution, abundance, and demography as they relate to landscape-scale change are urgently needed.

Occupancy analysis is an established modeling framework in wildlife biology that is often employed as a technique to determine resource selection among discrete populations or discrete landscape units (Olson et al. 2005, Nielsen et al. 2010). The framework uses maximum likelihood techniques that allow for heterogeneity in detection and occupancy parameters that can be modeled with environmental covariates. We conducted a three-year field study into the associations between freshwater turtles and the landscape by sampling small (0.1 – 1.8 ha) wetlands along a gradient of forest-cover. We targeted our sampling scheme on a subset of wetland types that could potentially contain spotted turtles. We used single-species, single-season occupancy models to elucidate relationships between landscape- and wetland-scale variables, and the occurrence of four species of freshwater turtles in Rhode Island. Our intent was to: (1) characterize the distribution and abundance of freshwater turtles across an urban gradient while testing the prediction that spotted turtles are a forest-associated species, (2) determine what landscape- and wetland-scale features and conditions freshwater turtles are selecting, and (3) improve our understanding of the conservation implications of landscape management for these species, especially spotted turtles.

Methods

Study area and species

Our study was conducted throughout the state of Rhode Island (including Aquidneck and Conanicut Islands, but excluding Block Island) located in southeastern New England, USA. At approximately 2,700 square kilometers (when excluding coastal waterways), Rhode Island is the smallest state geographically in the USA but ranks second highest in population density (U.S. Census 2010, Suitland, MD, USA. Available from www.census.gov, accessed March 2017). Mean elevation is approximately 60 m with a highest point of 247 m. The Wisconsin glaciation, which reached a maximum extent approximately 25,000 years ago and retreated in the area 10,000-12,000 years ago, is responsible for the dominant parent materials found in Rhode Island. These include glacial till, glacial outwash, and windblown silts (eolian mantle). Till soils are typically associated with higher elevation landforms, while outwash materials are located in valley landscape positions. A mantle of windblown silt can be found across various landscapes throughout the state (Rector 1981). Long-term (1981-2010) average annual temperature in Kingston, RI was 10.5 °C and long-term average annual precipitation was 134.3 cm. Long-term average monthly temperatures range from -1.4 °C in January, to 22.1 °C in July (National Oceanic and Atmospheric Administration, National Centers for Environmental Information [NOAA NCEI]. Available from www.ncei.noaa.gov, accessed March 2017).

Site selection

We selected sites using a stratified random design to capture the state-wide variability in landscape composition and configuration. To minimize confounding factors among sites, we focused our sampling on relatively small (0.1-1.8 ha; as measured via GIS polygons), isolated (i.e., discrete, non-riparian) wetlands. The minimum size was selected to ensure that the majority of wetlands had a hydroperiod that persisted throughout the turtle activity season in most years (Skidds and Golet 2005). We used ArcGIS version 10.1 to identify all freshwater wetlands 2 ha or less in size throughout the state using the National Wetland Inventory (NWI) dataset (Falls Church, VA, USA. Available from www.fws.gov/wetlands/index.html, accessed March 2013), the Land Cover and Land Use 2011, and the Lakes, Ponds, and Reservoirs datasets available from the Rhode Island Geographic Information System (RIGIS; Kingston, RI, USA. Available from www.rigis.org, accessed March 2013). To isolate the variable of greatest interest (i.e., forest cover) and minimize other potentially confounding variables, we excluded wetlands that: (1) were within 500 m of the coastline, (2) within 300 m of a federal or state highway or 10 m of a local road, or (3) contained > 50% manicured vegetation, such as lawn, along the perimeter.

We grouped retained wetlands as either small (0.1–0.4 ha) or large (< 0.4–1.8 ha) wetlands. The 0.4 ha breakpoint was the approximate median of the distribution of wetland size for all retained wetlands. We calculated percent forest cover within buffers of 300 m and 1 km from the wetland edge of all retained wetlands. At the 300-m scale, we grouped wetlands into eight 10% increments of forest cover (excluding 0–10% and 70–80%), and at the 1 km scale we grouped wetlands into four, partially overlapping, larger increments of forest cover (0–40%, 20–60%, 40–80%, 80–100%). These groups created a near-continuous gradient of sites from different forest cover conditions which captured the state-wide variation in landscape conditions. We assigned each retained wetland a random number, sorted them by random number in ascending order, and contacted property owners/managers in that order until we received permission to sample the desired number of wetlands in each stratification, with approximately equal numbers of small and large wetlands. We carried out this process in each of three consecutive years.

Turtle sampling and data collection

We carried out turtle sampling from May to October in 2013–2015 (Appendix 1). Each year we conducted up to four surveys, hydroperiod allowing, at each wetland. For each survey, we trapped turtles for an approximately 48-h period, with trap checks every 24 h, making for two trapping sessions per survey. We sampled sites using small (30.5 cm diameter collapsible minnow traps, Promar Nets, Gardena, CA) and large (91.4 cm single throated hoop traps, Memphis Net and Twine, Memphis, TN) traps baited with sardines placed inside perforated plastic containers. Alternating between small and large traps, we placed traps approximately 30 m apart around the perimeter of wetlands such that the perimeter of each wetland determined the number of traps deployed. We placed traps within 10 m of the wetted edge with a portion of the trap always staked or floated above the surface of the water to ensure turtles the opportunity to breathe. We opportunistically hand-captured a small number of turtles (<15) that were encountered when working with traps.

We collected data on all trapped turtles every 24 h. Each turtle was identified to species, sexed, measured and weighed, and marked along the marginal scutes with a unique code for each individual. We released all turtles immediately after processing. At each wetland, we made visual percent cover estimates of vegetation during the second or third survey after all vegetation had fully emerged. Estimates were made for each vegetation category while standing at the wetland edge, and all estimates were made by the same individual (S.B.) in the year that turtles were sampled. To assess water chemistry at each wetland, we collected samples from three distinct points within each wetland and combined them to form one 125 ml sample for subsequent laboratory analysis. We collected all water samples in the spring of 2015. We measured pH the same day as sample collection with a pH meter (model HI-902, Hanna Instruments Inc.,Woonsocket, RI). In the lab, we measured ammonia-nitrogen, nitrate-nitrogen, and dissolved phosphorous with a segmented flow nutrient autoanalyzer (Astoria Pacific Inc., Clackamas, OR). The limit of detection for ammonia and nitrate was 15 µg/l, and 4 µg/l for dissolved phosphorous. All analyses were measured against appropriate standards in the University of Rhode Island Watershed Watch state-certified laboratory.

We used aerial and digital imagery datasets available from RIGIS to quantify landscape features and wetland age. Road density (m/ha) was calculated using the TIGER Roads dataset. We examined historic aerial imagery taken at approximately 10-yr increments and dating back to 1939 to determine the age (up to >77 yr) of all sampled wetlands. We used the Forest Habitat dataset to determine percent cover of different landscape types and to quantify landscape metrics. We first reclassified attribute categories in this dataset to broader categories that included early-successional habitat (agriculture/grassland/upland shrubland), forest (all upland and wetland forest types), and wetland (freshwater lakes and rivers/wetland shrubland/wetland freshwater emergent), marine wetland/estuarine, and other (barren land, rock, sand). We then clipped buffers of this modified dataset at 300 m and 1 km from the perimeter of each wetland, converted these vector data to raster data with a 10 m cell size, extracted a separate raster file for each buffer, and used Fragstats (version 4.2, McGarigal et al. 2012) to quantify landscape composition. We selected spatial scales of 300 m and 1 km because of their relevance from an ecological and management standpoint (Semlitsch and Bodie 2003; Steen et al. 2012).

Statistical analysis

We made naïve estimates of abundance by calculating the total number of unique individuals caught divided by the total number of trap nights, for each forest cover class. Abundance estimates were compared only for common snapping turtles (hereafter, snapping turtles), eastern painted turtles (hereafter, painted turtles), and spotted turtles because of low sample sizes for the other species.

We used principal components analysis (PCA) to summarize relationships between presence of freshwater turtle species in wetlands and environmental covariates by reducing the dimensionality of our covariate dataset. We were primarily interested in using PCA as an exploratory technique to identify potential differences in explained variation among species (Everitt and Hothorn 2011). We built a data matrix of all site-specific covariates (Table 2.1; excluding geographic location) consisting of all instances in which a species was detected at a wetland, for each species (i.e., if two species were detected at the same wetland those data were entered twice in the matrix). Data were scaled and principal components were extracted from this correlation matrix using the 'stats' package in R (R Core Team). We constructed a graphical representation of the first two components using the R package 'ggbiplot.' Ellipses were drawn around mean values for each species encompassing one standard deviation of the variation along each axis.

We modeled heterogeneous detection probabilities using covariates that changed between surveys, including Julian date (day two of survey), survey number, temperature, and precipitation (Table 2.1). For each wetland, temperature and precipitation data were downloaded from the nearest of seven available weather stations (NOAA NCEI). For days one and two of each survey, we used mean maximum daily temperature for our temperature covariate and mean total daily precipitation for the precipitation covariate. To model heterogeneous occupancy probabilities, we used covariates that changed from site to site, including percent cover of different vegetation classes and water chemistry variables (Table 2.1).

We used a single-species, single-season occupancy modeling framework to estimate species detection probabilities (p) based on survey-specific covariates, and species occupancy probabilities (Ψ) based on site-specific covariates (MacKenzie at al. 2002; MacKenzie et al. 2006). Models were built using the occu function in the R package 'unmarked' (Fiske and Chandler 2011). This function fits the standard occupancy model based on zero-inflated binomial models (MacKenzie et al. 2006) using maximum likelihood techniques to estimate model parameters, and uses a logit link function to scale covariates to a sampling-history response of zeros (species absence) and ones (species presence). Using the logit link function, the probability of site i being occupied can be expressed as:

$$\text{logit}(\Psi_i) = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots + \beta_U x_{iU},$$

where U covariates are associated with site i and the $U + 1$ coefficients to be estimated (i.e., β_0 and U regression coefficients for each covariate). Using the same principles, the probability of detection can also be modeled as a function of covariates at site i during survey j, expressed as:

$$\text{logit}(p_{ij}) = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_U x_{iU} + \beta_{U+1} y_{ij1} + \ldots + \beta_{U+V} y_{ij\text{v}},$$

where x_{i1} ,…, x_{iU} represent the U site-specific covariates associated with site i, and y_{ij1} ,…., y_{ijv} are the V survey-specific covariates associated with survey j of site i (MacKenzie et al. 2006). We used a simulated annealing optimization process for all models. We used the R package ‘MuMIn’ to carry out model selection procedures and used the Bayesian Information Criterion (BIC) to select supported models from sets of candidate models (Burnham and Anderson 2002). Models with the lowest BIC score and fewest number of parameters within 2 BIC units of the lowest BIC score were considered most supported. All covariates were treated as continuous data and were standardized to a mean of zero and standard deviation of one prior to modeling (MacKenzie et al. 2006).

We conducted the following procedure for each species. We first modeled the probability of detection by keeping the occupancy parameter constant and allowing detection to vary as a function of the survey-specific covariates. For each covariate, we considered both a linear and quadratic functional form when building models. For model selection, we considered all subsets and used BIC to identify the most supported model. We retained the most supported model to serve as the detection parameter for all subsequent models for that species. Next, to model the probability of occupancy, we built an ‘initial’ additive global model consisting of the retained detection parameter and linear terms for each site-specific covariate (for landscape covariates these included only the 300-m scale). We considered all subsets and identified the most supported models using BIC. When subsetting, we limited the number of occupancy parameters (excluding the intercept) in any one model to five to limit the ratio of parameters to sample size. We retained all site-specific covariates included in any model within 2 BIC units of the top model and used these to build a ‘secondary’ global model. To determine which functional form to include in the secondary global model, for the appropriate covariates, we then built separate, single-covariate linear and quadratic models and compared them using

BIC. We retained the term from the most supported model. If the covariate was a landscape covariate, we compared both functional forms at both spatial scales (i.e., linear 300 m, quadratic 300 m, linear 1 km, and quadratic 1 km) and retained the term from the most supported model. If two remaining covariates were highly correlated (≥0.9 Pearson correlation coefficient; Appendix 3), we compared single covariate models containing each term using BIC and retained the term from the more supported model. With these retained terms, we then built the secondary global model, evaluated all subsets, and considered the most supported model as our top model. To assess fit of each top model, we used a MacKenzie-Bailey goodness-of-fit test with parametric bootstrapping employing 1000 simulations to approximate the distribution of the test statistic (MacKenzie and Bailey 2004). We used ArcGIS 10.1 to visualize spatial data.

Results

We sampled a total of 88 wetlands over three years (Fig. 2.1). Traps were deployed for a total of 5824 nights yielding 1661 unique individuals consisting of five species (Table 2.2). Painted turtles were the most abundant species and were detected in 84.1% of wetlands (1369 individuals; 74/88 wetlands). Snapping turtles were detected in 62.5% of wetlands (207 individuals; 55/88 wetlands), red-eared sliders were detected in 10.2% of wetlands (21 individuals; 9/88 wetlands), spotted turtles were detected in 7.9% of wetlands (52 individuals; 7/88 wetlands), and musk turtles were detected in 4.5% (12 individuals; 4/88 wetlands). We captured no wood turtles as we did not sample riparian wetlands, their primary habitat.

Painted turtle abundance was highest at the lowest forest cover class and generally decreased with increasing forest cover. Spotted turtle abundance was substantially higher in the highest forest cover class and only one individual was detected below the 60-70% forest cover class. Snapping turtle abundance exhibited relatively minor variation across most of the gradient of forest cover (Fig. 2.2). Non-native red-eared sliders did not occur in cover classes >50-60% forest cover.

We retained the first four principal component axes based on a scree plot of component variances (Cattell 1966; Everitt and Hothorn 2011). Collectively, these accounted for 55.6% of the variation in our data (Appendix 4). The first axis was positively correlated with road density and development and negatively correlated with forest cover. This axis accounted for 20.4% of the variation in our data. The second axis accounted for 15% of the variation and was positively correlated with percent woody vegetation and negatively correlated with wetland depth and percent open water. Ellipses for painted turtles and snapping turtles were similar in position and variance, with both ellipses centered near the middle of the plot. The spotted turtle ellipse was positioned towards the negative end of the first axis (more forest cover) and the positive end of the second axis (shallower wetlands with more woody vegetation). The red-eared slider ellipse was centered towards the positive end of the first axis (more development and roads) and approximately centered on the second axis (Fig. 2.3).

We modeled occupancy for four species of freshwater turtles. We did not consider musk turtle occupancy as detection probability fell below 5% (Cunningham and Lindermayer 2005; MacKenzie et al. 2006). One wetland, which yielded no turtle detections, was not included in occupancy models because of incomplete covariate data. There was evidence for lack of fit ($p < 0.05$) and overdispersion ($\hat{c} > 1$) in the top model for painted turtles, but all top models for other species exhibited evidence of model fit (p

> 0.05; Table 2.3; Fig. S.2.1). For snapping turtles, the naïve estimate of detection probability was 0.399 and the naïve estimate of occupancy probability was 0.776. The top model for snapping turtles included a negative quadratic relationship with Julian date for the detection parameter, and a positive logistic relationship with nitrate for the occupancy parameter. Painted turtles had the highest naïve estimates of detection and occupancy at 0.805 and 0.867, respectively. The top model for painted turtles included a negative logistic relationship with Julian date for the detection parameter, and a positive logistic relationship with wetland size and a negative logistic relationship with woody vegetation for the occupancy parameter. For spotted turtles, the naïve estimate of detection was 0.554 and the naïve estimate of occupancy was 0.086. The top model for spotted turtles included a positive logistic relationship with temperature for the detection parameter, and for the occupancy parameter included a positive logistic relationship with forest cover at the 1 km scale, and a negative logistic relationship with wetland depth. For red-eared sliders, the naïve estimate of detection was 0.407 and the naïve estimate of occupancy was 0.125. The detection parameter of the top model included a positive logistic relationship with air temperature, and a positive logistic relationship with road density at the 1-km scale for the occupancy parameter (Fig. 2.4).

Discussion

Spotted turtles and red-eared sliders were encountered far less frequently than painted turtles and snapping turtles. The fact that the introduced red-eared slider was found in a greater number of wetlands than the native spotted turtle is concerning. Principal component ellipses for these two species exhibited minimal overlap demonstrating strong differences in the types of habitats where they are found. There was strong evidence of an association between spotted turtles and forest cover. Spotted turtles were completely absent, except for a single individual, from wetlands surrounded by less than 60% forest cover, and abundance increased dramatically in wetlands with 90–100% forest cover. Similarly, the top spotted turtle occupancy model indicated a positive relationship with forest cover at the 1-km scale. The relatively low state-wide occupancy rate of spotted turtles is consistent with the idea that populations of this species are rare and that they are disproportionately affected by human disturbance (Enneson and Litzgus 2008; Anthonysamy et al. 2014). Spotted turtles are vulnerable to a variety of human impacts including habitat loss and fragmentation, road mortality, and collection (Ernst and Lovich 2009, van Dijk 2011). Forest cover at the 1-km scale was negatively correlated with road density (Pearson r = -0.889) and development (Pearson r = -0.901), indicating that human disturbances are generally reduced in areas of higher forest cover. Furthermore, all wetlands in which spotted turtles were detected belonged to the oldest age class (pre-1939), wetlands that are less likely to have been created or significantly altered by people. Our occupancy models also indicated that spotted turtles prefer shallow wetlands with abundant woody vegetation, results that are consistent with other studies of spotted turtle habitat selection (Milam and Melvin 2001; Ernst and Lovich 2009; Rasmussen and Litzgus 2010).

In southern New England, forest succession has greatly reduced the amount of early successional habitat on the landscape (Foster and Aber 2004, Buffum et al. 2011). The creation and maintenance of early successional habitat, primarily via clear-cutting and fire, is a management priority in the region (Buffum et al. 2014). The strong association between spotted turtles and forest habitat types may put them at greater risk to impacts from these types of forest management. We recommend surveys for spotted turtles at sites slated to undergo the creation of early successional habitat, and urge extreme caution in the implementation of clear-cuts if spotted turtles are present (Chapter 1). Furthermore, we recommend that existing conservation organizations that manage forested lands in the state conduct surveys for spotted turtles and, if present, put into place appropriate management practices.

Probability of red-eared slider occupancy increased with higher road density within 1 km of wetlands. Red-eared sliders have been introduced via the pet trade in many urban and suburban areas outside of their natural range, including parts of the northeastern USA (Carroll 2004, Winchell and Gibbs 2016, C. Raithel, personal communication), but there are few published data on the occurrence of red-eared sliders in Rhode Island. In this case, road density probably serves as a proxy for higher human population density and the red-eared sliders we caught are almost certainly former pets or the offspring of former pets. Whether the detected individuals constitute breeding populations remains unknown, but it is clear that the species is extant and widespread in the state. Red-eared sliders are listed as one of the world’s 100 most detrimental invasive species (Lowe et al. 2000) and future work should investigate if they are breeding in the state and whether they compete with native turtle species. A better understanding of their spatial distribution in the region (see Fig. 2.4) could help wildlife managers identify wetlands most likely to contain red-eared sliders and take appropriate mitigating action.

Painted turtles and snapping turtles exhibited relatively high occurrence and abundance in our study area and occupied remarkably similar niche space. These results support the idea that both species are habitat generalists with wide niche breadths (Ernst and Lovich 2009; Paterson et al. 2012; Anthonysamy et al. 2014). Painted turtle abundance was highest in the lowest forest cover class, where sites were heavily modified by either urban development or agriculture. Other studies have suggested that this species is not influenced by landscape fragmentation. In a landscape dominated by agriculture in Indiana, painted turtle abundance decreased with a higher proportion of agriculture within 100 m of wetlands, but exhibited different directional responses to landscape diversity at different spatial scales (Rizkalla and Swihart 2006). In New Hampshire, forest cover surrounding wetlands did not emerge as an important covariate for painted turtle abundance, but open nesting areas (measured in the field as suitable soils and open canopies) within 30 m of wetlands was positively correlated with abundance (Marchand and Litvaitis 2004). Freshwater turtles prefer open areas for nesting (Christens and Bider 1987; Janzen 1994; Kolbe and Janzen 2002) and it is likely that nesting habitat becomes more limited with increasing forest cover (Baldwin et al. 2004). Interestingly, wetlands on or immediately adjacent to golf courses, a highly altered landscape, produced some of the highest abundance estimates for painted turtles. Four of our sites occurred on golf courses and three of these were ranked in the top 12.5% of sites for painted turtle abundance. Recent studies have indicated that golf courses provide potentially important habitat for painted turtles (Failey et al. 2007; Foley et al. 2012) and that painted turtle abundance on golf courses is comparable to that in agricultural and conservation areas (Price et al. 2013; Winchell and Gibbs 2016). In North Carolina, freshwater turtle species richness was higher in golf course wetlands than in urban or rural wetlands, and the researchers concluded that the maintenance of green space connectivity (including golf courses) would be beneficial for freshwater turtle diversity in urban areas (Guzy et al.

2013). Additionally, connectedness of green spaces within 500 m of wetlands had no effect on painted turtle occupancy, but very high painted turtle occupancy rates may have precluded the detection of any relationship between occupancy and the covariates used in the models (Guzy et al. 2013).

Our top occupancy model for painted turtles suggests that they are associated with larger wetlands with little woody vegetation. However, for this model the observed chi-square test statistic is large relative to the boostrapped distribution, suggesting lack of fit. Therefore, this and other competing models for this species should be interpreted with caution, especially with respect to the precision of the estimates. Given that the MacKenzie-Bailey goodness of fit test has no power to assess heterogeneity in occupancy, the lack of fit probably stems from unmodeled detection heterogeneity (MacKenzie and Bailey 2004, MacKenzie et al. 2006). Incorporating additional covariates (unmeasured covariates or those that vary by site) in the detection component of the model likely would improve model fit. Rather than explore this option and risk confounding comparisons among species, we used the model overdispersion parameter ($\hat{c}$) to inflate parameter standard errors, thereby adapting our biological inference (MacKenzie and Bailey 2004). We think it is likely that larger, often more permanent, wetlands contain higher densities of painted turtles, which could be influencing the probabilities of both occupancy and detection from site to site. An alternative explanation is simply that painted turtles are cosmopolitan in Rhode Island and that none of the covariates we measured adequately captured variation in occupancy or detection (Chapter 3). Painted turtles are the most widespread North American turtle and populations appear to be resilient to intense alteration of habitats, perhaps owing to their ability to disperse and readily colonize modified and created wetlands and their affinity for open habitats. In Illinois, connectivity to other wetlands increased the probability of both occupancy and colonization of wetlands by painted turtles (Cosentino et al. 2010). Heavily modified habitat types (i.e., urban, suburban and agriculture) in southern New England may be beneficial to painted turtles, even at the extreme end of the gradient, by providing enhanced nesting habitat, basking habitat, and increased aquatic plant production resulting from nutrient runoff (Brinson et al. 1981, Marchand and Litvaitis 2004).

There was no apparent trend in snapping turtle abundance across the gradient of forest cover, but unlike painted turtles, snapping turtle abundance decreased in the lowest forest cover class. Snapping turtles are also widespread and considered capable of occupying almost every kind of freshwater habitat (Ernst and Lovich 2009). In Indiana, snapping turtle abundance was greatest in impoundments and marshes and was not strongly affected by fragmentation (Rizkalla and Swihart 2006). In North Carolina however, snapping turtle occupancy increased with connectedness of green spaces (Guzy et al. 2013). In Rhode Island, there is a strong negative relationship between forest cover and road density (Pearson r = -0.889 at 1-km scale). Roads pose a substantial threat to freshwater turtles due to vehicle strikes and this mortality has the potential to disproportionately kill nesting females, resulting in male-skewed sex ratios (Steen and Gibbs 2004; Aresco 2005; Gibbs and Steen 2005). Larger body size is thought to increase risk of road mortality in turtles, especially in the Northeast given above average traffic density (Gibbs and Shriver 2002). The sharp decline in snapping turtle abundance observed in the lowest forest cover class may come as a result of increased risk of road mortality due to their larger body size.

