Species Status Assessment (SSA) Report
for the
Massachusetts Population of the
Northern Red-bellied Cooter (*Pseudemys rubriventris*)

Version 1.0

Adult female northern red-bellied cooter from Plymouth, Massachusetts.

(Photo credit: Mike Jones)

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EXECUTIVE SUMMARY

This report summarizes a species status assessment (SSA) completed for the Massachusetts population of the northern red-bellied cooter (*Pseudemys rubriventris*). To assess the species' viability, we used the three conservation biology principles of resiliency, redundancy, and representation (the 3 R's). This report is intended to provide the biological support for the decision on whether the Massachusetts population northern red-bellied cooter should remain listed as endangered, warrants downlisting to threatened status, or no longer meets the statutory definition of endangered or threatened and warrants removal from listed status under the Endangered Species Act (Act) of 1973, as amended. The process and this SSA report do not represent a decision by the U.S. Fish and Wildlife Service (Service) whether or not to retain endangered status, downlist, or delist the species under the Act. Instead, this SSA report provides a review of the best available information related to the biological status of the northern red-bellied cooter in Massachusetts.

The northern red-bellied cooter is a large basking turtle in the family Emydidae that occupies a variety of aquatic habitats within a limited range in southeastern Massachusetts. The Massachusetts population is isolated from the rest of the species' range that spans several mid-Atlantic states from North Carolina to New Jersey. In 1980, the Massachusetts population was listed under the Act (formerly Plymouth redbelly turtle (*Pseudemys rubriventris bangsi*)) and was known to occur in only 12 ponds, with an estimated population of around 200 individuals. Following a 30-year recovery effort led by the Massachusetts Department of Fish and Wildlife, a study was conducted to determine the species' status (Regosin et al. 2017, entire). The study focused on a portion of the species' current range, located in Plymouth County, and estimated the population within to be around 933 individuals (Regosin et al. 2017 p. 29). We identified 43 analysis units (AUs) based on element occurrence and occupancy data that were used in this SSA, and calculated an overall current population estimate of 1950.73 individuals for the entire Massachusetts population by using existing population estimates (Regosin et al. 2017, p. 62) and newly calculated population estimates from headstart release data (MADFW, unpubl. data).

Some of the primary threats at the time of listing were a small population size and restricted range, habitat fragmentation and development of shoreline habitat, and nest predation. Many of these threats still continue to be relevant today and are expected to influence the species into the future. Some factors, such as predation, are known to be important but we lacked sufficient information to assess their impact on individual AUs or assess how impacts might change in the future. We considered a range of past, current, and future factors influencing species viability: water quality, habitat loss and fragmentation, predation, invasive species, road mortality, motorboat strikes, collection, harassment/disturbance, pathogens, effects of small population size, climate change, protected lands and regulatory mechanisms, and the headstart program.

We analyzed the resiliency of the northern red-bellied cooter relative to a subset of metrics related to demographics, habitat integrity, habitat degradation, and habitat protection, and translated this into an overall resiliency condition score for each AU. Because no consistent and comparable demographic data were available for each AU, we used the best available demographic information from each AU to determine a best estimate of number of individuals as a demographic metric. Habitat protection metrics we assessed included the percent of shoreline protected and the percent of protected land surrounding an AU. We assessed six habitat quality metrics that evaluated integrity and degradation for each AU including: the complexity of water body shapes, the percent of area surrounding and within the unit that had a high Index of Ecological Integrity score, whether an AU contained multiple occupied water bodies, the percent impervious surface, the average likelihood of road mortality, and the percent of area surrounding and within the unit that had a low Index of Ecological Integrity score.

We assessed the number of AUs with good and moderate resiliency condition scores across the range of the species to assess redundancy. To assess representation, we compared the species' current range with the extent at the time of listing and considered the variety of aquatic habitats occupied across the range due to limitations in available genetic information for the Massachusetts population. Overall, the Massachusetts population of the northern red-bellied cooter has medium resiliency with 11 AUs in high condition and 15 AUs in moderate condition. Current redundancy is considered good given the number of AUs in moderate or high condition and the number of extant AUs. Representation is also considered good, despite limited genetic diversity information, because the species range is far greater than the know historical limit and because it currently occupies a variety of aquatic habitat types including coastal plain ponds, large lakes, reservoirs, and rivers.

To evaluate the biological status of the northern red-bellied cooter in the future, we evaluated condition at the 2080 time step and calculated future resiliency condition scores using the same methodology that we used for current condition. When available, we used data outputs for the habitat metrics modeled at the 2080 time step, if modeled values at the 2080 time step were not available for a habitat metric, we used the same values used in our current condition analysis. We created future scenarios that examined how AU resiliency condition responded to changes in two variables.

The first variable examined use of a headstarting conservation action with three potential headstart program options. Since 1985, the Massachusetts Division of Fisheries and Wildlife has operated a headstart program that raises wild-born hatchlings in captivity for 9 months to maximize growth before release. Over 4,400 wild-born hatchlings have been released into at least 34 sites. The program has contributed to increasing the number of individuals and number of known occupied sites within Massachusetts. One headstart program option assumes that headstarts will be released in AUs that have historically received headstarts (HistoricalHS), a second headstart program option utilized a rule-based approach to determine which AUs were likely to receive headstarts in the future if a strategic release plan were developed (RuleHS), and finally, we considered a headstart program option where no headstarts are released (NoHS).

The second variable examined the influence of two different population growth rates, an optimistic (Opt) and a pessimistic (Pes) growth rate. The pessimistic growth rate (λ=0.98167) was calculated from demographic information available for Federal Pond. Recruitment information is not available for all AUs, and it is plausible that some AUs may have pessimistic growth rates. The optimistic growth rate we selected (λ=1.0) provides a representation of stability in a turtle population facing many stressors.

Each of the six future condition scenarios (Opt-NoHS, Opt-RuleHS, Opt-HistoricalHS, Pes-NoHS, Pes-RuleHS, Pes-HistoricalHS) examined a unique combination of the headstart programs and population growth rates that allowed us to project a plausible range of future conditions for each AU. Current and future resiliency condition results for each AU are summarized in table ES-1. Under all six plausible future scenarios, the species is expected to persist in Massachusetts into 2080. We projected that viability would improve slightly under the most optimistic scenario (Opt-RuleHS) which assumed a stable growth rate and the application of a rule-based headstart program. In our most pessimistic scenario (Pes-NoHS), we projected a loss of overall resiliency, redundancy, and representation, although some AUs are expected to maintain high resiliency condition scores.

Viability is supported by having multiple resilient AUs distributed throughout the geographical extent of the species range in a variety of aquatic habitats. Compared to the historical baseline, the northern red-bellied cooter in Massachusetts has improved redundancy, representation, and resiliency. The northern red-bellied cooter will continue to be exposed to a number of threats to viability in the future. A majority of AUs are expected to experience some level of decline in habitat quality compared to current condition. In addition, we were unable to examine some factors that may influence viability in our analysis due to lack of available information or uncertainty around the relationship between the factor and species viability. The northern red-bellied cooter has a life history characterized by a long life span and late sexual maturity, and adult individuals may persist for many years in suboptimal habitat even with limited or no annual recruitment. These factors may make it difficult for managers to recognize declines in populations over short periods of time. Although outside the scope of our analysis, we expect that some AUs may experience periods of both occupancy and extirpation and that natural dispersal or management programs could result in the creation of new occurrences in the future. Continued long-term monitoring, periodic intensive monitoring, research, and assessment and planning of conservation efforts is important for adaptive management of this species.

Table ES-1. Summary of AUs with current and future resiliency conditions under each scenario, sorted by current resiliency scores (CC). The six future scenarios are unique combinations of the growth rate variable (Opt – optimistic growth rate; Pes – pessimistic growth rate) and the headstart variable (NoHS – no headstarts; RuleHS – rule-based headstart program; HistoricalHS – headstart program based on historical releases).

AUID	CC	Opt-NoHS	Opt-RuleHS	Opt-HistoricalHS	Pes-NoHS	Pes-RuleHS	Pes-HistoricalHS
42	High	High	High	High	High	High	High
1	High	High	High	High	High	High	High
7	High	High	High	High	High	High	High
8	High	High	High	High	High	High	High
17	High	High	High	High	High	High	High
14	High	High	High	High	High	High	High
40	High	High	High	High	High	High	High
9	High	High	High	High	Moderate	High	High
39	High	High	High	High	High	High	High
37	High	Moderate	High	High	Moderate	High	Moderate

AUID	CC	Opt-NoHS	Opt-RuleHS	Opt-HistoricalHS	Pes-NoHS	Pes-RuleHS	Pes-HistoricalHS
4	High	Moderate	High	Moderate	Moderate	Moderate	Moderate
6	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
15	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
5	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
3	Moderate	Moderate	High	High	Moderate	Moderate	Moderate
2	Moderate	Moderate	High	Moderate	Moderate	Moderate	Moderate
36	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
11	Moderate	Moderate	High	Moderate	Low	High	Low
18	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
16	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
26	Moderate	Moderate	High	Moderate	Low	High	Low
35	Moderate	Moderate	High	Moderate	Low	High	Low
28	Moderate	Moderate	High	Moderate	Extirpated	High	Extirpated
29	Moderate	Moderate	High	Moderate	Extirpated	High	Extirpated
41	Moderate	Moderate	High	Moderate	Extirpated	High	Extirpated
23	Moderate	Low	Low	Low	Low	Low	Low
10	Low	Low	Low	Moderate	Low	Low	Moderate
24	Low	Low	Moderate	Low	Low	Moderate	Low
27	Low	Low	Moderate	Low	Extirpated	Moderate	Extirpated
19	Low	Low	Low	Low	Low	Low	Low
20	Low	Low	Low	Low	Low	Low	Low
21	Low	Low	Low	Low	Low	Low	Low
45	Low	Low	Moderate	Low	Extirpated	Moderate	Extirpated
12	Low	Low	Moderate	Low	Extirpated	Moderate	Extirpated
22	Low	Low	Moderate	Low	Extirpated	Moderate	Extirpated
38	Low	Low	Moderate	Moderate	Extirpated	Moderate	Low
25	Low	Low	Low	Low	Extirpated	Low	Extirpated
44	Low	Low	Low	Low	Extirpated	Extirpated	Extirpated
43	Low	Low	Low	Low	Extirpated	Extirpated	Extirpated
31	Low	Low	Low	Low	Extirpated	Extirpated	Extirpated
30	Low	Low	Low	Low	Extirpated	Extirpated	Extirpated
32	Low	Low	Low	Low	Extirpated	Extirpated	Extirpated
34	Low	Low	Low	Low	Extirpated	Extirpated	Low

CHAPTER 1 – INTRODUCTION

Background

This report summarizes the results of a species status assessment (SSA) conducted by the U.S. Fish and Wildlife Service (USFWS or Service) for the Massachusetts population of the northern red-bellied cooter (*Pseudemys rubriventris*). In 1980, the Service listed the species (formerly known as the Plymouth redbelly turtle (*Pseudemys rubriventris bangsi*)) as endangered, and designated critical habitat for the species in Plymouth County, Massachusetts (65 FR 21828). Subsequent to listing, a recovery plan (which included an assessment of the species' status) was completed in 1981 and revised in 1985 (USFWS 1981, entire; USFWS 1985, entire). In 1994, the recovery plan was revised a second time, and included updated information indicating the subspecific status of *P. r. bangsi* was no longer valid (USFWS 1994, pp. 7–9). In February 1997, the Service received a petition to delist the species due to taxonomic error (Gordon 1997, entire). In October 2006, we published a "substantial" 90-day finding in response to the petition that opened a 60-day public comment period and announced the initiation of a status review (71 FR 58363). In 2007, the Service completed a 5-year review of the species, which included a review of new genetic information regarding the species' taxonomic status, and concluded with a recommendation that the Massachusetts population of the northern red-bellied cooter be retained on the list of threatened and endangered species as a distinct population segment (DPS) of *Pseudemys rubriventris* (USFWS 2007, entire).

At the time of listing, northern red-bellied cooters in Massachusetts were known to occupy only 12 ponds, with an estimated population of approximately 200 individuals. The 1994 Recovery Plan states that reclassification to threatened status will be considered when the populations include 600 breeding-age turtles distributed among a minimum of 15 self-sustaining populations (USFWS 1994, entire). In addition, the 1994 Recovery Plan states that delisting will be considered when there are 1,000 breeding-age individuals distributed among 20 or more self-sustaining populations and sufficient habitat is protected to support the species' viability.

In 1985, to increase survival and recruitment by reducing predation rates of hatchling northern red-bellied cooters, the Massachusetts Division of Fisheries and Wildlife (MADFW), in partnership with the Service, began a headstarting program that raises wild-born hatchlings in captivity for 9 months to maximize growth before releasing them into suitable habitat in Massachusetts. This program continues today and is one of the longest and most intensive freshwater turtle headstarting program in existence. Since 1985, over 4,400 wild-born individuals have been headstarted and released at 34 sites, including 2 large river systems (MADFW, unpubl. data).

In partnership with scientists at the University of Massachusetts Amherst and MADFW, the Service conducted a study to assess the distribution of northern red-bellied cooters in Massachusetts and develop population estimates for a limited study area encompassing 45 ponds in the towns of Plymouth, Carver, and Wareham in Plymouth County, Massachusetts (Regosin et al. 2017, entire). The Plymouth Study Area did not include the entire range of the species in Massachusetts, which extends to other areas in Plymouth County and other occupied sites, including the Assawompset Pond Complex, the Burrage Pond Wildlife Management Area

(WMA), and the Weweantic and Taunton Rivers (Regosin et al. 2017, p. 12). During the study, 838 turtles were captured from 25 study ponds (Regosin et al. 2017, p. 23). Excluding recent headstarts (i.e., those released in 2013–2016), the study estimated there are 933 turtles distributed across seven subpopulations within the Plymouth Study Area (Regosin et al. 2017, p. 25). Additionally, the study detected interpond movements and evidence of breeding by mature headstarted females (Regosin et al. 2017, p. 26–27).

Use of this SSA in Decision Support

Under section 4(c)(2) of the Endangered Species Act (Act), the Service is responsible for reviewing the status of listed species every 5 years. In consideration of a species' updated status, "5-year reviews" make a recommendation on whether the Service should retain or change the species' current listing status. This SSA report provides biological information on the status of the Massachusetts population of the northern red-bellied cooter, and an assessment of the resources and conditions needed to maintain long-term viability. This SSA will be used to inform subsequent Service conservation actions. This SSA also provides information that will be used to support decisions concerning the listing status of the species; whether to retain the species as endangered, to downlist the species to threatened, or to propose removing the species from the Federal endangered species list. This SSA will be the primary support document for a 5-year review that will convey the Service's listing recommendation. Prior to any listing decisions, the Service will review this report and all relevant laws, regulations, and policies. Any decision to change the listing status of the Massachusetts population of the northern red-bellied cooter would be announced in the Federal Register with appropriate opportunities for public review and comment. Finally, if the Service determines the species meets the definition of a threatened species or determines the species still meets the definition of endangered, we will use this SSA to consider if revisions to the species' recovery plan are appropriate.

Analytical Framework

Using the SSA framework (figure 1), we consider what a species needs to maintain viability by characterizing the biological status of the species in terms of its resiliency, redundancy, and representation (USFWS 2016, entire; Smith et al. 2018, entire). For the purpose of this assessment, we generally define viability as the ability of the Massachusetts population of the northern red-bellied cooter to sustain populations in natural freshwater pond and riverine ecosystems over time. Resiliency, redundancy, and representation are defined as follows:

- **Resiliency** is the ability of a species to withstand environmental stochasticity (normal, year-to-year variations in environmental conditions such as temperature, rainfall), periodic disturbances within the normal range of variation (fire, floods, storms), and demographic stochasticity (normal variation in demographic rates such as mortality and fecundity). Simply stated, resiliency is the ability to sustain populations through the natural range of favorable and unfavorable conditions.

- **Redundancy** is the ability of a species to withstand catastrophes. Catastrophes are stochastic events that are expected to lead to population collapse regardless of population heath and for which adaptation is unlikely.

- **Representation** is the ability of a species to adapt to both near-term and long-term changes in its physical (climate conditions, habitat conditions, habitat structure, etc.) and biological (pathogens, competitors, predators, etc.) environments. This ability to adapt to new environments-- referred to as adaptive capacity--is essential for viability, as species need to continually adapt to their continuously changing environments. Species adapt to novel changes in their environment by either [1] moving to new, suitable environments or [2] by altering their physical or behavioral traits (phenotypes) to match the new environmental conditions through either plasticity or genetic change. The latter (evolution) occurs via the evolutionary processes of natural selection, gene flow, mutations, and genetic drift.

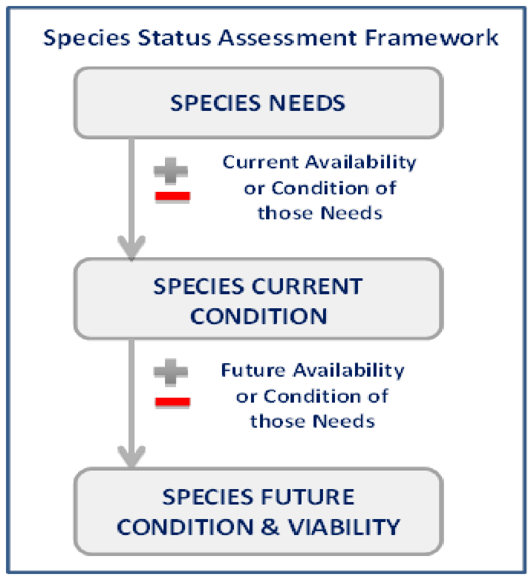

Figure 1. Species Status Assessment Framework

The decision on appropriate listing status for a species is based not on a prediction of the most likely future for the species, but rather on an assessment of the species' risk of extinction. Therefore, to inform this assessment of extinction risk, we describe the species' current biological status and assess how this status may change in the future under a range of scenarios to account for the uncertainty of the species' future. We evaluate the current biological status of the northern red-bellied cooter by assessing the primary factors negatively and positively affecting the species to describe its current condition in terms of resiliency, redundancy, and representation (together, the 3Rs). We then evaluate the future biological status of the northern red-bellied cooter by describing a range of plausible future scenarios representing a range of conditions for the primary factors affecting the species and forecasting the most likely future condition for each scenario in terms of the 3Rs. As a matter of practicality, the full range of potential future scenarios and the range of potential future conditions for each potential scenario are too large to individually describe and analyze. The scenarios in this SSA do not include all possible futures, but rather include specific plausible scenarios that represent examples from the continuous spectrum of possible futures. This SSA report provides a thorough assessment of northern red-bellied cooter biology and natural history and assesses demographic factors and stressors in the context of determining the viability and risk of extinction for the species.

CHAPTER 2 – LIFE HISTORY

2.1 Taxonomy and Genetics

The northern red-bellied cooter (*Pseudemys rubriventris*) belongs to the genus *Pseudemys* (cooter) within the family Emydidae, subfamily Deirochelyinae. The first specimen of the northern red-bellied cooter was collected near Trenton, New Jersey (Le Conte 1830, p. 101). The existence of a population of *Pseudemys* turtles in Massachusetts was first recognized in 1869 (Lucas 1916, pp. 98–100) and later described by Babcock (1937, p. 293) as a distinct subspecies (*Pseudemys* [= *Chrysemys*] *rubriventris bangsi*, Plymouth redbelly turtle) from the southern populations of the northern red-bellied cooter. Babcock's subspecific designation was based on observed differences in shell morphology between 8 Massachusetts specimens and 12 specimens from the southern range. The subspecific designation was questioned by several authors in the following decades (Conant 1951, pp. 287–288; Carr 1952, pp. 273-274; Graham 1969, p. 12). However, Graham (1978, as cited in 45 FR 21828) completed "an in-depth analysis of the shell dimensions of both subspecies of *Chrysemys rubriventris* [= *Pseudemys rubriventris*]" and concluded *P. r. bangsi* as morphometrically distinct from *rubriventris*. This study provided evidence corroborating the validity of the subspecific designation in the years leading up to the Service listing the subspecies as endangered in 1980.

Following the federal listing of the Plymouth redbelly turtle, further morphometric study by Iverson and Graham (1990, entire) examined more than 200 northern red-bellied cooter specimens collected throughout the species' entire range and found measurable differences in some physical characters for males (*e.g.,* plastron length at midline/maximum carapace length), but no obvious geographic patterns were documented among females. Iverson and Graham's findings led the authors to conclude that that no geographic population exhibited enough morphological distinction to warrant subspecific status. Additionally, genetic analysis of Massachusetts and New Jersey turtles (Haskell 1993, entire; Browne et al. 1996, entire) found that 8 of the 12 loci examined exhibited no variability between turtles from the 2 states, and only slight variability was observed in the other 4 loci. These genetic analyses suggested little genetic divergence between the Massachusetts and New Jersey populations. The Service addressed the recommended change in taxonomy in the 1994 recovery plan revision and concluded that the Plymouth redbelly turtle appeared insufficiently different from the mid-Atlantic turtles to warrant subspecific status (USFWS 1994, p. 9). In 2007, the Service completed a 5-year review of the species, which included a review of new genetic information regarding the species taxonomic status, and concluded with a recommendation that the Massachusetts population of the northern red-bellied cooter be retained on the list of threatened and endangered species as a DPS of *Pseudemys rubriventris* (USFWS 2007, entire). Current accepted taxonomy is *Pseudemys rubriventris*, as supported by the Integrated Taxonomic Information System (ITIS) Report (Integrated Taxonomic Information System [ITIS] 2020, entire) and the Turtles of the World Checklist (Turtle Taxonomy Working Group 2017, pp. 61–62). Although there are still some references to the original listed entity in the USFWS Environmental Conservation Online System and recent Federal Register documents such as a 2006 90-Day Finding (71 FR 58363), the Massachusetts population is no longer recognized as a subspecies and is referred to as the northern red-bellied cooter throughout the SSA.

Although the subspecific designation of the Massachusetts population was removed, there are important genetic difference that distinguish northern individuals from southern conspecifics. Genetic analysis of northern red-bellied cooters from Massachusetts, New Jersey, Maryland, and Virginia provides evidence that the Massachusetts population is isolated from southern populations (Bartron and Julian 2007, entire). The study found that genetic differences between Massachusetts and New Jersey and Massachusetts and Maryland were the largest. Differences in results between this study and earlier studies may be due to the limited number of polymorphic loci and low variability of markers sampled in studies conducted in the 1990s (Bartron and Julian 2007, p. 5). While the Massachusetts population of the northern red-bellied cooter is likely a remnant of a formerly continuous coastal plain population (Waters 1962, p. 650), this population is currently physically isolated from the rest of the species' range in the mid-Atlantic, and there is no known evidence of dispersal of individuals and therefore no evidence of recent genetic exchange between the Massachusetts population and other populations (USFWS 2007, p. 7; see section 2.6 Range and Distribution).