We found that probability of snapping turtle occupancy increased with nitrate levels. Sources of nitrates include fertilizers, human waste, and industrial pollution (Bouchard et al. 1992). Elevated levels of nitrates increase plant and algae production, which can lead to anoxic conditions after this material decays. Snapping turtles and painted turtles are highly tolerant of anoxic conditions when overwintering (Ultsch 2006). This may provide both species with the ability to occupy seasonally oxygen-poor wetlands that less tolerant species cannot. Though widespread and still abundant in many areas, snapping turtles are being harvested in the USA at unprecedented rates to meet demands from Asian markets (Luiselli et al. 2016; Colteaux and Johnson 2017). Exports of live snapping turtles have increased three orders of magnitude since 1999, exceeding 1.3 million individuals in 2014, and approximately 16% of these were wild caught (Colteaux and Johnson 2017). Small wetlands that occur in developed landscapes are likely to play an increasingly important role in maintaining snapping turtle meta-population structure if this demand persists.

Occupancy models indicated both temporal and environmental influence in detection probability. Spotted turtle and red-eared slider detection probability increased with higher temperature. Painted turtle and snapping turtle detection probabilities were influenced by Julian date, with painted turtle detection probability decreasing over the course of the activity season, and snapping turtle detection probability peaking in late June (see Fig. S.2.1). Temporal variation in the detection probability of a species has important implications for the design of sampling protocols and ecological studies. For example, in the case of painted turtles, our results suggest that study design should address waning detection as the season progresses, especially when sampling multiple sites for comparative purposes. In studies examining sex ratio, detection probability should be examined for each sex independently to ensure that estimates are not biased. Few published studies on turtles appear to confront detection issues explicitly. Our sampling period does not cover the entire turtle activity season and inference regarding early-season detection should be interpreted with caution.

We did not model naïve abundance with environmental covariates because these estimates of abundance were associated with a high degree of variation. Precise estimates of abundances of aquatic turtles are considered very difficult to obtain, without longer term mark-recapture studies, due to inherent variation in catchability and observability (Dorland et al. 2014). Moreover, when sampled sites include non-permanent wetlands it can be difficult to define the meaning of an abundance estimate even in the context of a meta-population. Although we marked individuals, recapture rates for most species (except for painted turtles) were too low to yield estimates of abundance via mark-recapture modeling, particularly because each wetland was sampled for only one season. Nonetheless, we report naïve abundance estimates for descriptive purposes and to compare to other studies. Occupancy modeling is more robust to these issues and can be interpreted in the context of presence/absence and habitat selection. Although the utility of occupancy modeling is limited in that it does not permit estimation of important population parameters such as density, survival, or recruitment, the technique contributes to knowledge of distribution and allows for the identification of habitat features associated with a particular species, when multiple species are compared (Nielsen et al. 2010). It is possible that there was some violation of the closure assumption of occupancy modeling, but as we sampled each wetland for only one season that concern is minimized.

Identifying habitat features at the landscape scale that are associated with species occurrence is a common goal in landscape ecology. Doing so can improve the ability of biologists to predict where sensitive species occur within a state or region and inform management decisions for those species. Amassing herpetological occurrence records, through herpetological atlases or natural heritage programs, is a priority among state biologists in the Northeast, and these occupancy models may be used by biologists to target areas for surveys.

An understanding of the relationships between landscape patterns and ecological processes remains elusive for freshwater turtles. Relatively few studies consider landscape change over space and time simultaneously, despite the fact that many areas in the United States have experienced dramatic shifts in landscape composition associated with sustained growth in the human population in the last 150 years. Studies that consider not only landscape composition and configuration, but also within-wetland and temporal variables, are needed to advance our understanding of the factors that influence distributions of freshwater turtle populations.

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Tables

Table 2.1. Detection and occupancy covariates considered for aquatic turtle occupancy models, Rhode Island, USA 2013-2015.

Covariate	Description
Survey-level (*p*)	
julian*	Julian date (1-365) of day two of each survey replicate
temp*	Mean of maximum daily temperature (from nearest weather station) for days one and two of each survey replicate
precip*	Mean of total daily precipitation (from nearest weather station) for days one and two of each survey replicate
time*	Survey replicate number (1,2,3, or 4)
Site-level (Ψ)	
wetland covariates	
wetland.age	Age of wetland as determined using historic imagery (continuous variable 1-77)
hectares	Surface area (ha) of wetland as measured via GIS
max.depth	Maximum detected (m) depth measured using a weighted measuring tape
ph*	pH
tds*	Total dissolved solids
nitrate*	Dissolved nitrate (ppb) as measured from the water column
phos*	Dissolved phosphorous (ppb) as measured in the water column
graminoid*	Percent of wetland surface containing emergent graminoid vegetation
herbaceous*	Percent of wetland surface containing emergent forbs and other non-woody vegetation (including Nymphaea)
open.water*	Percent of unvegetated wetland surface
surficial*	Percent of wetland surface containing floating algae or Lemnaceae
woody*	Percent of wetland surface containing woody shrubs and trees (including dead wood and *Decadon verticillatus*)
landscape covariates	
easting*	Longitude expressed in universal transverse mercator units (Zone 19N)
northing*	Latitude expressed in universal transverse mercator units (Zone 19N)
forest (300, 1000)*	Percent of forest within buffers of 300 m and 1 km
wetland (300, 1000)*	Percent of wetland within buffers of 300 m and 1 km
esh (300, 1000)*	Percent of early successional habitat (agriculture, grassland, upland shrubland) within buffers of 300 m and 1 km
develop (300, 1000)*	Percent of human development within buffers of 300 m and 1 km
road.dens (300, 1000)*	Road density (m/ha) within buffers of 300 m and 1 km

* indicates that both a linear and quadratic relationship were considered.

Table 2.2. Occurrence and abundance of freshwater turtle species by forest cover, Rhode Island, USA 2013-2015.

	Forest cover 1 km									
	0-40%		20-60%		40-80%		80-100%			
	Forest cover 300 m								Total no. of wetlands (% of total)	Total no. of individuals
	10-20%	20-30%	30-40%	40-50%	50-60%	60-70%	80-90%	90-100%		
No. of wetlands	8	12	11	12	12	11	10	12	88	
Chelydra serpentina										
Number of wetlands where species detected	2	10	8	9	9	4	8	5	55 (62.5)	-
Number of individuals detected	7	53	42	24	21	8	21	31	-	207
Chrysemys p. picta										
Number of wetlands where species detected	8	11	10	10	10	9	9	6	73 (82.9)	-
Number of individuals detected	209	206	204	204	196	129	103	118	-	1369
Clemmys guttata										
Number of wetlands where species detected	0	1	0	0	0	1	2	3	7 (7.9)	-
Number of individuals detected	0	1	0	0	0	3	4	44	-	52
Sternotherus odoratus										
Number of wetlands where species detected	1	0	1	1	0	0	1	0	4 (4.5)	-
Number of individuals detected	1	0	1	6	0	0	4	0	-	12
Trachemys scripta										
Number of wetlands where species detected	1	5	1	1	1	0	0	0	9 (10.2)	-
Number of individuals detected	2	11	3	4	1	0	0	0	-	21
Total	-	-	-	-	-	-	-	-	-	1661

Table 2.3. Occupancy models (from secondary global model subset) within 2 BIC units of top models (bolded) which show the strongest relationship between species presence and measured covariates in Rhode Island, USA, 2013-2015.

Chelydra serpentina												GOF statistics		
p intercept	*p* julian	*p* julian^2	Ψ intercept	Ψ nitrate				k	BIC	delta	weight	χ2	*P* -value	$\hat{c}$
0.20 (0.23)	-1.19 (0.22)	-0.71 (0.20)	0.91 (0.30)					4	346.15	0	0.716			
0.21 (0.23)	**-1.19 (0.22)**	**-0.73 (0.20)**	**0.93 (0.31)**	**0.52 (0.43)**				**5**	**347.65**	**1.50**	**0.284**	**28.1**	**0.53**	**0.86**
Chrysemys picta														
p intercept	*p* julian		Ψ intercept	Ψ hectares	Ψ wetland.300	Ψ woody	Ψ phos	k	BIC	delta	weight			
1.69 (0.19)	**-0.82 (0.18)**		**2.68 (0.66)**	**1.82 (0.84)**		**-1.35 (0.43)**		**5**	**316.64**	**0**	**0.552**	**64.19**	**0.027**	**2.06**
1.68 (0.19)	-0.78 (0.18)		2.72 (0.66)	1.59 (0.82)	0.86 (0.73)	-1.26 (0.43)		6	318.25	1.61	0.229			
1.74 (0.19)	-0.81 (0.19)		2.91 (0.79)	1.88 (0.89)		-1.53 (0.49)	1.86 (1.29)	6	318.51	1.87	0.219			
Clemmys guttata														
p intercept	*p* temp		Ψ intercept	Ψ forest.1000	Ψ max.depth	Ψ wetland.age	Ψ woody	k	BIC	delta	weight			
0.40 (0.59)	**1.97 (0.78)**		**-4.10 (1.06)**	**1.86 (0.68)**	**-2.52 (1.19)**			**5**	**74.36**	**0**	**0.398**	**16.82**	**0.814**	**0.64**
0.14 (0.64)	1.57 (0.72)		-4.11 (1.43)	2.20 (1.22)			1.65 (0.83)	5	75.09	0.73	0.217			
0.26 (0.69)	1.69 (0.77)		-4.01 (1.17)	1.80 (0.95)	-1.45 (1.17)		1.15 (0.72)	6	76.13	1.77	0.212			
-0.53 (0.48)	1.22 (0.58)		-11.00 (10.14)	6.15 (6.59)	-4.50 (4.79)	3.52 (6.16)	3.47 (3.27)	7	76.17	1.83	0.173			
Trachemys scripta														
p intercept	*p* temp		Ψ intercept	Ψ ph	Ψ road.dens.1000			k	BIC	delta	weight			
-0.86 (0.50)	**1.01 (0.41)**		**-2.57 (0.59)**		**1.39 (0.53)**			**4**	**101.06**	**0**	**0.608**	**16.87**	**0.742**	**0.52**
-0.88 (0.53)	0.85 (0.37)		-3.19 (0.95)	1.17 (1.15)	1.49 (0.69)			5	101.98	0.92	0.392			

values inside parentheses are SEs; *p* is detection probability; Ψ is occupancy probability; k is number of parameters in the model; BIC is Bayesian information criterion; GOF is goodness-of-fit; c-hat is the overdispersion parameter; covariate definitions can be found in Table 1

Figures

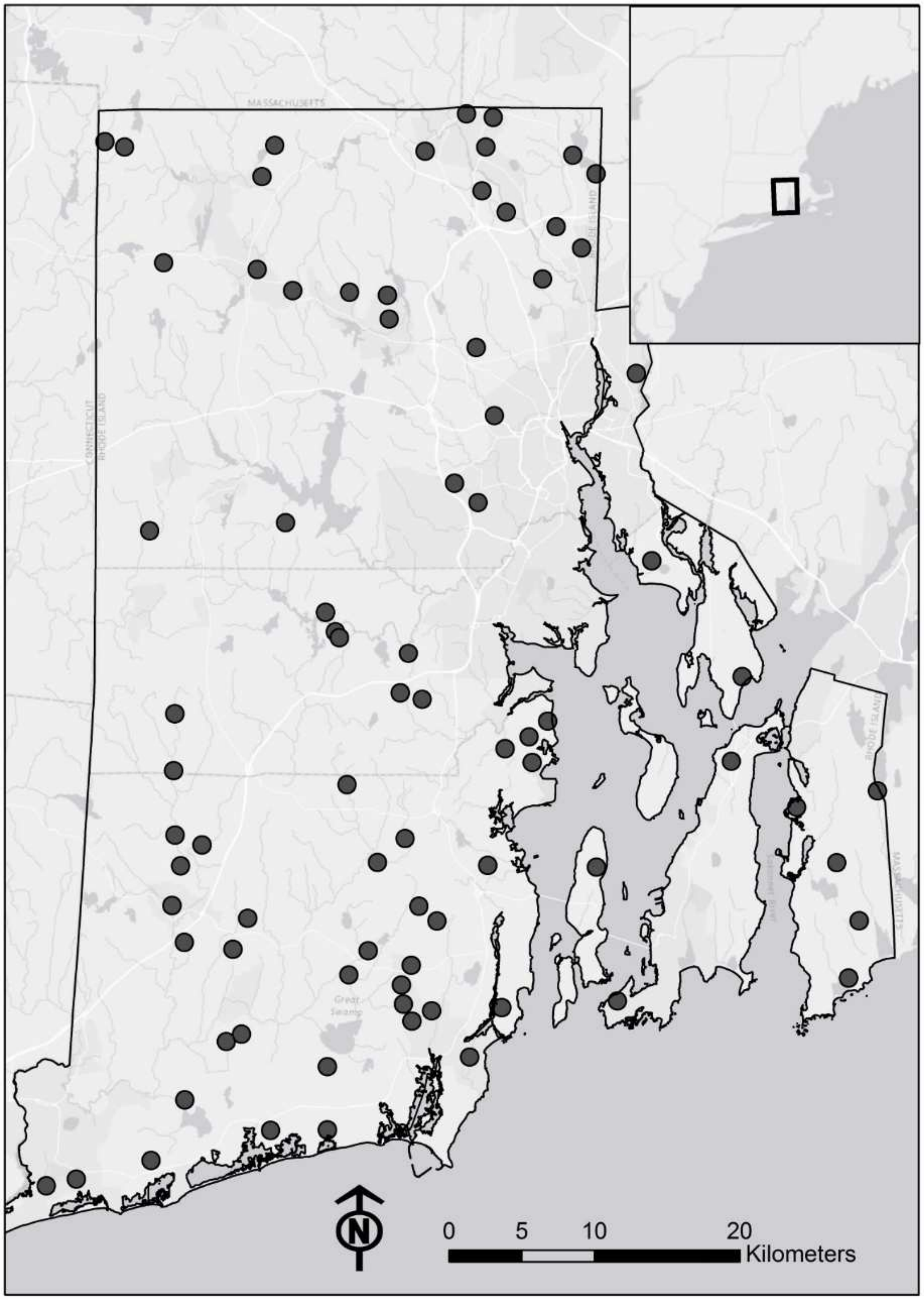

Figure 2.1 Map of Rhode Island, USA showing the locations of sampled wetlands 2013-2015. An additional seven sites are not pictured where spotted turtles where detected.

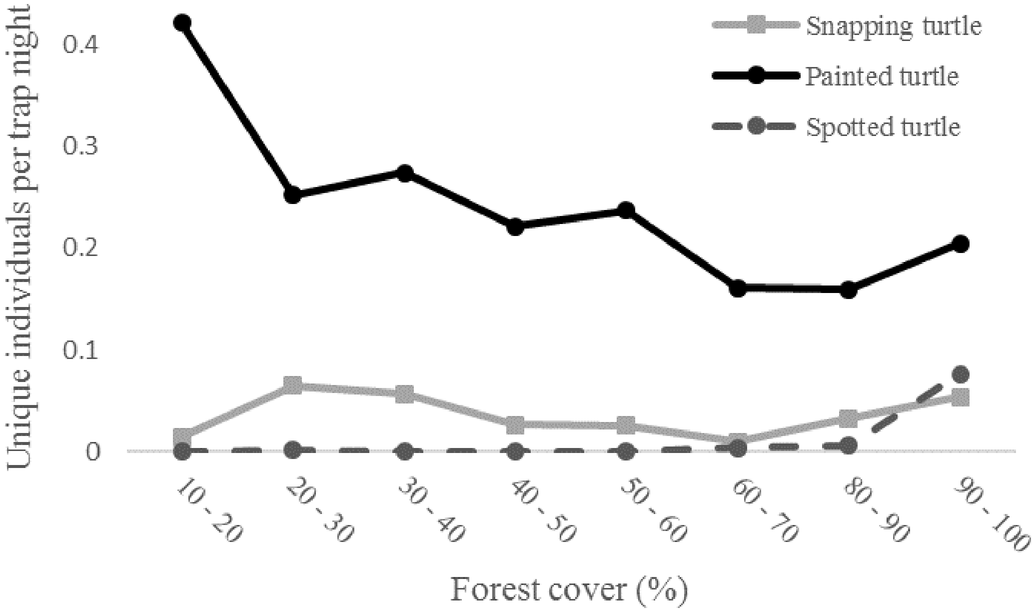

Figure 2.2 Number of unique individuals per trap night (naïve abundance) for each 300-m forest cover class for species of freshwater turtles trapped in Rhode Island 2013-2015. Red-eared sliders and musk turtles not presented because of low abundance estimates when visualized at this scale.

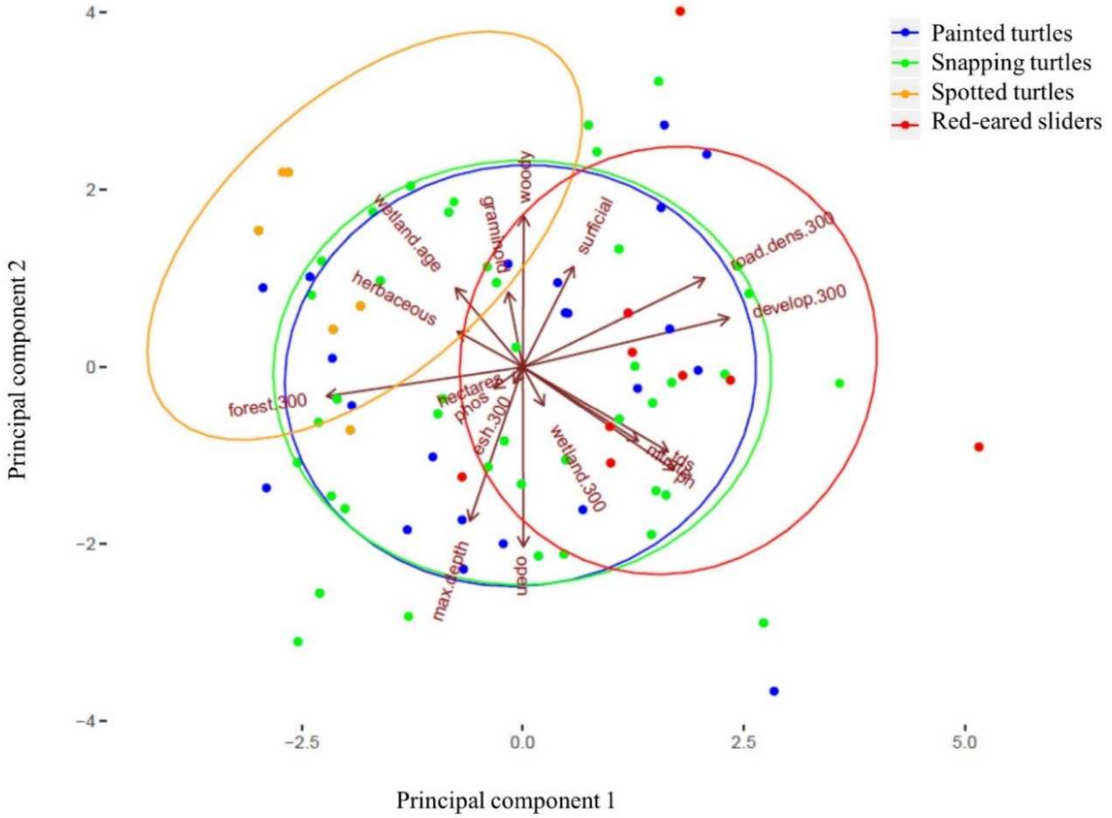

Figure 2.3 Principal components analysis plot showing relationships between presence of freshwater turtle species and environmental covariates in Rhode Island, USA. Principal component 1 explained 20.4% of the variation, and principal component 2 explained 15.0% of the variation. Arrows indicate the direction and magnitude of covariate loadings. Ellipses are centered on the mean values for each species and encompass one SD along each axis.

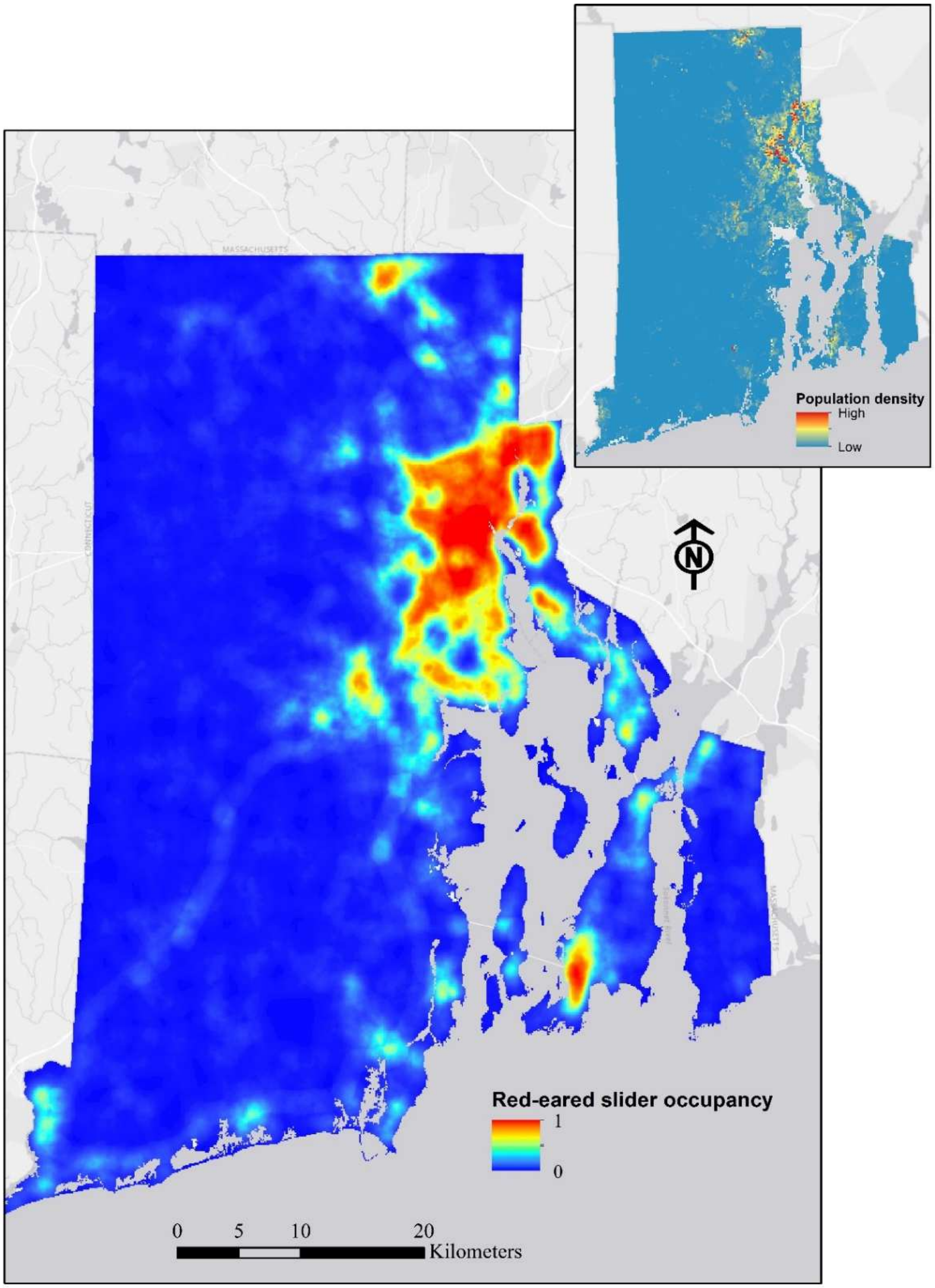

Figure 2.4 Predicted red-eared slider occupancy in Rhode Island, USA, developed from the top model at a 100-m cell size and based on detections from 2013-2015. Inset map shows human population density for comparison.