2.2 Species Description

The northern red-bellied cooter is a large basking turtle, growing to a length of 25.4 to 34.3 centimeters (cm) (10 to 13.5 inches (in)) and weighing up to 5.7 kilograms (kg) (12.7 pounds (lb)) (MADFW 2016, entire). Females are larger than males and have different plastron (portion of the shell covering the underside of turtles) coloration; males undersides are pale pink with dark mottling, and those of females are red with gray borders along the shell plate edges (Graham 1971a, p. 354). The carapace (portion of the shell covering the turtles back) of all adult northern red-bellied cooters is black to brown with reddish vertical bars on the laminae. The head, neck, limbs, and tail are black with yellow or ivory lines and a yellow arrow-shaped stripe along the throat and neck (Le Conte 1830, p. 102). Adult turtles develop a marbled reddish carapace with age, and some adult turtles, especially males, may develop melanistic coloration (USFWS 1981, p.1).

2.3 Life History

2.3.1 Demographics

Northern red-bellied cooters are a long-lived species and have a life expectancy of more than 50 years. Several old turtles originally captured by Terry Graham in Plymouth County, MA between 1968 and 1988 were recaptured during a 2014 to 2016 study (Regosin et al. 2017, entire). In this study, the following turtles were estimated to be over 60 years old: a female northern red-bellied cooter 'F15' initially captured in 1969 and recaptured in 2015 was estimated to be more than 70 years old; 'M151' was recaptured multiple times after initial capture in 1972, and was estimated to be 68 years old at the time of most recent recapture in 2015; and 'M140' was estimated to be 60 years old at the time of recapture in 2016 after initial capture in 1980 (Regosin et al. 2017, pp. 97–100).

Similarly, it is unknown exactly at what age northern red-bellied cooters become sexually mature. Females may not reach sexual maturity until 15–20 years of age (T.E. Graham, unpublished data as cited in USFWS 1994, p. 2), although headstarted females may reach sexual maturity as early as 8 years of age due to their accelerated growth rate in captivity and large size at release (Haskell 1993, p. 51; Regosin et al. 2017, p 34). Delayed sexual maturity is a trait shared among many long-lived turtle species (Ernst and Lovich 2009, entire), which is associated with benefits such as increased quality and quantity of young produced, as well as costs such as increased potential for death before first reproduction (Congdon et al. 1993, p. 827).

Generation time, a demographic variable that has been defined and calculated in a number of ways, estimates the average turnover rate of breeding individuals in a population (Pacifici et al. 2013, p. 88). Exact generation time for northern red-bellied cooters is unknown; however, due to their longevity, we can anticipate that their generation time may be as long as other long-lived turtles in the family Emydidae, such as Blanding's turtle (*Emyoidea blandingii,* 37 year generation time, Congdon et al. 1993, p. 829) and wood turtle (*Glyptemys insculpta,* ~50 year generation time, Weigel and Whiteley 2018, p. 20). However, the Blanding's turtle and wood turtle are in the subfamily Emydinae and the northern red-bellied cooter is in the subfamily Deirochelyinae, and it is important to consider the potential for differences between these two groups. There is evidence that some species within the Deirochelyinae subfamily reproduce earlier and have larger clutch sizes, which may result in a shorter generation time, such as the painted turtle (*Chrysemys picta*, 10–13 year generation time, Wilbur 1975, p. 75).

As is the case for many turtle species, low nesting success and high juvenile mortality limits population growth (USFWS 1994, p. 14). In turtles, annual survivorship is often lower in the egg and juvenile life stages, particularly within the first year of life, than in later sub-adult and adult life stages (Iverson 1991, pp. 385–386). Northern red-bellied cooter survival is positively correlated with size and age, and headstarted juveniles released in a Massachusetts study conducted by Haskell et al. (1996) were found to have the following survival rates in relation to their straight-line carapace lengths (SCL) in the first, second, and third year following release, respectively: 0.36, 0.60, and 1.00 when SCL is ≤65 millimeters (mm) (2.5 in); 0.66, 0.91, and 0.91 when SCL is 66–95 mm (2.6–3.7 in); and 0.92, 1.00, and 0.91 when SCL is ≥96 mm (3.8 in) (Haskell et al. 1996, p. 525–526). Nonheadstarted juveniles are typically smaller than equal age headstarted juveniles, and therefore, likely have lower survival rates (Haskell et al. 1996 p. 526). It is plausible that nonheadstarted juveniles with similar sizes to headstarted juveniles may have comparable survival rates, in which case 1-year-old nonheadstarted juveniles would have survival rates similar to headstarted juveniles with SCL ≤65 millimeters (2.5 in) (0.36) and 2-year-old nonheadstarted juveniles would have survival rates similar to headstarted juveniles with SCL 66–95 mm (2.6–3.7 in) (0.66) (Haskell 1993, p. 69).

To compensate for low egg and hatchling survivorship, turtles generally have high natural adult survivorship, resulting in long life-spans that allow for multiple opportunities to reproduce (Burke 2015, p. 300-301). Annual adult survivorship information for the northern red-bellied cooter is limited; however, in a study that examined three ponds and pond-complexes in Massachusetts that did not have naturally occurring populations, survivorship of headstarted turtles over multiple decades was estimated to be 0.91, 0.97, and 0.98 (Regosin et al. 2017, p.30). Although specific survivorship rates for nonheadstarted adult northern red-bellied cooters are unknown, overall adult survivorship likely falls somewhere within the range of available estimated values from headstarted individuals. Turtle populations are more sensitive to survivorship of later life stages than survivorship of younger life stages, which indicates the potential for population instability or decline when adult mortality is increased (Congdon et al. 1993, p. 832; Heppell 1998, p. 373)

Northern red-bellied cooters are assumed to have a sex ratio of 1:1, although some ponds have been found to have male-biased sex ratios that could be a result of temperature-dependent sex determination and the cooler summer temperatures found at the northern extent of the species range or a result of the sex of headstarted juveniles released into these ponds (Regosin et al. 2017, p. 33).

2.3.2 Reproduction

In late spring and early summer, northern red-bellied cooter females excavate shallow nests in sandy soil or other loose substrates, usually within 100 meters (m) (328 feet (ft)) of a pond, although they occasionally travel greater distances in search of suitable nest sites (USFWS 1994, p. 2). Nests located in a 2013–2015 study had a median distance of 22 m (72.2 ft) from the nearest pond (range 1–62 m (3.3–203.4 ft)) and were found in a variety of locations including roadsides, clearings, and borrow pits associated with active cranberry farms, gravel causeways, open pitch pine forest, residential yards and gardens, and natural shoreline habitat (Regosin et al. 2017, p. 32). Females lay an average of 12.6 eggs (range 5–17 eggs) in Massachusetts (Haskell 1993, p. 70) and an average of 12.2 eggs (range 4–22 eggs) in other parts of their range (Swarth 2003, p. 8). A small number of females produce two clutches of eggs in a year (Graham 1993 as cited in USFWS 1994; Swarth 2003, p. 8). The nesting season begins in late May and extends to mid-July, with the majority of egg laying occurring in June (Regosin et al. 2017, p. 25; Swarth 2003, pp. 6–7). Incubation takes 73–80 days at a temperature of 25˚C (77˚F) (Graham 1971b, p. 60). Incubation temperature can affect the sex ratio of hatchlings due to temperature dependent sex determination, with cooler nest temperatures producing more males and warmer temperatures favoring the development of females (USFWS 1994, p. 2). Northern red-bellied cooter hatchlings may emerge from nests in the late summer or overwinter in the nest chamber and emerge the following spring (USFWS 1994, p. 2; Swarth 2003, p. 8). Emergence in the fall is the norm while spring emergence is occasional; a pattern observed in other northern turtle species (Ultsch 1989, p. 464). Hatchlings emerge at an average of 30.1 millimeters (mm) (1.19 in) in carapace length (range 29.3–31.0 mm (1.15–1.22 in)) (Graham 1971b, p. 59).

2.3.3 Basking and Overwintering

Northern red-bellied cooters in Massachusetts are usually active from late March to October (USFWS 1994, p. 2) and require suitable basking and overwintering sites with good water quality that are free from disturbance (USFWS 1994, p. 12). Northern red-bellied cooters bask frequently to thermoregulate throughout the active season (April to October), and basking behavior may be more frequent earlier in the season (Gualco 2016, p. 37). Basking behavior results in increased body temperature and reduces fungal infections and parasites that may occur on the carapace or skin (Boyer 1965, p. 117). Habitat features utilized by northern red-bellied cooters for basking in Massachusetts include logs, rocks, artificial rafts, and vegetation mats (Regosin et al. 2017, p. 32).

Northern red-bellied cooters in Massachusetts overwinter submerged underwater and have been observed resting fully exposed on the sandy bottom of a pond rather than burrowed into substrate

(Graham and Guimond 1995, p. 473). Lower metabolism and oxygen demands due to cold temperatures allow overwintering northern red-bellied cooters to survive being submerged under solid ice by respiring aerobically via cutaneous (through the skin) and buccopharyngeal (through the tissues of the mouth and pharynx) gas exchange with the surrounding water (Graham and Guimond 1995, entire).

2.3.4 Diet

Northern red-bellied cooters are omnivorous and submergent aquatic vegetation is the primary diet for all age classes (USFWS 1994, p. 2). There is limited published information on the diet of northern red-bellied cooters in Massachusetts. Graham published notes on stomach contents and scat as well as direct observation which revealed that aquatic plants, such as milfoil (*Myriphyllum sp*.), are frequently consumed (Graham 1981, p. 123). In addition to milfoil, green algae (*Spirogyra sp*.), purple bladderwort (*Utricularia purpurea* Walt), slender arrowhead (*Sagittaria teres* S. Watson), and water-shield (*Brasenia schreberi* J.F. Gmel.) were found in stomach flushings of several adult and juvenile northern red-bellied cooters (Graham 1983, p. 5). Additionally, northern red-bellied cooters may consume snails, fish, tadpoles, and crayfish (Babcock 1938, p. 376). Turtle shell and bone fragments have also been identified in scat from northern red-bellied cooters captured in Massachusetts, which may be evidence of opportunistic scavenging (Graham 1984a, p. 50).

2.3.5 Movement/Dispersal

Immediately after hatching northern red-bellied cooters head for water, where they spend the majority of their time. Overland movements between water bodies and for nesting does occur. Regosin et al. (2017, p. 34) found that individuals appear to readily make short overland movements (< 40 m (131.2 ft)) between ponds, but long-distance movements may be more rare. They determined that 37 out of 72 individual turtles captured between 2015 and 2016 were captured in a pond different than their original capture/release site. Some ponds experienced more immigration or emigration events than others; evidence of a pattern of repeat turtle movements between certain ponds indicates that movement between some waterbodies might be more frequent than between others (Regosin et al. 2017, p. 31).

2.4 Environmental Settings

In Massachusetts, northern red-bellied cooters inhabit three broad categories of aquatic habitat (i.e., coastal plain ponds, reservoirs, and rivers) (Regosin et al. 2017, p. 11) in the seaboard lowland section of the New England physiographic province (Fenneman and Johnson 1946, entire). Coastal plain ponds occur in areas of glacially deposited outwash, till, or former glacial lake beds and are connected hydrologically to the underground aquifer and have sand and gravel shores which allow water levels to rise and fall with the water table and support a unique plant community (Barbour et al. 1998, p. 41).

Reservoirs are anthropogenic impoundments often associated with historical or current cranberry bog agricultural operations. Cranberry cultivation has been a major industry within the coastal plain region of Massachusetts since the 19th century. Reservoirs are used for irrigation and flooding to protect against cold and frost, eliminate pests or weeds, and facilitate harvesting

(Mason 1926, p. 64). If cranberry operations are actively using a reservoir, water levels may change rapidly during periods of high water use.

Rivers that northern red-bellied cooters are known to inhabit in Massachusetts are large, slow-moving, and winding. The Nemasket River in Middleborough, MA has its headwaters in a complex of ponds that include the largest natural bodies of water in Massachusetts: Lake Assawompsett, Pocksha Pond, Long Pond, Great Quittacus Pond, and Little Quittacus Pond. From its headwaters, to its confluence with the Taunton River, the Nemasket River falls 11.6 m (38 ft) over 19.3 kilometers (km) (12 miles (mi)) bordered by wetlands and woodland (Maddigan 2014, entire). The Weweantic River begins in the wetlands of Carver, MA and travels 27.4 km (17 mi) through pine and hardwood woodlands, the Tremont Dam and Horseshoe Dam, and joins the Sippican River before flowing into Buzzards Bay. Tidal influence extends as far north as the Horseshoe Dam, and the Weweantic is a mix of fresh and salt water from that point on (Wareham Land Trust 2011, entire).

The seaboard lowland section of the New England physiographic province is low in elevation, and consists of the sloping margin of the New England upland section as well as the ocean shoreline. Topographic relief is low, often less than 61 m (200 ft), with depositional features associated with glacial outwash and early-Holocene proglacial lakes. Climate in this area is strongly influenced by proximity to the Atlantic Ocean. The area in which the Massachusetts population of the northern red-bellied cooter can be found is composed of Precambrian and Paleozoic igneous and metamorphic rocks, and well drained sands, gravels, and soils with moderately fine to moderately coarse textures (Flanagan et al. 1999, pp. 3–13).

2.5 Habitat Needs

Northern red-bellied cooters in Massachusetts spend most of their time in aquatic habitats, primarily coastal plain ponds, river systems, cranberry bogs, and other wetlands. They rely on aquatic habitats for foraging, basking, and overwintering, and require nearby upland habitats for nesting and dispersal to other aquatic habitat.

All age classes of northern red-bellied cooters primarily feed on aquatic vegetation such as milfoil (Graham 1981 p. 123). Additionally, northern red-bellied cooters will feed on crayfish (Graham 1969, p. 12) and evidence from a diet study of northern red-bellied cooters in New Jersey in 1983 suggested they may scavenge on dead painted turtles (Graham 1984a, p. 50). Few diet studies have been conducted, but northern red- bellied cooters are often found in shallow inlet areas where vegetation is abundant. During a recent study of occupied ponds in Massachusetts, northern red-bellied cooters were often observed in shallow vegetated coves, and activity was most dense within these areas at some ponds (Regosin et al., pp. 87, 90), indicating that these shallow vegetated coves may be an important habitat feature. Patches of dense vegetation may be particularly important for juvenile northern red-bellied cooters (Gualco 2016, p. 79).

Adequate basking habitat within the aquatic environments is also a habitat need. Gualco (2016, p. 24, 26-34) surveyed 14 ponds between 2014 and 2015 in Plymouth and Carver Counties and evaluated availability and use of basking habitat by northern red-bellied cooters. In this study, cooters were observed basking 158 times on logs (49% of total observations), rocks (36%), artificial rafts (11%), and vegetation mats (2%) (Gualco 2016, p. 34). There was a greater probability of use of basking logs located in deeper water (up to 1.5 m (4.9 ft) average depth) and surrounded by a greater proportion of open-water (Gualco 2016, p. 36).

Northern red-bellied cooters generally require unforested, upland habitats with well-drained soils in proximity to aquatic habitat for nesting. Coastal plain ponds in southeastern Massachusetts with deep sand and gravel substrates, more readily offer nesting opportunities. Female northern red-bellied cooters select nesting sites in late spring and summer, usually in sandy soils within 90 m (295 ft) of their aquatic habitat, but they will occasionally travel farther in search of suitable nesting sites (J.D. Lazell, Conservation Agency, Conanicut Island, Rhode Island, in *litt.* 1980 *as cited in* USFWS 1994, p. 2). Suitable nesting habitat has enough solar exposure to meet the temperature needs required for egg development. Opportunistically observed nests found during a study by Regosin et al. (2017, pp. 32–33) typically had southern exposure. Nests were found in a variety of natural and developed areas, including clearings, borrow pits, and roadsides associated with cranberry bog operations; open pitch pine woodland; gravel causeways; residential yards and gardens; and natural shoreline habitat (Regosin et al. 2017, pp. 32–33). The relationship between quality habitat, nest site selection, incubation, and predation rates on the population was not well understood when the Recovery Plan (USFWS 1994, p. 13) was written, and to date, studies of northern red-bellied cooter nesting habitat site selection are still lacking.

In addition to the need for suitable nesting habitat, northern red-bellied cooters utilize upland habitat to move between aquatic habitats. Female turtles also require adequate upland connectivity between aquatic habitat and suitable nest sites. Northern red-bellied cooters have been found to move frequently between ponds located a short distance apart (<40 m), and less frequently between ponds located a larger distance apart (Regosin et al. 2017, p. 34). Individuals require adequate connectivity between bodies of water to emigrate. When the ability of individuals to successfully disperse is limited, this may result in lower genetic variability between populations (Gray 1995, p. 1251). Landscapes that include a combination of forested and wetland habitats that adequately link suitable aquatic and nesting habitat with only limited high-impact development may be best for aquatic turtle populations (Marchand and Litvaitis 2004a, p. 765).

In Massachusetts, northern red-bellied cooters overwinter on the bottoms of ponds under the ice in a state of hibernation or inactivity (USFWS 1994, p. 2). Graham and Guimond (1995, entire) found that the turtles rested directly on the sandy bottom rather than burrowed in the substrate like other turtle species, at a depth of 1–3 m (3.3-9.8 ft). While there are no studies that have described specific habitat needs for overwintering northern red-bellied cooters, a study by Ultsch (1989, p. 474) examined overwintering behavior in a variety of turtle species, and suggested the survival of all turtle species that overwinter under water is expected to be enhanced in normoxic water conditions as opposed to anoxic conditions. This study also suggested that hibernating in the water column rather than burrowed in substrate may result in less physiological stress due to anoxia. Therefore, overwintering northern red-belled cooters likely need water that is not depleted of dissolved oxygen.

2.6 Range and Distribution

2.6.1 Historical

The northern red-bellied cooter has a relatively continuous coastal plain distribution across seven mid-Atlantic states from eastern North Carolina to central New Jersey (Ernst and Lovich 2009, p. 393; Mitchell 1994, pp. 111–116). In addition, there is a disjunct population in southeastern Massachusetts that is separated from the more southern range of *P. rubriventris* by approximately 330 km (205 mi) (Ernst and Barbour 1972, p. 166; Iverson and Graham 1990, p. 2; B. Zarate, NJDFW, pers. comm. 2021). South of New England, the northern-most known population of northern red-bellied cooter occurs in central New Jersey (USFWS 1994, p. 3). Northern red-bellied cooters were reported historically from New York (Babcock 1938, p. 374; Carr 1952, p. 267), and an introduced population was reportedly established in Charleston, Staten Island, New York (R. Zappalorti, Herpetological Associates, Inc., *in litt*. 1992) (USFWS 1994, pp. 3, 7).

Waters (1962, p. 650) suggested that the Massachusetts population of northern red-bellied cooter may have descended from a once continuous, prehistoric distribution across the eastern coastal United States. The northern red-bellied cooter could have expanded its range when the continental shelf was emergent during the post-Wisconsin glacial period, later becoming isolated as the shelf was inundated following the retreat of the Laurentide ice sheet (USFWS 1994, p. 7). Paleoecological evidence suggests that the northern red-bellied cooter was more widely distributed in eastern Massachusetts during the mid- to late-Holocene than it is today. Subfossil remains have been reported from Vineyard Haven, Martha's Vineyard, Dukes County (Waters 1962, p. 650); Westborough, Worcester County (Rhodin 1992, p. 21); Shell Heap on the Sudbury River in Concord, Middlesex County (Rhodin and Largy 1984, p. 107); Wayland, Middlesex County (Rhodin 1992, p. 24); and Ipswich, Essex County, MA (Graham 1982, pp. 82–83; Waters 1962, p. 649). Reports of the northern red-bellied cooter from Naushon Island, Dukes County, MA (Lazell 1976, p. 114–117) were refuted by Graham (1982, pp. 82–83). A previously reported specimen from 1944 that was originally identified as *P. r. bangsi* (Waters 1962, p. 649), probably represented a specimen of river cooter (*P. concinna)* or Florida cooter (*P. floridana)* that was released on the island (Graham 1982, pp. 82–83). Although the presence of the northern red-bellied cooter was considered plausible by Rhodin (1992, p. 25); it is unlikely that the species has occurred on Naushon in recent decades (P. Elias, pers. comm. to M.T. Jones *as cited in* Regosin et al. 2017, p. 13).

The first formal reports of extant populations of the northern red-bellied cooter in Massachusetts were made in 1916, although observations by scientists had been made as early as the 1860s (Babcock 1916, entire; Lucas 1916, entire). The earliest known collections were made by Babcock (1916, entire) in 1911 and Lucas (1916, entire) in 1912. The MADFW has tracked occurrences of the northern red-bellied cooter since the 1970s (J. Cardoza, pers. comm. to M.T. Jones *as cited in* Regosin et al. 2017, p. 14).

At the time of listing in 1980, the distribution of the population was thought to be limited to about 12 ponds in Plymouth County, MA (45 FR 21828). By the time the 1994 Recovery Plan was written, a "large" population of cooters had been discovered in Federal Pond, Plymouth County, and a headstarting program had been underway for 10 years (USFWS 2007, p. 12). At that time the population was thought to occupy 18 sites in the towns of Plymouth and Carver, Plymouth County, MA. Based on mark-release-recapture data, the number of adult and subadult northern red-bellied cooters in Massachusetts, including both headstarted and nonheadstarted individuals, was estimated at 300–400 turtles. Ten of the ponds thought to support the turtle were within a 608-hectare (1500-acre) area (USFWS 1981, pp. 3–4) and nearly half of the known population at that time (not including headstarted turtles) was thought to be found in a single location, Federal Pond. Eight of the sites had no previous northern red-bellied cooter records before the initial release of headstarted hatchlings (including three ponds and one river site to which the turtles were first introduced in 1993): East Head Pond in Myles Standish State Forest, Muddy Pond in Carver (owned by the MADFW), Halfway Island Pond, Great South Pond, Halfway Pond, Little Long Pond, Forge Pond, and the Weweantic River (USFWS 1994, p. 3). The headstarting of approximately 100–200 hatchlings per year continued from 1994, and by the time the 5-Year Review was written in 2007, an estimated 400–600 breeding-age individuals occurred in more than 20 ponds, but fewer than half of those ponds were likely to contain more than 20 breeding-age animals. By 2007, headstarted juveniles had been released at 28 locations, the majority of which were ponds but also included the Taunton River and Weweantic River (USFWS 2007, p 12–13).