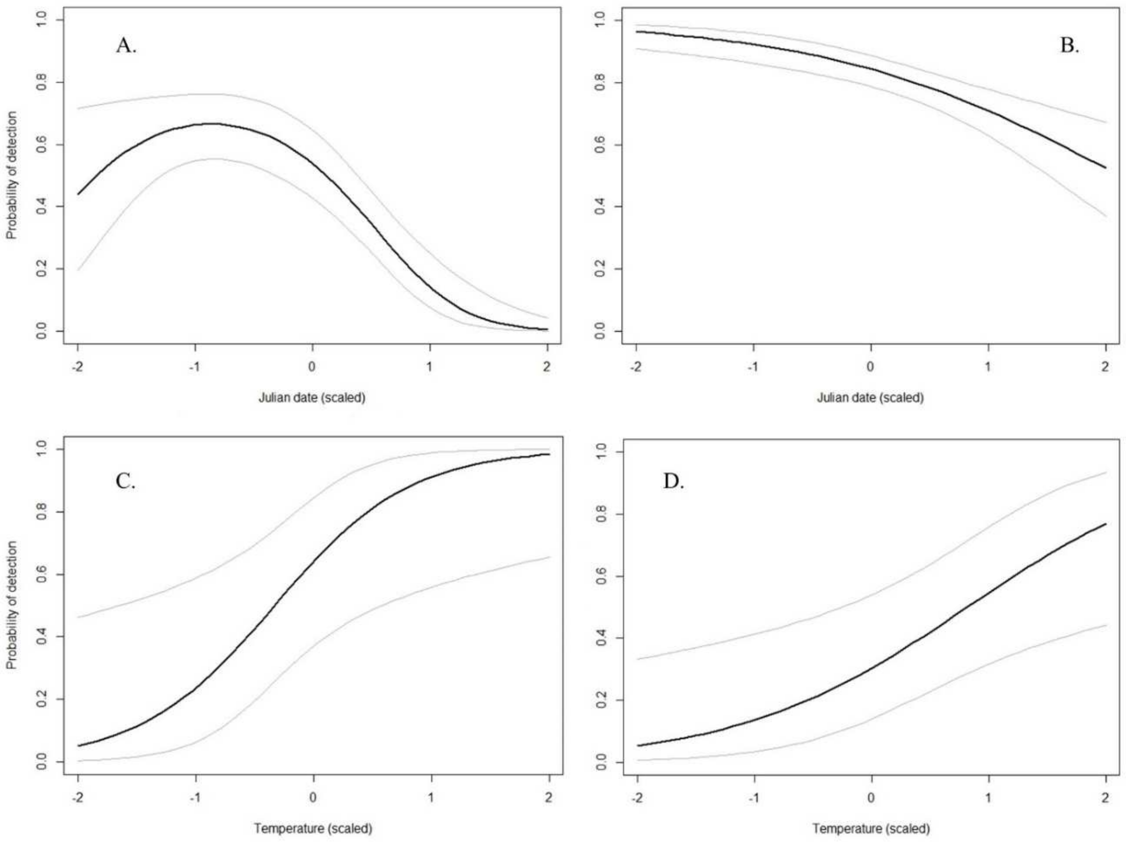

Figure S.2.1. Plots showing probability of detection estimates (p) by Julian date for A.) snapping turtles, B.) painted turtles, and by mean air temperature for C.) spotted turtles, and D.) red-eared sliders based on the top model. X-axes are scaled and centered on the mean of each covariate and each shows approximately 95% (±2 SD) of observed values. Mean Julian date is 30 July and the range shown is approximately the beginning of May to the end of October. Mean air temperature was 24.4˚C and the range shown is approximately 14.1 to 34.6˚C.

Manuscript III: Lack of Signal in Painted Turtle (*Chrysemys picta*) Abundance and Sex Ratio across a Landscape Gradient in Rhode Island

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ABSTRACT

Painted turtles (*Chrysemys picta*) are one of the most well-studied species of freshwater turtle, but our understanding of the ways in which populations respond to human-induced landscape alteration remains lacking. We sampled eastern painted turtles (*Chrysemys p. picta*) from 88 randomly selected wetlands across a range of landscape conditions in Rhode Island, USA. Turtles were systematically and intensively sampled for one year at each wetland to estimate abundance, sex ratio, juvenile ratio, and body mass index. We compared demographic traits between natural and manmade wetlands, used model selection to determine which environmental and within-wetland covariates best explained abundance, and tested whether increasing road density surrounding wetlands resulted in more male-skewed populations. There was no difference in abundance or any demographic trait between natural and manmade wetlands. A negative relationship between abundance and forest cover surrounding wetlands emerged as the most parsimonious model, but explained exceedingly little variation. Contrary to expectations, there was a significant, but very weak relationship between increasing road density and the proportion of females in a population. Collectively, these results suggest that eastern painted turtles are exhibiting little to no detectable variation in population demography across the range of landscapes found in Rhode Island and are resilient in the face of human-induced landscape change.

Habitat degradation and loss are considered leading global causes of population declines in turtles (Gibbons et al. 2000, van Dijk 2000). Therefore, human-induced landscape alteration is usually associated with instability in turtle populations (Dudgeon et al. 2006, Bohm et al. 2013). New England, in the northeastern United States, has experienced tremendous changes to the landscape since the time of European settlement (Dahl 1990, Foster and Aber 2004), but the legacy of these changes on populations of freshwater turtles is poorly understood. The sensitivity of certain species of freshwater turtles to landscape change is clear. For example, as habitat specialists, bog turtles are particularly susceptible to habitat loss and fragmentation (U.S. Fish and Wildlife Service 2001, Shoemaker and Gibbs 2013). But how populations of more generalist species, like painted turtles (*Chrysemys picta*), respond to landscape change remains an open question.

Due to their widespread geographic range, relative abundance, and generalist habitat requirements (Ernst and Lovitch 2009), painted turtles are one of the most well-studied species of freshwater turtle. Despite a relatively expansive literature, our understanding of how past and current landscapes influence the distribution, abundance, and demography of this species is lacking. Studies have posited a number of different landscape-scale factors as influencing painted turtle abundance within populations. These include a positive relationship with more open area for nesting (Baldwin et al. 2004, Marchand and Litvaitis 2004), a negative relationship with land use diversity (Rizkalla and Swihart 2006), and a negative relationship with increasing road density (Winchell and Gibbs 2016). But there is little consensus as to what features of landscape composition and configuration influence painted turtle distribution and abundance, even for the most conspicuous and ostensibly impactful types of alteration.

Roads are one of the most ubiquitous ways in which humans alter the landscape (Laurance and Balmford 2013). Aside from being implicated in direct population declines (Nafus et al. 2013), roads are thought to skew sex ratios in turtle populations by disproportionately affecting females as they cross roads while seeking nesting habitat (Steen et al. 2006). Direct observations of turtles dead on roads have supported this idea (Wood and Herlands 1997, Haxton 2000), and studies examining this question experimentally have documented proportionally fewer females in wetlands surrounded by more roads (Marchand and Litvaitis 2004, Steen and Gibbs 2004, Aresco 2005a). Other studies have documented sex ratios that have shifted over time to become male-biased and implicated roads as a probable cause (Gibbs and Steen 2005, Browne and Hecnar 2007).

That some turtle populations are threatened by road mortality is not in question (Ashley and Robinson 1996, Wood and Herlands 1997, Aresco 2005b). This problem is compounded by delayed sexual maturity and low recruitment rates in turtles, making populations particularly susceptible to the removal of adult females (Brooks et al. 1991, Doak et al. 1994). But under what conditions and to what extent the threat of road mortality applies to painted turtles is unclear. Studies have reported associations between roads and both skewed sex ratios (Marchand and Litvaitis 2004, Steen and Gibbs 2004), and reduced reproductive success (Browne and Hecnar 2007, Laporte et al. 2013) in painted turtles. However, other studies have failed to find evidence of a relationship between roads and abundance (Failey et al. 2007) or sex ratio (Dorland et al. 2014, Reid and Peery 2014) for the species. The question remains as to whether even the most conspicuous landscape alterations are responsible for consistent effects on painted turtle abundance and sex ratio.

Eastern painted turtles (*Chrysemys p. picta*) are one of four recognized subspecies of painted turtles. Collectively, these four subspecies have the largest geographic range of any turtle in North America, stretching across the continent (Ernst and Lovich 2009). Eastern painted turtles occupy the eastern part of this range, stretching from Georgia, USA to New Brunswick, Canada along the Atlantic seaboard. Using data collected during a broader study of freshwater turtle occurrence and habitat selection, we set out to determine if there were detectable trends in eastern painted turtle abundance and demography across a gradient of landscape alteration. Specifically, we (1) compared measures of abundance and demographic traits between natural and manmade wetlands, (2) modeled abundance using a number of landscape and within-wetland covariates, and (3) tested the prediction that sex ratio would be male-biased in wetlands with higher surrounding road density.

STUDY AREA

Our study was conducted throughout the state of Rhode Island located in southeastern New England, USA. At approximately 2,700 square kilometers (when excluding coastal waterways), Rhode Island is the smallest state geographically in the USA but ranks second highest in population density (U.S. Census 2010, Suitland, MD, USA. Available from www.census.gov, accessed March 2017). Approximately 54% of the state is forested, with pine, oak, and maple forests dominating the western part of the state (Butler 2013) and the highest human population densities occurring along the ocean coast and along Narragansett Bay. Intensive landscape alteration since Europeans settlement has occurred for centuries including immense alteration to the composition of freshwater wetlands (Magilligan et al. 2016). Drainage, filling, damming, and channelization have resulted in the loss of an estimated 37% of the wetlands in Rhode Island between 1780 – 1980 (Dahl 1990). The creation of wetlands for drinking water and agriculture has further altered the landscape. More recently, post-World War II economic growth led to a construction boom and the creation of the interstate highway system, both of which consumed and fragmented large areas of the landscape.

Mean elevation in the state is approximately 60 m with a highest point of 247 m. Long term (1981-2010) average annual temperature in Kingston, RI was 10.5 °C and long-term average annual precipitation was 134.3 cm. Long term average monthly temperatures range from -1.4 °C in January to 22.1 °C in July (National Oceanic and Atmospheric Administration, National Centers for Environmental Information [NOAA NCEI]. Available from www.ncei.noaa.gov, accessed March 2017).

METHODS

In an effort to capture the state-wide variability in landscape composition and configuration, we used a stratified random design to select sites. To minimize confounding factors among sites, we focused our sampling on relatively small (0.1–1.8 ha), non-riparian wetlands. To further reduce confounding variables, we excluded wetlands that were within 500 m of the coastline, or within 300 m of a federal or state highway, or 10 m of a local road. We used ArcGIS version 10.1 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) to perform landscape and spatial analyses. We identified all freshwater wetlands 2 ha or less in size throughout the state using the National Wetland Inventory (NWI) dataset (Falls Church, VA, USA. Available from www.fws.gov/wetlands/index.html, accessed March 2013), the Land Cover and Land Use 2011, and the Lakes, Ponds, and Reservoirs datasets available from the Rhode Island Geographic Information System (RIGIS; Kingston, RI, USA. Available from www.rigis.org, accessed March 2013). We grouped each wetland into a small (0.1–0.4 ha) or large (< 0.4–1.8 ha) category, with a 0.4 ha breakpoint that was the approximate median of the distribution of wetland size for all wetlands. We calculated percent forest cover within buffers of 300 m and 1 km from the wetland edge of all retained wetlands. At the 300-m scale, we grouped wetlands into eight classes representing 10% increments of forest cover (excluding 0–10% and 70–80%), and at the 1 km scale we grouped wetlands into four, partially overlapping, larger stratifications of forest cover (0–40%, 20–60%, 40–80%, 80–100%). These groups created a near-continuous gradient of sites from different forest cover conditions. We assigned each retained wetland a random number, sorted them by random number, and contacted property owners/managers in ascending order until we received permission to sample the desired number of wetlands in each forest cover and size stratification. We carried out this process in each of three consecutive years.

Turtle Sampling and Data Collection

We sampled turtles from May to October in 2013–2015, sampling between 28-30 wetlands each year. At each wetland, we conducted up to four survey occasions (hydroperiod allowing) that lasted approximately 48 hours each. Survey occasions consisted of two, consecutive trapping sessions in which traps were checked and data were collected approximately every 24 hours.

We sampled sites using small (30.5 cm diameter collapsible minnow traps, Promar Nets, Gardena, CA, USA) and large traps (91.4 cm single throated hoop trap, Memphis Net and Twine, Memphis, TN, USA), baited with sardines that were placed inside perforated plastic containers. Alternating between small and large traps and always setting an even number of traps, we placed traps approximately 30 m apart around the perimeter of wetlands such that the perimeter of each wetland determined the number of traps deployed. The number of traps set ranged from four in the smallest wetland (0.10 ha) to 24 in the largest wetland (1.73 ha). We placed traps within 10 m of the wetted edge with a portion of the trap always staked or floated above the surface of the water to ensure turtles the opportunity to breathe. We opportunistically hand-captured a small number of turtles (<10) that were encountered when working with traps. Each turtle was identified to species, sexed, measured and weighed, and marked along the marginal scutes with a unique code. Turtles were sexed using secondary sexual characteristics and considered juveniles if these characteristics were not well developed, or unknown if they were ambiguous. We released all turtles immediately after processing.

At each wetland, we made visual percent cover estimates of vegetation during the second or third survey after all vegetation had fully emerged. There were five categories including emergent graminoid, emergent forbs and other non-woody vegetation (including Nymphaeaceae), woody shrubs and trees, surficial algae and Lemnaceae, and open water. Estimates were made for each vegetation category while standing at the wetland edge, and all estimates were made by the same individual (S.B.) in the year that turtles were sampled. To assess water chemistry at each wetland, we collected samples from three distinct points within each wetland and combined them to form one 125 ml sample for subsequent laboratory analysis. We collected all water samples in the spring of 2015. We measured pH (model HI-902, Hanna Instruments Inc.,Woonsocket, RI, USA) and total dissolved solids (EcoTestr TDS Low, Oakton Instruments, Vernon Hills, IL, USA) on the same day as sample collection. We measured nitrate-nitrogen and dissolved phosphorous with a segmented flow nutrient autoanalyzer (Astoria Pacific Inc., Clackamas, OR, USA). The limit of detection for nitrate was 15 μg/l, and 4 μg/l for dissolved phosphorous. All analyses were measured against appropriate standards in the University of Rhode Island Watershed Watch state-certified laboratory.

We used aerial and digital imagery datasets available from RIGIS to quantify landscape features and wetland age. Road density (m/ha) was calculated using the TIGER Roads dataset. Wetland age was determined using historic aerial imagery taken at approximately 10-yr increments and dating back to 1939 to determine the age (up to > 77 yr) of all sampled wetlands. We used the Forest Habitat dataset to determine percent cover of different landscape types and to quantify landscape metrics. We first reclassified attribute categories in this dataset to broader categories that included early-successional habitat (agriculture/grassland/upland shrubland), forest (all upland and wetland forest types), and wetland (freshwater lakes and rivers/wetland shrubland/wetland freshwater emergent), marine wetland/estuarine, and other (barren land, rock, sand). We then clipped buffers of this modified dataset at 300 m and 1 km from the perimeter of each wetland, converted these vector data to raster data with a 10-m cell size, extracted a separate raster file for each buffer, and used Fragstats (version 4.2, McGarigal et al. 2012) to quantify landscape composition. We selected spatial scales of 300 m and 1 km because of their precedent and ecological relevance (Semlitsch and Bodie 2003; Steen et al. 2012).

Statistical Analysis

We estimated abundance for each wetland as captures per unit effort by calculating the total number of unique individuals caught divided by the total number of trap nights. To estimate sex ratios at each wetland, we calculated the proportion of females among all unique adults captured. To explore the possibility of temporal bias in estimates of sex ratio, we also calculated the proportion of female captures by each sampling occasion, separately for newly caught individuals and recaptures. We calculated the proportion of juveniles among all unique individuals caught for each wetland. We calculated a body mass index (BMI) for all turtles by dividing weight (g) by straight-line carapace length (mm). We classified all wetlands as either "natural" (i.e., ≥ 77 years old) or "manmade" (i.e., < 77 years old) and compared each demographic measure described above between both groups. We used two-sample independent *t*-tests to compare population means of each demographic measure between both groups.

We used linear regression to estimate the effect of road density on sex ratio. As a response variable, we used wetland-specific sex ratio estimates and performed identical tests on a 'full' dataset including all wetlands, and a 'restricted' dataset limited to wetlands where at least 10 individuals were caught. Shapiro-Wilk tests were used to assess normality in these response variables.

We used generalized linear models (GLM) to estimate the effect of environmental covariates on unique individuals per trap night as a measure of abundance. We used a Gamma distribution to model error in the abundance distribution, adding nominal values (0.0001) to wetlands with no eastern painted turtle detections to address large numbers of zeros in the dataset that would otherwise limit analyses. We used an information criterion framework to compare models composed of different combinations of covariates with the aim of identifying the model(s) that most parsimoniously described abundance (Burnham and Anderson 2002). As a means of variable reduction, for landscape covariates, we first compared single-covariate models at both spatial scales (i.e., 300 m and 1 km) and retained the term from the most supported model based on a Bayesian Information Criterion (BIC; Burnham and Anderson 2002). We built an 'initial' additive global model consisting of the linear terms for each retained landscape covariate as well as all other wetland covariates. We considered all subsets and identified the most supported models using BIC. When subsetting, we limited the number of covariates (excluding the intercept) in any one model to five to limit the ratio of parameters to sample size. We retained all covariates included in any model within 4 BIC units of the top model and used these to build a 'secondary' global model. To determine which functional form to include in the secondary global model, for the appropriate covariates, we then built separate, single-covariate linear and quadratic models and compared them using BIC. We retained the term from the most supported model. If remaining covariates were highly correlated (≥0.9 Pearson correlation coefficient; Appendix 3), we compared single-covariate models containing each term using BIC and retained the term from the more supported model. With these retained terms, we then built the secondary global model, evaluated all subsets of covariates included in this model, and considered the most supported model as our top model. A pseudo-R^2 was used to assess model fit.

We report means ± one standard error (SE), and we defined statistical significance as $P \leq 0.05$. Statistical analyses were performed using R (version 3.4.1, www.r-project.org, accessed 1 October 2017).

RESULTS

We made a total of 1841 eastern painted turtle captures at 88 wetlands over three years (5824 trap nights). These captures consisted of 1369 unique individuals. Eastern painted turtles occurred in 73/88 wetlands (82.9%) and averaged 15.6 (SE = 2.06, n = 88 wetlands, range = 0–94 individuals) unique individuals/wetland. The proportion of females in the total catch was 0.433 (516 females/1191 adult individuals), and averaged 0.465 (SE = 0.024, n = 73, range = 0–1) when measured by wetland, but the proportion of new females caught declined after sampling occasion one (Figure 1). When limiting data to the first sampling occasion, the proportion of females in the total catch was 0.483 (265 females/549 adult individuals), and averaged 0.549 (SE = 0.03, n = 66, range = 0–1) when measured by wetland. There were no differences in abundance, sex ratio, juvenile ratio, or BMI between natural and manmade wetlands (all P-values > 0.10; Table 1).

Mean road density within 300 m of sampled wetlands was 37.8 m/ha (SE = 3.2, n = 88, range = 0–132.2 m/ha). We found no relationship ($R^2 = 0.020$, $P = 0.119$) between the proportion of females and road density for all wetlands, but identified a positive relationship ($R^2 = 0.072$, $P = 0.039$; Figure 2) for wetlands in which >10 turtles were captured.

Females averaged 140.5 mm (SE = 0.75, n = 516, range = 81.2–181 mm) in length and 380.9 g (SE = 5.10, range = 85–755 g) in weight. Males averaged 124.1 mm (SE = 0.48, n = 675, range = 85.5–160.7 mm) in length and 242.2 g (SE = 2.52, range = 70–570 g) in weight. We identified two top models for eastern painted turtle abundance based on BIC scores (Table 2). The first was the null model that contained only an intercept and no covariates. The second was a model that included a single coefficient for forest cover within 300 m (Figure 3). The pseudo-R^2 for the forest cover model was 0.027.

DISCUSSION

We found no evidence that eastern painted turtle abundance was influenced strongly by any landscape or within-wetland covariate. Nor was there evidence that sex ratio was male-skewed in wetlands surrounded by more roads. In fact, contrary to expectations we found a very slight, but significant positive relationship between female-skewed sex ratios and road density. Furthermore, all demographic measures, including abundance, sex ratio, proportion of juveniles, and BMIs, were similar between natural and manmade wetlands. Collectively, these results suggest that eastern painted turtles are cosmopolitan in Rhode Island and are exhibiting little to no detectable variation in population demography related to covariates measured along a gradient of landscape types.

The trend of decreasing abundance with greater forest cover was significant in the top model, but the amount of variation explained was very low (< 3%). There was a recognizable pattern of reduced abundance at the highest end of the forest cover gradient, however. We detected no painted turtles at six of 11 wetlands surrounded by > 90 % forest cover, and four of the five remaining wetlands contained values of abundance that were below the mean. Painted turtles prefer open areas for nesting and the limited availability of nesting habitat in fully forested areas may reduce abundance (Baldwin et al. 2004, Marchand and Litvaitis 2004). Painted turtles are often found in high abundance relative to other species of freshwater turtles, even in areas of major human disturbance (Congdon and Gibbons 1996, Gamble and Simons 2004). Other studies have found significant relationships between painted turtle abundance and a variety of different factors. In New Hampshire, greater abundance was explained by decreasing distance to the nearest wetland and less herbaceous vegetation in wetlands, among other factors (Marchand and Litvaitis 2004). These authors concluded that populations of painted turtles were under threat by habitat alterations related to human development, namely increased road density and a greater density of generalist predators like raccoons (*Procyon lotor*). Our results do not support a similar conclusion for our study area.

We chose not to model other demographic measures with our suite of covariates as preliminary analyses suggested very little pattern in the data. Instead, we classified each wetland as natural or manmade based on historic aerial imagery that dates back to 1939 and inspected these groups for differences. We found no measurable differences in abundance, sex ratio, proportion of juveniles, or body mass indices between groups. All manmade wetlands were either created by excavation, or formed by restricting the flow of a stream in the last 77 years. The fact that there were no differences in populations of eastern painted turtles between these sites and natural sites suggests that turtles actively colonized the novel habitats shortly after it was created and began successfully breeding. Painted turtles are known to readily disperse from one wetland to another via terrestrial movements on the order of kilometers (Zweifel 1989, Bowne 2008) and readily colonize wetlands where they do not occur (Tuberville et al. 1996, Cosentino et al. 2010). Colonization and extinction of small wetlands by painted turtles is a dynamic process that is heavily influenced by wetland size, hydroperiod, and landscape connectivity to other wetlands (Cosentino et al. 2010).

Our overall estimate of sex ratio was near 1:1, but detectability varied substantially over the course of the activity season. The sex ratio detected in the first sampling occasion (May–June) may be most reflective of the actual sex ratio within the population. Alternatively, the first sampling occasion may have occurred during the portion of the activity season when females are most active and most likely to enter traps, thus representing an inflated estimate of sex ratio. Similarly, the reduced proportion of new females detected in subsequent sampling occasions may have resulted from reduced activity, or may be more reflective of the actual sex ratio. Either way, it is clear that there is a strong seasonal effect on the detection of females, which has major implications for the estimate of sex ratios. Without knowing the characteristics of this trapping bias, only by censusing an entire population can we confidently estimate sex ratio. In Long Island, an 18-year study of painted turtles at a complex of small ponds found that estimates of adult sex-ratio varied greatly from year to year, but averaged close to 1:1 over the course of the study (Zweifel 1989). It remains common for many ecologists to ignore both stochasticity and temporal shifts in detection when sampling reptiles and estimating demographic measures like sex ratio, even though these issues have been recognized for decades.