2.6.2 Current

Currently, the northern red-bellied cooter is known to occur in at least 26 ponds in 15 pond complexes and 2 rivers (Regosin et al. 2017, p. 27) in Plymouth and Bristol Counties, MA. Most of the known occurrences of northern red-bellied cooters lie within an area bounded by Routes 3, 25, 58, and 44 in Plymouth County, MA. A current population estimate for this entire area does not exist, but many ponds (Billington Sea Complex, Crooked Pond, East Head Pond Complex, Federal Pond, Great South Complex, Island Pond, Halfway Pond Complex, Sampsons Pond, West Pond Complex, and Wenham Pond), were intensively sampled between 2014 and 2016, and estimated to have a combined total population of 933 individuals (not including recently released headstarted turtles) (Regosin et al. 2017, pp. 11, 29, 33).

Additionally, introduced populations of northern red-bellied cooters occur in two additional areas: (1) the Lakeville Ponds Complex in Lakeville and Freetown and the lower Nemasket River upstream from the Assawompset Pond, which received headstarts beginning in the late 1990s, and (2) the Burrage Pond WMA in Hanover and Halifax, which has received headstarts in most years since 2011. MADFW continues to gather sightings annually, which have recently suggested additional northern red-bellied cooter occurrences within Plymouth County as well as occurrences in Bristol County, MA (Regosin et al. 2017, p. 48). Additionally, in 2020 reports from the public revealed a previously unknown occurrence in the Neponset Reservoir, Foxborough, Norfolk County, MA (MADFW, unpublished data).

CHAPTER 3 – FACTORS INFLUENCING VIABILITY

In this chapter, we evaluate the past, current, and future influences that are affecting or could be affecting the current and future condition of the northern red-bellied cooter population in Massachusetts. Stressors and threats can influence one or more species needs. Habitat factors influence the demographics of a population, such as growth and recruitment. Demographic factors of healthy populations can offset some effects of threats, but the current and potential extent and magnitude of the threats influence overall viability. Conservation measures, population management, and regulatory management can mitigate negative effects to increase population resiliency, redundancy, and representation to increase viability.

The ESA requires consideration of five factors when making listing determinations. In broad terms, those factors include issues relevant to habitat; overutilization; disease and predation; existing regulatory mechanisms, and other natural or manmade.

3.1 Water Quality

Many environmental factors have contributed to the current status of northern red-bellied cooters, but limiting factors related to reduced water quality that have been previously noted include: siltation resulting from land clearing adjacent to ponds; pollution and excess nutrient flow into ponds; and pollution of ground water or reduction in the water levels of ponds from ground water withdrawals (pumping). These influences can adversely affect aquatic invertebrate and vegetation communities, which provide food and shelter for northern red-bellied cooters. Draining or filling of wetlands adjacent to occupied ponds, shoreline modifications such as filling, dredging, construction of dikes, and urban development can all exacerbate reduced water quality and quantity (45 FR 21832; USFWS 1994, p. 12).

In particular, the impacts of the cranberry (*Vaccinium macrocarpon*) industry on water quality and quantity, and potential resultant impacts to northern red-bellied cooters, is multifaceted. The area of farmed cranberry bogs has increased greatly during the last century, and today the cranberry industry collectively owns and manages more than 5,260 hectares (13,000 acres) in Massachusetts, making cranberries the largest agricultural crop in Massachusetts. The majority of farmed cranberry bogs occur in Plymouth County in just five towns: Carver, Wareham, Middleboro, Plymouth, and Rochester, MA (Cape Cod Cranberry Growers Association 2020, entire). In the late 1990s, the cranberry industry was also the single largest water user on Cape Cod and in southeastern Massachusetts (using 13.2 billion liters (3.5 billion gallons) of water annually) (Barbour et al. 1998, p. 43). Many of the northern red-bellied cooter populations occurring near cranberry bog operations are dependent on the same ponds and reservoirs used by cranberry growers for irrigation and harvest (USFWS 2007, p. 19). In particular, northern red-bellied cooters occupy many coastal plain ponds in southeastern Massachusetts which have well drained, sandy and gravelly shorelines and are hydrologically connected to the underground aquifer (Barbour et al. 1998, pp. 41–42). Therefore, actions which impact the water quality and quantity of that aquifer may also impact northern red-bellied cooters.

Herbicides used in conjunction with cranberry production may affect water quality and food resources for northern red-bellied cooters (USFWS 1994, p. 14). Coastal plain ponds that collect runoff from cranberry bog operations are exposed to additional nutrients and pesticides and subject to alterations of natural water level fluctuations and shoreline dynamics (MADFW 2015, p. 302). The increased nutrients and pesticides can change the pond and shoreline environment such that some species of algae and vascular plants may thrive, when they otherwise would not (MADFW 2015, p. 302). The immediate and long-term effects of chemicals and insecticides applied for cranberry production, other agricultural purposes, forestry, and mosquito control, are largely unknown. A substantial amount of organochlorine-based and other pesticides was applied by the cranberry industry in Plymouth County from the late 1940s to 1960s, but no studies have been done to determine the long term impacts on northern red-bellied cooters. It is unknown whether these pesticides accumulate in northern red-bellied cooters. Safer chemical agents (insecticides, herbicides, fungicides, and fertilizers) have been used by cranberry growers, as well as in mosquito control and silviculture, since 1970. Although these chemical treatments are likely less toxic to wildlife than chemical treatments prior to 1970, the synergistic effects of multiple chemicals in the system are unknown (USFWS 1994, p. 14). Other studies investigating the effects of chemicals on turtles suggest several possible negative impacts. For example, high levels of glyphosate-based herbicides in water induces stress in turtles (Heritier et al. 2017, pp. 3345–3346), and exposure to organochlorine pesticides may suppress turtle immune systems (Tangredi and Evans 1997, pp. 97–99).

An increase in residential and commercial development, including conversion of existing cranberry bog operations, could mean an increased threat to coastal plain pond health. High-nutrient leachate from failing or unmaintained septic systems could lead to pond eutrophication. Increased water withdrawal from municipal wells could lower coastal plain pond water levels, or result in more unnatural water level fluctuations. Increased roads and impervious surfaces could result in subsurface compaction and altered groundwater flow, and more runoff and contaminants (particularly salt and chloride) entering the ground and surface waters (MADFW 2015, p. 302).

Detailed water quality information is not available for all the surface waters that northern red-bellied cooters inhabit in Massachusetts; however, some information about water quality can be gleaned from the Massachusetts Department of Environmental Protection's periodic status updates (Massachusetts Department of Environmental Protection [MassDEP] 2017, entire).

3.2 Habitat Loss/Fragmentation

3.2.1 Upland Habitat Loss

Upland habitat is an important resource need for northern red-bellied cooters, and long-term changes in land use practices may cause loss of suitable nesting and dispersal habitat. Historically, naturally created nesting habitat around occupied ponds may have been more abundant. The pitch pine/scrub oak ecosystem surrounding the aquatic habitat utilized by northern red-bellied cooters periodically burned due to lightning strikes and fires set by Native Americans to create fields or clear undergrowth (Cronon 1983, pp. 48–49). These burns created openings in the forested landscape and as a result, sandy, dry soil with adequate sunlight for incubation was available for nesting. Fires have been suppressed in recent history due to residential and agricultural use of the land and no longer regularly take place in the region. As a result of fewer fires occurring on the landscape, canopies have closed over and undergrowth has thickened, reducing the amount of naturally occurring nesting habitat available (USFWS 2007, pp. 19–20).

Loss of nesting habitat could result from development of any upland habitat with sandy soil near to ponds, reservoirs, and rivers occupied by northern red-bellied cooters. The Plymouth County area in southeastern Massachusetts, where the majority of known occupied sites are located, underwent a period of rapid residential and commercial development during the 1970s and 1980s (USFWS 1994, p. 13). The town of Plymouth, one of two towns containing original occupied sites at the time of Federal listing, ranked fourth in an analysis of forest loss to residential development in Massachusetts towns from 1985–1999 (Breunig 2003, p. 5). As of 2020, the town of Plymouth and the surrounding areas was still considered to be one of the most rapidly developing areas in Massachusetts (Ricci et al. 2020, p. 4). Currently, the landscape around many coastal plain ponds in southern Massachusetts consists of residential homes and cranberry agricultural operations, and availability of nesting habitat varies depending on how privately owned properties are managed. Loss of natural shoreline habitat around coastal plain ponds has been one outcome of residential and agricultural land use. However, some upland watershed areas managed by the cranberry industry may provide limited nesting habitat or connectivity between water bodies where undeveloped habitat remains

Despite the potential impacts of the cranberry industry to northern red-bellied cooters, some of these areas in southeastern Massachusetts have become increasingly important to the conservation of this species as other surrounding habitat is lost to development or habitat quality declines related to forest succession (USFWS 1994, p. 13–14). Some aspects of cranberry agriculture may help maintain the aquatic and nesting habitats used by northern red-bellied cooters in this increasingly developed landscape. Northern red-bellied cooters also nest on open areas of sandy levees, water control structures, and other infrastructure where vegetation is managed (USFWS 2007, p. 19), and there are known areas, such as Federal Pond, where successful nesting occurs. The conversion of commercial cranberry lands into residential developments in the future could ultimately pose a greater threat to northern red-bellied cooters than the cranberry operations. Residential developments could result in an overall loss of high quality pond and surrounding upland habitat, and increased road mortality due to vehicular volume (USFWS 2007, p. 19–20).

While residential and agricultural landscapes may provide areas of suitable nesting habitat (e.g. lawns, roadsides, sandy levees) attractive to northern red-bellied cooters, these areas are also prone to disturbance and nests may be at a greater risk of being destroyed. For one population of diamondback terrapins (*Malaclemys terrapin*) in New Jersey, when nesting habitat was lost to development, sandy shoulders of heavily trafficked roads became the only available nesting substrate and the population experienced increased road mortality (Wood and Herlands 1997, p. 47). Blanding's turtles (*Emydoidea blandingii*) may experience lower hatchling survivorship in residential areas in northeastern Massachusetts (Jones and Sievert 2012, entire). Female turtles may be more vulnerable than males to mortality and injury in upland habitats as a result of more encounters with vehicles, machinery, and predators during nesting season (Marchand and Litvaitis 2004a, p. 764; Steen et al. 2006, entire).

In addition to loss of nesting habitat, development or change in land use practices have the potential to impact this species in other ways. Northern red-bellied cooters may experience difficulty dispersing through the dense vegetated undergrowth that results from fire suppression. In addition, the increased availability of anthropogenic food sources in developed areas may support higher populations of raccoons (*Procyon lotor*) and other generalist predators (Oehler and Litvaitis 1996, p. 2078; Prange et al. 2003, pp. 324, 330) that may predate turtle eggs and hatchlings. Increased presence of humans may result in disturbance of turtles during basking, dispersal, or nesting, and pet dogs may dig up turtle nests (USFWS 2007, p. 22). Continued modification of upland habitat is expected to negatively impact the northern red-bellied cooter.

3.2.2 Aquatic Habitat Loss

Aquatic habitat is essential for northern red-bellied cooters in all seasons (see section 2.5 Habitat Needs). During the active season, northern red-bellied cooters spend most of their time in water or basking on partially submerged objects. In addition, northern red-bellied cooters feed almost exclusively on vegetation that is found in aquatic habitats. In the winter, northern red-bellied cooters hibernate on the bottoms of ponds and rivers in deep water that is not completely frozen. Aquatic habitat utilized by northern red-bellied cooters in Massachusetts includes coastal plain ponds, reservoirs, and some rivers. Loss of aquatic habitat could include loss of basking habitat, severe reduction in water level, loss of aquatic vegetation, dam removal, or other alterations that would make aquatic habitat unsuitable or unavailable.

Most coastal plain ponds do not have surface water inlets or outlets and, as a result, water levels are influenced by precipitation, evaporation, and the level of the underlying ground-water aquifer (Sorrie 1994, p. 225). Water levels in coastal plain ponds fluctuate seasonally and inter-annually due to natural variations in the underlying aquifer; a trait that has led to the development of unique pond and pondshore vegetation communities (McHorney and Neill 2007, p. 366; Schneider 1994, p. 253). Lowering the aquifer has the potential to change the hydrologic regimes of coastal plain ponds and endanger the rare plant communities that are found there (Schneider 1994, p. 260). Increased water withdrawal from the aquifer to support residential and agricultural use has the potential to influence water levels (McHorney and Neil 2007, p. 367), which could alter aquatic habitat and negatively impact aquatic vegetation. Northern red-bellied cooters could be impacted by reduced availability of aquatic vegetation or alteration of suitable aquatic habitat. Changes in coastal plain pond hydrologic regimes as a result of municipal ground-water pumping is a conservation issue that will become increasingly important as demands on ground-water grow with development (Barbour et al. 1998, p. 43).

Some aquatic habitat in southeastern Massachusetts has been altered to create cranberry bogs and reservoirs for use in growing and harvesting cranberries. Although the cranberry bogs themselves are a monoculture and are considered to be of low value to the northern red-bellied cooter, agricultural reservoirs may be utilized by this species (USFWS 1994, p. 14). Northern red-bellied cooters often inhabit the natural ponds and human-enhanced reservoirs that are used and maintained as important water sources for cranberry bog irrigation and harvest. However, these agricultural reservoirs are subject to changes in water level, may experience high levels of disturbance, and likely vary in their year-round suitability as northern red-bellied cooter aquatic habitat.

3.2.3 Habitat Fragmentation

Habitat modification often creates barriers to wildlife movement by transforming contiguous habitat into a patchwork of habitat types with reduced connectivity, resulting in habitat fragmentation. Habitat fragmentation can occur as a result of residential or agricultural development, construction or paving of roads, a change in land use, timber harvest, stream channel alteration, or natural processes such as fire or wind events. As a result, connectivity between aquatic habitats and between aquatic habitat and nesting habitat may become restricted (USFWS 1994, p. 13).

Creation of new roads, widening roads, paving of dirt roads, or increased use of roads can contribute to habitat fragmentation. Roads have the potential to act as barriers to wildlife dispersal and migration, especially for slow, terrestrial species. Large roads and highways with heavy traffic volumes are effective boundaries to turtles and prevent individuals from moving between suitable habitats (Congdon et al. 1993, p. 832; Gibbs and Shriver 2002, p. 1647). Blanding's turtles have been demonstrated to avoid crossing roads, a behavior that may result in isolation (Proulx et al. 2014, p. 269). Roads can also be a significant source of direct mortality in turtles (see section 3.5 Motorboat Strikes/Road Mortality). In addition, road creation provides increased human access to turtle habitat and may increase the chance of collection and disturbance (Boarman et al. 1997, p. 55; Regosin et al. 2017, p. 12).

Fragmented wildlife populations with limited dispersal ability can experience genetic drift that leads to loss of genetic variation over time (Gray 1995, p. 1251; Templeton et al. 1990, p. 20). Restricted connectivity can result in small, fragmented populations with increased risk of inbreeding depressions or localized extinctions from catastrophic or stochastic events (Quinn and Hastings 1987, pp. 200, 206; Templeton et al. 1990, p. 20). Connectivity between ponds is important to allow for movement between water bodies, and protection of upland areas and minimization of road mortality are conservation priorities for the northern red-bellied cooter (Regosin et al. 2017, p. 36).

Although we know that suitable habitat for nest sites is an important habitat need for this species, the effects of a scarcity of good nesting habitat on nest site selection, incubation, and predation rates on the population as a whole are not well understood. It is also unclear what effect fire suppression is having on the quality of northern red-bellied cooter nesting habitat.

3.3 Predation

Predation of northern red-bellied cooter nests and high hatchling mortality are believed to be important factors limiting the Massachusetts population (USFWS 1994, pp. 14–15). Eggs and small juvenile turtles are most vulnerable to predation, with the threat decreasing once individuals reach a larger carapace size (Frazer et al. 1990, p. 196; Haskell et al. 1996, entire). By the time northern red-bellied cooters reach maturity predation is not considered a likely mortality factor (USFWS 2007, p. 21), though loss of limited numbers of adult turtles to predation is possible (Karson et al. 2018, entire). Raccoons, striped skunks (*Mephitis mephitis*), red fox (*Vulpes vulpes*), coyotes (*Canis latrans*), and crows (*Corvus spp.*) are common predators of northern red-bellied cooter nests (Christensen 2008 pp. 66–67; USFWS 1994, pp. 15–16; USFWS 2007, p. 21). Owls (order: Strigiformes) and small rodents like eastern chipmunks (*Tamias striatus*) are also likely to be opportunistic predators of hatchling turtles on land (Jones and Sievert 2012, pp. 91–92; Swarth 2003, p. 8). Once northern red-bellied cooter hatchlings reach the water, they are suspected to face predation from a variety of small to medium animals including bullfrogs (*Lithobates catesbeianus*) (Graham 1984b, entire), predatory fish including chain pickerel (*Esox niger*) (Haskell et al. 1996, p. 526) and bass (*Micropterus* sp.) (unpubl. USFWS data), snapping turtles (*Chelydra serpentina*), wading birds, and raccoon (unpubl. USFWS data, as cited in USFWS 2007, p. 21). Based on archaeological data, predation by pre-colonial humans is suggested to have been a factor contributing, at least in part, to the contraction of the northern red-bellied cooter's historical distribution in Massachusetts (Rhodin and Largy 1984, entire), however, intentional harvest by humans is no longer considered to be a threat to the species (see section 3.6. Collection).

Predation rates for unprotected northern red-bellied cooter nests can be spatially variable but are generally believed to be high at a majority of sites in Massachusetts. At Federal Pond, once the largest pond population in Massachusetts, predation of nests at the main nesting location is thought to approach 100 percent of unprotected nests in some years (USFWS and MADFW unpubl. data, as cited in USFWS 2007, p. 21). At Crooked Pond within Massasoit National Wildlife Refuge, nest monitoring efforts between 2013 and 2019 documented predation of all confirmed nests left unexclosed (n=25), with raccoons documented or suspected to be the culprit of eight nest predation events (unpubl. USFWS data provided by S. Koch 2019). Sources of mortality for hatchling and small juvenile northern red-bellied cooters in Massachusetts are understudied, but predation is presumed to contribute at least in part to generally low survival rates observed for this demographic. Northern red-bellied cooters often nest in fragmented residential and agricultural landscapes adjacent to ponds (MADFW 2016, p. 2; USFWS 1994, p. 23) which may increase exposure to several species of predators (Jones and Sievert 2012, pp. 91–92; Haskell et al. 2001, p. 251; Marchand and Litvaitis 2004b, p. 248) and could result in higher rates of egg and hatchling predation.

Permanently decreasing the number of generalist, egg predator species that occur along the 20+ coastal plain ponds in Plymouth County, Massachusetts is considered infeasible (USFWS 2007, p. 22). In an effort to increase nest success, a selection of ponds is monitored annually for northern red-bellied cooter nests and wire exclosures are erected to preclude access by medium-sized predators (S. Koch and M.T. Jones pers. comm. 2019). Nest protection is effective at increasing nest success; however, it is presumed that only a small percentage of total northern red-bellied cooter nests laid in a given year are found during monitoring and not all nests have predator exclosures installed (unpubl. USFWS data provided by Koch 2019); therefore, nest predation will continue to be a threat. Similarly, to increase recruitment rates of juveniles, a headstarting program for northern red-bellied cooters was initiated in 1984 which produces larger yearling turtles that are presumably less vulnerable to predation upon release. However, given that such efforts are only addressing a symptom of high predation rates, high nest and hatchling predation rates will continue to be a factor limiting the northern red-bellied cooter population at most ponds (USFWS 2007, p. 22).

3.4 Invasive Species

Invasive and nonnative animal and plant species may impact northern red-bellied cooters through direct predation or by reducing the quality of the habitat. Northern red-bellied cooters have many predators when they are small (see section 3.3 Predation for more details), and survival of newly hatched northern red-bellied cooters is thought to be very low (USFWS 2007, p. 14). The extensive introduction and translocation of predatory sport fish in Massachusetts, including smallmouth and largemouth bass (*Micropterus dolomieu* and *Micropterus salmoides;* both nonnative to southeastern Massachusetts), chain pickerel, brown bullhead (*Ameiurus nebulosus*), and white perch (*Morone americana*), may be an important factor contributing to low hatchling turtle survivorship (USFWS 1994, p. 15). The Service's biologists have observed bass (*Micropterus* sp.) eating northern red-bellied cooter hatchlings when released into the water. In September 2017, Service biologists released 37 northern red-bellied cooter hatchlings at several locations along the Crooked Pond shoreline at Massasoit NWR; bass were subsequently observed to eat at least 3 of the released hatchlings, likely more. It is suspected that predation of northern red-bellied cooter hatchlings by bass is underreported given that predation events are seldom observed, especially those that occur under water (USFWS 2017, unpubl. data).

Although we found no studies on the impacts of nonnative invasive fauna on habitat quality for northern red-bellied cooters, the Massachusetts State Wildlife Action Plan notes several potential problematic species in lakes and ponds including, common carp (*Cyrinus carpio*), northern snakehead fish (*Channa argus*), and Asian clam (*Corbicula fluminea*), which may reduce native fauna and disrupt the natural community structure (MADFW 2015, p. 237). Red-eared sliders (*Trachemys scripta*), a nonnative deirochelyine turtle, are present in several northern red-bellied cooter ponds in the Plymouth area (M.T. Jones, unpubl. data). In some circumstances, competition could occur between red-eared sliders and northern red-bellied cooters over shared resources (Pearson et al. 2013, p. e62891). Additionally, the increasing nonmigratory geese populations grazing along pond shorelines may alter the shoreline vegetation and habitats, and over wintering geese may contribute enough nutrient source in their excrement which could result in overgrowth of algae and nonnative plants on lakes and ponds. Nonnative invasive plants, such as common reed (*Phragmites australis* var. *australis*) and gray willow (*Salix cinerea, S. atrocinerea*, and probable hybrids) thrive in areas of disturbed habitats and soils, and are found in some of the coastal plain ponds of southeastern Massachusetts. Lastly, submerged aquatic invasive plants, such as fanwort (*Cabomba caroliniana*) and hydrilla (*Hydrilla verticillata*), are increasingly occupying coastal plain ponds. Control of these species is difficult and typically involves the use of herbicide (MADFW 2015, p. 304).