Our results make clear that there is no pattern of male-skewed sex ratios with increasing road density for eastern painted turtles in Rhode Island. Given our large sample size of 88 wetlands distributed broadly across the state and intensive sampling regime, we believe this is a robust result. However, we must consider our scope of inference and place this result in the proper context. The location and traffic density of a road are important factors to be considered as well. When choosing sites, we selectively excluded wetlands within 300 m of federal or state highways. Collectively, these roads account for a very small percentage of total linear distance in the state, but these are the roads with some of the highest traffic volumes. This is likely to have excluded a number of wetlands where turtles experience the highest rates of road mortality (Gibbs and Shriver 2002, Litvaitis and Tash 2008). A number of studies have examined the effects of high traffic volume roads immediately adjacent to wetlands and there is evidence that the majority of turtle road mortality occurs at severe hotspots with these characteristics (Aresco 2005b, Langen et al. 2012) and that mortality spikes at particular times of year (Ashley and Robinson 1996, Glista et al. 2007, Beaudry et al. 2009). In New York, the proportion of painted turtle females decreased with higher surrounding density of high volume roads, but overall sex ratio was not different than 1:1 (Winchell and Gibbs 2016). The disproportionate susceptibility of females to road mortality is thought to be more pronounced in freshwater turtles than terrestrial turtles, as male freshwater turtles are expected to spend more of their time in the water and less exposed to the dangers of roads. However, male painted turtles are known to move across land between wetlands and are certainly susceptible to road mortality as well. Road density alone may be an insufficient predictor of sex ratio in painted turtle populations. Future studies should integrate information on traffic volume, when possible.

Our sampling was limited to one year at each wetland making our estimates of abundance subject to error associated with inter-annual variation. However, eastern painted turtles are highly aquatic and so inter-annual abundance is likely less variable in this species than in a more terrestrial freshwater species such as the spotted turtle (*Clemmys guttata*). In addition, we made a substantial effort to limit potential bias. By distributing traps in a spatially homogenous way at each wetland, we were able to ensure coverage of the entire wetland and not rely on the assumption that turtles are distributed evenly throughout the wetland at all times. By sampling intensively up to four times, with sampling occasions fairly evenly spaced across the activity season, we were able to limit temporal bias associated with peaks of activity throughout the year. Surprisingly few studies take both of these things into account. Future studies should be sure to sample in a systematic way and, when possible, collect data for multiple years.

MANAGEMENT IMPLICATIONS

At least two factors give painted turtles an advantage over sympatric species of freshwater turtles when it comes to the impacts of road mortality and other disruptive forces that occur in areas of greater human disturbance. Small body size reduces the probability of vehicle strikes when crossing roads (Gibbs and Shriver 2002), and relatively rapid reproductive parameters (i.e., shorter generation time and higher fecundity) allow populations to rebound from declines in shorter periods of time. If we can identify which species are most susceptible to road mortality and which are not, based on these parameters, this could greatly help to steer limited management resources to those species that are most susceptible.

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Tables

Table 3.1. Comparison of *Chrysemys picta* abundance and demography between "natural" (≥77 years old) and "manmade" (< 77 years old) wetlands, Rhode Island, USA, 2013-2015. Numbers in parentheses are SE. *P*-values are from a two-sample *t*-test.

	"Natural"	"Manmade"	Combined	*P*-value
Number of wetlands	53	35	88	-
Unique individuals per trap night	0.212 (0.021)	0.216 (0.031)	0.214 (0.021)	0.923
Proportion female (adults only)	0.437 (0.039)	0.503 (0.025)	0.465 (0.025)	0.153
Proportion juvenile (all individuals)	0.103 (0.018)	0.089 (0.022)	0.097 (0.014)	0.608
Female body mass index	2.663 (0.051)	2.708 (0.079)	2.684 (0.045)	0.629
Male body mass index	1.979 (0.034)	1.89 (0.044)	1.939 (0.027)	0.116
Sexes combined body mass index	2.263 (0.033)	2.303 (0.054)	2.280 (0.029)	0.528

Table 3.2. Generalized linear models within 2 BIC units of top model for *Chrysemys picta* abundance, Rhode Island, USA, 2013-2015.

intercept	forest.300	log Likelihood	k	BIC	delta	weight	pseudo r^2
4.685		68.647	1	-128.4	0	0.241	0
2.238	0.0519	70.238	2	-127.1	1.28	0.127	0.027

Figures

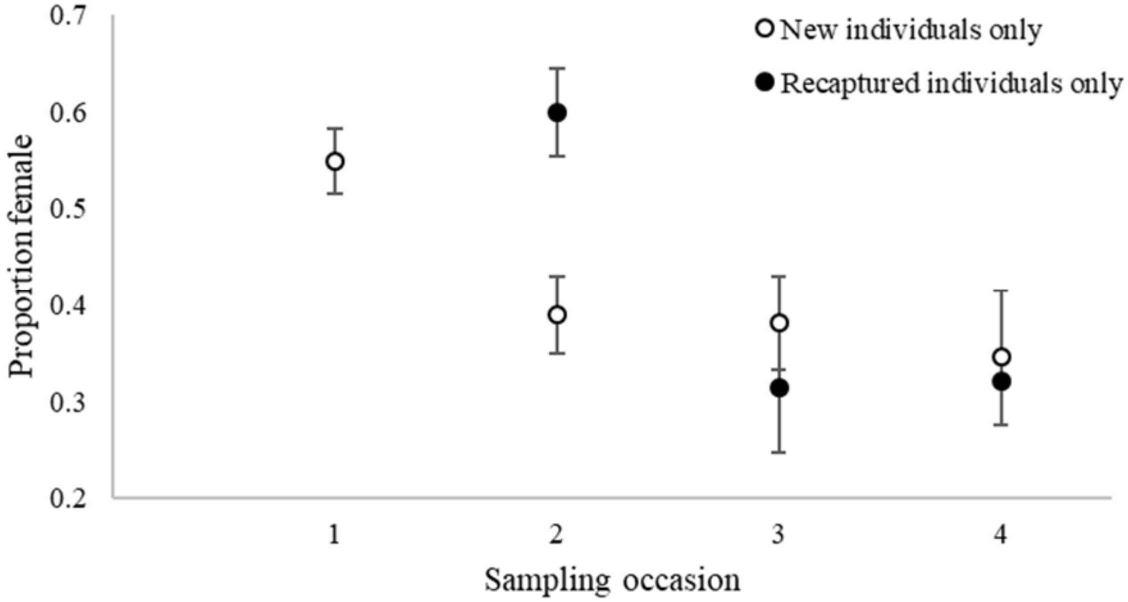

Figure 3.1. Proportion of female captures by sampling occasion for adult *Chrysemys p. picta*, Rhode Island, USA, 2013-2015. Open circles are means derived from all wetlands with captures during that sampling occasion using only new individuals. Closed circles are means derived from all wetlands with captures during that sampling occasion using only recaptured individuals. Error bars are standard errors.

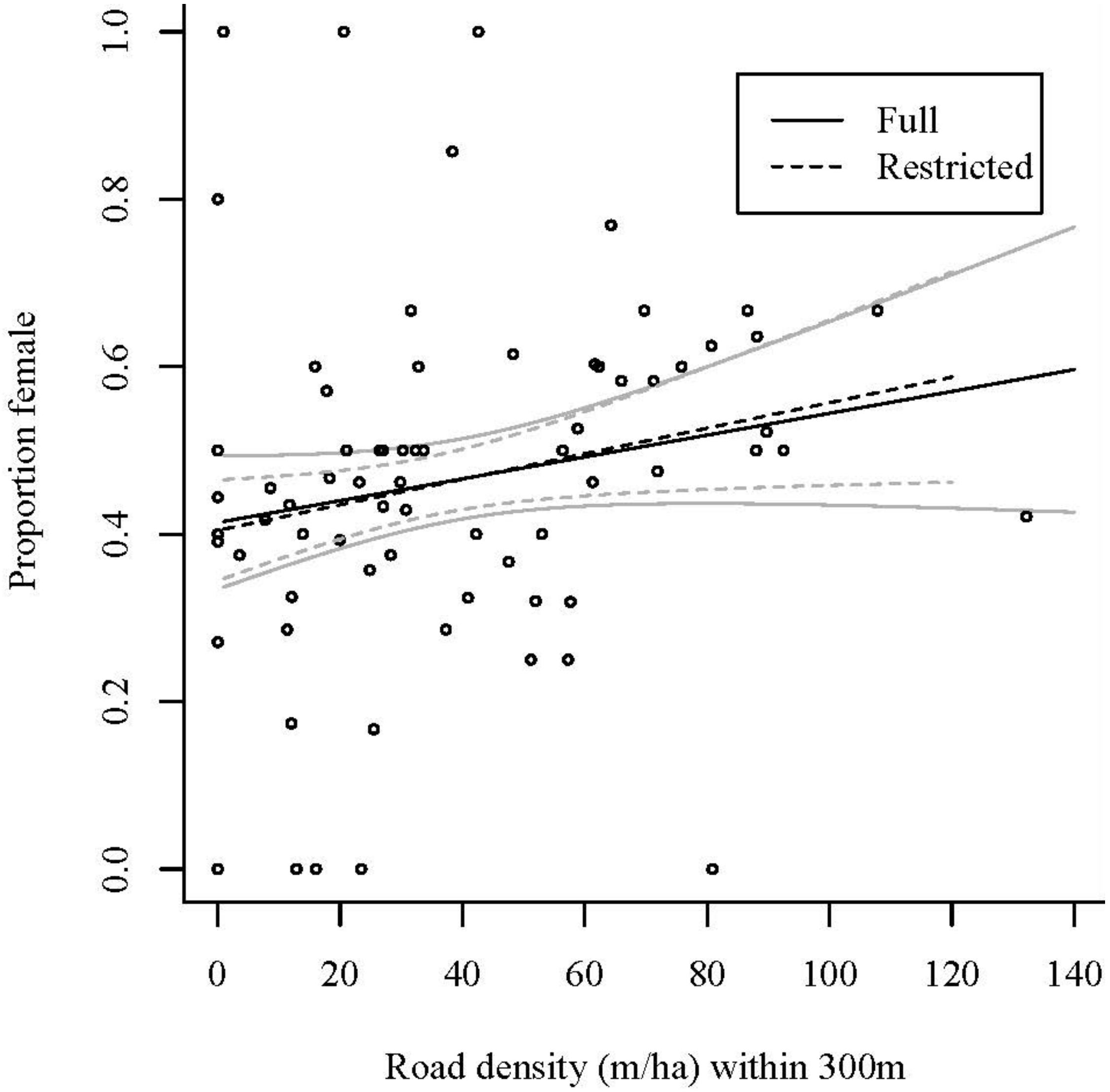

Figure 3.2. Linear model showing the proportion of female *Chrysemys p. picta* by road density within 300 m of wetlands, Rhode Island, USA, 2013-2015. The full dataset (P = 0.119, $r^2 = 0.020$) includes all observations and the restricted dataset (P = 0.039, $r^2 = 0.072$) is limited to wetlands with 10 or more individuals. Gray lines are 95% confidence intervals. Points show values for each wetland.

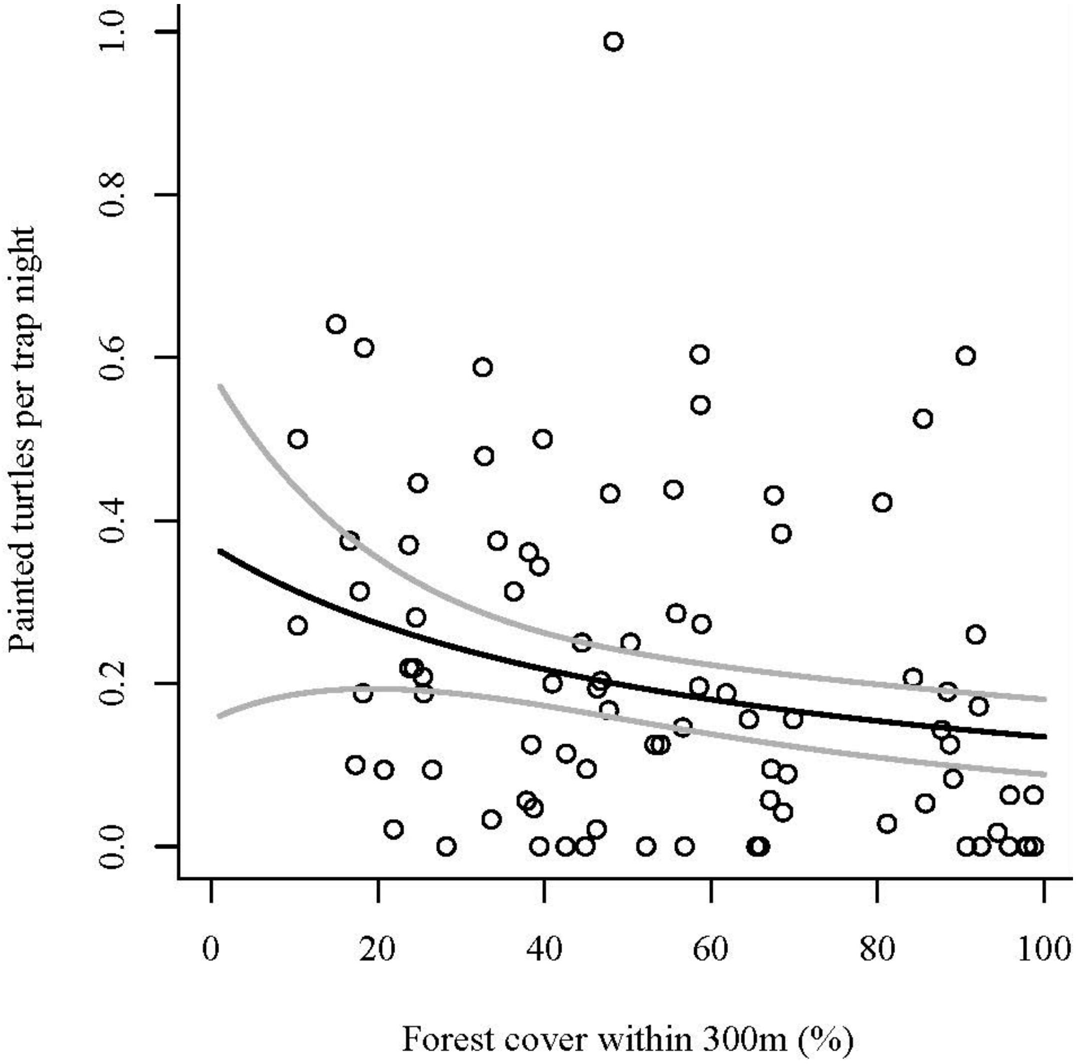

Figure 3.3. Top model for *Chrysemys p. picta* abundance showing unique individuals per trap night by forest cover within 300 m, Rhode Island, USA, 2013-2015. Gray lines are 95% confidence interval. Points show values for each wetland.

Manuscript IV: A Comparison of Population Genetic Structure and Diversity between a Common (*Chrysemys p. picta*) and Endangered (*Clemmys guttata*) Freshwater Turtle

Formatted for Conservation Genetics

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Abstract

The northeastern United States has experienced dramatic alteration to the landscape since the time of European settlement. This alteration has had major impacts on the distribution and abundance of wildlife populations, but the legacy of this landscape change remains largely unexplored for most species of freshwater turtles. We used microsatellite markers to characterize and compare population genetic structure and diversity for a generalist species, the eastern painted turtle (*Chrysemys p. picta*), and a rare, more specialized species, the spotted turtle (*Clemmys guttata*). We predicted that *C. guttata* was more likely to have experienced the detrimental effects of habitat loss and fragmentation associated with landscape change, and that these effects would manifest in the form of more inbreeding, reduced diversity, and greater population genetic structure. As expected, *C. p. picta* exhibited little population genetic structure, showing no evidence of inbreeding or strong differentiation among sampling sites. For *C. guttata* however, results were consistent with certain predictions and inconsistent with others. We found tentative evidence of recent population declines in *C. guttata*, as well as a greater degree of inbreeding in this species compared to *C. p. picta.* Genetic diversity and differentiation among sites were comparable between species, however. As our results do not suggest any major signals of genetic degradation in *C. guttata*, the southern region of Rhode Island may serve as a regional conservation reserve network where the maintenance of population viability and connectivity is prioritized.

Introduction

Intensive and large-scale landscape alteration by European settlers dates back several centuries in Rhode Island. Clearing of the land for timber and agriculture began in the 17th century and peaked in the mid-19th century, when approximately 70% of the state was deforested (Foster and Aber 2004). Only after agriculture from western states began to outcompete farms in New England did the landscape begin to regenerate to early successional habitat and secondary forest. Today, approximately 54% of the state is forested, with pine, oak, and maple forests dominating the western part of the state (Butler 2013). Freshwater wetlands have undergone immense alteration in the previous centuries as well. Drainage, filling, damming, and channelization occurred for centuries without regulation, resulting in the loss of approximately 37% of the wetlands in Rhode Island between 1780 – 1980 (Dahl 1990, Magilligan et al. 2016). The creation of novel wetlands for drinking water and agriculture has further altered the landscape. More recently, post-World War II economic growth led to a construction boom and the creation of the interstate highway system, both of which consumed and fragmented large areas of the landscape in Rhode Island. Undoubtedly, these human activities have had major impacts on the abundance, demography, and connectivity of populations of wildlife throughout the state and region, but for most species the legacy of landscape change remains largely anecdotal or completely unexplored.

Populations of freshwater turtles in the region have certainly been impacted by these alterations, but not necessarily in a uniform fashion across species. Certainly, some species have experienced declines due primarily to historic habitat loss and fragmentation. True habitat specialists, like the bog turtle (*Glyptemys muhlenbergii*), have probably experienced the most dramatic declines (USFWS 2001, Rosenbaum et al. 2007). Habitat generalists however, that have the ability not only to acclimate to new conditions, but subsist in heavily altered wetlands or colonize newly-created wetlands, have maintained comparable distributions and abundances, and in some cases may have benefited from changes (Price et al. 2013, Winchell and Gibbs 2016). The common snapping turtle (*Chelydra serpentina*) is an example of a generalist species that remains abundant throughout most of its range (Paterson et al. 2012, Anthonysamy et al. 2014). The eastern painted turtle (*Chrysemys p. picta*) and the spotted turtle (*Clemmys guttata*) are two species that are thought to have experienced very different responses to recent anthropogenic landscape change, with the former having remained abundant, and the later having experienced substantial declines.

Chrysemys p. picta often occurs in high abundance even in areas of major human disturbance (Congdon and Gibbons 1996, Gamble and Simons 2004, Chapter 3) and has been shown to occur in much higher densities than *C. guttata,* where they co-occur (Ernst 1976). *Chrysemys p. picta* is one of four recognized subspecies of *C. picta*, a small (carapace length up to 25.4 cm) freshwater turtle with a large geographic range that spans across North America (Ernst and Lovich 2009). *Chrysemys p. picta* occupies the eastern part of this range, stretching from Georgia, USA to New Brunswick, Canada along the Atlantic seaboard. They occur in all types of freshwater wetlands including riparian systems. Sexual maturity usually occurs in 2-4 years in males, and 6-10 years in females (Ernst and Lovich 2009). Precise data are limited and variable across the range, but generation time is thought to be in the range of 10-20 years (Wilbur 1975, Ernst and Lovich 2009). They are known to readily disperse from one wetland to another via terrestrial movements on the order of kilometers (Zweifel 1989, Bowne 2008) and readily colonize uninhabited wetlands (Tuberville et al. 1996, Cosentino et al. 2010).

In contrast to *C. picta*, *C. guttata* is believed to have experienced severe population declines throughout its range in the last two centuries due primarily to habitat loss, alteration, and fragmentation (Gibbons et al. 2000, Lewis et al. 2004, van Dijk 2011). *Clemmys guttata* is a small (carapace length up to 14.3 cm) freshwater turtle native to the eastern United States and Great Lakes region (Ernst and Lovich 2009). Sexual maturity usually occurs between 7–15 years (Ernst and Lovich 2009) and tends to be at the higher end of this range in northern populations (Litzgus and Brooks 1998). Estimates of generation time are usually considered to be between 20–30 years, but may be as high as 40 years in the northern latitudes (Ernst and Lovich 2009, COSEWIC 2014). They are often described as semi-aquatic because they use both wetland and upland habitats for extended periods (Beaudry et al. 2009). Throughout their range, *C. guttata* occur in a variety of wetland types, but do exhibit habitat selection for bog-like wetlands (Milam and Melvin 2001, Rasmussen and Litzgus 2010). In Rhode Island, *C. guttata* are rare relative to other species of freshwater turtles and are strongly forest-associated (Chapter 2). Dispersal is limited and fidelity to wetlands is high, with individuals often overwintering in the same hibernaculum each year (Haxton and Berrill 1999, Litzgus et al. 1999, Chapter 1). They are a species of increasing conservation concern, especially in the northeastern United States where six of the seven states in which it occurs have designated it with some type of conservation protection. The International Union for the Conservation of Nature (IUCN) currently lists *C. guttata* as Endangered (van Dijk 2011), and it is currently under review by the U.S. Fish and

Wildlife Service (USFWS) for federal listing under the U.S. Endangered Species Act (USFWS 2015). In this study, we characterize and compare population genetic diversity and population genetic structure and diversity of this relatively rare species with that of the more widespread and abundant *C. p. picta*.

The population genetic structure of endangered species is of fundamental interest to conservation biologists. Genetic diversity and inbreeding have implications for a population's vulnerability to environmental and demographic stochasticity, thus affecting the probability of extinction (Brook 2008, Frankham et al. 2010). A loss of genetic diversity can reduce the ability of a population to adapt to changing environmental conditions, and inbreeding depression can have deleterious effects on the reproductive fitness of offspring (Ralls et al. 1988, Frankham 2005, O'Grady et al. 2006). Genetic differentiation among subpopulations is in part a product of gene flow, and measures of differentiation can help identify subpopulations that may be genetically isolated due to barriers associated with habitat fragmentation. Maintaining gene flow to counteract the loss of genetic diversity due to inbreeding and genetic drift is important to ensure genetic viability, especially for species that occur in small, isolated subpopulations (Frankham et al. 2010).

Our primary objective was to assess whether *C. guttata* is experiencing elevated risk of extirpation due to increased levels of inbreeding, reduced genetic diversity, and increased population genetic structure due to isolation, which collectively we refer to as genetic degradation. We made several predictions based on the insight that *C. guttata* occur in smaller, more isolated populations, and that they probably exhibit reduced rates of gene flow compared to *C. p. picta*. We predicted that *C. guttata* would 1) exhibit less genetic diversity, 2) exhibit more inbreeding, 3) exhibit more differentiation among sites and 4) were more likely to have undergone recent reductions in effective population size (i.e., a population bottleneck), as compared to *C. p. picta.*

Materials and methods

Study area and sampling

Our study was conducted throughout the state of Rhode Island located in southeastern New England. Rhode Island is the smallest state geographically in the United States (approximately 2,700 square kilometers when excluding coastal waterways), but ranks second highest in population density (U.S. Census 2010). Highest levels of land development and human population densities occur along the south coast and around Narraganset Bay in the eastern part of the state. Mean elevation is approximately 60m with a highest point of 247m. Rhode Island experienced repeated glaciation during the Pleistocene Epoch, the most recent of which was the Laurentide Glacier. This glacier reached a terminus about 20 km south of Block Island around 20,000 years ago, and subsequently retreated northward leaving Rhode Island ice free by 16,000 years before present (Sirkin 1996, Uchupi et al. 2001). Today, Block Island is a 284 km^2 island located approximately 15 km south of the Rhode Island coast. Block Island has existed as an island for approximately 15,000 years since sea level rise associated with the retreat of the Laurentide Glacier caused the catastrophic drainage of glacial lakes along the southern New England terminal moraine (Uchupi et al. 2001).