3.5 Motorboat Strikes/Road Mortality

3.5.1 Road Mortality

Mortality due to vehicle strikes is a major threat to some turtle populations. While many collisions on roads are accidental, there is evidence that some people will intentionally turn their vehicles towards turtles to hit them (Boarman et al. 1997, p. 54). Road mortality is a limiting factor for some turtle populations due to the high adult survival rates and delayed sexual maturity that characterize turtle life histories, a trait that makes it difficult for populations to recover from loss of adults (Congdon et al. 1993 p. 832; Gibbs and Shriver 2002, p. 1649). Population decline occurs as a result of adult mortality in turtle populations because reproductive output and juvenile recruitment do not increase to compensate for the loss of those individuals (Bennett and Litzgus 2014, p. 262). A decrease in genetic diversity may indicate population decline as a result of high road mortality in Alabama red-bellied turtles (*Pseudemys alabamensis*; Hieb et al. 2014, p. 259) and road mortality has been identified as one of the most significant threats to continued survival of this closely related species (Nelson et al. 2009, p. 72).

Road mortality or injury resulting from a collision may occur when northern red-bellied cooters are moving between aquatic habitats at any time during the active season, during the nesting season when females move between aquatic habitat and nest sites, or when hatchlings are moving from nests to aquatic habitat. Substrates along roadsides that are similar to natural nesting habitat may attract female turtles and increase the possibility of road mortality occurring (Aresco 2005, p. 37). Female turtles nesting along roadsides are at a greater risk of being injured or killed by a vehicle, as are hatchlings emerging from nests located along roadways (Hieb et al. 2014, p. 254; Wood and Herlands 1997, p. 47). Female turtles make more overland movements, resulting in females having a greater probability of road mortality and likely leading to male biased populations (Aresco 2005, p. 41). In addition, greater road densities have been found to be associated with pond turtle populations containing a higher proportion of males and adult turtles than females and juveniles (Marchand and Litvaitis 2004a, p. 763). Land areas with >2 km (1.2 mi) if roads/km^2 with traffic volumes of >200 vehicles/lane/day are predicted to contribute excessively to the annual adult mortality rates of large-bodied pond turtles (Gibbs and Shriver 2002, p. 1649). In Massachusetts, road mortality is evidenced through DOR (dead on road) reports received in recent years and documented in the state Natural Heritage and Endangered Species Program database (MADFW 2020, unpubl. data), as well as anecdotal reports of northern red-bellied cooters killed on roads (Regosin et al. 2017, p. 12).

3.5.2 Boat Strikes

The effects of boat strikes on freshwater turtles are not well studied, but there is evidence that recreational boating may cause significant mortality (Bennett and Litzgus 2014, p. 262). In Ontario, Canada, one study found that 28.5 percent of female northern map turtles (*Graptemys geographica*) and 12.8 percent of males had injuries resulting from propeller strikes (Bennett and Litzgus, p. 263). Another study in Jug Bay, Maryland, found that 11 of 78 northern red-bellied cooters had scars from propeller strikes (Swarth 2003, p. 4). Many turtles that are hit by boats may die and sink to the bottom of the waterway; therefore, direct mortality due to boat strikes may be underreported (Selman et al. 2013, p. 883). Boat strike mortality has been reported in the northern red-bellied cooters in Massachusetts (Regosin et al. 2017, p. 34).

3.6 Collection

Turtles worldwide have traditionally been utilized for meat, oil, medicines, pets, and other products, however, commercial trade in freshwater turtles is unsustainable, and has been cited as one of the major causes of population decline for some species (Ceballos and Fitzgerald 2004, p. 881; Gibbons et al. 2000, p. 658; Rhodin et al. 2011, entire). Due to life histories characterized by late maturation, low reproduction rates, and high juvenile mortality, turtle populations are vulnerable to commercial exploitation.

In North America, over collection of turtles for the pet trade or foreign food trade are a major cause of decline (Ernst and Lovich 2009, p. 26–27). The diamondback terrapin experienced severe population decline and was nearly overharvested to extinction for the domestic food industry from the late 1880s and early 1900s (Gibbons et al. 2000, p. 658). Although demand for terrapin meat has not been a major threat to the species since the 1930s, the genetic variability and structure of the species has likely been impacted by the over-collection of individuals during that period of history (Drabeck et al. 2014, p. 125). Likewise, alligator snapping turtle (*Macrochelys temminckii*) populations were severely depleted from the 1960s to 1980s as a result of commercial trapping for the food industry and have been slow to recover (Roman et al. 1999, p. 140).

People have historically used the northern red-bellied cooter as a food source. The northern red-bellied cooter is known to have been commonly sold at food markets in the Chesapeake Bay area as recently as the early 20th century, and a bone fragment found at an archeological site in Massachusetts shows that this species was a food source as early as pre-colonial times (Rhodin 1992, p. 24). Babcock (1919/1971, p. 51; citing Ditmars) indicated that the northern red-bellied cooter was regularly traded as a food item in the early 1900s. There is no evidence that there is a current culinary demand for northern red-bellied cooters.

Northern red-bellied cooters are not believed to be a species commonly taken for the commercial pet trade in freshwater turtles at this time (USFWS 2007, p. 21), although this is a significant threat facing other species of freshwater turtles (Stanford et al. 2020, entire). In 2009 a young northern red-bellied cooter found in a Florida state park was suspected to be a released pet (Munscher and Weber 2012, p. 219), suggesting that this species may not be completely immune to the threat of collection for the pet trade.

3.7 Harassment/Disturbance

Northern red-bellied cooters may experience intentional or unintentional anthropogenic harassment, disturbance, or injury. Disturbance occurs when human or predator presence causes a change in behavior. Any activity that brings humans close to basking or nesting turtles has the potential to cause disturbance.

While basking, turtles are exposed and may abandon basking sites quickly when humans are nearby, even with minimal disturbance (Peterman and Ryan 2009, pp. 634–635). The visual presence of humans or boats may startle basking turtles, or wakes from passing boats may sweep them off of basking structures (Selman et al. 2013, p. 883). When turtles are disturbed and abandon basking sites, activity levels are increased as they dive into water, swim away, and eventually pull themselves back onto basking structures (Selman et al. 2013, p. 884). Increases in disturbance rates may increase the time spent responding to disturbance and can result in loss of body mass due to increased energy expenditure (Houston et al. 2011, p. 598). In addition, lower body temperature and metabolic rate as a result of loss of basking time may impact food digestion and development of eggs (Moore and Siegel 2006, p. 392). Decreased basking duration as a result of disturbance may alter physiological processes such as scute shedding, capacity for immune response, and ability to defend against pathological organisms (Selman et al. 2013, p. 884). Although no difference in stress levels was observed in one study of two populations of painted turtles (*Chrysemys picta*) exposed to different levels of disturbance (Polich 2016, p. 6), disturbance was found to negatively affect stress levels of a population of yellow-blotched map turtles (*Graptemys flavimaculata*) that experienced high levels of disturbance (Selman et al. 2013, p. 883).

Nesting behavior of turtles may be altered by human activity or presence. If disturbed, a nesting female may abandon the nesting attempt and either seek another nest site or return at a later time. In one study, disturbance of yellow-blotched map turtles by recreational activity was found to alter nesting behavior to an extent that may impact the number of clutches females are able to lay and alter what habitat is selected for nesting (Moore and Seigel, p. 391).

Many coastal plain ponds in Massachusetts are surrounded by residential or agricultural development, which results in more opportunities for disturbance. While human encroachment may not result in complete loss of upland or aquatic habitat, wildlife will be exposed to the presence of increasing numbers of people (Polich 2016, p. 6). In addition, these ponds are popular for recreation both on the shore and in the water, increasing the likelihood that this species could experience disturbance while basking, dispersing, or nesting. Northern red-bellied cooters in Massachusetts are sensitive to disturbance and may be negatively affected by increased human presence. While shooting of turtles was identified as a threat in the 1978 listing proposal, this threat has likely been eliminated due to educational efforts (USFWS 2007, p. 21).

3.8 Pathogens

Few observations of severely diseased (*i.e.*, requiring veterinary intervention) northern red-bellied cooters have been made in Massachusetts since the species was listed (MADFW, unpubl. data). Similarly, literature on pathogens impacting northern red-bellied cooters elsewhere in the species' range (*i.e.,* outside of Massachusetts) is also sparse. Northern red-bellied cooters are assumed to be affected by, or susceptible to, diseases typical of other freshwater turtle species, including various bacterial infections (Sidor 2014, p. 1; Wallach 1975, p. 27), fungal infections (Hunt 1957, p. 20), viral infections (Cox et al. 1980, p. 447; Shender 2019, p. 1), and internal parasites such as nematodes (Holliman and Fisher 1968, p. 316; Sidor 2014, p. 1). On an annual basis, a small number of captive-reared northern red bellied cooter hatchlings for the headstart program occasionally present with minor health issues related to husbandry (MADFW, unpubl. data).

Disease is not believed to be a major factor currently influencing the species' viability in Massachusetts (USFWS 2007, p. 21). However, disease may play a more significant role in the viability of the species in the future. Emerging pathogens like *Ranavirus* spp., *Mycoplasma* spp., and Herpesvirus are associated with significant mortality in turtles (De Voe et al. 2004, p. 535; Jacobson et al. 2014, entire; Johnson et al. 2008, p. 859). *Ranavirus* in particular is a pressing concern for chelonians and has been documented to cause acute, rapidly lethal infection in free-ranging eastern box turtles (*Terrapene carolina carolina*) in the northeastern U.S. (Johnson et al. 2008, p. 859). Nonnative turtles such as red-eared sliders (*Trachemys scripta elegans*) are susceptible to *Ranavirus* (Johnson et al. 2007, pp. 293–294) and may serve as a vector to transmit this pathogen, or other pathogens common in captive turtles, to habitats occupied by the northern red-bellied cooter (Hidalgo-Vila et al. 2009, entire). Though prohibited in Massachusetts (MADFW 2014, entire), red-eared sliders are still believed to be widely possessed by members of the public and releases of turtles from captivity into the wild continue to occur (Trufant 2016, entire). Potential for disease transmission between red-eared sliders and northern red-bellied cooters likely exists given overlap in basking habitat use at ponds where both species occur (Stone 2010, p. 11; M. Jones, MADFW, unpubl. data).

Outside of Massachusetts, ulcerative disease of the shell ("shell rot") has been documented to be a problem for northern red-bellied cooters in the Rappahannock River, Virginia between 1993 and 1996 (Ernst et al. 1999, p. 214) and in Tony Tank Lake, Maryland from 1997 to 1999 (Green 1997, entire; S. Smith, Maryland DNR, pers. comm. 2019). More recently in 2019, severe necrotic lesions were observed on the shell plastron of several northern red-bellied cooter individuals from five lakes in Gloucester County, New Jersey (B. Zarate, NJDFW, pers. comm. 2019). Lesions observed on the turtles in the New Jersey and Maryland were similar to those described in Lovich et al. (1996, entire) and Garner et al. (1997, entire) on river cooters (*Pseudemys concinna*) and yellow-bellied turtles (*Trachemys scripta*) from Lake Blackshear, Georgia. Various bacteria, fungi, algae, and trematode parasites were found associated with the diseased turtles observed in Virginia, Maryland, and Georgia, but it could not be discerned whether any one of the organisms observed was the causal agent or if they represented secondary opportunistic infection (Ernst et al. 1999, p. 214; Garner et al. 1997, p. 85; Green 1997, p. 3). Investigation is still ongoing for the recent New Jersey cases (B. Zarate, NJDFW, pers. comm. 2019). It is suspected that exposure to some systemic toxin or visceral infectious disease, followed by damage to the shell epidermis could result in the plastron lesions (Garner et al. 1997, entire). Exposure to caustic chemicals is unlikely to result in the pattern of lesions observed, but immunosuppression from chemical contaminants and secondary infection by opportunistic pathogens is plausible (Garner et al. 1997, entire). Vascular disease resulting from an infection or blood clotting due to acute or chronic exposure to a contaminant could also be a potential cause of the lesions (Green 1997, p. 3). Low levels of mortality were observed to be associated with the disease event in Maryland despite a high percentage of turtles captured from the lake over 3 years exhibiting shell lesions (S. Smith, Maryland DNR, pers. comm. 2019).

There is substantial uncertainty regarding the likelihood of introducing new pathogens to northern red-bellied cooters in Massachusetts. Natural movement of pathogens over long distances are rare, but human activities facilitate transmission of pathogens via movement of soil, water, and animals between places (Jancovich et al. 2005, pp. 220–222; St-Amour et al. 2008, entire). Significant human activity occurs in and around northern red-bellied cooter habitat, including movement of gear (*e.g.,* boats, equipment for cranberry cultivation), which increases the likelihood of moving a pathogen that is able to persist in environment. If introductions of pathogens are occurring in Massachusetts as a result of one or more of the above-described factors, the frequency of such introduction events is unknown. Some illnesses, such as the shell lesions observed in northern red-bellied cooters, have not had the causative agent identified, so the risk of future disease events cannot be meaningfully assessed.

There is also significant uncertainty regarding the susceptibility of northern red-bellied cooters to various potential pathogens and the long-term fitness consequences illness may have on affected individuals. Susceptibility to a pathogen like *Ranavirus* can vary a great deal between turtle species, with some species demonstrating no ill effects from exposure (Brunner et al. 2015, p. 87) while others experience severe morbidity (Johnson et al. 2008, p. 859). Other environmental stressors such thermal extremes or resource limitations due to climate change or contaminant exposure may have a synergistic effect increasing the species vulnerability to disease (Hing et al. 2016, pp. 52–54; Tangredi and Evans 1997, entire). Additionally, the potential long-term consequences of a pathogen or illness on the fitness of individual northern red-bellied cooters is often unknown.

3.9 Effects of Small Population Size

In Massachusetts, the northern red-bellied cooter's small population size and restricted range are foremost among the factors limiting its long-term viability. As a small, isolated population, the northern red-bellied cooter in Massachusetts may be subject to inbreeding and genetic drift, which can reduce genetic diversity and potentially decrease survivorship (USFWS 1994, p. 12). Populations with reduced genetic diversity have less ability to adapt to changes in the environment and therefore have increased rates of extinction (Markert et al. 2010, p. 11).

Both mark-recapture (T.E. Graham unpubl. data *as cited in* USFWS 1994, p. 12) and genetic analyses of turtles from Federal Pond and nearby Island Pond (Haskell 1993, pp. 15–16) indicate that these ponds represent disjunct breeding populations, possibly affecting intrapopulation genetic variability. Genetic exchange and movement (immigration and emigration) may be necessary to sustain small populations through periods of natural demographic fluctuation (USFWS 1994, p. 12).

While the current minimum population estimate of 933 individuals within the greater Plymouth region indicates a significant increase since 1980, when there were an estimated 200 to 300 individuals in the state (Regosin et al. 2017, pp. 13, 33), the Massachusetts population remains small and isolated in comparison to the rest of the range. In addition, the genetic viability of populations augmented by the headstart program in Massachusetts has not been assessed, and there is the possibility that factors such as a skewed sex ratio may lead to instability over time if not addressed (Regosin et al. 2017, p. 35). The timing of hatchling collection in the early years of the headstart program could have led to a skewed sex ratio in headstarts due to temperature dependent sex determination in northern red-bellied cooters, although it is possible that being at the northern extent of the species range has some impact on sex ratio due to cooler summer temperatures overall (Regosin et al. 2017, p. 33). Evidence suggests that genetic factors should be considered to ensure that extinction risk is not underestimated and appropriate recovery strategies are implemented (Frankham 2005, entire).

3.10 Climate Change

3.10.1 Temperature and Precipitation

The Northeast climate is already changing in ways that are likely to impact our biological resources. The annual average temperature has been rising since 1900, and much of this warming has occurred in the last few decades, with temperatures increasing more than 2.2° C (4°F) from 1970 to 2000 (Frumhoff et al. 2007, p. 3). The number of extremely hot days (32.2° C (90°F) or hotter) doubled from about 1961 to 2006 (Frumhoff et al. 2007, p. 6). Annual precipitation has also been increasing in the Northeast since 1900, with increases observed mostly in the spring, summer, and fall. Additionally, with winter temperatures rising, more precipitation in the winter is in the form of rain. Extreme precipitation events (more than 5.1 cm (2 in) of rain in 48 hours) had also become more common by the end of the 20^{th} century (Frumhoff et al. 2007, p. 8).

The Intergovernmental Panel on Climate Change (IPCC) developed two scenarios that represent the highest and lowest projections of continued human emissions of heat-trapping gases to assess future climate change (Frumhoff et al. 2007, p. 3). Temperatures are expected to continue rising in the Northeast, with an increase of 1.4° C to 2.2° C (2.5°F to 4°F) in the winter and an increase of 0.8° C to 1.9° C (1.5°F to 3.5°F) in the summer, over the next several decades. There is a higher level of uncertainty during the second half of this century, dependent on different emission scenarios, but even under the lower emission scenario, temperatures will continue to rise with a resulting increase of 2.8° C to 4.4° C (5°F to 8°F) in winter and an increase of 1.7° C to 3.9° C (3°F to 7°F) in summer by the end of the century (Frumhoff et al. 2007, p. 3). The number of extremely hot days is also expected to increase from less than 20 days to 60 or more days annually, by the end of the century (Frumhoff et al. 2007, p. 6). Annual precipitation is expected to increase in the Northeast by about 10 percent this century, but winter precipitation could increase by 20 percent or more, with more winter precipitation as rain and less as snow. The frequency of extreme precipitation events is also expected to increase (Frumhoff et al. 2007, p. 8). Despite projections for increasing rainfall, the Northeast will still likely experience droughts. "Rising winter temperatures will melt snow faster and earlier, likely increasing runoff and soil moisture in winter and early spring. These increases could be followed by reductions in soil moisture in late summer and early fall as warmer temperatures drive evaporation rates higher" (Frumhoff et al. 2007, pp. 8–9).

3.10.2 Impacts to Lakes, Ponds, and Rivers

Changes in climate and local weather patterns will likely affect aquatic systems such as lakes and ponds and may especially threaten the healthy functioning of coastal plain ponds. Although there is uncertainty with respect to the degree that climate will continue to change in the future, the Massachusetts State Wildlife Action Plan notes several possibilities regarding lakes, ponds, and coastal plain ponds (MADFW 2015, pp. 238, 304–305). A warming climate and increasing air temperatures will result in coastal plain pond waters warming faster than normal during the year and creating a habitat that may not be livable to some of the current species. Surface and groundwaters will also warm with an increasingly warming climate, and this may create more favorable habitat and provide longer growing seasons for invasive species and harmful algae. The projected increase in severe rain and snowfall events will increase the amount of pollutants entering the coastal plain ponds from the surrounding landscape, including agricultural and urban areas, and increased rain events will also lead to higher loads of atmospheric nitrogen deposition (MADFW 2015, pp. 304–305). Lastly, extended droughts may impact the littoral habitat of coastal plain ponds, negatively impacting the fish and invertebrate species that depend upon them (MADFW 2015, p. 238).

Changes in air temperature, as well as in the amount, timing, and type of precipitation, affect streamflows and drought characteristics (Frumhoff et al. 2007, pp. 9–10). With more winter precipitation as rain and less as snow, and increasing winter temperatures, there is likely to be more runoff during the winter and early spring (Frumhoff et al. 2007, p. 8). This phenomenon, along with the increased air temperatures resulting in earlier snowmelt and ice breakup, would cause streamflow to peak earlier in the year; up to 10 to 14 days earlier by the end of the century. A decrease in duration and thickness of ice cover on lakes in the Northeast has been documented over the past century and this trend is expected to continue (Frumhoff et al. 2007, p. 85). Changes in precipitation and runoff can have a substantial impact on aquatic systems. Drought is related to soil moisture, which in turn is related to evapotranspiration, rainfall, temperature, drainage, and climatic changes. Stream flows would be lower in the summer months, especially under the high emissions scenario, as a result of higher evapotranspiration (Frumhoff et al. 2007, pp. 9–10).

3.10.3 Impacts to Northern Red-bellied Cooters

The northern red-bellied cooter population is geographically separate and distinct from the more southern species and an increasingly warmer climate could have several effects on this northern population. Warmer temperatures in spring and summer may be beneficial, providing more opportunities for basking, and more favorable conditions for feeding and nesting. Warmer temperatures may also increase hatching success (absent predation) and result in more female hatchlings. However, our changing climate may also bring about shifts in other species' ranges, resulting in the arrival or expansion of new competitors, pathogens, and invasive species, all of which could negatively impact northern red-bellied cooters (USFWS 2007, p. 23). Longer growing seasons may result in increased growth of nonnative aquatic vegetation which could contribute to anoxic conditions in winter when ice cover is present (Regosin et al. 2017, p. 41). Warmer winters could also result in ponds not icing over or icing over for a shorter duration and may change the winter hibernation pattern of northern red-bellied cooters. In addition, warmer winters may allow northern red-bellied cooter hatchlings to overwinter in nest chambers more frequently. More research is needed to better understand direct impacts of climate change on northern red-bellied cooters.

3.11 Headstart Program

In 1985, the MADFW established a headstart program to increase the size and extent of the northern red-bellied cooter population in Massachusetts (Regosin et al. 2017, p. 14). This program was established because predation is a leading cause of egg and juvenile mortality and it is infeasible for managers to permanently decrease the number of generalist, egg predator species that occur near coastal plain ponds in Plymouth County, Massachusetts (USFWS 1994, p. 18).

Headstarting is a conservation measure used to increase turtle numbers by offsetting the high mortality rate of first-year turtles in the wild. Eggs and/or hatchling turtles are brought into captivity, and hatchlings are held in aquariums at above ambient water temperatures and fed a diet of red leaf or romaine lettuce supplemented with Repto-min®. After 8 to 9 months in captivity, headstarted turtles can be either returned to their natal pond or translocated to new habitat to support recovery objectives (USFWS 1994, p. 18). Headstarted hatchlings grow rapidly and can generally attain sizes (carapace length) two to six times that of similar-aged turtles in the wild. When released, headstarted turtles are expected to experience reduced mortality from predators due to their larger size (USFWS 1994, p. 18; see section 3.3 Predation).

Federal Pond has served as the primary source for hatchlings throughout the program's three decades of operation because the breeding population of turtles there was much more robust than in any other pond (USFWS 1994, p. 18). However, a population status assessment in 2017 found that the population has not increased despite the addition of 156 headstarted turtles to Federal Pond between 1987 and 2002 (Regosin et al. 2017, p. 41). A winter die-off of 10 individuals at Federal Pond in 2015, possibly as a result of unusual winter ice-cover conditions that year, may be one potential reason why this population is stable or declining rather than increasing (Regosin et al. 2017, p. 35). There are limited data available on the degree of genetic variability among subpopulations of the northern red-bellied cooter in Massachusetts; however, Haskell (1993, pp. 15–16) found that there is less heterozygosity of sampled allozymes within Federal Pond turtles than within Island Pond, another pond population nearby. Therefore, it is possible that the vast majority of northern red-bellied cooters in the Massachusetts population today (Federal Pond northern red-bellied cooters and virtually all of the headstarted hatchlings released in other ponds) may be genetically less diverse, and possibly less fit, than the nonheadstarted turtles present in the ponds. An additional factor is the likelihood that a majority of the headstarted turtles released during a multi-year period, 1985–1998, are likely to be males. For example, Boot Pond received 66 headstarted hatchlings from 1987 to 1991, and Graham and Graham (2001, pp. 6–8) later found that 72 percent of turtles captured at that pond in 2001 were males. Thus, the effective population size (the number of animals contributing gametes) of ponds receiving these headstarts is much less than if the sex ratio of headstarts was 1:1 (USFWS 2007, p. 23).

Since 1985, over 4,000 headstarted hatchlings have been released at over 30 sites in Massachusetts including 11 of the 14 original ponds that supported natural occurrences of the species as well as several ponds and rivers without evidence of historical occupancy (Regosin et al. 2017, pp. 14, 16). The northern red-bellied cooter population has increased from around 200 individuals in 1980 to a recent minimum estimate of 933 individuals within ponds sampled in a study area in Plymouth County, MA between 2014 and 2016 (Regosin et al. 2017, pp. 13, 33). As a result of the headstart program, the Massachusetts northern red-bellied cooter population has increased and new, reproducing occurrences have been created in at least five locations (Regosin et al. 2017, p. 12, 30).

The headstart program continues to be successful in supporting a stabilized population of northern red-bellied cooters in Massachusetts; however, for populations to become self-sustaining, threats need to be better understood and mitigated (Regosin et al. 2017, p. 12). The continued operation of the headstart program is expected to maintain populations at stable levels, but population models suggest that closure of the program may result in a slow decline of populations over several decades (Regosin et al. 2017, p. 37). In addition, the possibility of sourcing future headstarts from East Head Pond, which now is confirmed to have a larger population than Federal Pond, should be considered as an alternative to sourcing the majority of headstarts from Federal Pond (Regosin et al. 2017, p. 40). A strategy for releases that targets ponds that are expected to stabilize over time with the addition of headstarts could be developed to prioritize release sites and improve the program (Regosin et al. 2017, p. 37, 62).

There is uncertainty around the long-term future of the headstart program if Federal listing status were to change as the Federal listing status of the species has provided support for continued operation of the program for more than three decades. Without the input of headstarts into subpopulations, it is likely that some would decline over time; however, the exact number of subpopulations that would experience decline is unknown, and effects are not likely to be measurable for many years (Regosin et al. 2017, p. 37). More information is needed on the age at which headstarted individuals reach sexual maturity; ongoing observations of an introduced population will continue over the next several years to determine the mean age when headstarts reach sexual maturity (Regosin et al. 2017, p. 49).

3.12 Protected Lands/Regulatory Mechanisms

3.12.1 Protected Lands

Habitat protection at ponds with existing or introduced northern red-bellied cooter populations is a high priority management activity (USFWS 1994, p. 16), and one of the recovery objectives listed in the species' Recovery Plan (second revision) is protection of sufficient habitat "to allow long-term maintenance of the population" (USFWS 1994, p. 25). This objective directly addresses the need to protect aquatic feeding, resting, breeding, and over wintering habitats, in addition to adjacent upland habitats used for nesting and dispersal. Protection of upland areas will contribute towards preserving connectivity between ponds and is a conservation priority for the northern red-bellied cooter (Regosin et al. 2017, p. 36).

Level of protection of suitable shoreline and upland habitat for the northern red-bellied cooter varies from total protection from human activity to no protections at all. Upland habitat is a mix of privately and publicly owned residential, agricultural, and conserved land. However, most of the habitat for this species is in private ownership (USFWS 1994, p. 17). Conservation easements and protections range from properties protected under agricultural easements that do not allow development but do allow agricultural activities to occur, to properties with more limited land use allowances. Some land uses allowed under certain conservation easements may not achieve the goal of protecting northern red-bellied cooter habitat. Only a few ponds, including East Head Pond (Myles Standish State Forest) and Crooked Pond (Massasoit National Wildlife Refuge), are entirely within state or Federal conservation ownership.

At present there are no known major nesting areas that are completely protected by the Service, MADFW, municipal, or nongovernmental organizations. Inadequate protection of major nesting areas is a key threat to this species (Regosin et al. 2017, p. 37). Establishing protection of known nesting habitat is a priority conservation measure critical to the recovery of this species.

The lack of protection to more of the uplands surrounding northern red-bellied cooter occupied water bodies is an important concern, because turtles utilize uplands for nesting and to disperse to other water bodies. It is also important because development of the uplands can lead to direct mortality of turtles due to vehicles striking turtles on roads or indirectly increase pets and other predators, which dig up turtle nests. Upland development may have more subtle deleterious effects such as degradation of water quality and displacement of turtles from favored basking and nesting sites by increased levels of human presence (USFWS 2007, p. 21). Suggested measures to protect areas delineated as existing or recent populations as well as nearby potential habitat, including corridors for interchange among pond populations include fee acquisition from willing sellers, easements, zoning, registry agreements or other methods (USFWS 1994, pp. 30–31).

3.12.2 Regulatory Mechanisms

Regulatory mechanisms provide some level of protection to the species and its habitat. In addition to Federal listing as endangered and the designation of about 10 ponds and 3,269 acres as critical habitat, the northern red-bellied cooter is also listed as an endangered species by the Commonwealth of Massachusetts under the Massachusetts Endangered Species Act (MESA; Mass. General Law, chapter 131 A, and Code of Massachusetts Regulations 321 CMR 10.00). This state designation prohibits the taking and possession of northern red-bellied cooters without a permit and provides a regulatory framework for review of non-exempt projects within "Priority Habitat". Further protections for some habitats are provided by the Massachusetts Wetland Protection Act (WPA; Mass. General Law, chapter 131, sections 40 and 40A), and subsequent regulations (1987), which provides relatively strong protection to aquatic habitats that are mapped as Estimated Habitat of State-listed Rare Wetlands Wildlife, published by the Massachusetts Natural Heritage and Endangered Species Program (NHESP) in accordance with 321 CMR 10.12: Delineation of Priority Habitat of State-listed Species. Proposed development that is within the mapped estimated habitat (M.G.L. c131A) and requires the filing of a Notice of Intent subject to the WPA, requires environmental review by the MADFW NHESP (USFWS 2007, p. 22).

Aquatic habitats where northern red-bellied cooters occur are protected to some extent by Federal regulations such as the Clean Water Act (CWA), which requires that an applicant for a federal license or permit provide a certification that any discharges from the facility will not degrade water quality or violate water-quality standards, including state-established water quality standard requirements. The discharge of dredged and fill material is also regulated through the CWA. Additionally, the U.S. Army Corps of Engineers reviews and permits projects that propose to impact wetlands and other aquatic habitat (e.g., fill, re-alignment, culverts, bridges) and may implement minimization or mitigation measures.

3.12.3 Uncertainty

It is unclear what level of land protection is needed to consider land protected for the purposes of northern red-bellied cooter recovery. It may be that a wide range of land protection statuses achieve sufficient protection to satisfy this need, or it may be possible that only the most restrictive land protections actually benefit the species and some land uses allowed under conservation easements are incompatible. We don't know how far from a water body protection status is still important for the northern red-bellied cooter, although we can reasonably assume that more protection is beneficial no matter what area around a water body is considered. In one study, 275 m (902.2 ft) beyond the Federal delineation of a water body was found to be a biologically meaningful buffer within which full protection of upland sites is important for the three species of turtles observed (Burke and Gibbons 1995, p. 1368). Likewise, the percentage of nest sites that must be protected to ensure long-term viability of the northern red-bellied cooter is unknown. However, protecting terrestrial areas and limiting development within these areas is expected to result in reduced risk of individual mortality for female turtles and hatchlings (Steen et al. 2012, p 125). Further, we note that key nesting areas are likely to disproportionately influence the persistence of the northern red-bellied cooter. Part of the uncertainty for this species is whether key nesting areas that can be feasibly managed can be sufficiently protected.

Another source of uncertainty is the level of regulatory certainty afforded by the MESA and the Massachusetts WPA. These two state acts, and their supporting regulations, interact to provide a layer of protection for the northern red-bellied cooter in Massachusetts. The MESA is probably the stronger of the two acts as it relates to protecting key upland features such as nesting areas and migration corridors. Many known areas of confirmed occurrence are mapped as "Priority habitat" and are subject to regulatory review by the MADFW NHESP, and mapping is regularly updated. The WPA provides protection for the ponds and rivers themselves, as well as several classes of wetland resource areas such as bordering vegetated wetland. The WPA further protects some water bodies known to be occupied by northern red-bellied cooters, which are mapped as "Estimated Habitat," by requiring environmental review by the MADFW NHESP. Two prominent exceptions, at present, are the Nemasket River and Burrage Pond WMA, which are headstart populations that have not been confirmed by MADFW to have been successful. Burrage Pond WMA is essentially protected as a state WMA, and the Nemasket River receives some protections under the Riverfront provisions of the WPA. In addition, it is unknown whether this includes locations where headstarts have been introduced to previously unoccupied water bodies or have colonized areas on their own, including rivers.

CHAPTER 4 – CURRENT CONDITION

4.1 Methodology

To assess the current condition of the northern red-bellied cooter in Massachusetts, we used the best available information, including peer reviewed scientific literature, academic reports, and survey data provided by state and Federal agencies.

Fundamental to our analysis of the northern red-bellied cooter was the determination of scientifically sound analysis units at a scale useful for assessing the species. In previous documents, individual ponds have been considered to be discrete populations; however, movement patterns suggest that metapopulation dynamics may exist in some complexes consisting of several ponds (Regosin et al. 2017, p. 36). Analysis units (AUs) were developed using element occurrence (EO) data (i.e., areas of land or water in which a species is or was present) provided by the MADFW NHESP to identify occupied water bodies and rivers. Water bodies from the National Hydrography Dataset (NHD) (U.S. Geological Survey [USGS] 2019) that overlapped with EO data were treated as standalone AUs unless they were within 400 m (1312 ft) of another occupied water body, in which case an AU made up of multiple water bodies was created. The 400-m (1312-ft) buffer was selected based on a natural break in regular movement of individual northern red-bellied cooters between water bodies, as based on expert opinion and a study conducted in Plymouth County (Regosin et al. 2017, entire). River AUs were created by identifying river flowlines from the NHD data layer, where EO data indicated occurrence, and selecting a segment that extended 2,000 m (6,561.7 ft) upstream and downstream of each EO data point, combining AUs where they overlapped (See appendix A for detailed AU methodology). We identified a total of 43 AUs that span the range of the Massachusetts population of the northern red-bellied cooter (figure 2). We assumed that natural breaks in movement patterns and gaps in observation data indicate meaningful disruptions in connectivity but do not assume that they represent total barriers to movement. The AUs developed for this SSA provide a mechanism by which to evaluate the species based on functional units of occurrences but do not represent discrete populations.

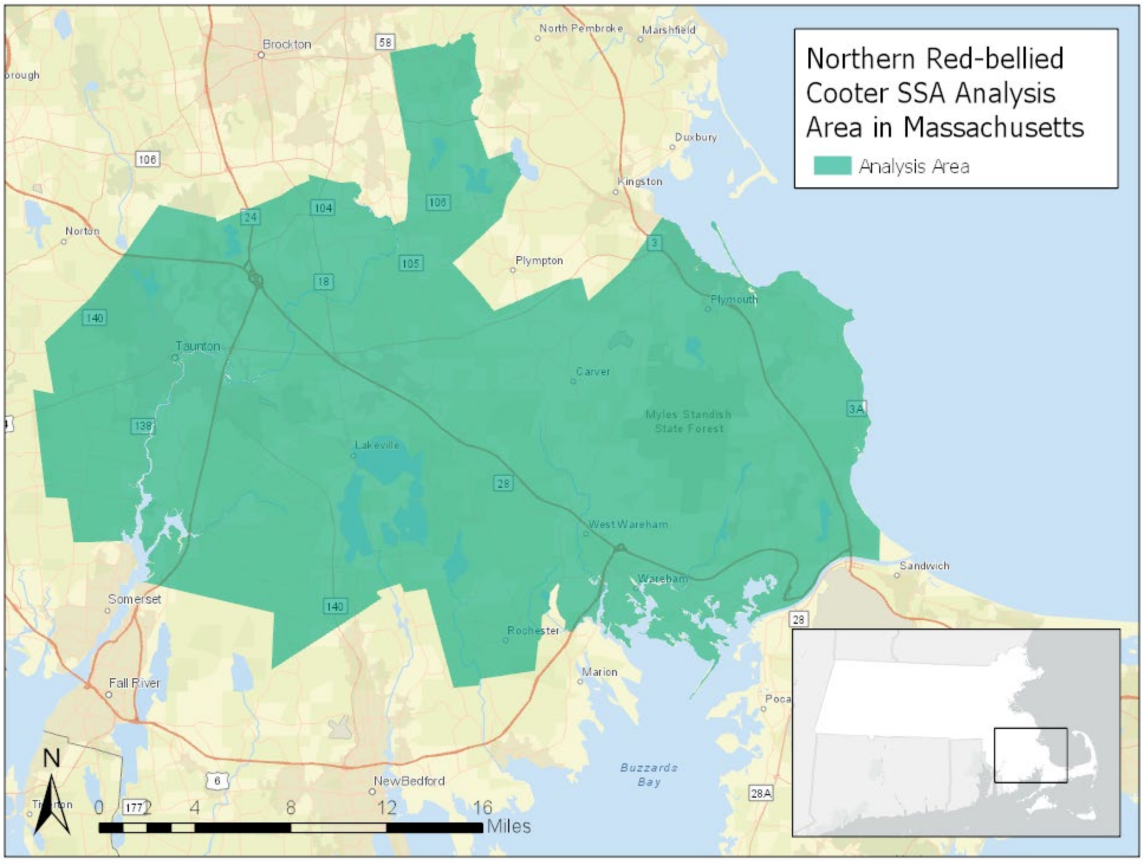

Figure 2. Area in which analysis units for the northern red-bellied cooter SSA were distributed in Massachusetts.

We used the best available data to assess several demographic and habitat parameters using a geospatial analysis and then used percentile ranking and weighting to reach a final resiliency condition score for each AU, which was then categorized as one of four resiliency condition tiers. An initial list of metrics was reduced through discussion, surveys, and use of a correlation matrix to identify redundant variables, resulting in nine final metrics. See appendix A for more details.

4.1.1 Demographic Metric – Best Estimate

We developed the Best Estimate metric in the absence of consistent and comparable demographic data available for each AU. We used the best available information for each of the AUs from four possible metrics: total population estimate, an estimate of headstarts accounting for survivorship and recruitment, the maximum occupancy total from a three-survey occupancy assessment, and the maximum number of individuals recorded in the EO data. When a total population estimate was available, this was selected as the value for Best Estimate. If a total population estimate was not available, we selected either the headstart estimate or maximum occupancy total, whichever was highest. Finally, if all other demographic data sources were not available for an AU, the highest number of individuals observed at one time in any one water body within an AU, as documented in the EO dataset, was used as the Best Estimate value.

Total population estimates and maximum occupancy assessment information came from Regosin et al. (2017, pp. 62–63). Headstart estimates were calculated using summarized annual headstart release data from 1985-2019 (MADFW, unpubl. data) and adjusted with an estimate of survivorship and recruitment. We then applied an average survivorship rate of 0.9509, calculated from available data from three ponds within the Massachusetts population (Regosin et al. 2017, p. 62), to adjust the headstart estimate. Finally, we applied an average recruitment corrective factor of 1.3929 calculated from the same three ponds and applied it to all survivorship corrected headstart estimates to calculate our final headstart estimates for each AU that has received headstarts (see appendix A).

While we do not consider AUs to be representative of standalone populations, we assumed that higher Best Estimate values, as our best available measure of number of individuals in an AU, have a positive impact on the resiliency of an AU and the Massachusetts population as a whole. As discussed in section 3.9. Effects of Small Population Size, small population size may result in increased risk of reduced genetic diversity and lack of ability to withstand stochastic events, while larger population sizes may be at less risk from these factors.

4.1.2 Habitat Quality Metrics – Protection

Habitat protection reflects the on-the-ground protected status of aquatic and upland habitat within and surrounding AUs. Protected land is assumed to provide some level of protection of resources and habitat needed for breeding, feeding, and sheltering. Protected land may also result in less human disturbance and allow for increased opportunity to put conservation measures in place to protect northern red-bellied cooters (see section 3.12.1 Protected Lands). A composite dataset of protected lands was developed by combining relevant categories of protected lands from three separate datasets documenting protected lands within Massachusetts (see appendix A). This dataset was then used to calculate the percentage of protected land within two areas associated with each AU.

<u>Percent Shoreline Protected</u>

We assessed the percent of shoreline protected to capture the level of protection of the water bodies within each AU. We calculated the total percentage of the shoreline length that was in a protected status as determined by our composite protected lands dataset. We buffered the protected lands dataset by 50 m (164 ft) to correct for any discrepancies in how the AUs and protected lands data layer shapefiles were mapped along shorelines. Higher levels of protected shoreline surrounding water bodies are assumed to be beneficial to northern red-bellied cooters by providing some level of protection for activities such as feeding, hibernating, and basking. Protected shorelines may provide more opportunities for natural basking habitat to form and remain in place, and for northern red-bellied cooters to access upland habitat used for nesting and dispersal.

<u>Percent Protected Land</u>

We calculated the percent of land within a 400-m (1312-ft) buffer surrounding AU water bodies to assess the level of protection within the contextual landscape surrounding each AU. Higher levels of protected land within the landscape surrounding AUs are assumed to be beneficial to northern red-bellied cooters. Protected landscapes surrounding occupied water bodies may provide some level of protection for individuals dispersing to other aquatic habitat. The upland area around water bodies may also be used for nesting when appropriate sandy or gravelly substrate is present, and protected status is assumed to provide some security against human disturbance. Protected upland landscapes also may provide the opportunity for beneficial habitat management, habitat restoration, or nest protection.

4.1.3 Habitat Quality Metrics – Integrity

Water Body Shape Complexity – Fractal Dimension Index

Water Body Shape Complexity was used as a proxy for estimating the presence of shallow vegetated coves, a habitat feature that has been associated with areas of high density northern red-bellied cooter occurrence in some ponds (see section 2.5 Habitat Needs). Although it is unclear exactly what role these habitat features play in northern red-bellied cooter life history, they appear to be important in some way, and therefore we assumed increased presence of shallow vegetated coves was beneficial. We assumed more complex water body shapes would have more sinuous shorelines and shallow vegetated coves and measured the complexity of water body shapes within each AU by calculating the fractal dimension index (see appendix A). Higher fractal dimension index scores were assumed to be beneficial.

Percent High Index of Ecological Integrity (IEI)

The IEI dataset incorporates a variety of metrics related to landscape resiliency and intactness. It depicts the ecological integrity of the landscape in 30 m (98.4 ft) grid cells, on a scale from 0 to 1 (North Atlantic Landscape Conservation Cooperative [NALCC] 2017, entire), where an index score of 1 indicates a higher level of integrity. Habitat fragmentation, and loss of upland and aquatic habitat are potential threats to northern red-bellied cooters throughout their range in Massachusetts (see section 3.2. Habitat Loss/Fragmentation). Using the IEI, we developed a metric to assess the amount of aquatic or terrestrial habitat within or surrounding an AU with a high level of ecological integrity and expected that landscapes containing more area categorized as high ecological integrity would be more resilient and less fragmented. We developed a Percent High IEI metric by reclassifying IEI index scores into three categories: low (0–0.33), medium (0.33–0.66) and high (0.66–1). We then calculated the percentage of an AU plus a 400-m (1312-ft) buffer surrounding the AU that was categorized as being within the high IEI category, or top 3rd of possible IEI scores (0.66–1).

Multi-pond Complex

The presence of multiple water bodies within an AU provides additional aquatic habitat and likely increases resilience of the northern red-bellied cooter population within that AU. Therefore, an AU containing only one water body was assumed to be less beneficial to northern red-bellied cooters than an AU with multiple water bodies. An AU with only one water body may be at more risk of being negatively influenced by stochastic events or other risk factors if they impact a single water body (see chapter 3. Factors Influencing Viability). In addition, a single water body may provide less opportunity for hibernating, basking, feeding, and other aquatic habitat needs (see section 2.5 Habitat Needs) than multiple water bodies. We based this metric on known occupied ponds that made up each AU and did not attempt to estimate how much additional suitable habitat may be available in nearby water bodies without any record of occupancy. We determined whether or not an AU contained multiple water bodies through visual analysis of aerial imagery and shapefiles for each AU. AUs with multiple water bodies were given a score of 1 (presence) and AUs with only one water body were given a score of 0 (absence).

4.1.4 Habitat Quality Metrics – Degradation

Percent Impervious Surface

We measured the percent impervious surface within a 400-m (1312-ft) buffer of all water bodies within an AU using the Massachusetts Bureau of Geographic Information (MassGIS) Data: 2016 Land Cover/Land Use dataset (MassGIS 2019, entire). We used this metric to characterize the level of threat of human influence on the landscape surrounding AUs, including buildings, roads, and other infrastructure. A higher percent of impervious surface was assumed to negatively impact resilience of an AU, where increased human presence and influence may lead to increased threat from a variety of factors (see sections 3.2 Habitat Loss/Fragmentation, 3.3 Predation, 3.4 Invasive Species, 3.5 Motorboat Strikes/Road Mortality, 3.6 Collection, and 3.7 Harrassment/Disturbance).

Average Likelihood of Road Mortality

The mean probability of road mortality within a 400-m (1312-ft) buffer of the water bodies that make up an AU was calculated using a road-crossing wildlife mortality dataset (Grand 2014, entire). The dataset is based on a model developed specifically for turtles that uses traffic rate and wildlife mortality data to assess probability of road-crossing mortality (Gibbs and Shriver 2002, entire). Road mortality is a significant threat facing many turtle species, including the northern red-bellied cooter (see section 3.5.1. Road Mortality), and we assumed that higher probability of road mortality in the landscape surrounding an AU would negatively influence resiliency of turtle populations.

Percent Low IEI

We used the IEI dataset a second time to generate a metric to assess the amount of habitat within or surrounding an AU with a low level of ecological integrity (NALCC 2017, entire). We assumed that landscapes containing a larger amount of area categorized as having a low level of ecological integrity would be less resilient. We developed a Low IEI metric by reclassifying IEI index scores into three categories: low (0–0.33), medium (0.33–0.66), and high (0.66–1). We then calculated the percentage of an AU plus a 400-m (1312-ft) buffer surrounding the AU that was categorized as being within the low IEI category, or bottom 3rd of possible IEI scores (0–0.33).