From 2013-2015, small (0.1 – 1.8 ha), hydrologically isolated (i.e., discrete, non-riparian) wetlands throughout the state were randomly selected across a gradient of forest cover for a mark-recapture study focusing on occupancy and demography (Chapters 2 and 3). Genetic tissue collection took place concurrently at a subset of these wetlands. Because *C. p. picta* were relatively common, tissue was collected only at wetlands with high densities of turtles that would ensure an adequate number of individuals for population genetics analysis (Hale et al. 2012). One additional wetland was sampled for *C. p. picta* on Block Island to serve as an outlier group. Because *C. guttata* were relatively rare, tissue was collected from all individuals encountered during the study, and several additional wetlands known to contain *C. guttata* were also sampled in order to augment the dataset for this species. Two of these additional wetlands deviated from the other wetlands in notable ways. Site 24 was a slow-moving riparian wetland with peripheral marshes and adjacent forested vernal pools. Turtles were sampled from within an approximately 15 ha area that contained both the vernal pools and the riparian wetlands. Site 29 consisted of a matrix of permanent bog and forested vernal pools within a 2.5 ha area. Using historic aerial imagery, we determined that several of the wetlands sampled were manmade since 1939, the year of the oldest available imagery (Rhode Island Geographic Information System [RIGIS]; www.rigis.org). We used imagery, available at intervals of approximately every ten years, to determine the age (up to at least 77 years) of all sampled wetlands.

For all individuals, less than 1 ml of blood was collected from the sub-carapacial vein using a 25-gauge sterile needle and a 3 ml syringe, and placed immediately on a Whatman FTA sample collection card (GE Healthcare, Buckinghamshire, United Kingdom). These cards were stored at room temperature and used for subsequent DNA extraction. All individuals were released at the site of capture.

Genotyping

We used the DNEasy Blood and Tissue Kit (Qiagen Corporation, Valencia, CA, USA) to extract DNA using the standard protocol. For both species, we amplified previously described microsatellite loci (Pearse et al. 2001, King and Julian 2004). We amplified 18 loci for *C. p. picta* and 17 loci for *C. guttata*, organizing these into 6 and 5 multiplexes, respectively. We carried out polymerase chain reaction (PCR) using the Qiagen Type-it Microsatellite PCR Kit under conditions recommended in King and Julian (2004), but with a modified initial denaturing step of 95℃ for 5 minutes. We used negative controls on PCR plates to identify any potential contamination. Fragment size analysis of PCR products was conducted at the DNA Analysis Facility on Science Hill at Yale University on a 3730xl DNA Analyzer with a 96-capillary 50cm array, using GeneScan 600 LIZ dye size standard (Applied Biosystems, Foster City, CA, USA). Allele peaks were visualized and called using Geneious 7.0.6 (Kearse et al. 2012). We used Geneious and MICRO-CHECKER (van Oosterhaut et al. 2004) to search for genotyping errors. We re-ran PCR for approximately 4% of our samples to calculate a genotyping error rate.

Genetic diversity and differentiation

We used a variety of packages developed for the R statistical platform v.3.3.3 (R Core Team 2017) to estimate population genetic statistics, for each species. We used the poppr package (Kamvar et al. 2014) to quantify missing data and to test for linkage disequilibrium among loci. We used the pegas package (Paradis 2010) to test for deviations from Hardy-Weinberg Equilibrium (HWE) for each locus, and for each combination of locus and sampling site, using an exact test based on 10,000 Monte Carlo permutations of alleles. *P*-values were assessed after Bonferroni correction in which alpha (0.05) was divided by the number of tests. We used the popgenreport package (Adamack and Gruber 2014) to estimate the frequency of null alleles for each locus (Brookfield 1996), private alleles per site, and mean allelic richness per site using the rarefaction method to correct for variation in sample size (Kalinowski 2004). We calculated expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficients (F_{IS}) for each site, and calculated 95% confidence intervals for F_{IS} estimates using 10,000 bootstrap iterations, all using the diveRsity package (Keenan et al. 2013).

We used the diveRsity package to calculate global measures of F_{IT}, F_{IS}, and F_{ST}, and to calculate pairwise F_{ST} values for all sites. All *F*-statistics used the bias-corrected formulation of Weir and Cockerham (1984). As an alternative measure of population differentiation and to maximize comparability with other studies, we also used the diveRsity package to calculate pairwise values of the bias-corrected Jost's D_{est} (Jost 2008, Gerlach et al. 2010). The diveRsity package was used to estimate 95% confidence intervals for all measures of differentiation using 10,000 bootstrap iterations. We used the poppr package to perform an analysis of molecular variance (AMOVA). We conducted the test with two stratifications such that variance of allele frequencies was partitioned within sites and among sites (Excoffier et al. 1992). For the global *F*-statistics and AMOVA analyses, we excluded the Block Island site for *C. p. picta,* and included only the five *C. guttata* sites with sample sizes >4 to limit confounding factors such as outliers and small sample size (Kalinowski 2005), and thereby maximize the comparative inference between the two species.

Population structure

We used the ade4 package to perform a Mantel test with 10,000 permutations to test for genetic isolation by distance. We used Nei's (1972) measure of genetic distance to create the genetic matrix, and geographic locations centered on individual wetlands or on a geographic mean when turtles were sampled from multiple wetlands, to create the Euclidean distance matrix. For *C. p. picta*, we did not include the Block Island site, and for *C. guttata* included only the five sites with sample sizes >4 to avoid falsely inflating measures of genetic distance.

We used program STRUCTURE v.2.3.4 (Pritchard et al. 2000) to characterize population genetic structure for both species (Porras-Hurtado et al. 2013) and to test our prediction of a greater degree of subpopulation structure in *C. guttata*. STRUCTURE allows for the identification of genetic clusters within a dataset by detecting differences in allele frequencies and assigning individuals to those clusters based on analysis of likelihood. For all runs, we assumed an admixture model with correlated allele frequencies and employed the LOCPRIOR parameter using sampling location as the additional sample information. The LOCPRIOR parameter is informative in situations of weak population structure such as that to be expected given the spatial scale of our study (Hubisz et al. 2009, Porras-Hurtado et al. 2013). In all cases, we performed 20 independent iterations of runs consisting of a burn-in of 200,000, followed by 500,000 MCMC repetitions, which was sufficient for all runs to reach convergence. For *C. p. picta* we ran an initial analysis with all individuals included (hereafter complete analysis) and a second analysis with a maximum of 25 individuals selected randomly (hereafter subset analysis) from each site to ensure that sample size unevenness was not influencing results (Puechmaille 2016). We specified the range of *K* as 1-10 for both runs. For *C.*

guttata we ran an initial analysis with all individuals from all sites (i.e., complete analysis), and a second analysis with only sites with more than 9 individuals, while also limiting site 29 to only 30 randomly selected individuals (i.e., subset analysis). We specified the range of *K* as 1-11 for the complete analysis, and 1-4 for the subset analysis. We considered both the ln Pr(X|*K*) and the Δ*K* method (Evanno et al. 2005) with STRUCTURE Harvester (Earl and vonHoldt 2012) to evaluate the most likely number of clusters. We used CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007) and distruct v.1.1 (Rosenberg 2004) software for post-hoc data processing and visualization.

Population bottleneck

We used program BOTTLENECK v.1.2.02 (Piry et al. 1999) to test the prediction that *C. guttata* were more likely than *C. p. picta* to have undergone recent reductions in effective population size. To test for the signature of heterozygosity excess, we considered results from both a two-tailed sign test (Luikart and Cornuet 1998) and a one-tailed Wilcoxon signed-rank test using the two-phase mutation model (TPM) with 10,000 iterations used to generate a distribution of expected equilibrium heterozygosity (H_{eq}). Following the recommendations of Peery et al. (2012), we used a value of 3.1 for the mean size of multi-step mutations, which was used to specify a variance for the TPM (Williamson-Natesan 2005). We then conducted separate tests using values of 0.12, 0.22, and 0.32 for the proportion of multi-step mutations in the TPM. We conducted tests for all sampling sites with at least 20 individuals, and for all individuals combined, for both species.

Results

Sampling and genotyping

We collected tissue samples from 647 *C. p. picta* from 22 sites (mean = 29.7 individuals / site, SE = 2.2, n = 22), and 148 *C. guttata* from 11 sites, but only five of the 11 sites from which *C. guttata* were sampled yielded enough individuals for the majority of population genetics analyses (mean = 27.4 individuals / site, SE = 6.4, n = 5; Figures 4.1 and 4.2). We retained 12 of 18 microsatellite loci for *C. p. picta* (Table 4.S.1). Removed loci included GmuB67 and GmuA32, which were monomorphic, loci GmuD87 and Cp10, which had high levels of missing data (>13%) and high frequencies of null alleles (0.120 and 0.219, respectively), and loci GmuD121 and Cp2, which had high frequencies of null alleles (0.205 and 0.158, respectively). GmuD87, GmuD121, and Cp10 deviated most consistently from HWE among sampling sites (Figure 4.S.1). For retained loci, the total missing data was 3.6%. We retained 16 of 17 loci for *C. guttata* (Table 4.S.1). We removed loci GmuD28, which had a high frequency of null alleles (0.174). For retained loci, the total missing data was 0.6%. There was no evidence of linkage disequilibrium among retained loci for either species. The genotyping error rate was approximately 2.3%.

Genetic diversity and population structure

For *C. p. picta,* both global F_{IT} (-0.0031, CI = -0.0147 – 0.0087) and F_{IS} (-0.0220, CI = -0.0341 – -0.0099) overlapped zero, but F_{ST} (0.0185, CI = 0.0143 – 0.0231) did not. For this species, mean H_e of all retained loci was 0.659 (SE = 0.085) and mean H_o was 0.659 (SE = 0.084; Table 4.S.1). Mean allele richness ranged from 5.54 – 6.59 among sites (Table 4.1). Pairwise F_{ST} values for *C. p. picta* (not including Block Island) ranged from 0.002 – 0.058 (mean = 0.018, SE = 0.001, n = 210), with 143/210 (68.1%) values containing a 95% confidence interval that did not overlap zero. Only the Block Island site

(F_{ST} = 0.008 – 0.081) and sites 12 (F_{ST} = 0.019 – 0.058) and 21 (F_{ST} = 0.019 – 0.055) contained all values that did not overlap zero (Table 4.2). Pairwise Jost's D_{est} values for *C. p. picta* ranged from 0.001 – 0.102 (mean = 0.020, SE = 0.001, n = 210), with 51/210 (24.3%) of values containing a 95% confidence interval that did not overlap zero.

For *C. guttata*, global F_{IT} (0.0503, CI = 0.0256 – 0.0758), F_{IS} (0.0364, CI = 0.0094 – 0.0628), and F_{ST} (0.0144, CI = 0.0045 – 0.0264) did not overlap zero. Mean H_e of all retained loci was 0.697 (SE = 0.044) and mean H_o was 0.677 (SE = 0.052; Table 4.S.1). Mean allele richness ranged from 4.78 – 4.97 among sites (Table 4.1). Pairwise F_{ST} values for *C. guttata* ranged from -0.002 – 0.025 (mean = 0.012, SE = 0.003, n = 10) and 3/10 (30%) values containing a 95% confidence interval that did not overlap zero. Pairwise Jost's D_{est} values ranged from 0 – 0.031 (mean = 0.010, SE = 0.003, n = 10), with 1/10 values (10%) containing a 95% confidence interval that did not overlap zero (Table 4.3).

The vast majority of genetic variance occurred within sites for both species (AMOVA: *C. p. picta* = 96.5%; *C. guttata* = 97.9%), with the remaining variance attributed among sites, and we found no evidence for isolation by distance in *C. p. picta* (r = 0.097, P = 0.128) or *C. guttata* (r = -0.454, P = 0.926). STRUCTURE results for *C. p. picta* clearly distinguished the Block Island site from all mainland sampling locations in all runs. In the complete analysis, the ΔK method suggested two clusters, and the ln Pr(X|K) method suggested six clusters. In the subset analysis, both K selection methods suggested four clusters (Figure 4.3A-B; Figure 4.S.2). In both analyses in which $K \geq 4$, the majority of sites showed a lack of definitive assignment of individuals to a particular cluster, but several sites did show a relatively high probability of assignment to a particular cluster. In the complete analysis, sites 2, 12, 15, 18, and 21 exhibited the highest probabilities of belonging to the purple, red, yellow, green, and blue clusters, respectively. In the subset analysis, sites 12, 15, and 21 exhibited the highest probabilities of belong to the green, blue, and red clusters, respectively. STRUCTURE results for *C. guttata* suggested two ancestral clusters in the complete analysis, with site 29 distinguished from the other sites. In the subset analysis, the relationship did not persist and the ΔK and ln Pr(X|K) methods suggested different numbers of clusters (Figure 4.3C-D; Figure 4.S.2). The ln Pr(X|K) method suggested no structure (i.e, $K = 1$), and the ΔK method suggested three ancestral clusters with a greater amount of admixture in sites 24 and 27.

Population bottleneck

None of the *C. p. picta* sites showed evidence of recent genetic bottlenecks, but site 18 did yield significant results in the two-tailed sign test ($\alpha < 0.05$) at more than one TPM level. The one-tailed Wilcoxon test returned a *P*-value approaching one, suggesting heterozygous deficiency, the signal of a recent population expansion (Table 4.4). For *C. guttata,* site 29 had a significant sign test result at multiple TPM levels, and site 24 had a significant Wilcoxon test result ($\alpha < 0.05$) at multiple TPM levels. Site 30 also had a significant Wilcoxon test result at the highest (i.e., 0.32 proportion of multi-step model) TPM level. When all the sites were grouped together and considered one population, the Wilcoxon test returned a significant result at the highest TPM level (Table 4.4). All *C. guttata* results had reduced *P*-values in the Wilcoxon tests suggesting heterozygous excess, the signal of a recent population decline.

Discussion

Overall, *C. p. picta* exhibited weak population genetic structure. We found no evidence of isolation by distance, and global F_{IT} and F_{IS} both overlapped zero, suggesting a lack of overall population structure, and a lack of inbreeding, respectively. In *C. guttata*, there was no evidence of isolation by distance or strong differentiation among sites, but global F_{IS} and the consistency of private alleles among sites suggested the presence of some population structure consistent with inbreeding.

Results were consistent with some predictions and inconsistent with others, which we interpret as limited evidence that *C. guttata* is at greater risk due to genetic degradation in our study area. In line with predictions, *C. guttata* exhibited a greater degree of inbreeding than *C. p. picta* and there was tentative evidence of recent population declines in *C. guttata*, whereas there was no evidence for declines and even some evidence for population expansion in *C. p. picta*. Genetic diversity and differentiation among sites were similar for both species, however.

Genetic diversity

A lower mean allelic richness in *C. guttata* suggested less genetic diversity compared to *C. p. picta*, but mean expected heterozygosity was higher in *C. guttata*. For both species, estimates of observed and expected heterozygosity and allelic richness were comparable to those from other studies of turtles using microsatellites (Vargas-Ramirez et al. 2012, see Table 4) and probably do not indicate significant depletion of genetic diversity. However, long-lived species can mask declines in genetic diversity even after prolonged population declines, making interpretation difficult (Kuo and Janzen 2004). A comparison of genetic diversity in fragmented populations of *C. guttata* and *C. picta* *marginata* in Indiana found lower diversity in *C. guttata* (Parker and Whiteman 1993). The authors identify smaller habitat patch size, lower population density, and greater isolation of *C. guttata* populations as potential factors, but low sample sizes and the possibility of different mutation rates of the genetic markers used for the different species limit strong conclusions from this study. An investigation of population genetic structure found that genetic diversity was highest in *C. picta*, intermediate in *C. serpentina*, and lowest in Blanding's turtles (*Emydoidea blandingii)* in Wisconsin (Reid et al. 2017). This was consistent with the prediction that genetic diversity would decrease with reduced mobility and greater habitat specialization among these turtle species. A comparison of population genetic structure among the same three species in Illinois yielded similar results, with populations of *E. blandingii, C. serpentina*, and *C. picta* exhibiting increasing allelic richness and heterozygosity (Anthonysamy 2012). This study did not detect intraspecific differences between fragmented and relatively undisturbed sites, however. While some studies have demonstrated strong empirical evidence of a relationship between genetic diversity, life history and the landscape, it remains difficult to compare genetic diversity directly between species when different loci are used, as these can influence estimates (Rubinsztein et al. 1995, Väli et al. 2008). Standardized approaches for comparing population genetic diversity among species and studies are needed so that conservation scientists can better resolve causality for this important measure.

Population structure

We documented weak, but existing differentiation among *C. p. picta* sampling sites. The Block Island site was only moderately differentiated despite limited or no opportunity for gene flow with the mainland since the Pleistocene (Sirkin 1996). The post-glacial colonization of the northeastern United States by *C. p. picta* occurred as populations expanded from southern refugia after glaciers retreated (Starkey et al. 2003). *Chrysemys picta* are physiologically well adapted to cold climates (Storey et al. 1988, Churchill and Storey 1992) and, along with *C. serpentina,* were the first turtles to expand northward into formerly glaciated areas (Holman and Andrews 1994). The exact time at which these species first colonized what is now Block Island and mainland Rhode Island is not known, but it probably took place between 10,000 – 15,000 years ago (Holman and Andrews 1994, Starkey et al. 2003). A characteristic reduction in genetic diversity associated with this relatively recent post-glacial range expansion (Hewitt 2000, Weisrock and Janzen 2000), along with high rates of contemporary gene flow, may be responsible for the lack of pronounced population genetic structure.

STRUCTURE results indicated that the majority of the mainland sites were assigned to multiple genetic clusters, a common signature of weak population structure (Porras-Hurtado et al. 2013). However, sites 12, 15, and 21 did exhibit consistent signals of substructure, both in pairwise measures of differentiation and in STRUCTURE results. Under both scenarios where $K \geq 4$ these sites contained the highest probabilities of belonging to the distinct clusters (Figure 4.3A-B). Interestingly, these three sites are all manmade or heavily modified. Sites 12 and 15 are both about 44 years old, whereas site 21 predates the earliest available aerial imagery (>77 years old), but is clearly a pool that formed when a former stream was bisected by a road. In total, at least 6/22 *C. p. picta* sites were manmade or heavily modified. The three sites also contain plentiful nesting habitat immediately adjacent to the wetland. Site 12 is located at the end of a commercial/military airport where much of the grounds next to the wetland are maintained as grassland, site 15 is located on an urban golf course with manicured lawns and sand traps, and site 21 is on private property with sandy soils maintained as lawn (Figure 4.2). Together, the recent creation of novel habitat and beneficial habitat characteristics could have facilitated recent population expansions at these sites, causing allele frequencies to differ from the population at-large. However, there is no signature of a reduction in heterozygosity in any of the sites as would be expected after a founder event (Frankham et al. 2010). An alternative explanation could be limited gene flow due to isolation. This is plausible at sites 12 and 15 that both occur in highly developed landscapes, but unlikely at site 21 where nearby riparian and permanent wetlands located in a relatively undisturbed landscape are likely to contain *C. p. picta*. Ultimately, we cannot say with certainty what is causing the observed differentiation.

Contrary to predictions, we detected similarly modest differentiation among sites of *C. guttata*. In fact, a smaller percentage of sites exhibited differentiation compared to *C. p. picta*, but direct comparison is difficult because of the disparity in sample size (*C. guttata* = 20 comparisons, *C. p. picta* = 420 comparisons). All significant *C. guttata* pairwise comparisons included site 29 and this site was also differentiated in the complete STRUCTURE analysis. Adults from site 29 were radiotracked for two years as part of another study and were found to exhibit limited movements and high levels of home range fidelity (Chapter 1). Given that dispersal is a requisiste process for gene flow, if dispersal rates to neighboring wetlands are indeed low, limited gene flow could explain the higher differentiation. The *C. guttata* STRUCTURE subset analysis resulted in a more ambiguous pattern of differentiation and the fact that the ΔK and ln $\Pr(X|K)$

methods resulted in disparate results make this difficult to interpret. Overall, there is little evidence that *C. guttata* exhibits appreciably greater population genetic structure than *C. p. picta.*

Population bottleneck

We documented tentative evidence of recent population declines in *C. guttata*. Bottleneck tests can be difficult to interpret, but results comparable to ours have been interpreted in a similar way as those for other species of turtles (Kuo and Janzen 2004). We ran multiple tests under a range of different multi-step mutation model proportions to assess the robustness of results (Peery et al. 2012). Statistical evidence for population bottlenecks tended to occur at the higher proportion of multi-step model in the TPM, where the test is most vulnerable to Type I error (Williamson-Natesan 2005). Thus, our results should be interpreted with some caution. Site 18 was the only *C. p. picta* site that exhibited a significant heterozygosity deficiency, indicating evidence of a recent population expansion. This, coupled with the fact that all other sites of *C. guttata* indicated population declines, suggests that any population fluctuations that may have occurred were in opposite directions for the two species.

Scope and limitations

For both species, the genetic structure that we detected was modest. Given the limited spatial scale of our study (especially for *C. guttata*) and the fact that we expected these sampling sites to be admixing to some degree, it should be emphasized that we were indeed seeking fine-scale genetic structure. Moreover, in our study area the impact of human activities on turtle populations has occurred in the evolutionarily recent past (~250 years) and has intensified only in the last ~75 years. The number of *C. p. picta* generations since the more intense period of human influence began is probably between 4-7 generations, and between 12-25 generations for the longer period. The number of *C. guttata* generations is probably between 2-4 for the shorter period, and 8-12 for the longer period or nearly half the number of generations of *C. p. picta*. As it can be difficult to detect the effects of genetic drift in long-lived organisms, the spatial and temporal scales (i.e., time since habitat loss and fragmentation) of our investigation may have limited our ability to detect genetic differentiation and demographic events that have occurred in the recent past, particularly for *C. guttata*. Simulation studies have demonstrated that F_{ST} is relatively insensitive to disruptions to gene flow, especially when dispersal is limited in the organism of study, and that other population-based metrics may be superior in detecting changes that have occurred in the recent past (Landguth et al. 2010). Compounding the issue, turtle DNA evolves slowly relative to that of other vertebrates (Avise et al. 1992, Shaffer et al. 2013). Other studies of population genetics in freshwater turtles have failed to detect predicted genetic structure, even when there is strong empirical evidence of the effects of historic habitat fragmentation (Bennett et al. 2010, Anthonysamy 2012). Nonetheless, the ability to detect strong genetic structure among sites only a few generations after fragmentation has been demonstrated in reptiles (Blair et al. 2010), birds (Delaney et al. 2010), and mammals (Munshi-South and Kharchenko 2010). Given an ample number of generations, the same should be possible in turtles, but it is not yet clear how many generations are necessary and this likely varies among species. When working on such limited spatial and temporal scales, adequate sample size, number of markers used, and mutation rates of markers need to be considered to maximize the resolution of analyses (Kalinowski 2005, Hale et al. 2010, Spinks et al. 2014, Elbers et al. 2016). Direct comparisons among studies can be difficult as well, and standardized approaches and accepted minimums of markers and sample sizes would be helpful in improving interpretability and context of individual studies.

We did not perform statistical tests between species because it was unclear if the data would meet assumptions about uniform error between groups. Rather, we calculated measures of genetic diversity, inbreeding, and genetic structure independently and with sound sample sizes for each site (Hale et al. 2012) and an adequate number of markers, and compared them qualitatively. Our study is limited by the fact that the differences we detected between the two species are qualitative and offer only empirical evidence of a relationship between genetic structure and the combination of landscape and life history factors. Finding causal relationships here remains difficult, but nonetheless using a comparative multiple species approach can help strengthen inference (Anthonysamy 2012, Reid et al. 2017).

Clemmys guttata has undergone severe declines in many parts of its range, but their status in Rhode Island is unclear. In the recent revision of the Rhode Island Wildlife Action Plan, *C. guttata* is listed as a "Species of Greatest Conservation Need," but is given an S5 ranking indicating it is considered widespread and abundant in the state (RIDEM 2015). While a recent state-wide sampling effort disputes the idea that they are common (Chapter 2), it is possible that *C. guttata* in our study area have not experienced population declines at the same level of severity as in other places throughout its range, and are not representative of the experiences of the species at large. Moreover, it is possible the sites we sampled were inherently biased towards being those least affected by population declines and isolation given that they are places where they were known to occur in relatively robust numbers and where our sampling yielded the most individuals.

Concluding remarks and conservation implications

Chrysemys p. picta is one of the most well-studied freshwater turtle species, largely because they are widespread and abundant. Our analysis confirms that they exhibit little population genetic structure across Rhode Island, making for an appropriate contrast with a far less abundant species. Some sites do exhibit modest genetic differentiation, but the reasons why remain elusive and warrant further investigation. Our analysis provides some evidence that *C. guttata* exhibit a greater degree of inbreeding and may have experienced population declines in the recent past. Overall, population genetic structure remains comparable to that of *C. p. picta* though, suggesting the sites studied have not experienced serious genetic degradation at this point. As we were unable to find strong evidence of genetic degradation in *C. guttata*, the southern region of Rhode Island may be well suited to serve as a regional conservation reserve network where the maintenance of gene flow among wetlands occupied by the species is prioritized (Kautz et al. 2006, Shoemaker and Gibbs 2013). Relatively little is known about *C. guttata* population genetics and how genetic structure varies range-wide. Much of what has been inferred is derived from studies of different species of freshwater turtles considered ecologically similar. Understanding the legacy of habitat loss and fragmentation on population genetic structure is critical for effective management and conservation of this species, at both regional and local spatial scales. Additional population genetics studies of *C. guttata* at multiple spatial scales will help improve our understanding of the potential vulnerabilities to environmental and genetic stochasticity in this declining species.

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Tables

Table 4.1. Site characteristics and genetic diversity measures for all sampled sites. Geographic coordinates are in decimal degrees (locations withheld for *Clemmys guttata*). H_e is expected heterozygosity, H_o is observed heterozygosity, and F_{IS} is the inbreeding coefficient. Bolded F_{IS} indicate a bootstrapped 95% confidence interval that does not overlap zero. Diversity measures (except private alleles) calculated only for sites with >3 individuals for C. *guttata* .

Site	Geographic coordinates (latitude, longitude)	Age	No. of individuals	H_e	H_o	Private alleles	Mean allele richness	F_{IS}
			Chrysemys p. picta					
1	41.153247, -71.604493	>77	40	0.63	0.63	2	5.83	0.014
2	41.346108, -71.789074	>77	39	0.61	0.60	0	6.21	-0.012
3	41.380141, -71.630523	>77	19	0.61	0.67	1	5.82	**-0.101**
4	41.42052, -71.585525	~28	34	0.63	0.66	0	6.29	**-0.062**
5	41.494763, -71.706265	>77	18	0.61	0.66	1	5.69	-0.076
6	41.53227, -71.385252	>77	24	0.59	0.59	0	5.54	-0.003
7	41.548007, -71.458184	~19	17	0.59	0.63	0	5.75	-0.076
8	41.547675, -71.549071	>77	26	0.63	0.64	2	6.59	-0.022
9	41.555359, -71.694297	>77	19	0.63	0.71	1	6.33	**-0.127**
10	41.560943, -71.716856	>77	23	0.63	0.64	0	6.22	-0.018
11	41.600674, -71.719651	>77	27	0.65	0.68	0	6.21	**-0.058**
12	41.612399, -71.42399	~44	38	0.64	0.69	0	5.96	**-0.073**
13	41.615407, -71.685647	>77	57	0.62	0.63	1	6.34	-0.029
14	41.739527, -71.329793	>77	17	0.62	0.67	0	5.79	**-0.065**
15	41.826773, -71.463335	~44	40	0.62	0.60	2	6.02	0.034
16	41.855324, -71.346786	>77	41	0.64	0.63	1	6.54	0.016
17	41.900587, -71.633623	>77	20	0.63	0.69	0	6.25	**-0.100**
18	41.912204, -71.426565	~54	34	0.61	0.61	0	6.18	-0.005
19	41.944757, -71.416485	>77	35	0.65	0.71	0	6.29	**-0.090**
20	41.965696, -71.478915	>77	24	0.62	0.67	0	6.12	**-0.078**
21	41.970512, -71.66193	>77	31	0.64	0.68	0	5.94	**-0.064**
22	41.98915, -71.527097	>77	24	0.59	0.61	2	5.98	-0.018
			Clemmys guttata					
23	-	>77	2	-	-	1	-	-
24	-	>77	22	0.65	0.63	2	4.78	0.040
25	-	>77	9	0.67	0.68	2	4.81	-0.028
26	-	>77	4	-	-	1	-	-
27	-	>77	28	0.67	0.67	3	4.86	-0.008
28	-	>77	1	-	-	1	-	-
29	-	>77	51	0.67	0.64	5	4.90	0.033
30	-	>77	23	0.68	0.67	6	4.97	0.007
31	-	>77	3	-	-	1	-	-
32	-	>77	3	-	-	1	-	-
33	-	>77	2	-	-	1	-	-

Table 4.2. Pairwise F_{ST} (below diagonal) and Jost's D_{est} (above diagonal) measures of differentiation for all combinations of sampling sites of *Chrysemys p. picta* . Bolded values indicate a bootstrapped 95% confidence interval that does not overlap zero.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	-	**0.047**	**0.053**	**0.043**	**0.075**	**0.051**	**0.081**	**0.043**	0.008	**0.041**	0.021	**0.070**	**0.036**	**0.047**	**0.060**	**0.039**	**0.044**	**0.069**	**0.075**	**0.053**	**0.081**	0.026
2	**0.027**	-	0.014	0.002	**0.059**	**0.029**	**0.035**	0.018	**0.030**	0.014	0.015	**0.077**	**0.018**	0.015	**0.026**	0.013	0.012	**0.020**	0.017	0.016	**0.053**	0.011
3	**0.034**	**0.015**	-	0.008	**0.045**	0.027	0.007	0.026	0.007	0.007	0.006	**0.050**	0.011	0.002	0.020	0.018	0.014	0.025	0.022	0.006	**0.074**	**0.040**
4	**0.028**	0.006	**0.012**	-	**0.041**	0.010	0.020	0.014	0.000	0.003	0.001	**0.046**	0.002	0.004	0.006	0.000	0.004	0.008	0.005	0.000	**0.060**	0.005
5	**0.049**	**0.042**	**0.047**	**0.040**	-	0.005	**0.045**	**0.050**	0.009	0.025	0.010	**0.042**	0.023	0.015	0.027	**0.033**	0.012	**0.057**	**0.036**	0.013	**0.047**	**0.050**
6	**0.032**	**0.020**	**0.024**	**0.014**	0.020	-	0.008	0.008	0.001	0.003	0.019	**0.028**	0.004	0.008	0.021	0.011	0.014	0.015	0.029	0.002	**0.036**	0.030
7	**0.046**	**0.024**	**0.014**	**0.019**	**0.044**	0.010	-	0.019	0.024	0.003	0.024	0.021	0.013	0.014	0.022	0.017	0.026	0.032	0.027	0.006	**0.102**	0.035
8	**0.025**	**0.016**	**0.015**	**0.016**	**0.038**	0.013	**0.017**	-	0.005	0.005	0.011	**0.054**	0.013	0.003	0.018	0.005	0.003	0.008	0.019	0.002	**0.069**	0.021
9	**0.013**	**0.019**	0.017	**0.015**	0.013	0.003	**0.018**	0.007	-	-0.001	0.000	0.022	-0.001	0.004	0.016	0.001	0.002	0.020	0.009	0.000	0.018	0.013
10	**0.022**	**0.010**	**0.010**	**0.010**	**0.027**	0.008	0.006	0.007	0.001	-	0.000	0.027	0.000	0.001	0.011	0.001	0.009	0.014	0.009	-0.001	**0.067**	0.013
11	**0.021**	**0.014**	**0.014**	0.011	**0.016**	**0.019**	**0.021**	**0.015**	0.009	0.005	-	**0.038**	0.001	-0.001	0.005	0.001	0.001	0.020	0.000	-0.001	0.028	0.007
12	**0.048**	**0.045**	**0.036**	**0.032**	**0.031**	**0.029**	**0.022**	**0.038**	**0.022**	**0.022**	**0.023**	-	**0.033**	**0.032**	**0.067**	**0.028**	**0.045**	**0.069**	**0.037**	0.022	**0.088**	**0.098**
13	**0.021**	**0.013**	**0.014**	0.005	**0.026**	0.005	0.011	**0.011**	0.001	0.000	**0.008**	**0.024**	-	0.009	**0.019**	0.002	0.003	**0.023**	0.017	0.000	**0.063**	0.016
14	**0.033**	**0.019**	0.011	0.015	0.018	**0.015**	**0.018**	0.009	0.009	0.003	-0.002	**0.029**	**0.011**	-	0.004	0.000	0.005	0.003	0.001	0.000	0.035	0.014
15	**0.033**	**0.017**	**0.012**	**0.011**	**0.031**	**0.021**	**0.016**	**0.013**	**0.014**	**0.008**	**0.009**	**0.039**	**0.017**	0.006	-	**0.021**	0.008	0.017	0.018	0.003	**0.070**	0.016
16	**0.020**	0.009	**0.011**	0.002	**0.027**	**0.011**	**0.015**	0.006	0.006	**0.007**	0.006	**0.025**	0.004	0.005	**0.013**	-	0.003	0.005	0.010	0.000	**0.051**	0.011
17	**0.030**	**0.014**	0.014	0.013	**0.027**	**0.017**	**0.027**	0.005	**0.013**	**0.015**	0.012	**0.044**	0.013	0.009	0.012	0.006	-	0.008	0.007	0.002	**0.056**	0.005
18	**0.036**	**0.016**	**0.015**	**0.011**	**0.048**	**0.017**	**0.020**	**0.009**	**0.026**	**0.015**	**0.018**	**0.046**	**0.015**	0.014	**0.015**	**0.008**	0.008	-	0.012	0.007	**0.073**	0.010
19	**0.039**	**0.020**	**0.022**	**0.018**	**0.026**	**0.027**	**0.022**	**0.019**	**0.016**	0.012	0.002	**0.023**	**0.016**	0.004	**0.015**	**0.015**	**0.022**	**0.023**	-	0.003	**0.036**	0.029
20	**0.030**	**0.015**	0.012	0.016	0.022	0.016	0.011	0.012	0.005	0.001	0.001	**0.019**	0.009	0.004	0.004	0.011	0.015	**0.021**	0.003	-	**0.037**	0.006
21	**0.051**	**0.045**	**0.045**	**0.041**	**0.031**	**0.037**	**0.055**	**0.046**	**0.026**	**0.039**	**0.019**	**0.045**	**0.036**	**0.025**	**0.040**	**0.033**	**0.035**	**0.049**	**0.020**	**0.025**	-	**0.055**
22	**0.025**	**0.012**	**0.022**	0.009	**0.049**	**0.028**	**0.030**	**0.018**	**0.024**	**0.015**	0.015	**0.058**	**0.015**	**0.020**	**0.017**	**0.010**	0.012	0.011	**0.029**	0.022	**0.049**	-

Table 4.3. Pairwise F_{ST} (below diagonal) and Jost's D_{est} (above diagonal) measures of differentiation for all combinations of sampling sites of *Clemmys guttata* . Bolded values indicate a bootstrapped 95% confidence interval that does not overlap zero.

	24	25	27	29	30
24	-	0.002	0.012	0.009	0.002
25	0.013	-	0.013	0.007	0.000
27	0.018	0.023	-	**0.031**	0.008
29	**0.016**	0.006	**0.025**	-	0.008
30	0.004	-0.001	0.012	0.008	-

Table 4.4. *P* -values of sign tests and Wilcoxon signed-rank tests under varying proportions of multi-step mutation model included in the two-phase model. Only sampling sites with >20 individuals included.

Site	Proportion of multi-step mutation model					
	0.12		0.22		0.32	
	sign test	Wilcoxon test	sign test	Wilcoxon test	sign test	Wilcoxon test
			Chrysemys p. picta			
1	0.592	0.311	0.572	0.311	0.582	0.151
2	0.087	0.898	0.423	0.715	0.579	0.633
4	0.483	0.319	0.476	0.183	0.254	0.087
6	0.462	0.416	0.456	0.350	0.463	0.350
8	0.575	0.575	0.569	0.396	0.571	0.311
10	0.241	0.120	0.233	0.074	0.236	0.062
11	0.539	0.517	0.459	0.319	0.463	0.183
12	0.450	0.350	0.464	0.139	0.462	0.062
13	0.102	0.741	0.451	0.575	0.467	0.485
15	0.412	0.545	0.430	0.485	0.425	0.367
16	0.552	0.339	0.553	0.259	0.543	0.190
17	0.090	0.830	0.353	0.545	0.340	0.515
18	0.024	0.912	0.025	0.830	0.090	0.741
19	0.443	0.339	0.440	0.285	0.337	0.151
20	0.237	0.232	0.244	0.160	0.239	0.139
21	0.436	0.604	0.555	0.455	0.147	0.235
22	0.410	0.867	0.434	0.765	0.425	0.633
All	0.058	0.715	0.352	0.575	0.608	0.425
			Clemmys guttata			
24	0.129	0.065	0.128	0.037	0.122	0.017
27	0.550	0.569	0.551	0.410	0.557	0.202
29	0.129	0.116	0.047	0.072	0.046	0.052
30	0.341	0.264	0.455	0.072	0.122	0.017
All	0.481	0.202	0.476	0.079	0.048	0.017

Table 4.S.1. Summary statistics for all loci for *Chrysemys p. picta* and *Clemmys guttata* . Bolded loci are those that were excluded from population analyses. Asterisks (*) denote loci that significantly deviated from HWE (after Bonferroni correction) in an exact test based on 10,000 Monte Carlo permutations of alleles. He is expected heterozygosity and Ho is observed heterozygosity.

Locus	Multiplex	Fragment size range	No. of alleles	H_e	H_o	Evenness
Chrysemys p. picta						
GmuA18*	1	107 - 123	4	0.051	0.036	0.335
GmuD21*	1	140 - 172	4	0.432	0.552	0.816
GmuD87*	1	207 - 251	9	0.190	0.068	0.400
GmuD114	2	90 - 134	12	0.877	0.867	0.883
GmuB67	2	147	1	0	0	NA
GmuD79	2	171 - 215	12	0.871	0.841	0.823
GmuB08	2	215 - 254	14	0.875	0.897	0.853
GmuD93	3	123 - 195	13	0.823	0.837	0.746
GmuD121*	3	128 - 164	9	0.659	0.378	0.640
GmuB12	3	175 - 184	3	0.209	0.212	0.587
GmuD70	4	134 - 366	50	0.949	0.933	0.702
GmuD55	4	170 - 206	10	0.626	0.635	0.503
GmuA32	5	128	1	0	0	NA
GmuD90	5	114 - 162	12	0.811	0.815	0.821
GmuA19	5	122 - 154	11	0.841	0.834	0.810
GmuB21*	5	207 - 231	6	0.542	0.452	0.593
Cp2*	6	172 - 210	13	0.838	0.581	0.838
Cp10*	6	187 - 239	14	0.751	0.437	0.675
Mean of retained loci	-	-	12.58	0.659	0.659	0.706
Standard error	-	-	3.58	0.085	0.084	0.048
Clemmys guttata						
GmuD21	1	141 - 177	10	0.789	0.865	0.780
GmuD87	1	200 - 288	22	0.939	0.905	0.900
GmuA18	1	98 - 116	6	0.691	0.669	0.850
GmuD88*	2	112 - 148	7	0.368	0.293	0.470
GmuD90	2	113 - 121	3	0.549	0.446	0.810
GmuD40	2	124 - 184	17	0.908	0.884	0.850
GmuD121	3	130 - 174	11	0.736	0.719	0.640
GmuD16	3	139 - 183	13	0.855	0.898	0.820
GmuD55	3	179 - 207	8	0.790	0.784	0.790
GmuB08	3	196 - 205	3	0.366	0.365	0.750
GmuD114	4	83 - 107	5	0.571	0.520	0.800
GmuD79	4	163 - 207	11	0.798	0.770	0.740
GmuB21*	4	185 - 206	6	0.574	0.458	0.770
GmuA19	5	126 - 132	4	0.628	0.555	0.900
GmuA32*	5	130 - 140	5	0.721	0.830	0.860
GmuD70	5	145 - 237	16	0.876	0.868	0.800
GmuD28*	5	183 - 259	14	0.893	0.613	0.860
Mean of retained loci	-	-	9.19	0.697	0.677	0.783
Standard error	-	-	1.38	0.044	0.052	0.026

Figures

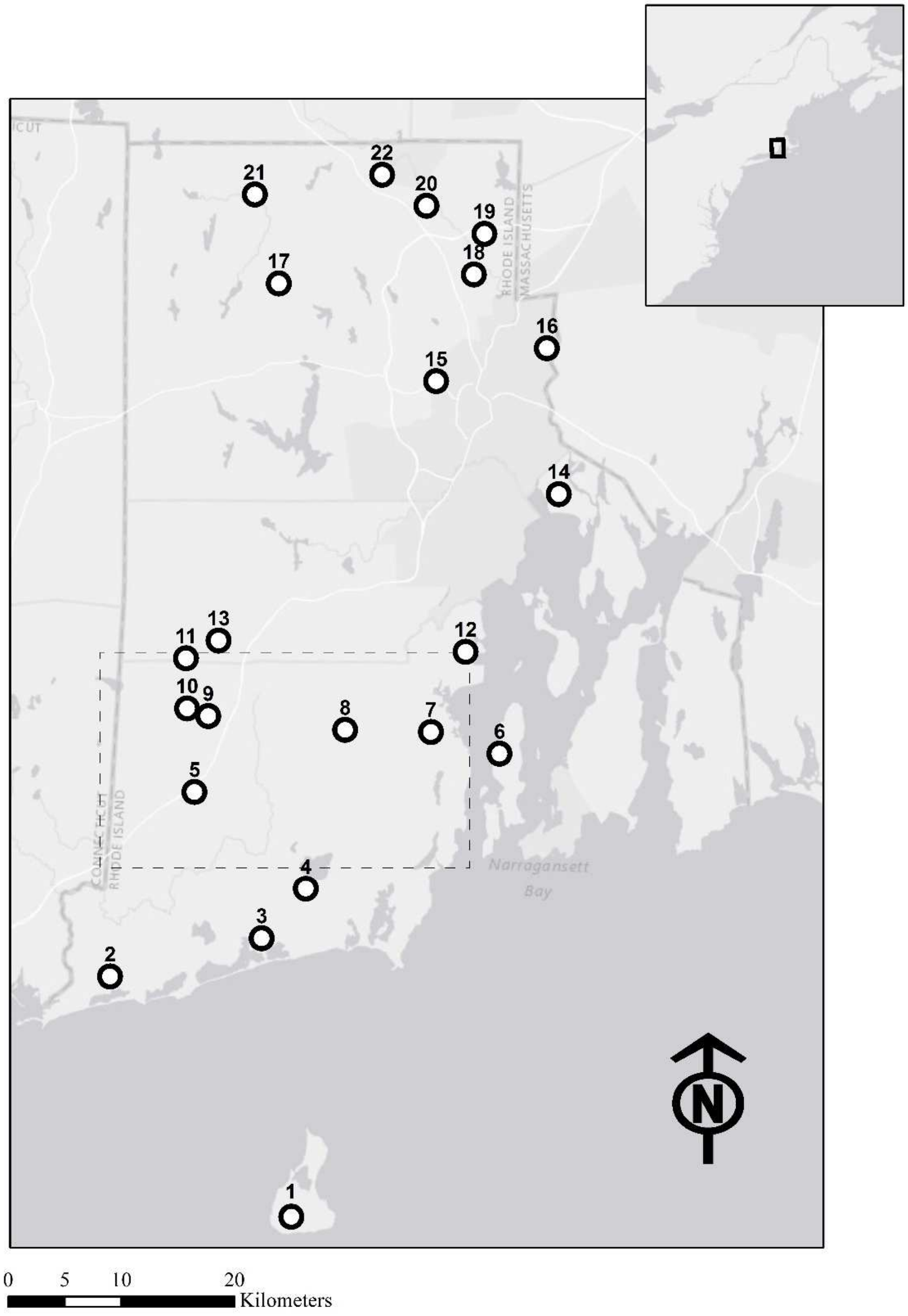


Figure 4.1. Map showing all sampling sites for *Chrysemys p. picta* (circles) and the sampling extent of all sites with >4 individuals for *Clemmys guttata* (dashed polygon).

Figure 4.2. Aerial photographs of representative sites selected for sampling. White arrows indicate specific wetland sampled. Wetlands ranged in size between 0.1 – 1.8 ha and were predominantly hydrologically isolated (i.e., non-riparian) with the exception of site 24 (not pictured). Clockwise from top left are wetlands 8, 12, 15, and 21.

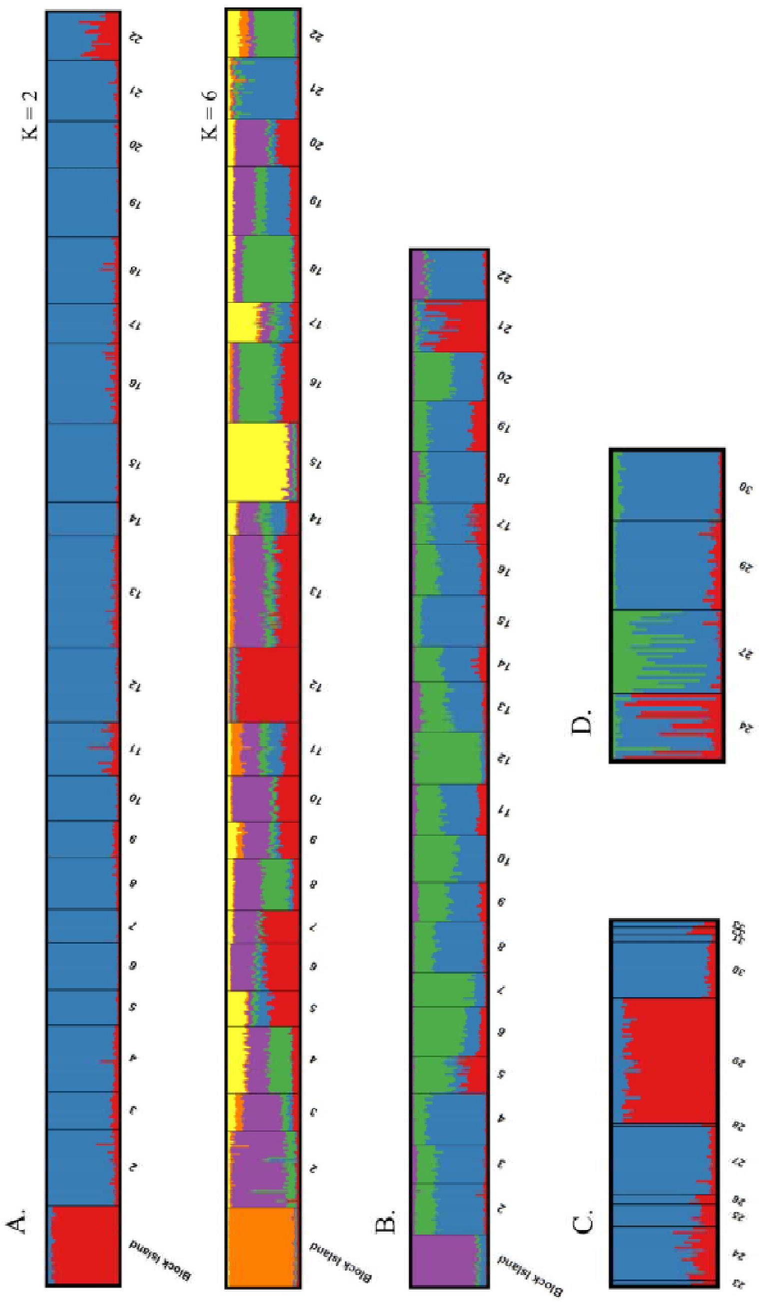


Figure 4.3. Program STRUCTURE bar plots for A) All *Chrysemsy p. picta* sites with all individuals, showing results for *K* = 2 and *K* = 6; B) all *C. p. picta* sites limited to 25 individuals per sampling locality (*K* = 4); C) *Clemmys guttata* with all individuals from all sites (*K* = 2); and D) *C. guttata* with sites with > 9 individuals and with site 29 limited to 30 individuals (*K* = 3).