4.1.5 Final Current Resiliency Condition Score

Final scores for each of the AUs were calculated by first converting the raw values of each of the metrics to a percentile rank based on the distribution of the values across all analysis units. For the binary metric assessing the presence or absence of multiple water bodies, we scored AUs with multiple water bodies as 100 and AUs with single water bodies as 0. We inverted percentile ranks for the three Habitat Quality-Degradation metrics to indicate that lower raw values for these metrics were beneficial. Next, we weighted each metric through a process of core team surveys and focus group discussions (see appendix A). We then multiplied the percentile ranks by our final weights for each metric and summed the products for each AU to get a final AU current resiliency condition score of 0–100. We used natural breaks in the distribution of current resiliency condition scores to designate two thresholds and divide the final resiliency condition scores into three tiers: high, moderate, and low. A fourth tier, extirpated, was added for instances in which the final resiliency condition score was 0 for an AU (table 1).

Table 1. Analysis unit (AU) resiliency condition categories for northern red-bellied cooter. Current and future resiliency condition scores were categorized into High, Moderate, and Low categories based on natural breaks in the distribution of all final current resiliency condition scores. AUs with a final score of 0 were considered to be Extirpated.

T1 – High	**T2 – Moderate**	**T3 – Low**	**T4 – Extirpated**
An AU in the high resiliency condition tier is likely to have high quality habitat and we have confidence that all or the majority of individuals are able to complete their life functions and breeding is successful. The population is likely able to withstand stochastic events or recover from stochastic events from connected populations.	An AU in the moderate resiliency condition tier could have high, moderate, or low quality habitat and some individuals can complete life functions and have some successful breeding. In general these populations are expected to be relatively stable, although numbers may increase or decrease. Populations or portions of populations are expected to withstand some stochastic events or but may or may not be able to recover through the immigration.	An AU in the low resiliency condition tier may have low quality habitat and a population where only some or few individuals can complete life functions and have successful breeding. The population is not likely to be able to withstand stochastic events and is not able to recover through the immigration of connected populations.	A population with no resilience is one that might be extirpated completely.

4.2 Current Condition

We used the SSA Framework and a current condition analysis to describe the current viability of the Massachusetts population of the northern red-bellied cooter. Viability refers to the ability of a species to sustain a healthy population within a biologically meaningful timeframe. Our results are described in terms of Resiliency, Redundancy, and Representation.

4.2.1 Resiliency

Resiliency describes the ability of a population to withstand stochastic disturbance and is often positively related to population size and growth rate, habitat availability and quality, and connectivity between occupied habitats. Disturbances such as fluctuations in birth rate (demographic stochasticity), length of winter ice cover on ponds (environmental stochasticity), or the effects of anthropogenic activities may impact less resilient populations to a higher degree. Small populations limited in geographic area, like the Massachusetts population of the northern red-bellied cooter, are often limited in their ability to recover following stochastic events. We examined the resiliency of this population of northern red-bellied cooters by considering demographic and habitat factors in each AU.

The AUs were scored based on the metrics described above and ranked according to the following overall resiliency condition categories: high, moderate, low, extirpated (appendix C, tables C1 and C2). Of the 43 AUs, 11 were in high condition, 15 were in moderate condition, and 17 were in low condition (figure 3).

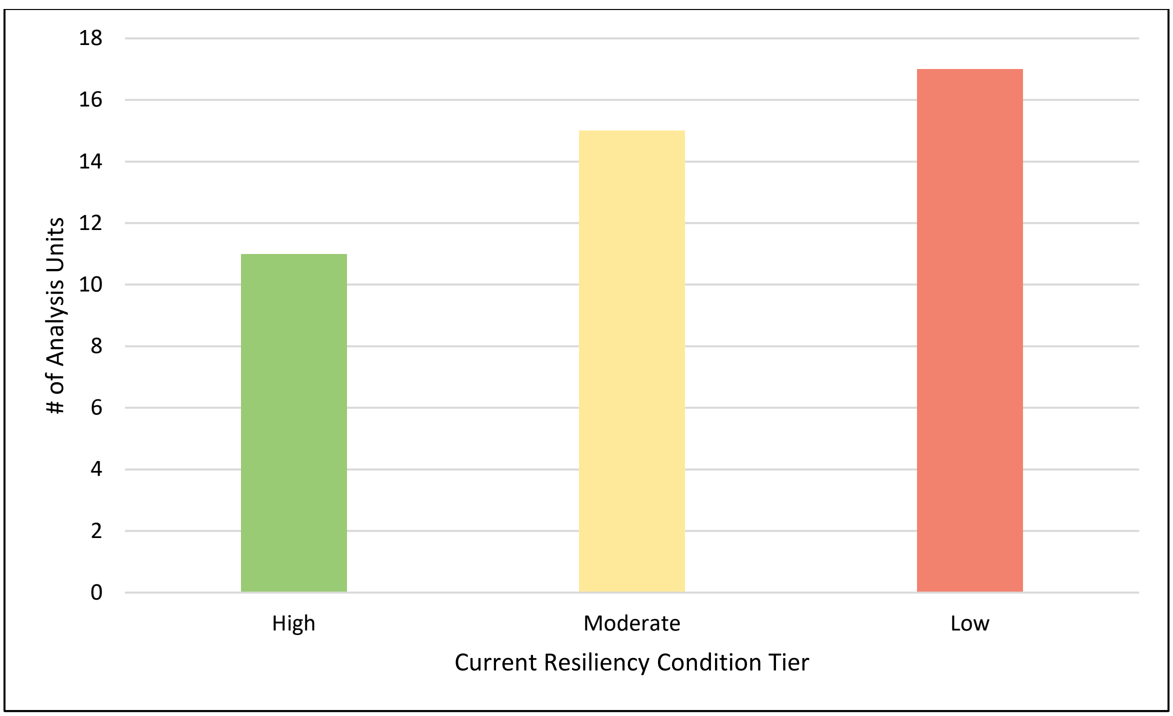

Figure 3. Current condition results by number of AUs in three resiliency condition tiers.

No AUs are currently considered extirpated because we assumed each AU had a minimum of 1 individual for our Best Estimate metric, which is plausible. We used the best available information, including historical observations, in our resiliency condition assessment because current information about the number of individuals present was not available for each AU.

In addition to assessing the resiliency condition of individual AUs, we estimated a total population size to support our analysis of overall current condition of the Massachusetts northern red-bellied cooter population. The most recent combined population estimate is around 933 individuals from ponds within a study area in Plymouth County, MA, excluding headstarts released from 2013–2016 (Regosin et al. 2017, p. 29). We estimated a total Massachusetts population size of 1950.73 individuals by summing existing population estimates for AUs from Regosin et al. (2017, table 5, p. 62) and newly calculated population estimates for AUs that had available headstart release data from 1985-2019 (MADFW, unpubl. data). We applied a survivorship rate of 0.91 when calculating population estimates from headstart release data because it represents the most conservative estimated survivorship of headstarted turtles from a headstart-only pond complex (Regosin et al. 2017, table 5, p. 62). We do not know whether reproduction is occurring in all AUs, however, there is evidence that reproduction is occurring to some degree at most of the water bodies assessed in a recent study of the Plymouth, MA area (Regosin et al. 2017, p. 33). Although the northern red-bellied cooter population in Massachusetts is small compared to populations in the southern portion of the species' range, and habitat and demographic condition vary between AUs, the population as a whole seems to have medium resiliency throughout its range.

4.2.2 Redundancy

Redundancy refers to the number of populations of a species and their distribution across the landscape, reflecting the ability of a species to survive catastrophic events. The greater the number of populations/subpopulations, and the more widely they are distributed, the lower the likelihood a single catastrophic event will cause a species to become extinct.

At the time of listing, the northern red-bellied cooter was known to exist in only 12 water bodies in southeastern Massachusetts, and by the time the Recovery Plan was updated in 1994, that number had increased to around 18 water bodies located in the towns of Plymouth and Carver, MA (see section 2.6 Range and Distribution). In developing AUs for this species status assessment, we identified 43 unique AUs, some of which are complexes containing multiple water bodies. The AUs we assessed were located in the Massachusetts towns of Hanson, Bridgewater, Taunton, Raynham, Dighton, Lakeville, Freetown, Rochester, Middleborough, Halifax, Carver, Rochester, Wareham, Plymouth, and Bourne. Although this species likely had a larger prehistoric range in Massachusetts, currently it appears to be well distributed throughout and beyond its recent historical range. Although it is possible that a catastrophic event, such as severe drought, a large storm, or an extended winter ice over may impact several AUs at one time, the impacts to individual aquatic habitats would likely vary based on their unique characteristics such as depth, water source, and other factors. Additionally, we have no evidence of any catastrophic disease currently impacting the species. While some AUs are connected hydrologically, it is unlikely that disease would impact AUs throughout the species' range. Because the northern red-bellied cooter has 26 AUs in moderate or high condition, we consider the species to have good redundancy in Massachusetts.

4.2.3 Representation

Representation refers to the ability of a species to adapt to changing environmental conditions over time and is characterized by the range of genetic or environmental diversity within and among populations. The greater the diversity, the more successfully a species should be able to respond to changing environmental conditions.

We are aware of three studies that have assessed the genetics of the Massachusetts population of northern red-bellied cooters (Bartron and Julian 2007, entire; Browne et al. 1996, entire; Haskell 1993, entire) (see section 2.1 Taxonomy and Genetics). In the most recent study, Bartron and Julian (2007; entire) examined samples from multiple family groups originating from Federal Pond in Massachusetts. Genetic diversity within the Massachusetts population may be lower than that of other northern red-bellied cooter populations, as evidenced by lower intrapopulation genetic variability, and the lowest number of alleles observed relative to other sampled populations in a recent analysis (Bartron and Julian 2007, p. 6). However, because samples were collected from a single pond, this study did not examine differences between water bodies within the larger Massachusetts population. Earlier genetic analyses of northern red-bellied cooter genetics in Massachusetts found evidence of different allele frequencies between samples taken from two ponds, suggesting that the level of genetic variability may differ between some water bodies (Browne et al. 1996, p. 194; Haskell 1993, pp. 13, 15). Low genetic diversity is an anticipated effect of genetic drift, a problem faced by small, isolated populations (see section 3.9 Effects of Small Population Size). However, mean relatedness estimates between samples from the Massachusetts population have been found to be consistent with estimates from other populations, and management efforts aimed at increasing reproduction, connectivity between water bodies, and population size may help to reduce the rate of loss of diversity (Bartron and Julian 2007, p. 6). The larger current population size estimate and frequency of interpond movements observed suggests that genetic diversity within and among water bodies is likely maintained or improved from what it may have been at the time of listing.

Northern red-bellied cooters in Massachusetts occupy several types of aquatic habitat, including coastal plain ponds, reservoirs, and rivers (see section 2.4 Environmental Settings). Prior to the start of the headstart program in 1985, northern red-bellied cooters were only known to occur in coastal plain ponds in Massachusetts (USFWS 1994, p. 5). However, this species is now known to occupy additional aquatic habitat types including human-made reservoirs and rivers. Northern red-bellied cooters have been introduced to or have dispersed to several rivers in Massachusetts, such as the Nemasket, Taunton, and Weweantic rivers (USFWS 2007, p. 13). At least five of the AUs we examined contained riverine habitat while the remainder of AUs included a combination of other water body types such as ponds, lakes, reservoirs, and wetlands.

Because the northern red-bellied cooter occupies a variety of aquatic habitat types within its restricted range in Massachusetts, we consider the species to have good representation. In addition, although we have limited information about the current genetic diversity of the overall Massachusetts population, we expect that an increased population size and expanded number of water bodies occupied by this species may contribute to stable or increasing genetic diversity compared to the small population size and limited distribution of individuals in the 1980s.

CHAPTER 5 – FUTURE CONDITION

5.1 Methodology

To assess the future condition of the northern red-bellied cooter in Massachusetts, we used the same AUs and metrics that were used for the current condition analysis to analyze current condition (see chapter 4. Current Condition) and modeled six scenarios to assess the potential viability of the northern red-bellied cooter 60 years in the future (2080). This 60-year time step (2020–2080) was chosen, in part, due to availability of 2080 modeled data for several of the habitat metrics we assessed. In addition, 60 years represents a time step between approximately one and two generation times of long-lived turtle species such as the northern red-bellied cooter (see section 2.3.1 Demographics). Due to the long length of the expected generation time, the effects of changes to habitat, resources, or impacts from stressors or conservation actions may not be observable in northern red-bellied cooter populations at earlier time steps.

We calculated final future resiliency condition scores using the same methodology for calculating the final current resiliency condition scores and used the same metric weights and natural breaks for tier designation as those used in our current condition analysis (see section 4.1.5 Final Current Resiliency Condition Score).

The metrics we assessed in the future condition analysis are the same as those used in the current condition analysis. When available, we used data outputs for habitat quality metrics modeled at the 2080 time step by the UMass Designing Sustainable Landscapes (DSL) Project (McGarigal et al. 2017b, entire). Modeled outputs for 2080 were available for the Percent High IEI, Percent Low IEI, Percent Impervious Surface, and Average Likelihood of Road Mortality metrics. For the remaining habitat quality metrics, we carried forward the values calculated for our current condition analysis. We assumed that the Water Body Shape Complexity and Multi-Pond Complex metric values would not change between 2020 and 2080. We did not have modeled data outputs at the 2080 time step for the Percent Shoreline Protected and Percent Protected Land metrics.

We considered creating scenarios in which we varied the habitat quality metrics up or down by one standard deviation to indicate worst case or best case habitat quality-related scenarios. However, while these scenarios are possible, they were determined to be less plausible than scenarios created using the modeled output results available for some metrics at the 2080 time step and focusing the future condition assessment on how AU resiliency condition responded to several demographic-related scenarios that would influence the Best Estimate metric. We created future condition scenarios using two variables that we selected because we determined that variations in these two variables were all plausible and that therefore it was important to analyze how AU resiliency condition might respond to these variables.

5.1.1. Headstart Variable

The use of headstarting as a conservation action for northern red-bellied cooter is well-established and the current headstart program in Massachusetts has been in operation since the 1980s (see section 3.11 Headstart Program). As a conservation action that we believe has strongly influenced the current condition Best Estimate metric values for many AUs, we chose to examine the effects of implementing three plausible headstart program options into the future. While all of the three headstart program options are plausible, we assume the rule-based headstart is the most likely scenario in the short term (next 5 to 10 years), based on MADFW interest for continuing the program (M. Jones, pers. comm. 2021). In the long term (at the 2080 time step), the rule-based and no headstarts scenarios are most likely, but absolute certainty is untenable as resources and interest for continuing the work for the next 60 years is uncertain. We considered the historical approach to headstarting the least likely, as it is doubtful that mangers would make decisions that didn't use the understanding gained from past monitoring efforts.

<u>No Headstarts</u>
Although the headstart program has operated for over 35 years with relative stability, there is the potential that this program could be terminated or phased down in the future and within our 60-year timestep. Therefore, we included scenarios in which headstarting does not occur between our current condition and future condition time steps. Additionally, we wanted to evaluate the future condition of the species in the absence of this important conservation work that has contributed to its recovery.

<u>Rule-based Headstarts</u>
A recent population study recommended development of a comprehensive strategy for future headstart releases that targets ponds most likely to be stable over time with added headstarts (Regosin et al. 2017, p. 37). While developing a headstart strategy was beyond the scope of this SSA, we did a rapid assessment using expert judgment of potential factors that might be considered when determining whether a water body should receive headstarts or not in the future. We created a set of rules that determined which AUs were likely to receive headstarts. By applying these rules, we selected 29 AUs that would receive a total of 263 headstarts each (global average of 127 headstarts released per year multiplied by 60 years and divided by 29 AUs) at the 2080 time step in scenarios with the Rule-based Headstart variable applied (see appendix B).

<u>Historical Headstarts</u>
In addition to considering a rule-based approach, we also looked at how headstarts have been allocated to ponds throughout the history of the headstart program and assumed that it was plausible that headstarts could be released only to those locations that have received headstarts in the past. We used headstart release data (see appendix B) to determine which AUs have received headstarts in the past. In scenarios where the Historical Headstarts variable is applied, each of the 23 AUs determined to be a historical headstart location received a unique number of headstarts based on historical site-specific release averages multiplied by 60 years (see appendix B).

5.1.2. Population Growth Rate Variable
We explored the impact of varying the rate of population growth (lambda (λ)) in future condition scenarios by selecting two plausible population growth rates.

Optimistic Population Growth Rate
We chose an optimistic population growth rate of λ=1.0 to represent scenarios in which population numbers remained stable 60 years into the future. Although this plausible population growth rate is indicative of stability rather than an increasing population, we believe that this population growth rate is optimistic for a turtle population facing many stressors.

Pessimistic Population Growth Rate
We considered several sources for a plausible pessimistic population growth rate, including population growth rates calculated from populations of other turtle species, but ultimately chose to use the best available information from the Massachusetts population of northern red-bellied cooters. Of four potential ponds in Massachusetts where enough data were available to estimate population growth rates of northern red-bellied cooters, we selected Federal Pond, because it represented the pond with the most pessimistic growth rate (λ=0.98167). This pessimistic population growth rate was calculated based on the largest population estimate prior to the initiation of headstarting (158 individuals in 1981) (Regosin et al. 2017, p. 62) and a population estimate from 2015 (84.25 individuals—derived by subtracting the estimated surviving number of headstarts (47.85 individuals) from the total individuals observed in 2015 (132.1)). An average annual survivorship (0.95) was calculated from survivorship estimates from three pond complexes (Regosin et al. 2017, p. 62) and was used with annual headstart release data to calculate the estimated surviving number of headstarts at Federal Pond in 2015.

5.2 Future Scenarios

We assessed six plausible future scenarios that explored a range of ways in which two variables, population growth rate and application of headstarting as a conservation action, might influence the Best Estimate metric and overall resiliency condition for each of the 43 AUs (table 2).

Table 2. Summary of six future scenarios considered and the variations of the headstart and population growth rate variables that were applied for each.

	No headstarts	**Rule-based headstarts**	**Historical headstarts**
Optimistic population growth rate λ = 1.0 (no population decline)	Scenario Opt-NoHS	Scenario Opt-RuleHS	Scenario Opt-HistoricalHS
Pessimistic population growth rate λ = 0.98167 (approximately 66% population decline)	Scenario Pes-NoHS	Scenario Pes-RuleHS	Scenario Pes-HistoricalHS

These six scenarios capture a range of plausible viability outcomes that the northern red-bellied cooter could exhibit within 60 years. As described in the methodology, all future condition scenarios used the same values for habitat quality metrics as were used in the current condition analysis except when modeled outputs at the 2080 time step were available to replace current condition habitat quality metric values (appendix C, table C3). See appendix B for more details on methodology.

5.2.1. Scenario Opt-NoHS

In this scenario, we assessed the future condition of AUs without additional inputs from the headstart program, and assumed population stability with no decline or increase (λ=1.0). Table C3 in appendix C shows the resulting resiliency condition scores for Scenario Opt-NoHS, in addition to the raw values used for metrics at the 2080 time step, prior to percentile ranking. See table C4 in appendix C for percentile ranks of habitat and demographic metrics. Differences between AU current resiliency condition scores and Scenario Opt-NoHS resiliency condition scores are the result of changes in habitat metrics that were modeled at the 2080 time step.

Resiliency

Under this scenario, of the 43 AUs, 9 were in high condition, 16 were in moderate condition, and 18 were in low condition (figure 4). Three AUs decreased in resiliency condition tier under this scenario compared to current condition (figure 5). Decreases in AU resiliency condition score or tier in this scenario can be attributed to a negative change in one or more of the four habitat quality metrics that were able to be modeled at the 2080 time step. At the 2080 time step, the Percent High IEI metric decreased for 74 percent of AUs, the Percent Impervious Surface metric increased for 88 percent of AUs, the Average Likelihood of Road Mortality increased for 98 percent of AUs, and the Percent Low IEI increased for 91% of AUs, each representing a negative change from current condition (table C1 and table C3 in appendix C). However, because change in habitat quality resulted in only three AUs decreasing in resiliency condition tier, we project the northern red-bellied cooter population in Massachusetts to continue to have medium overall resiliency under Scenario Opt-NoHS.

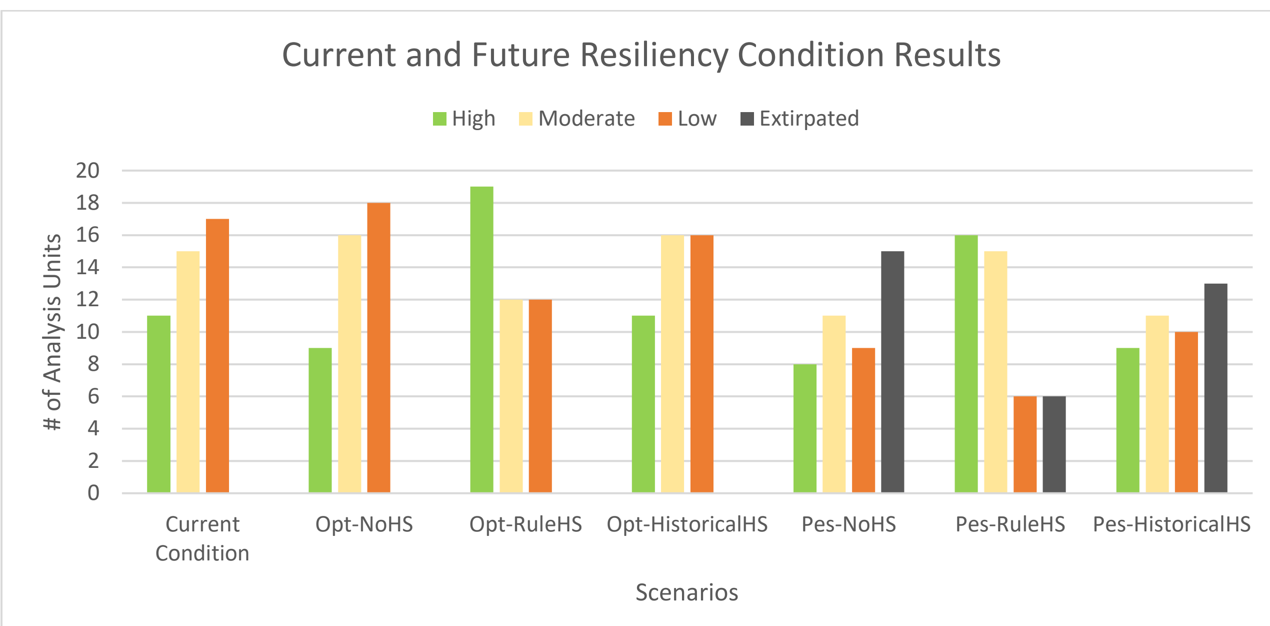

Figure 4. Massachusetts population of the northern red-bellied cooter current and future resiliency condition results by resiliency condition category

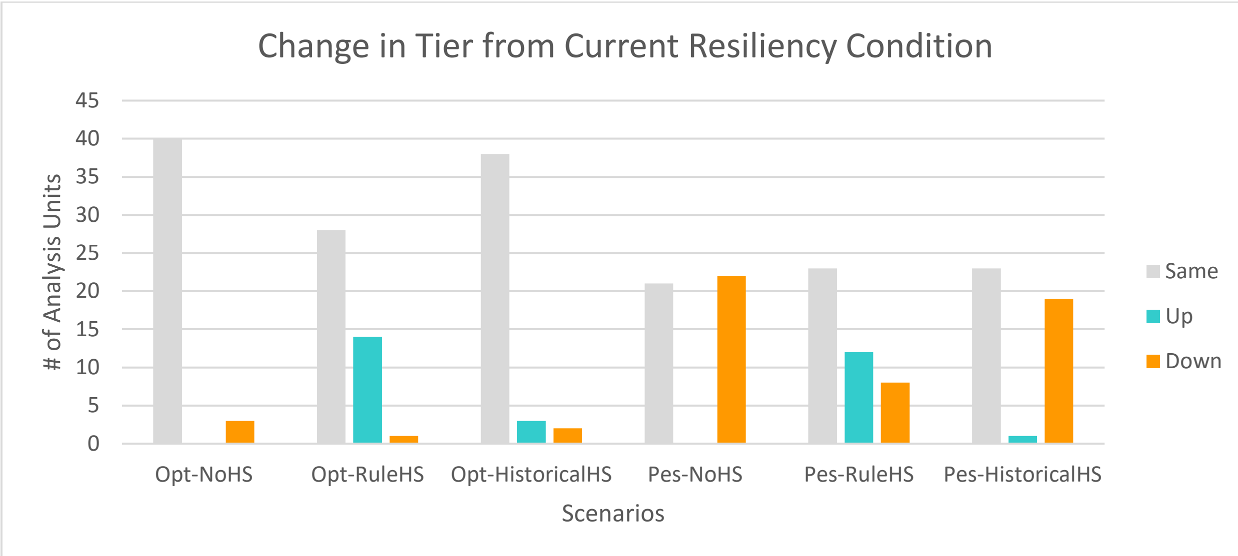

Figure 5. Number of northern red-bellied cooter SSA AUs that went up a resiliency condition category tier, down tier, or remained in the same tier in each future condition scenario as compared to current condition.

<u>Redundancy</u>
In Scenario Opt-NoHS, all AUs remained extant; therefore, we expect the species to continue to have good redundancy across its range.

<u>Representation</u>
In Scenario Opt-NoHS, the population continues to be well distributed throughout its restricted range and is expected to continue to occupy a variety of aquatic habitat types. Under this scenario, the Massachusetts population of the northern red-bellied cooter has good representation across its range

5.2.2. Scenario Opt-RuleHS
In this scenario we applied the rule-based headstart variable to the Best Estimate metric and assumed population stability with no decline or increase (λ=1.0) (appendix C, table C5).

<u>Resiliency</u>
Under this scenario, of the 43 AUs, 19 were in high condition, 12 were in moderate condition, and 12 were in low condition (figure 4). Compared to current resiliency condition, 14 AUs increased in resiliency condition tier under this scenario compared to current condition, while only 1 AU decreased. Under this scenario, the Massachusetts population of the northern red-bellied cooter has good overall resiliency.

<u>Redundancy</u>
In Scenario Opt-RuleHS, all AUs remained extant and the resilience of many AUs increased, therefore, we expect the species to continue to have good, and even increased, redundancy across its range.

Representation
In Scenario Opt-RuleHS, the population continues to be well distributed throughout its restricted range. Increased resilience of many AUs under this scenario may enhance the likelihood that this species will continue to occupy a variety of aquatic habitat types and maintain good, and even increased, representation throughout its range in Massachusetts.

5.2.3. Scenario Opt-HistoricalHS
In this scenario we applied the historical headstart variable to the Best Estimate metric and assumed population stability with no decline or increase (λ=1.0) (appendix C, table C6).

Resiliency
Of the 43 AUs, 11 were in high condition, 16 were in moderate condition, and 16 were in low condition (figure 4). Compared to current condition, 3 AUs increased in resiliency condition tier, and 2 decreased. Under this scenario, we expect the Massachusetts population of the northern red-bellied cooter to continue to have medium overall resiliency.

Redundancy
In Scenario Opt-HistoricalHS, all AUs remained extant; therefore, we expect the species to continue to have good redundancy across its range.

Representation
In Scenario Opt-HistoricalHS, the population continues to be well distributed throughout its restricted range and is expected to continue to occupy a variety of aquatic habitat types. Under this scenario, the Massachusetts population of the northern red-bellied cooter has good representation across its range

5.2.4. Scenario Pes-NoHS
In this scenario we applied a pessimistic population growth value of λ=0.98167 and assumed that no additional inputs from the headstart program would occur (appendix C, table C7).

Resiliency
In Scenario Pes-NoHS, 8 AUs were in high condition, 11 AUs were in moderate condition, 9 AUs were in low condition, and 15 were considered extirpated (figure 4). Of the 43 AUs, 22 decreased in resiliency condition tier and none increased compared to current condition. Given that so many AUs declined in resiliency condition, we project the northern red-bellied cooter population in Massachusetts to decline to poor overall resiliency under this scenario.

Redundancy
Under Scenario Pes-NoHS, the northern red-bellied cooter population in Massachusetts is expected to lose redundancy, with the extirpation of 15 populations within the next 60 years. Of the remaining 28 AUs, 9 are expected to be in low resiliency condition. The decreased number of extant populations puts the population at increased risk of catastrophic events. The redundancy is expected to decrease to poor under this scenario.

Representation

Under Scenario Pes-NoHS, representation of the northern red-bellied cooter population in Massachusetts is expected to decrease. Although the population will continue to occupy a variety of habitat types, the extirpation of 15 AUs may include some AUs in less common habitat types occupied by this population, such as rivers. Extirpation of some AUs has the potential to reduce some genetic diversity; however, there is no evidence that genetic diversity is likely to be less than at the time of listing or less than the current condition. Therefore, we expect that overall representation will either remain good or decrease to a medium level.

5.2.5. Scenario Pes-RuleHS

In this scenario we applied a pessimistic population growth rate of λ=0.98167 and applied the rule-based headstart variable to the Best Estimate metric (appendix C, table C7).

Resiliency

In Scenario Pes-RuleHS, of the 43 AUs, 16 were in high condition, 15 were in moderate condition, 6 were in low condition, and 6 were considered extirpated (figure 4). Twelve AUs increased in resiliency condition tier and 8 AUs decreased, compared to current condition. Overall, this population of northern red-bellied cooters is expected to have medium overall resiliency.

Redundancy

Under Scenario Pes-RuleHS, the northern red-bellied cooter population in Massachusetts is expected to lose redundancy, with the extirpation of 6 populations within the next 60 years. Of the remaining 28 AUs, 6 are expected to have be in low resiliency condition. The decreased number of extant populations puts the population at risk of catastrophic events. The redundancy is expected to decrease to medium under this scenario.

Representation

Under Scenario Pes-RuleHS, representation of the northern red-bellied cooter population in Massachusetts is expected to decrease. Although the population will continue to occupy a variety of habitat types, the extirpation of 6 AUs may include some AUs in less common habitat types occupied by this population, such as rivers. Extirpation of some AUs has the potential to reduce some genetic diversity; however, there is no evidence that genetic diversity is likely to be less than at the time of listing or less than the current condition. Therefore, we expect that overall representation will either remain good or decrease to a medium level.

5.2.6. Scenario Pes-HistoricalHS

In this scenario we applied a pessimistic population growth rate of λ=0.98167 and applied the historical headstart variable to the Best Estimate metric (appendix C, table C7).

Resiliency

In Scenario Pes-HistoricalHS, of the 43 AUs, 9 were in high condition, 11 were in moderate condition, 10 were in low condition, and 13 were considered extirpated (figure 4). One AU increased in resiliency condition tier compared to current condition, and 19 AUs decreased in condition tier. Overall resiliency of this population is expected to decline to poor in this scenario.

Redundancy

Under Scenario Pes-HistoricalHS, the northern red-bellied cooter population in Massachusetts is expected to lose redundancy, with 13 AUs considered extirpated. Of the remaining AUs, 10 are anticipated to be in low condition. A medium or poor level of redundancy is anticipated under this scenario where a reduced number of extant AUs and a high level of low condition AUs will result in a decrease in redundancy compared to current condition.

Representation

Under Scenario Pes-HistoricalHS, representation is expected to decrease for this species in Massachusetts. Although the population will continue to occupy a variety of habitat types, the extirpation of 13 AUs may include some AUs in less common habitat types and therefore reduce diversity of environmental conditions. Extirpation of some AUs has the potential to reduce some genetic diversity; however, there is no evidence that genetic diversity is likely to be less than at the time of listing or less than the current condition. We therefore expect that overall representation will either remain good or will decrease to a medium level.

5.3 Overall Summary of Species Viability

This species status (SSA) report describes a comprehensive review of the available data and analytical process used to assess the viability of the endangered population of the northern red-bellied cooter in Massachusetts. During this process, the resiliency, representation, and redundancy were evaluated using the best available information associated with the species' biological and environmental needs and relevant threats that may adversely impact the species to determine current condition and the future condition of the species for the next 60 years.

Overall, in the future the species will continue to be exposed to a number of threats to viability including habitat loss, fragmentation, road mortality, and human disturbance. The area in which this species occurs is expected to continue to experience land development and land use change as a result of proximity to urban areas and the coast. Modeled results available at the 2080 time step for four habitat quality metrics showed that a majority of AUs would experience some level of decline in habitat quality compared to current condition, although significance of these declines is unknown. In addition, there are other factors that may influence viability that were not included in our analysis due to lack of available information or uncertainty around the relationship between the factor and species viability.

Conservation management efforts such as population augmentation through a headstart program can help to maintain species persistence. Current condition of the northern red-bellied cooter in Massachusetts is better than the historical baseline, with improved redundancy, representation, and resiliency largely as a result of recovery efforts including a long-running headstart program. However, despite the program's success, the likelihood of conservation efforts such as this continuing for the foreseeable future is uncertain. Additional turtle conservation measures such as aquatic habitat protection, upland habitat protection to improve connectivity and protect nesting areas, habitat restoration including management of nesting areas, individual nest protection from predation and other disturbance, and efforts to reduce the likelihood of adult mortality or collection may be important strategies in the future and should be considered by managers.

Under all of our plausible future scenarios, the species is expected to persist in Massachusetts into 2080 (table C8, appendix C). In the three optimistic scenarios (Opt-NoHS, Opt-RuleHS, and Opt-HistoricalHS), AUs could not become extirpated in our model as a result of the fixed population growth rate (λ=1.0); however, this allowed us to analyze the relative shift in resiliency condition based on habitat quality metrics and the headstart conservation programs. We projected that viability would improve slightly under the most optimistic scenario, Scenario Opt-RuleHS, which is contingent on the application of a rule-based headstart program and assumes that recruitment is equal to the number of deaths. Although recruitment has been documented at some AUs, it is unknown whether recruitment occurs at all AUs, and a pessimistic growth rate at some AUs is plausible. In our three most pessimistic scenarios (Pes-NoHS, Pes-RuleHS, and Pes-HistoricalHS) a loss of viability is expected. However, even in our most pessimistic scenario, Scenario Pes-NoHS, some AUs are expected to maintain high resiliency condition scores despite the loss of overall resiliency, redundancy, and representation when a pessimistic growth rate is assumed and the headstarting program does not continue. The number of AUs that are expected to persist in high condition in our most pessimistic scenario (n=8; table C7, appendix C) is greater than the number of AUs that include the original ponds with known occurrences documented prior to 1985 (n=7; table C5, appendix C). Although this portrays a better scenario than existed at the time of listing in the 1980s, it does reflect a population in decline. The life history of turtle species such as the northern red-bellied cooter, which are characterized by long life spans and late sexual maturity, may make it difficult for managers to recognize declines in populations over short periods of time as adult individuals may persist for many years or decades in suboptimal habitat even with little or no annual recruitment. Although our scenarios did not account for establishment of new AUs or natural recolonization of extirpated AUs through dispersal, we expect that some AUs with a low number of individuals and a pessimistic population growth rate may experience periods of both occupancy and extirpation and that new occurrences outside of our AUs could be established through either natural dispersal or management programs. Assessing a range of optimistic and pessimistic scenarios allowed us to project a plausible range of future conditions for each AU as well as overall viability.

This concludes our assessment of the needs, current condition, and future condition of the northern red-bellied cooter population in Massachusetts. To better assess the status of the species in the future, continued long-term monitoring, periodic intensive monitoring, and research of the species is needed. Assessment of current and planning for future conservation efforts, including the long-running headstart program, is important for adaptive management of this species. This SSA should be updated as new information becomes available.

CHAPTER 6 – KEY UNCERTAINTIES

Through the course of this analysis, it was necessary to make certain assumptions. These assumptions introduce uncertainty into our assessment of current condition and our projections of future conditions under a variety of scenarios. The following are uncertainties recognized in this report:

- *Analysis units*—AUs in our analysis include at least one occupied water body. We assumed that any water body which overlapped with a known occurrence record was an occupied water body; however, it is unknown whether or not all of the occupied water bodies identified represent established subpopulations because some occurrence records may represent individuals dispersing through aquatic environments or crossing roads nearby to water bodies. In identifying occupied water bodies we did not distinguish between well-known populations and occurrences with less information. We assumed, based on our current understanding of individual's movement patterns, that pond complexes were appropriate AUs, when occupied water bodies were less than 400 m (1312 ft) apart, despite historical treatment of ponds as individual populations. Additionally, we assumed it was appropriate to aggregate demographic information from individual water bodies up to the AU level, and as such, lost some ability to look at detailed information available for individual water bodies when we combined multiple water bodies into the same AU. Another source of uncertainty includes the potential for undocumented natural colonization of water bodies and the presence of undetected natural populations. These assumptions may have resulted in under- or overestimation of the number of AUs.
- *Multi-pond metric*—We only examined whether or not multiple occupied water bodies existed in an AU and did not take into account that suitable habitat may be present in ponds with no occupancy record surrounding or nearby to AUs. This approach allowed us to focus on known suitable habitat for this metric. Another possible approach would have been to consider any surrounding or nearby water body to be potentially suitable habitat, which would likely have increased the number of AUs that scored highly in the multi-pond metric.
- *Best Estimate*—We assumed that comparing the best available demographic information available for each AU was appropriate given that we had detailed information for some AUs and almost no demographic information available for others. We also assumed that there was no upper limit to population size after which condition would decline and always considered larger Best Estimate values to be better when calculating final resiliency condition scores. It is possible that some AUs with only a single historical observation are no longer currently occupied; however, we assumed that every AU had at least one individual present for our current condition analysis. Other sources of error in our Best Estimate include immigration from other water bodies, emigration away from occupied water bodies, and potential for recolonization after extirpation.
- *Age at first reproduction*—It is unknown at what age this species can begin breeding, and headstarts may be able to reproduce earlier due to their larger size at release than nonheadstarted individuals of the same cohort. If headstarts do reproduce at a significantly younger age, we could have overestimated the number of recruits per female

if some of the recruits were actually from headstarts breeding at an earlier age. This would have affected our calculations for Best Estimate for those AUs where headstarting data were used.

- *Population growth rate (λ)*—When deciding on population growth rates and how to apply them to our Best Estimate metric for our future condition analysis, we applied a single population growth rate to all AUs in each scenario, and acknowledge that in reality, population growth rates are not likely to be the same across all subpopulations and may not be the same from year to year. We assumed the pessimistic population growth rate we calculated for the future condition analysis was plausible and a good representation of a real pessimistic population growth rate calculated from Massachusetts population data. However, this population growth rate may actually be optimistic because we can't remove recruits from headstarted individuals. Therefore, it does not represent the population growth rate of a population without the added influence of a headstarting conservation action. We considered using an even more pessimistic population growth rate for a hypothetical scenario in which no recruitment was occurring, in which case lambda would be equal to the adult survivorship rate of a declining population in Massachusetts. While this is a possible population growth rate for some portions of the population, we decided to move forward with the more plausible pessimistic population growth rate derived from Federal Pond data.
- *Shallow vegetated coves*—We are uncertain exactly what role these habitat features play in northern red-bellied cooter life history; however, we assumed that they are important in some way and used Water Body Shape Complexity as a proxy for the presence of these coves.
- *Winter kill*—We know that winter kill has occurred in some ponds in recent years, but do not know what the minimum pond depth is to avoid this. Our uncertainty led us to leave this known factor influencing viability out of our analysis.
- O*ne-time stochastic events*—we were unable to identify any plausible stochastic events for which we could estimate or model the northern red-bellied cooter's response with an acceptable level of certainty.
- *Habitat quality metrics in future scenarios*—In our future condition analysis, we assumed that some habitat quality metrics would not change over time based on available data and our evaluation of what factors were likely to change within the time step and be most important to future condition of the population. Metrics that did not change between current and future condition include those that measured the complexity of water body shapes in an AU (Water Body Shape Complexity), whether AUs consisted of multiple water bodies (Multi-pond Complex), the percent of water body shoreline of each AU that was protected (Percent Shoreline Protected), and the percent of protected land surrounding AUs (Percent Protected Land).
- *Conservation efforts*—Future conservation efforts are dependent on funding availability, available conservation opportunities, and the willing cooperation of our partners, so likelihood of continuation of them, including the headstart program, or how they will be implemented in the future is unknown.
- *Climate Change*—We are uncertain of how climate change may impact northern red-bellied cooters or their habitat, and do not have enough information to estimate the relationship between this factor and species viability. Although there are a variety of ways in which climate change may influence precipitation and aquatic habitat or expose

northern red-bellied cooters to increased pressures from pathogens or invasive species, it is unclear to what extent these factors may affect the Massachusetts population.

- *Disease/Pathogens*—We are uncertain of how susceptible northern red-bellied cooters are to various potential pathogens and what the long-term fitness consequences of illness may be. Therefore, while we know that disease and pathogens are a potential factor influencing viability, we were unable to assess differences between AUs or make predictions around future impacts.
- *Predation*—High nest and hatchling predation rates are a factor that is known to directly impact northern red-bellied cooters and are expected to continue to be a factor limiting the northern red-bellied cooter population at most ponds. However, we assume that predation pressures are similar across all ponds. We are uncertain about whether predation rates may change in the future.
- *Contaminants/water quality*—Water quality or contaminants in aquatic environments are a potential concern for any aquatic species, although we are uncertain about what water quality attributes may impact species viability or what responses to contaminants might be. Water quality data are not available for all water bodies where this species occurs, so we did not attempt to assess this factor in our analysis.
- *Collection*—Although collection does not seem to be a major threat to this species currently, the illegal trade in freshwater turtles is a rapidly changing crisis that may cause demand to shift to new species in the future as global turtle populations decline. Overexploitation of this species may be hard to detect but any threat resulting in loss of adults from the population through collection or mortality has the potential to influence the overall viability.

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APPENDIX A Current Condition Analysis Methodology

A.1. Units of Analysis

To create the analysis units (AUs) for northern red-bellied cooter, we used element occurrence (EO) data provided by the Massachusetts Natural Heritage and Endangered Species Program (NHESP) to identify occupied water bodies in Plymouth County and Bristol County. These data include well-known populations as well as occurrences that we have less knowledge about, and we did not distinguish between EO types while identifying occupied water bodies and the resulting AUs. Occupied water bodies were identified and selected from the National Hydrography Dataset (NHD) (USGS 2019, entire) water body layer, where polygons or points in the EO layer overlapped with water body polygons in the NHD layer. We did not select for specific types of water bodies in the NHD layer while identifying occupied water bodies. In addition to the areas identified in the EO layer as evidence of occupancy, we also included five water bodies (Dunham Pond, College Pond, Curlew Pond, East Head Bog Pond South, and Little Herring Pond) that are known to be occupied based on a recent occupancy survey (Regosin et al. 2017, entire). In two instances, water bodies were manually combined in order to be treated as a single system to correct for inconsistencies between the water body polygons and expert knowledge of the sites (East Head Pond and East Head Bog Ponds; and Burrage Lower and Upper Reservoirs). Any EOs that occurred on land and did not overlap with a water body in the NHD data layer were not included or 'attached' to a nearest water body for analysis and were not included in the creation of AUs. A 400-m (1312-ft) buffer was applied to all selected water bodies, and water bodies that had overlapping 400-m (1312-ft) buffers were grouped into AUs. The 400-m (1312-ft) buffer was selected based on a natural break in regular movement between water bodies as based on expert opinion and a study conducted in Plymouth County (Regosin et al. 2017, entire). Individuals are expected to move between water bodies at greater distances apart within a longer timeframe. One water body polygon in AU 35 did not include the entirety of the underlying larger water body identified from base maps and was delineated manually.

A separate rule was created for EO points that occurred in rivers. For these rivers, occupied flowlines from the NHD (USGS 2019, entire) were used instead of occupied water body polygons to establish riverine AUs. Segments of a flowline were selected that extended 2000 m (6562 ft) upstream and downstream from an EO in these rivers, and then a 1-m (3-ft) buffer was applied to this selected segment to create a river AU. Although NatureServe suggests a separation distance of at least 20 km (12 mi) for aquatic/wetland habitat for *Pseudemys* species in general (NatureServe 2021, entire) and we expect that northern red-bellied cooters may move more easily through aquatic habitat than upland habitat, we don't have much information about regular movements of northern red-bellied cooters in rivers. Therefore, the 2000 m (6562 ft) distance was selected as a biologically appropriate and conservative distance by which we could examine habitat surrounding river occurrences. If resulting AUs overlapped, the units were combined into a single AU.

A.2. Metrics

Final Metric Selection Process

Before final metrics were selected, a series of surveys and focus group discussions were used to rank the original list of a variety of potential metrics (table A1) and reduce the list to a smaller number of potential metrics (table A2). The shortened metric list was then further reduced through examining correlation and information availability. See figure A1 for a correlation matrix used to eliminate redundant variables from the short list of candidate variables.

Table A1. Survey results ranking original list of potential metrics from 1-10 (least to most important). R1-R5 indicate individual survey respondents.