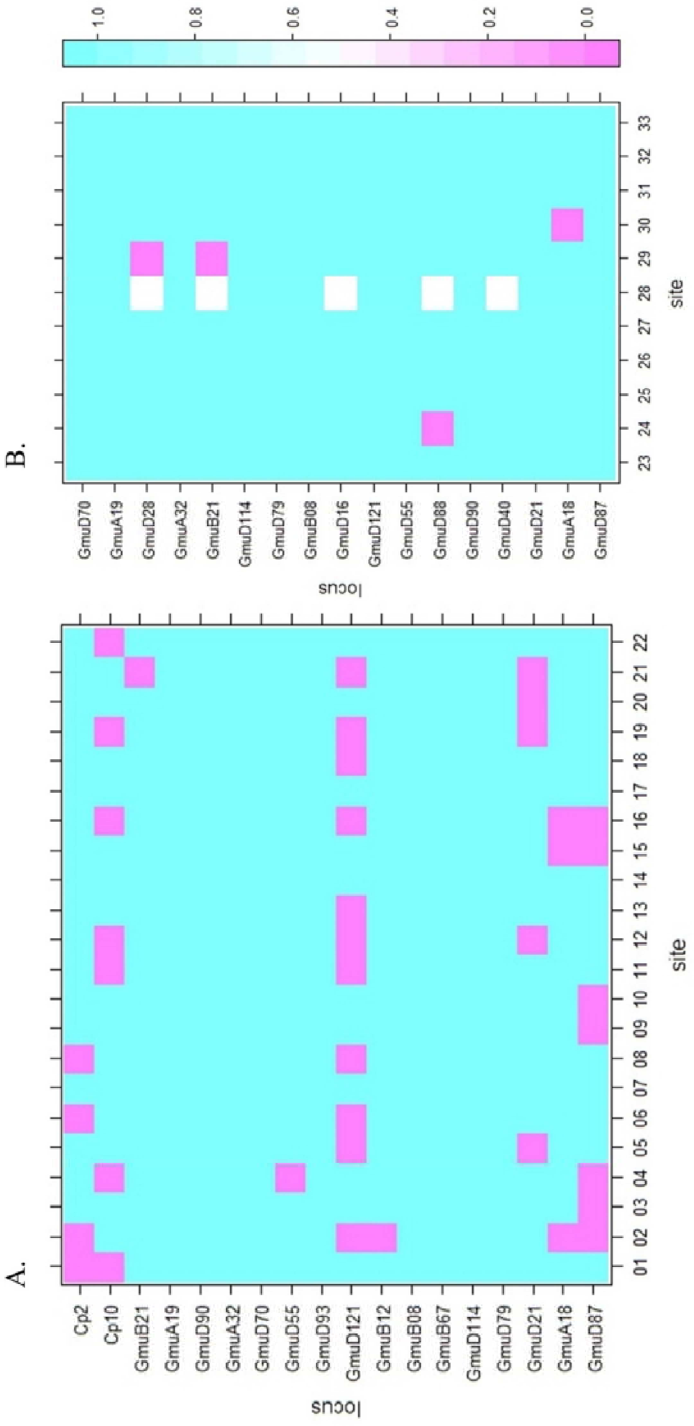
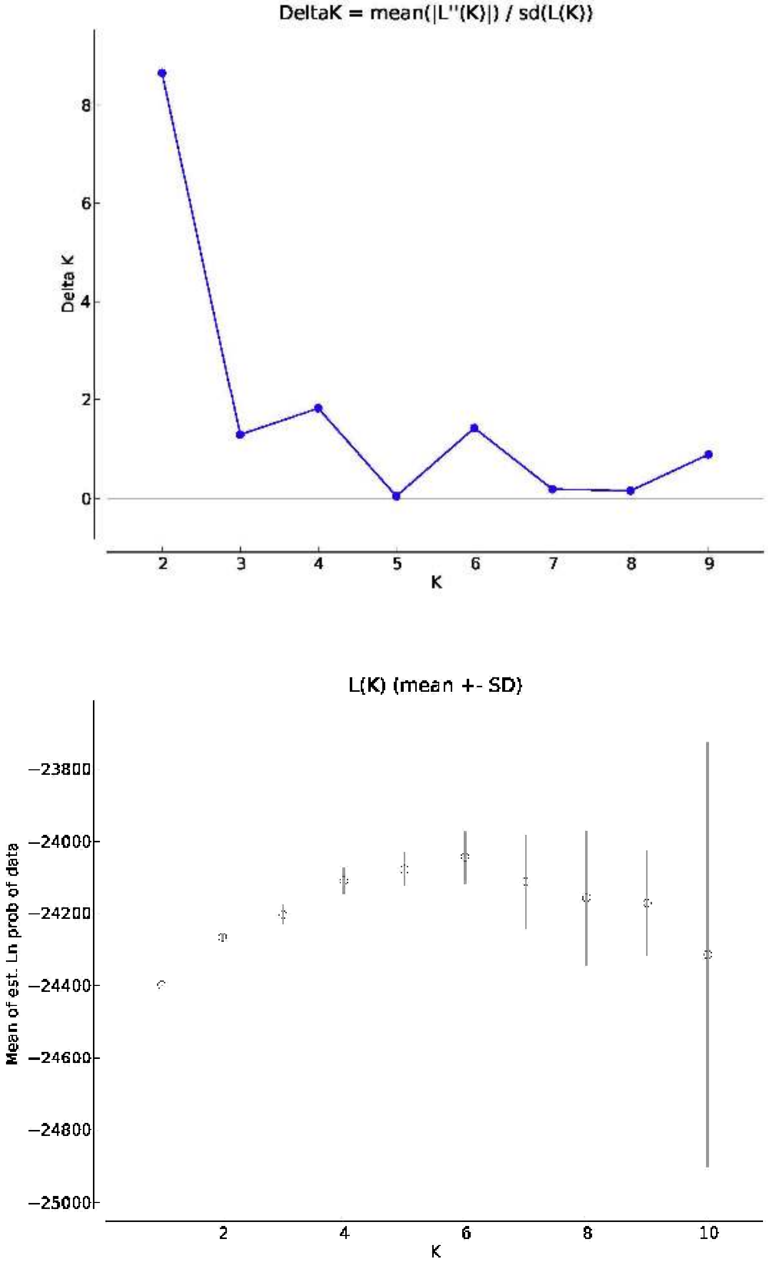
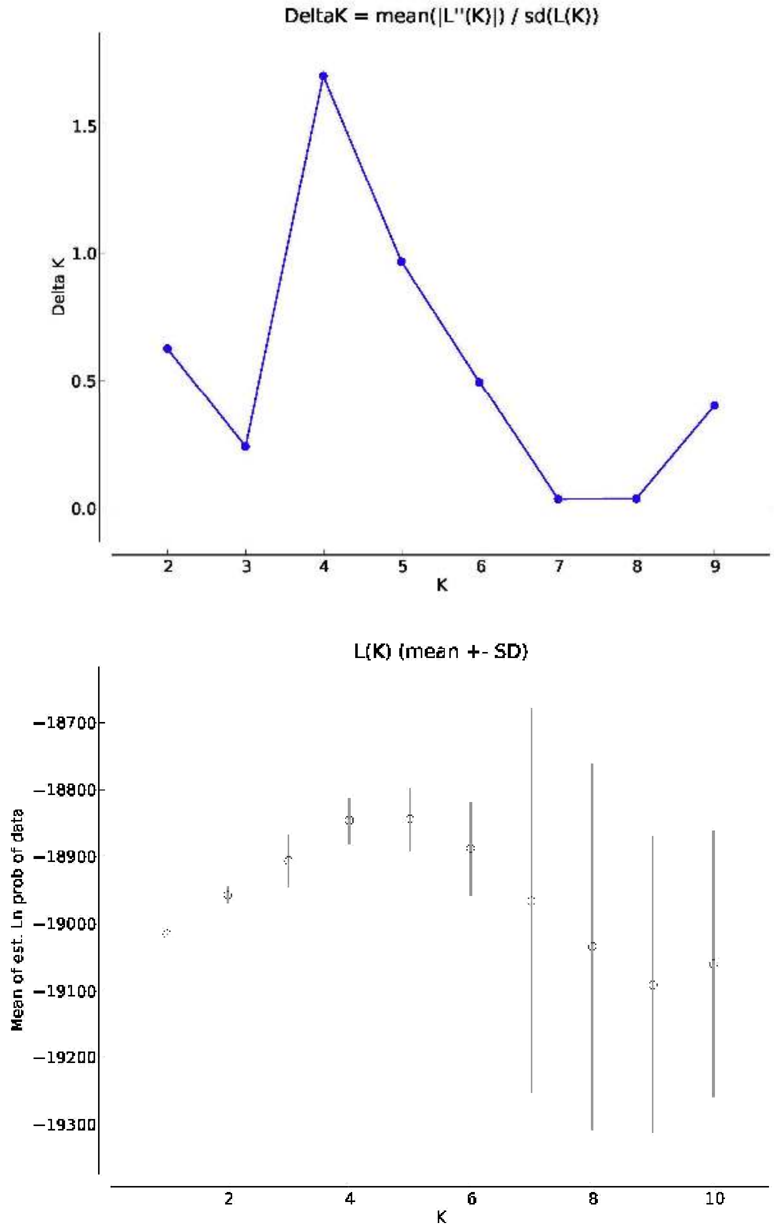


Figure 4.S.1. *P*-values for all loci by sampling site combinations for (A) *Chrysemys p. picta*, and (B) *Clemmys guttata*. Pink boxes indicate instances in which loci were found to significantly deviate from Hardy-Weinberg Equilibrium using an exact test based on 10,000 Monte Carlo permutations. White boxes indicate no information available due to too few alleles to calculate. Bonferroni corrections applied to data from both *C. picta* ($\alpha = 0.05/396 = 0.00012$) and *C. guttata* ($\alpha = 0.05/170 = 0.00029$).

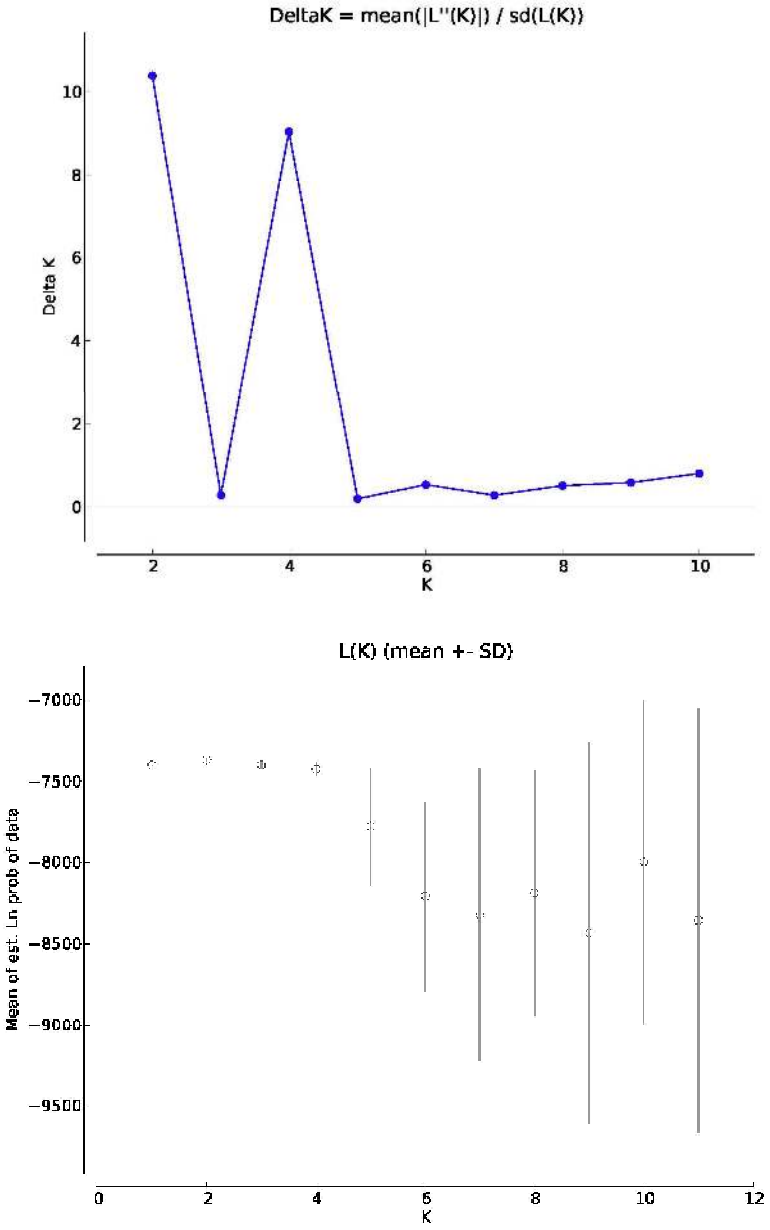
A.


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
8
6
4
2
0
2
3
4
5
6
7
8
9
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
−23800
−24000
−24200
−24400
−24600
−24800
−25000
2
4
6
8
10
K

B.


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
0.0
0.5
1.0
1.5
K
2
3
4
5
6
7
8
9
L(K) (mean +- SD)
Mean of est. Ln prob of data
−18700
−18800
−18900
−19000
−19100
−19200
−19300
K
2
4
6
8
10

C.


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
0
2
4
6
8
10
K
2
4
6
8
10
L(K) (mean +- SD)
Mean of est. Ln prob of data
−7000
−7500
−8000
−8500
−9000
−9500
K
0
2
4
6
8
10
12

D.

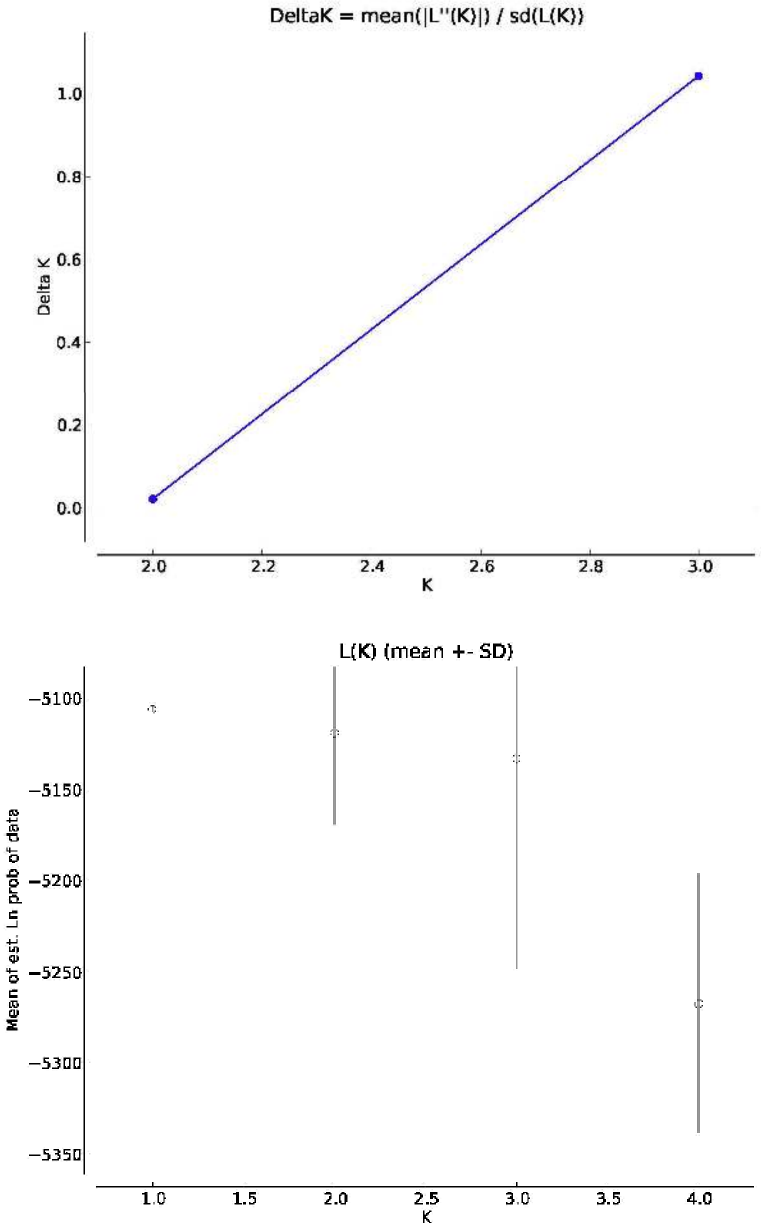


Figure 4.S.2. Δ*K* and ln Pr(X|*K*) STRUCTURE Harvester results for A) *Chrysemsys p. picta* with all individuals from all sites; B) *C. p. picta* with maximum 25 individuals per site; C) *Clemmys guttata* with all individuals from all sites; and D) *C. guttata* from sites > 9 individuals and with site 29 limited to 30 individuals.

Appendices

Appendix 1 . Wetlands sampled for aquatic turtles, Rhode Island, USA, 2013-2015. UTMs for Zone 19N. Location information removed for wetlands containing *Clemmys guttata* . Asterik indicates site not included in occupancy analysis due to missing covariate information.

	Site	Year sampled	Easting	Northing	Wetland age	Wetland area (ha)	Maximum depth (m)	Proportion forested 300m	Total trap nights	Site visit 1	Site visit 2	Site visit 3	Site visit 4
Albion Field, Lincoln	1	2013	296241	4647470	54	0.16	0.53	0.206	32	14-May	25-Jun	9-Aug	21-Sep
Angell Rd., Cumberland	2	2015	299693	4646467	77	0.49	1.95	0.558	80	28-May	14-Jul	1-Sep	6-Oct
Arcadia Rd., Richmond	3	2013	275308	4603916	77	0.60	1.95	0.586	112	8-May	23-Jul	24-Aug	28-Sep
Arcadian Fields, Hope Valley	4	2014	273832	4602473	77	0.46	2.61	0.857	76	4-Jun	15-Jul	8-Aug	9-Sep
Arnold Dr, Cumberland	5	2013	302431	4650113	77	0.36	0.96	0.445	64	14-May	25-Jun	9-Aug	21-Sep
Arrow Way, Cranston	6	2013	292689	4628824	77	0.36	1.27	0.284	60	24-May	16-Jul	20-Aug	NA
Ayoho Campgrounds Rd., Coventry	7	2014	283812	4619939	77	0.27	0.94	0.467	64	4-Jul	30-Jul	21-Aug	3-Oct
Beach Pond State Park, Exeter	8	2015	273352	4609014	77	0.43	2.21	0.803	64	19-May	22-Jul	25-Aug	14-Oct
	9	2014			77	1.00	0.96	0.243	96	4-Jun	26-Jun	8-Aug	7-Oct
Blitzkrieg Trail, Arcadia	10	2013	273446	4604595	77	0.98	5.81	0.919	96	29-May	9-Jul	6-Aug	30-Aug
Bristol Ferry Rd., Portsmouth	11	2015	311747	4609661	44	0.16	4.05	0.103	48	16-Jun	31-Jul	9-Sep	20-Oct
	12	2015			77	0.31	0.51	0.686	24	23-Jun	10-Jul	NA	NA
Buck Hill One	13	2014	268620	4652325	35	0.13	2.20	0.987	32	28-May	15-Jul	15-Aug	16-Sep
Buck Hill Two	14	2015	269978	4651967	77	0.48	1.05	0.978	20	22-May	NA	NA	NA
Buckeye Brook Rd., Charlestown	15	2013	274130	4586385	77	0.24	0.75	0.980	28	11-May	18-Jun	2-Aug	NA
	16	2014			77	0.21	0.62	0.877	56	17-May	30-Jul	20-Aug	NA
Burlingame Rd., Glocester	17	2015	272669	4643990	77	0.32	0.73	0.811	72	19-Jun	7-Aug	4-Sep	23-Oct
Buttonhole Golf, Johnston	18	2013	295433	4633477	44	1.00	0.50	0.149	92	8-May	16-Jul	20-Aug	14-Sep
Candy Apple Lane, NK	19	2014	291483	4598679	77	0.28	0.35	0.656	16	14-May	NA	NA	NA
Canyon Dr., Westerly	20	2013	266654	4580933	77	0.64	0.83	0.483	80	8-Jun	28-Jun	24-Aug	5-Oct
Carolina Back Rd.	21	2013	278035	4590900	77	0.65	1.19	0.944	60	12-Jun	28-Jun	30-Jul	NA
Carolina Nooseneck Rd., Richmond	22	2015	278456	4598860	77	0.16	0.50	0.988	16	19-May	7-Jul	NA	NA
Catalpa Way, Coventry	23	2015	284487	4618599	77	0.47	0.60	0.477	60	23-Jun	4-Aug	NA	9-Oct
	24	2013			77	0.39	0.40	0.958	60	29-May	20-Jun	24-Jul	30-Aug
Chase Farm, Lincoln	25	2013	298755	4642876	54	1.21	1.56	0.327	160	14-May	25-Jun	9-Aug	21-Sep
Cranston High School West	26	2014	294302	4627491	54	0.18	0.42	0.174	40	18-Jul	2-Aug	24-Aug	3-Oct
Curtis Corner School, South Kingstown	27	2015	289186	4592976	44	1.49	2.41	0.583	128	9-Jun	7-Jul	14-Aug	29-Sep
CVS Dr., Woonsocket	28	2014	294847	4651954	28	0.52	0.53	0.412	80	20-May	7-Jul	12-Aug	30-Sep
Deerfield Park, Smithfield	29	2015	288188	4640110	77	0.15	0.85	0.430	44	19-Jun	28-Jul	26-Aug	14-Oct
Division Rd., East Greenwich	30	2015	288947	4614359	35	0.40	2.71	0.639	64	1-Jul	4-Aug	15-Sep	9-Oct
East Ave., Burrillville	31	2015	279435	4649930	77	0.35	1.75	0.678	72	10-Jul	7-Aug	16-Sep	23-Oct
Eight Rod Way, Tiverton	32	2015	319012	4602683	28	0.13	1.05	0.557	42	17-Jun	31-Jul	9-Sep	20-Oct
Evans Rd., Glocester	33	2014	285475	4641971	77	0.19	0.61	0.908	20	20-Jun	NA	15-Aug	NA
Fairgrounds Rd., SK	34	2014	286740	4596634	35	0.19	4.90	0.425	48	14-May	25-Jun	5-Aug	19-Sep
Fairview Ave, Richmond	35	2013	273232	4599745	77	0.16	1.61	0.384	48	12-Jun	16-Jul	2-Aug	28-Sep
Fletcher Rd., North Kingstown	36	2014	297825	4611337	77	0.46	1.05	0.568	96	18-Jun	23-Jul	21-Aug	19-Sep
Great Neck Rd., South Kingstown	37	2014	285422	4594971	77	0.10	0.85	0.585	24	25-Jun	23-Jul	15-Aug	NA
Hamilton Allenton Rd., North Kingstown	38	2013	294977	4602512	19	1.73	0.78	0.692	192	15-Jun	12-Jul	30-Jul	7-Sep
Harrison Ave, Newport	39	2013	303914	4593175	28	0.23	1.51	0.185	64	21-May	19-Jul	27-Aug	19-Oct
High St., Lincoln	40	2015	294585	4648940	77	1.36	1.71	0.440	124	28-May	14-Jul	1-Sep	6-Oct
Hines Rd., Cumberland	41	2014	301444	4645008	44	0.10	1.70	0.396	32	1-Jul	2-Aug	3-Sep	14-Oct
	42	2014			77	1.53	1.02	0.906	128	17-May	10-Jul	20-Aug	23-Sep
Johnson Rd., Foster	43	2013	271712	4625552	54	0.40	2.43	0.537	80	12-Jun	23-Jul	23-Aug	5-Oct
Kenyon Hill Trail, Carolina	44	2013	277431	4596724	28	0.20	2.15	0.530	48	29-May	9-Jul	6-Aug	30-Aug
Leisure Dr., South Kingstown	45	2013	283932	4588667	28	0.89	3.85	0.686	112	15-Jun	11-Jul	2-Aug	28-Sep
Liena Rose Way, Coventry	46	2015	284752	4618196	28	0.15	1.01	0.264	32	9-Jun	4-Aug	15-Sep	9-Oct
Long Highway, Little Compton	47	2015	320537	4598695	77	0.14	1.05	0.464	48	16-Jun	31-Jul	9-Sep	20-Oct
Lynn Circle, East Greenwich	48	2013	290476	4613913	44	0.11	1.46	0.618	32	15-May	25-Jun	6-Aug	16-Sep
Maple Ave., Barrington	49	2015	306261	4623480	77	0.79	0.80	0.238	92	28-May	14-Jul	1-Sep	6-Oct
Mount Hope Farm, Bristol	50	2015	312490	4615482	77	0.67	1.35	0.340	80	1-Jul	4-Aug	9-Sep	20-Oct
Narragansett High School	51	2015	293726	4589320	13	0.16	0.35	0.376	72	9-Jun	7-Jul	14-Aug	29-Sep
Narrow Ave., Tiverton	52	2014	321797	4607626	77	0.50	1.10	0.569	56	13-Jun	5-Aug	24-Aug	7-Oct
North Niantic Dr., Charlestown	53	2013	280035	4584297	77	0.47	2.28	0.179	80	17-May	21-Jun	30-Jul	19-Oct
	54	2015			77	0.89	0.53	0.955	32	26-Jun	28-Jul	14-Aug	NA
Old Mill Rd., Charlestown	55	2014	276979	4590386	77	0.61	0.30	0.922	20	4-Jun	NA	NA	NA
Old North Rd., South Kingstown	56	2014	289713	4595641	54	0.11	3.90	0.524	32	7-Jun	10-Jul	8-Aug	19-Sep
Old Switch Rd., Richmond	57	2014	274099	4597219	77	0.53	1.11	0.857	40	7-Jun	30-Jul	NA	NA
Pardon Joslin Rd., Exeter	58	2015	285295	4608062	77	0.15	2.06	0.885	42	19-May	10-Jul	11-Aug	13-Oct
Patton Rd., East Providence	59	2013	305199	4636376	77	0.68	0.71	0.259	144	24-May	16-Jul	20-Aug	14-Sep
Plain Meeting House Rd., W. Greenwich*	60	2014	273436	4612933	44	0.17	*NA*	0.832	48	28-May	10-Jul	20-Aug	23-Sep
Potter Rd., N. Kingstown	61	2013	296165	4610531	77	0.48	2.26	0.502	80	24-May	19-Jul	20-Aug	7-Sep
Priscilla Rd., Woonsocket	62	2014	295355	4654006	54	0.75	0.55	0.219	96	20-May	7-Jul	12-Aug	30-Sep
Providence St., Woonsocket	63	2014	290669	4651661	77	0.34	1.55	0.249	56	1-Jul	2-Aug	3-Sep	30-Sep
Putnam Pike, Glocester	64	2015	281542	4642093	77	0.83	2.20	0.845	92	19-Jun	7-Aug	4-Sep	13-Oct
Quidnessett Country Club	65	2013	299094	4612413	44	0.11	0.40	0.104	32	17-May	21-Jun	30-Jul	7-Sep
Quonset Airport	66	2014	298030	4609581	44	0.75	1.01	0.183	116	23-May	23-Jul	24-Aug	7-Oct
Rainbow Pond, Jamestown	67	2013	302451	4602373	28	1.21	2.07	0.451	168	21-May	19-Jul	27-Aug	19-Oct
Reservoir Rd., Cumberland	68	2014	300837	4651394	44	0.40	1.76	0.382	36	13-Jun	2-Aug	3-Sep	NA
School Landwood Rd., Exeter	69	2015	287395	4602695	77	0.16	0.71	0.585	48	26-Jun	28-Jul	26-Aug	9-Oct
Seabury Dr., Westerly	70	2013	264594	4580486	77	0.41	1.75	0.395	80	7-Jun	28-Jun	23-Aug	5-Oct
Seapowet Ave., Tiverton	71	2015	316251	4606486	77	0.17	0.17	0.169	24	26-Jun	31-Jul	NA	NA
Secluded Dr., Narragansett	72	2014	295940	4592732	19	0.22	1.11	0.243	64	17-Jun	23-Jul	21-Aug	23-Sep
Shaw Rd., Little Compton	73	2014	319819	4594766	77	0.11	0.55	0.233	32	13-Jun	5-Aug	24-Aug	7-Oct
Shelter Harbor Golf Course	74	2014	271796	4582230	77	0.42	1.05	0.669	84	1-Jul	30-Jul	9-Sep	NA
Slocum Rd., North Kingstown	75	2015	290246	4599702	13	0.12	1.65	0.390	32	19-May	7-Jul	11-Aug	29-Sep
South County Sand and Gravel	76	2013	291108	4592504	28	0.25	1.95	0.258	64	17-May	21-Jun	23-Jul	27-Aug
South Rd, South Kingstown	77	2013	289034	4594279	44	0.33	0.50	0.657	32	12-May	18-Jun	NA	NA
South Rd., Exeter	78	2015	289297	4604347	77	1.47	0.74	0.314	60	1-Jul	11-Aug	15-Sep	9-Oct
Spring Lake Rd., Burillville	79	2015	280328	4652078	44	1.15	2.40	0.891	132	22-May	22-Jul	4-Sep	23-Oct
St. Leon Ave., Woonsocket	80	2015	293525	4654249	77	0.88	0.85	0.256	48	23-Jun	11-Aug	NA	6-Oct
	81	2015			77	0.16	1.30	0.888	24	22-May	10-Jul	NA	NA
Sweet Allen Farm	82	2013	289763	4591797	28	0.27	1.00	0.477	48	17-May	28-Jun	7-Aug	16-Sep
Thibeault Trail, Smithfield	83	2014	288067	4641770	44	0.11	1.72	0.698	32	20-May	4-Jul	12-Aug	3-Oct
Tiogue Ave, West Warwick	84	2014	289513	4617095	77	0.23	0.30	0.451	92	18-Jul	5-Aug	9-Sep	3-Oct
Trustom Pond, South Kingstown	85	2013	283938	4584324	77	0.29	1.64	0.363	80	18-Jun	9-Jul	2-Aug	30-Aug
Tunk Hill Rd., Foster	86	2015	281069	4626092	44	0.38	2.21	0.921	64	19-Jun	28-Jul	25-Aug	13-Oct
Wendi Dr., North Providence	87	2014	294176	4638166	77	0.45	0.73	0.385	64	7-Jun	7-Jul	12-Aug	14-Oct
White Horse Dr., Glocester	88	2014	279117	4643531	77	1.27	2.80	0.672	140	17-May	15-Jul	15-Aug	16-Sep

Appendix 2. *Chrysemys picta* abundance and demography, Rhode Island, USA, 2013-2015. Location information removed for wetlands containing *Clemmys guttata* .

Wetland	Site number	Unique individuals	Individuals per trap night	Female ratio (adults only)	Total juveniles	Juvenile ratio (all individuals)
Albion Field, Lincoln	1	3	0.094	0.667	0	0
Angell Rd., Cumberland	2	35	0.438	0.433	5	0.143
Arcadia Road, Richmond	3	22	0.196	0.286	0	0
Arcadian Fields, Hope Valley	4	4	0.053	0.000	0	0
Arnold Dr, Cumberland	5	16	0.250	0.636	3	0.188
Arrow Way, Cranston	6	0	0.000	NA	0	NA
Ayoho Campgrounds Road, Coventry	7	13	0.203	0.462	0	0
Beach Pond State Park,Exeter	8	27	0.422	0.391	3	0.111
	9	27	0.281	0.421	8	0.296
Blitzkrieg Trail, Arcadia	10	25	0.260	0.435	2	0.08
Bristol Ferry Rd., Portsmouth	11	13	0.271	0.615	0	0
	12	1	0.042	0.000	0	0
Buck Hill One	13	2	0.063	0.500	0	0
Buck Hill Two	14	0	0.000	NA	0	NA
Buckeye Brook Road, Charlestown	15	0	0.000	NA	0	NA
	16	8	0.143	0.375	0	0
Burlingame Rd., Glocester	17	2	0.028	0.500	0	0
Buttonhole Golf, Johnston	18	59	0.641	0.475	18	0.305
Candy Apple Lane, NK	19	0	0.000	NA	0	NA
Canyon Drive, Westerly	20	79	0.988	0.603	4	0.051
Carolina Back Road	21	1	0.017	0.000	0	0
Carolina Nooseneck Rd., Richmond	22	0	0.000	NA	0	NA
Catalpa Way, Coventry	23	26	0.433	0.583	2	0.077
	24	0	0.000	NA	0	NA
Chase Farm, Lincoln	25	94	0.588	0.325	11	0.117
Cranston High School West	26	4	0.100	0.667	0	0
Curtis Corner School, South Kingstown	27	35	0.273	0.400	5	0.143
CVS Drive, Woonsocket	28	16	0.200	0.357	1	0.063
Deerfield Park, Smithfield	29	5	0.114	0.600	0	0
Division Rd., East Greenwich	30	10	0.156	0.375	2	0.2
East Ave., Burrillville	31	31	0.431	0.667	1	0.032
Eight Rod Way, Tiverton	32	12	0.286	0.417	0	0
Evans Road, Glocester	33	0	0.000	NA	0	NA
Fairgrounds Road, SK	34	0	0.000	NA	0	NA
Fairview Ave, Richmond	35	6	0.125	0.400	1	0.167
Fletcher Road, North Kingstown	36	14	0.146	0.500	0	0
Great Neck Road, South Kingstown	37	13	0.542	0.500	3	0.231
Hamilton Allenton Rd., North Kingstown	38	17	0.089	0.571	3	0.176
Harrison Ave, Newport	39	12	0.188	0.600	2	0.167
High St., Lincoln	40	24	0.194	0.250	4	0.167
Hines Road, Cumberland	41	16	0.500	0.600	1	0.063
	42	77	0.602	0.271	16	0.208
Johnson Road, Foster	43	10	0.125	0.400	0	0
Kenyon Hill Trail, Carolina	44	6	0.125	0.500	0	0
Leisure Drive, South Kingstown	45	43	0.384	0.324	6	0.14
Liena Rose Way, Coventry	46	3	0.094	0.667	0	0
Long Highway, Little Compton	47	1	0.021	1.000	0	0
Lynn Circle, East Greenwich	48	6	0.188	0.500	0	0
Maple Ave., Barrington	49	34	0.370	0.500	4	0.118
Mount Hope Farm, Bristol	50	30	0.375	0.393	2	0.067
Narragansett High School	51	4	0.056	0.500	0	0
Narrow Ave., Tiverton	52	0	0.000	NA	0	NA
North Niantic Drive, Charlestown	53	25	0.313	0.320	0	0
	54	2	0.063	0.500	0	0
Old Mill Road, Charlestown	55	0	0.000	NA	0	NA
Old North Road, South Kingstown	56	0	0.000	NA	0	NA
Old Switch Road, Richmond	57	21	0.525	0.500	1	0.048
Pardon Joslin Rd., Exeter	58	8	0.190	0.000	3	0.375
Patton Rd., East Providence	59	69	0.479	0.367	8	0.116
Plain Meeting House Rd., W. Greenwich	60	0	0.000	NA	0	NA
Potter Rd., N. Kingstown	61	20	0.250	0.526	1	0.05
Priscilla Road, Woonsocket	62	2	0.021	0.500	0	0
Providence Street, Woonsocket	63	25	0.446	0.522	2	0.08
Putnam Pike, Glocester	64	19	0.207	0.467	4	0.211
Quidnessett Country Club	65	16	0.500	0.769	3	0.188
Quonset Airport	66	71	0.612	0.319	2	0.028
Rainbow Pond, Jamestown	67	16	0.095	0.250	0	0
Reservoir Road, Cumberland	68	13	0.361	0.462	0	0
School Landwood Rd., Exeter	69	29	0.604	0.462	1	0.034
Seabury Drive, Westerly	70	0	0.000	NA	0	NA
Seapowet Avenue, Tiverton	71	9	0.375	0.286	1	0.111
Secluded Drive, Narragansett	72	14	0.219	0.583	2	0.143
Shaw Road, Little Compton	73	7	0.219	0.167	1	0.143
Shelter Harbor Golf Course	74	8	0.095	0.800	3	0.375
Slocum Rd., North Kingstown	75	11	0.344	0.400	6	0.545
South County Sand and Gravel	76	12	0.188	0.600	2	0.167
South Rd, South Kingstown	77	0	0.000	NA	0	NA
South Rd., Exeter	78	2	0.033	1.000	1	0.5
Spring Lake Rd., Burillville	79	11	0.083	0.455	0	0
St Leon Ave., Woonsocket	80	10	0.208	0.625	2	0.2
	81	3	0.125	1.000	0	0
Sweet Allen Farm	82	8	0.167	0.857	1	0.125
Thibeault Trail, Smithfield	83	5	0.156	0.500	0	0
Tiogue Ave, West Warwick	84	0	0.000	NA	0	NA
Trustom Pond, South Kingstown	85	25	0.313	0.174	1	0.04
Tunk Hill Rd., Foster	86	11	0.172	0.444	2	0.182
Wendi Drive, North Providence	87	3	0.047	0.000	0	0
White Horse Dr., Glocester	88	8	0.057	0.429	1	0.125
Sum	-	1369	-	-	155	-
Mean	-	15.56	0.211	0.465	1.76	0.097

Appendix 3. Pearson correlation coefficients for all combinations of covariates measured for occupancy and abundance analyses, Rhode Island, USA, 2013-2015.

	easting	northing	wetland.age	hectares	develop.300	road.dens.300	esh.300	forest.300	wetland.300	develop.1000	road.dens.1000	esh.1000	forest.1000	wetland.1000	ammonia	nitrate	phos	ph	tds	max.depth	graminoid	herbaceous	open	sav	woody	surficial
easting	1.000																									
northing	0.143	1.000																								
wetland.age	-0.497	-0.007	1.000																							
hectares	-0.250	0.126	0.074	1.000																						
develop.300	0.664	0.382	-0.450	-0.079	1.000																					
road.dens.300	0.618	0.388	-0.325	-0.106	0.983	1.000																				
esh.300	0.585	-0.330	-0.316	-0.258	-0.077	-0.135	1.000																			
forest.300	-0.895	-0.178	0.551	0.207	-0.868	-0.817	-0.412	1.000																		
wetland.300	0.368	-0.072	-0.113	-0.143	0.178	0.097	0.239	-0.391	1.000																	
develop.1000	0.688	0.366	-0.460	-0.029	0.990	0.966	-0.028	-0.883	0.186	1.000																
road.dens.1000	0.674	0.384	-0.443	-0.017	0.981	0.958	-0.025	-0.873	0.162	0.996	1.000															
esh.1000	0.619	-0.373	-0.359	-0.355	0.008	-0.048	0.975	-0.483	0.288	0.040	0.032	1.000														
forest.1000	-0.882	-0.178	0.541	0.207	-0.885	-0.836	-0.370	0.995	-0.390	-0.901	-0.889	-0.449	1.000													
wetland.1000	0.296	-0.018	-0.081	-0.343	0.047	-0.004	0.239	-0.254	0.818	0.017	-0.023	0.305	-0.262	1.000												
ammonia	0.105	0.011	0.182	-0.209	0.084	0.163	-0.004	-0.048	-0.198	0.043	0.033	0.029	-0.039	-0.126	1.000											
nitrate	0.426	0.142	-0.556	-0.183	0.582	0.510	-0.023	-0.541	0.313	0.581	0.564	0.045	-0.553	0.205	-0.084	1.000										
phos	-0.224	-0.026	-0.109	0.019	-0.197	-0.221	-0.067	0.231	-0.214	-0.181	-0.189	-0.044	0.193	-0.095	-0.149	-0.249	1.000									
ph	0.802	0.289	-0.674	-0.061	0.720	0.619	0.414	-0.859	0.321	0.749	0.752	0.425	-0.835	0.138	-0.053	0.635	-0.142	1.000								
tds	0.528	0.352	-0.479	0.032	0.782	0.713	-0.073	-0.680	0.210	0.790	0.791	-0.042	-0.679	-0.006	0.021	0.720	-0.223	0.803	1.000							
max.depth	-0.295	-0.107	-0.294	0.214	-0.331	-0.415	0.107	0.257	-0.079	-0.327	-0.309	0.041	0.296	-0.043	-0.270	-0.042	0.152	0.079	-0.051	1.000						
graminoid	0.239	-0.238	0.254	-0.413	-0.101	-0.056	0.310	-0.080	0.209	-0.076	-0.069	0.327	-0.088	0.125	-0.054	0.062	-0.230	-0.056	-0.153	-0.530	1.000					
herbaceous	-0.172	-0.171	0.355	-0.018	-0.457	-0.430	0.153	0.334	-0.007	-0.434	-0.419	0.125	0.332	-0.029	-0.247	-0.350	-0.093	-0.379	-0.448	-0.241	0.572	1.000				
open	-0.187	0.111	-0.329	0.164	-0.105	-0.191	-0.053	0.128	-0.059	-0.109	-0.081	-0.098	0.158	-0.072	-0.196	0.132	0.154	0.213	0.209	0.800	-0.541	-0.318	1.000			
sav	-0.288	-0.125	-0.052	0.236	-0.184	-0.242	-0.085	0.185	0.128	-0.185	-0.200	-0.100	0.198	-0.006	-0.232	-0.177	0.067	-0.117	-0.060	0.034	-0.117	0.143	-0.199	1.000		
woody	-0.285	-0.212	0.482	0.128	-0.122	-0.003	-0.418	0.335	-0.317	-0.144	-0.163	-0.385	0.292	-0.242	0.422	-0.379	0.054	-0.621	-0.375	-0.510	0.009	0.115	-0.507	-0.083	1.000	
surficial	0.557	0.309	-0.057	-0.297	0.468	0.522	0.226	-0.522	0.029	0.465	0.444	0.273	-0.540	0.204	0.220	0.160	-0.095	0.272	0.096	-0.476	0.167	-0.185	-0.575	-0.343	0.074	1.000

Appendix 4. Loadings and variances for each principal component, Rhode Island, USA, 2013-2015.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17
wetland.age	-0.155	0.212	-0.020	-0.246	0.131	-0.652	-0.270	-0.228	-0.183	0.023	-0.161	0.370	-0.251	0.096	-0.094	0.159	0.001
hectares	-0.013	-0.006	0.248	-0.252	0.545	0.302	-0.473	0.094	-0.039	-0.144	0.200	-0.223	-0.308	-0.180	-0.120	0.007	0.000
develop.300	0.476	0.132	0.183	-0.059	0.031	-0.037	0.169	-0.099	0.007	0.144	0.189	-0.066	-0.012	0.050	-0.001	0.473	-0.625
road.dens.300	0.421	0.238	0.210	-0.021	0.040	-0.158	0.160	-0.124	0.054	0.033	0.186	0.096	-0.093	-0.024	-0.216	-0.742	0.002
esh.300	-0.021	-0.044	-0.499	0.310	0.395	0.047	0.120	0.054	0.156	-0.296	-0.222	0.236	-0.083	-0.184	-0.219	-0.096	-0.403
forest.300	-0.453	-0.078	0.190	-0.053	-0.271	-0.001	-0.282	0.013	0.082	0.064	-0.042	-0.060	0.040	0.087	0.130	-0.361	-0.651
wetland.300	0.049	-0.103	-0.233	-0.372	-0.005	0.037	0.202	0.207	-0.796	-0.081	-0.028	-0.083	0.101	-0.058	0.018	-0.157	-0.151
nitrate	0.267	-0.199	-0.074	-0.106	-0.456	0.258	-0.191	0.328	0.041	0.004	-0.029	0.443	-0.481	0.074	-0.128	0.042	0.001
phos	-0.067	-0.057	0.125	0.353	-0.093	0.380	-0.072	-0.680	-0.411	0.018	0.013	0.198	-0.087	-0.061	-0.102	0.024	0.000
ph	0.350	-0.277	-0.212	0.068	0.213	-0.017	-0.260	-0.173	-0.011	-0.073	-0.089	-0.083	-0.019	0.587	0.468	-0.154	0.003
tds	0.336	-0.228	-0.020	-0.226	-0.082	-0.029	-0.402	-0.152	0.114	0.011	-0.231	0.148	0.556	-0.437	-0.051	-0.013	0.000
max.depth	-0.123	-0.414	0.159	0.153	0.180	-0.173	0.023	0.173	-0.069	-0.067	0.624	0.441	0.269	0.076	-0.005	0.032	0.000
graminoid	-0.034	0.201	-0.406	-0.216	-0.288	-0.017	-0.064	-0.283	0.144	-0.443	0.517	-0.074	-0.072	-0.178	0.228	0.018	-0.004
herbaceous	-0.151	0.095	-0.288	-0.363	0.240	0.312	0.139	-0.137	0.168	0.626	0.118	0.297	0.023	-0.004	0.179	-0.074	0.001
open	0.002	-0.483	0.192	-0.070	0.047	-0.204	0.323	-0.149	0.079	0.011	-0.121	-0.040	-0.402	-0.447	0.411	-0.041	-0.001
woody	0.002	0.405	0.345	-0.053	0.097	0.228	0.108	0.140	-0.026	-0.374	-0.211	0.423	0.141	-0.018	0.487	0.012	0.001
surficial	0.117	0.269	-0.158	0.483	-0.019	-0.143	-0.321	0.273	-0.228	0.347	0.142	-0.030	-0.080	-0.357	0.357	-0.043	-0.005
Standard deviation	1.862	1.599	1.425	1.186	1.087	1.020	1.009	0.965	0.942	0.816	0.737	0.645	0.606	0.485	0.409	0.290	0.015
Proportion of variance	0.204	0.150	0.119	0.083	0.070	0.061	0.060	0.055	0.052	0.039	0.032	0.024	0.022	0.014	0.010	0.005	0.000
Cumulative variance	0.204	0.354	0.474	0.556	0.626	0.687	0.747	0.802	0.854	0.893	0.925	0.950	0.971	0.985	0.995	1.000	1.000