Metric	R1	R2	R3	R4	R5	Mean
Percent of shoreline protected	9	10	9	8	8	8.8
Total estimated population size	9	10	5	9	10	8.6
Number of individuals	8	10	4	9	10	8.2
Average likelihood of road mortality within 400 m (1312 ft) buffer	6	10	8	7	10	8.2
Percent of protected land within 400 m (1312 ft) buffer	9	9	9	8	5	8
Percent of pond + 400 m (1312 ft) buffer in top 3rd of Index of Ecological Integrity	7	10	8	8	5	7.6
Percent juveniles	8	6	4	9	10	7.4
Average Road Density within 400 m (1312 ft) buffer	7	10	8	9	1	7
Perimeter: Area ratio	7	10	8	5	4	6.8
Percent of pond + 400 m (1312 ft) buffer in bottom 3rd of Index of Ecological Integrity	8	5	8	8	5	6.8
Impaired for aquatic life use (1) or not impaired/not assessed/not enough info (0)	7	8	8	2	8	6.6
SHAPE index-(perimeter/ minimum circumference given pond area)	7	10	8	3	4	6.4
Headstart totals	7	8	4	3	10	6.4
Percent of 400 m (1312 ft) buffer consisting of impervious surfaces	7	6	9	8	1	6.2
Fractal dimension index-(2 * ln (perimeter) / ln(area))	7	10	8	2	4	6.2
Total area of protected land within 400 m(1312 ft) buffer	9	9	6	5	1	6
Total area or proportion of pond surface vegetation based on aerial imagery classification or visual examination	7	8	6	4	2	5.4
Presence of pond surface vegetation (Y/N) based on aerial imagery classification or visual examination	8	2	7	7	2	5.2

Metric	R1	R2	R3	R4	R5	Mean
Percentage area of wetlands within pond + 400 m (1312 ft) buffer	7	1	5	8	5	5.2
Number of headstarts 2013-2016	3	5	4	4	10	5.2
Average landscape diversity in 400 m (1312 ft) buffer	6	8	4	4	3	5
Percent of 400 m (1312 ft) buffer that is above average landscape diversity (+ 1 SD)	6	8	4	4	3	5
Female : Male ratio	8	6	3	6	1	4.8
Element occurrences	7	1	2	4	10	4.8
Total counts occupancy assessment	8	8	2		5	5.75
Number of juveniles	8	1	4	8	1	4.4
Average likelihood of road mortality within 1 km (0.6 mi) buffer	3	8	5	3	3	4.4
Number of natives	8	6	2	1	5	4.4
Number of previously un-notched	8	6	1	5	1	4.2
Average Road Density within 1 km (0.6 mi) buffer	4	8	5	3	1	4.2
Percent native captures	4	5	1	8	1	3.8
Percent previously notched	4	6	1	4	1	3.2
Number of females	7	1	1	5	1	3
Shannon diversity index of wetland type	7	1	4		3	3.75
Number of males	7	1	1	4	1	2.8
Shannon evenness index of wetland type	6	1	4		3	3.5
Number notched pre-2013	2	6	1	2	1	2.4
Total captures	4	1	1	2	1	1.8
Number of notched unknown	1	1	1	1	1	1
Average recapture rate	1	1	1	1	1	1
Presence of invasive species*						
Soil type*						
Diversity of water body depth*						

*Excluded pre-survey due to data limitations

Table A2. Survey results weighting final metrics from 1-10 (least to most important). The three lowest scoring metrics were not used in current or future condition analyses. R1-R5 indicate individual survey respondents.

Metric	R1	R2	R3	R4	R5	Mean
Percent of shoreline protected	10	10	9	10	8	9.4
Best Estimate	9	8	9	10	10	9.2
Percent protected land within 400 m (1312 ft) buffer	9	8	9	10	7	8.6
Percent impervious surface within 400 m (1312 ft) buffer	7	6	7	9	0	5.8
Average likelihood of road mortality within 400 m (1312 ft) buffer	7	6	7	7	6	6.6
Fractal dimension index- shape complexity (2* ln (perimeter) / ln(area))	5	4	7	7	9	6.4
Percent of analysis unit + 400 m (1312 ft) buffer in top 3rd of Index of Ecological Integrity	2	7	5	8	7	5.8
Multi-pond complex (Y/N) based on visual examination	5	3	8	4	5	5
Percent of analysis unit + 400 m (1312 ft) buffer in bottom third of Index of Ecological Integrity	2	5	7	5	6	5
Presence of pond surface vegetation (Y/N) based on visual examination	1	1	6	1	4	2.6
Impaired for aquatic life use (Y/N)	2	3	2	1	2	2
Percent of analysis unit + 400 m (1312 ft) buffer consisting of wetland area	2	2	2	1	3	2

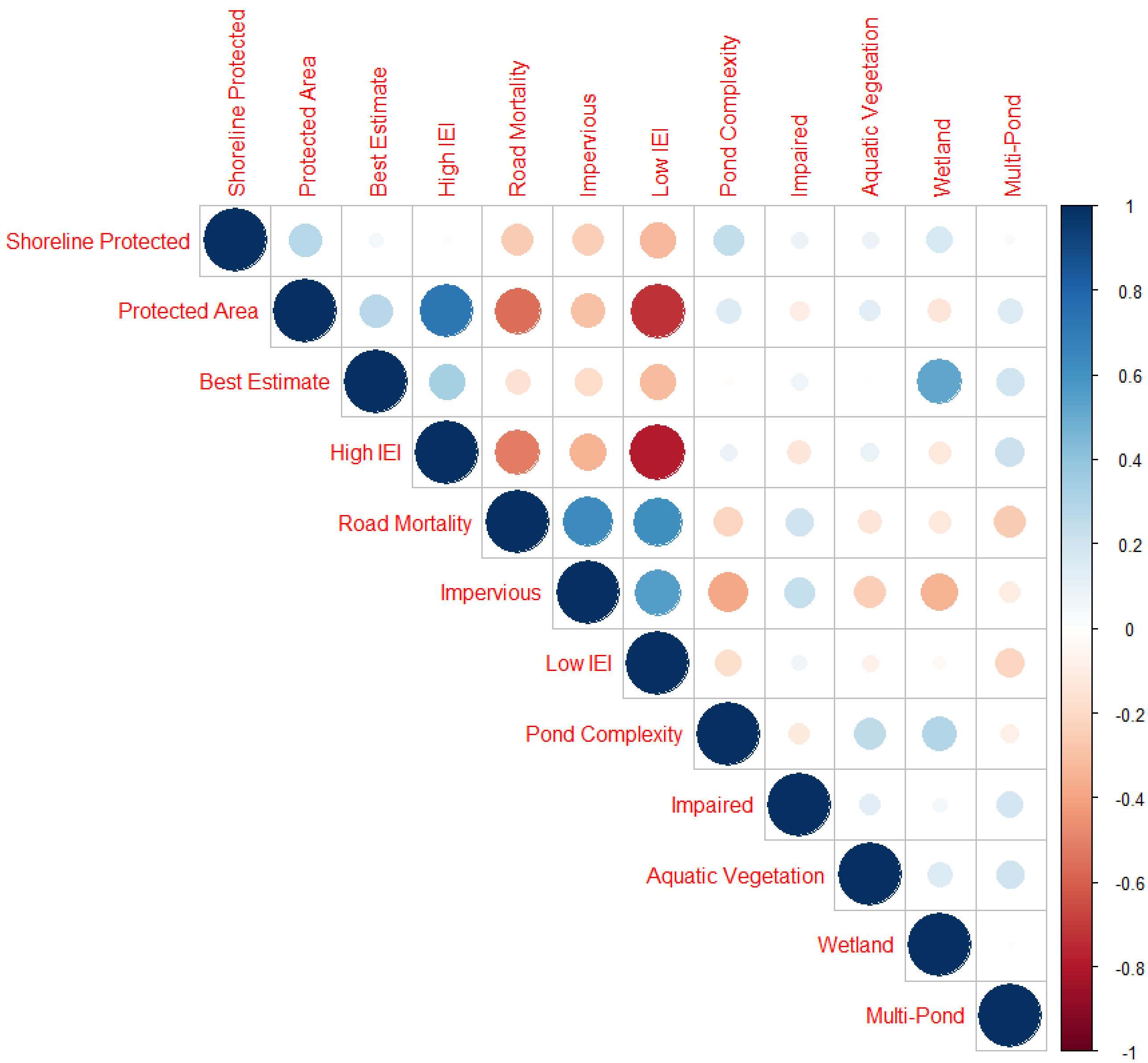

Figure A1. Correlation matrix used to assess redundant or strongly correlated variables. Red indicates negative correlation, blue indicates positive correlation and size of the circle indicates strength of correlation

Three semi-final metrics (impairment, aquatic vegetation, and wetland) were considered but were eliminated from our final list of metrics due to lack of available information or uncertainty around the relationship between the metric and northern red-bellied cooter resiliency.

The impairment metric was generated using the Integrated List of Waters 303(b)/303(d) (MassDEP 2014, entire). A spatial join was conducted to determine if any of the water bodies in each AU were designated as 'Not Supporting,' specifically for the designated use of 'Aquatic Life,' as described in the 'IL_ADB_2014' lookup table. If so, the AU was designated as impaired for aquatic life. If the water bodies in the AU were designated as 'Fully Supporting,' 'Not Assessed,' or 'Insufficient Information,' the AU was designated as not impaired. The impairment metric was eliminated from the candidate list because of its data deficiency. See table A3 below for the designation breakdown for the 119 water bodies comprising the AUs.

Table A3. Integrated List of Waters (MassDEP 2014, entire) designations for water bodies in AUs.

Integrated List of Waters Designation	Number of water bodies
Not Supporting	23
Fully Supporting	4
Not Assessed	45
Insufficient Information	9
Not in dataset	38

The aquatic vegetation metric was generated by visual examination of National Agricultural Inventory Program (NAIP) composite imagery (Farm Service Agency [FSA] 2016–2019, entire). The images consisted of four NAIP tiles that contained the near-infrared band: two from July 4, 2016 and two from Sept 29, 2018. Each AU was examined in the NAIP imagery for presence (1) or absence (0) of surface vegetation on any of its component water bodies. The aquatic vegetation metric was eliminated from the candidate list due to expert opinion that this metric did not accurately or adequately capture food availability in shallow vegetated coves.

The wetland metric was generating using the National Wetlands Inventory (USFWS 2019, entire) layer for Massachusetts. All wetland types were included, including agricultural or cranberry bogs (NWI Classification Codes). The wetland metric was derived by calculating the total area of wetlands within a 400 m (1312 ft) buffer of water bodies in the AU, including water bodies themselves. The total area of wetlands was divided by the total area of the buffer zone and multiplied by 100 to derive percent wetland. The percent wetland metric was eliminated from the candidate list due to the unclear relationship with protection status as well as high-quality habitat/food availability for the species.

<u>Demographic Metric</u>
Demographic information from the Regosin et al. (2017, entire) report, headstart release table, and element occurrence data table were appended to each water body and aggregated up to the AU scale using the Dissolve tool. The total population estimate was taken from table 5 in Regosin et al. (2017, p. 62) which describes estimates for pond complexes and individual ponds. In either case, the total population estimate was attributed to the entire AU comprising the pond complex or individual ponds. Occupancy assessment estimates were taken from table 6a in Regosin et al. (2017, p. 63). The occupancy estimates were aggregated to the AU by summing the maximum of the three survey results for each water body in the AU. The headstart estimates

(accounting for survivorship and recruitment—see below for details) were aggregated to the AU by summing the headstart estimates for each water body in the AU. The EO data were aggregated by attributing to the AU the maximum number of individuals sighted in any one EO in any of the water bodies in the AU.

The following four variables were used to populate a 'Best Estimate' metric for each AU:

1. Total population estimate from Regosin et al. (2017, p. 62)
2. Headstart numbers accounting for survivorship and recruitment
    a. An average survivorship rate of 0.9509 from Regosin et al. (2017, table 5, p.62) derived from three ponds (East Head, Halfway, Sampsons) was applied over each year in the summarized headstart release table from 1985-2019 (MADFW, unpubl. data), to derive a headstart estimate corrected for survivorship.
        i. Exception: 22 headstart individuals released in 1998 in the Nemasket River were re-assigned to Great Quittacas in to reflect where releases occurred in relation to AUs.
    b. An average recruitment corrective factor of 1.3929 from Regosin et al. (2017, table 5, p. 62) derived from three ponds (East Head, Halfway, Sampsons) was then applied to the survivorship-corrected headstart estimate, to derive the final headstart attribute, accounting for survivorship and recruitment
        i. The recruitment corrective factor was calculated as: (Estimated total population without recent headstarts)/(estimated total number of presumed headstarts)
3. Maximum occupancy total from a three-survey occupancy assessment from Regosin et al. (2017, table 6a., p. 63)
4. Element occurrence data (MADFW, unpubl. data)

The Best Estimate metric was calculated as follows:

1. If a total population estimate existed for the analysis unit, this was used as the Best Estimate value.
2. If no total population estimate existed, and headstart and/or occupancy assessment information was available, the maximum of the annualized headstart attribute and the occupancy assessment attribute was used as the Best Estimate value.
3. If no total population estimate, headstart, or occupancy assessment information was available, the maximum element occurrence data—observations at any one point of time—was used as the Best Estimate value.
    a. Exception: AU 41 manual edit—a maximum observed number of one individual was selected because the larger occurrence was associated with AU 42.
    b. Exception: AU 19 and AU 21—the second highest observation (two individuals) was selected for each because the larger number of observed individuals recorded was associated with the AU 18 and AU 17, respectively.

<u>Habitat Protection Metrics</u>
Two metrics related to protected area were included in the current condition analysis: 1) Percent Shoreline Protected, to capture the level of protection of the water bodies in each AU, and 2) Percent Protected Land in a 400 m (1312 ft) buffer, to capture the level of protection of the

contextual landscape surrounding the AU. A composite dataset of protected lands was created by combining a selection of each of three datasets:

1. Recreational open space dataset (Massachusetts Bureau of Geographic Information [MassGIS] 2020, entire) where the primary purpose was listed as 'Conservation', 'Recreation and Conservation', 'Water Supply Protection', or 'Flood Control.'
2. Secured lands dataset (The Nature Conservancy 2018, entire) where protected status Gap Code was equal to 1 or 2, indicating the land is 'managed for biodiversity'
3. Protected Areas Database of the United States (PAD-US 2.0, USGS 2018, entire) where protected status Gap Code was equal to 1 or 2, indicating the land is 'managed for biodiversity'

The Percent Shoreline Protected metric was derived by calculating the length of total shoreline of the water bodies in each AU that is within 50 m (164 ft) of a protected area. The 50 m (164 ft) buffer was generated to account for the slight discrepancies between the boundaries in the protected lands composite dataset and the water body boundaries. The total length of shoreline within the protected area buffer zone was then divided by the total length of shoreline of the water bodies and multiplied by 100 to derive the percentage of the total shoreline protected.

The Percent Protected Land metric was derived by calculating the total area of the protected lands composite dataset falling within a 400 m (1312 ft) buffer of water bodies in the AU, excluding water bodies themselves. The total area of protected land was divided by the total area of the buffer zone and multiplied by 100 to derive Percent Protected Land.

Habitat Integrity Metrics

A metric for Water Body Shape Complexity was included in the current condition analysis to indicate sinuosity of the shoreline, as a proxy for presence of shallow vegetated coves. The fractal dimension index (FDI) was used to capture water body complexity within each AU. The index is a measure of shape complexity, given the total perimeter and area of all water bodies in AU and was calculated with the following equation:

$$FDI = 2 * \ln(\text{perimeter}) / \ln(\text{area})$$

The index value approaches 1 for shapes with very simple perimeters and approaches 2 for shapes with highly convoluted perimeters.

The Index of Ecological Integrity (IEI) dataset (NALCC 2017, entire) used to generate the Percent High IEI metric to indicate the degree of high habitat integrity of the AU and its surrounding landscape. IEI includes a multitude of ecological metrics that pertain to two main categories of landscape metrics: 1) intactness, or freedom from human influences and 2) resiliency, or the capacity to recover from disturbance (McGarigal et al. 2017a, entire). The index ranges from 0 to 1 and was reclassified into three categories, low (0-0.33), medium (0.33-0.66), and high (0.66-1) ecological integrity. The Percent High IEI habitat integrity metric was derived by calculating the percentage of area categorized as high ecological integrity within each AU plus a 400 m (1312 ft) buffer of the water bodies.

The multi-pond complex binary attribute was added to capture whether or not there are multiple water bodies within the AU to provide additional habitat and resiliency to the northern red-bellied cooter population. Multi-pond complex was coded as presence (1) or absence (0) of multiple ponds in the complex based on a visual examination of each AU.

<u>Habitat Degradation Metrics</u>
A Percent Impervious Surface metric was included in the current condition analysis to characterize the threat of human influence (roads, buildings, etc.) on the landscape surrounding each AU. The metric was derived by taking the percentage of the total land area within a 400 m (1312 ft) buffer of the water bodies in each analysis unit, excluding the water bodies themselves, that is comprised of impervious surfaces using a 2016 Land Cover/Land Use Dataset (MassGIS 2019, entire).

Threat from roads specifically was also incorporated as a metric, using a road-crossing wildlife mortality model (v3.0, Grand 2014, entire) developed by Gibbs and Shriver (2002, entire), specifically for turtles. To create an Average Likelihood of Road Mortality metric, the average probability of road mortality was calculated over the land area within a 400 m (1312 ft) buffer of the water bodies within each AU, excluding the water bodies themselves.

The Index of Ecological Integrity was again used to generate a metric indicating the degree of low habitat integrity of the AU and its surrounding landscape. See ‘Habitat Integrity Metrics’ section above for further details. The Low IEI habitat degradation metric was derived by calculating the percentage of area categorized as low ecological integrity within each AU plus a 400 m (1312 ft) buffer of the water bodies.

A.3. Final Scoring

<u>Weights</u>
Final weights for the final list of metrics were obtained through a survey process of allocating 100 points among metrics that collected responses from 6 individuals (table A4). See table A5 for final weights.

Table A4. Survey results allocating 100 points among metric groups from 6 respondents (R1-R6) for the purpose of assigning weights to each metric.

Metric	Metric Category	Final Weights	Final Group Weights	R1	R2	R3	R4	R5	R6	Mean
Best Estimate	demographic	40	40	40	30	30	40	50	40	38
Percent Shoreline Protected	habitat quality-protection	13	20	15	25	25	20	10	20	19
Percent Protected Land		7								
Water Body Shape Complexity	habitat quality-integrity	8	20	20	15	25	20	15	10	18
Percent High IEI		4								
Multi-pond Complex		8								
Percent Impervious Surface	habitat quality-degradation	8	20	25	30	20	20	25	30	25
Average Likelihood of Road Mortality		8								
Percent Low IEI		4								

Table A5. Final weights for all Species Status Assessment metrics.

Metric Name	Metric Description	Metric Category	Final Weights	Final Group Weights
Best Estimate		demographic	40	40
Percent Shoreline Protected	Percent of shoreline protected	habitat quality-protection	13	20
Percent Protected Land	Percent of protected land within 400 m (1312 ft) buffer		7	
Water Body Shape Complexity	Fractal dimension index- measure of shape complexity (2* ln (perimeter) / ln(area))	habitat quality-integrity	8	20
Percent High IEI	Percent of analysis unit + 400 m (1312 ft) buffer in top 3rd of Index of Ecological Integrity		4	
Multi-pond Complex	Multi-pond complex (Y/N) based on visual examination		8	
Percent Impervious Surface	Percent impervious surface within 400 m (1312 ft) buffer surrounding AU	habitat quality-degradation	8	20
Average Likelihood of Road Mortality	Average likelihood of road mortality within 400 m (1312 ft) buffer around AU		8	
Percent Low IEI	Percent of analysis unit + 400 m (1312 ft) buffer in bottom third of Index of Ecological Integrity		4	

Scoring

For each of the AUs, the raw values of each of the final metrics were converted to a percentile rank based on the distribution of the values across all AUs. For the multi-pond complex binary variable, multi-pond complexes were scored as 100 and single pond complexes were scored as 0. For the three metrics under the habitat quality-degradation category, the percentile ranks were inverted to indicate the inverse relationship (higher raw value → lower score for resiliency condition). Each of the converted percentile rank metrics were then multiplied by their corresponding weight and added together to get a final current resiliency condition score from 0-100.

Tier Designation
The natural breaks in the distribution of current resiliency condition scores were used to designate two thresholds to divide the AUs into three tiers of resiliency condition. Final scores greater than 58.773 were Tier 1(High), final scores from 43.926–58.773 were Tier 2 (Moderate), and final scores less than 34.926 were Tier 3 (Low).

A.4. Sensitivity Analysis

We tested the sensitivity of varying model weights on index scores using the current condition analysis modeling framework. We used the current condition model weights, index scores, and three-tier cutpoint values as benchmarks when comparing results from the sensitivity analysis. We tested a range of variable weights while holding the variable values for each AU constant. We let weight values range ± 10 around selected current condition model weights that were ≥ 10 (e.g., Best Estimate weight value ranged from 30-50), and let weights range from 0-20 for variables that had selected weights <10. As we increased or decreased a variable's weight value, we applied the difference evenly to all other variable weights; therefore, the sum of all weights always equaled 100 (e.g., if we increase by 1 we subtract 0.125 from the remaining eight variable model weights). Weight values were then divided by 100 and applied in a linear model to the AU variable values. This method allowed us to test 21 different variable weight scenarios per variable per AU and measure the difference in the model results to the current condition model's benchmark values. We summarized the absolute change in index values per AU per variable to visually inspect which variables exhibited the greatest change in index values over the range of weights tested (figure A2). We summarized how many AUs changed tiers over the range of weights tested for each variable (table A6, figures A3 and A4). We plotted the number of AUs in each tier for each weight scenario tested figure A5).

We found that the variables multi-pond and shoreline complexity exhibited the greatest influence on model results (figures A3 and A4). There were 33 AUs that did not change tiers over the range of weights tested for all variables. The remaining 10 AUs that did change tiers were generally those that had index values near the three-tier cutpoint values, and the majority of AUs only changed tiers for ≤ 3 variables (table A6). The number of units that changed a tier over the range of weights tested for each variable ranged from 3-6 (figure A3). There were two AUs that changed a tier over the range of weights tested for each variable, and four units that changed a tier for only one variable (table A6). Model output was surprisingly stable over the range of values tested for each variable, and there was more variation in the number of AUs in the middle and low tiers for the weight scenarios tested (figures A4 and A5).

Table A6. The 10 AUs that changed a tier over the range of weights tested for at least one variable, and the number of variables where a change in tier was observed.

Analysis Unit	Number of Variables in Which an AU Changed Tiers Over the Range of Weights Tested
AU 40	1
AU 12	1
AU 4	1
AU 10	1
AU 20	2
AU 27	3
AU 29	5
AU 8	6
AU 23	9
AU 37	9

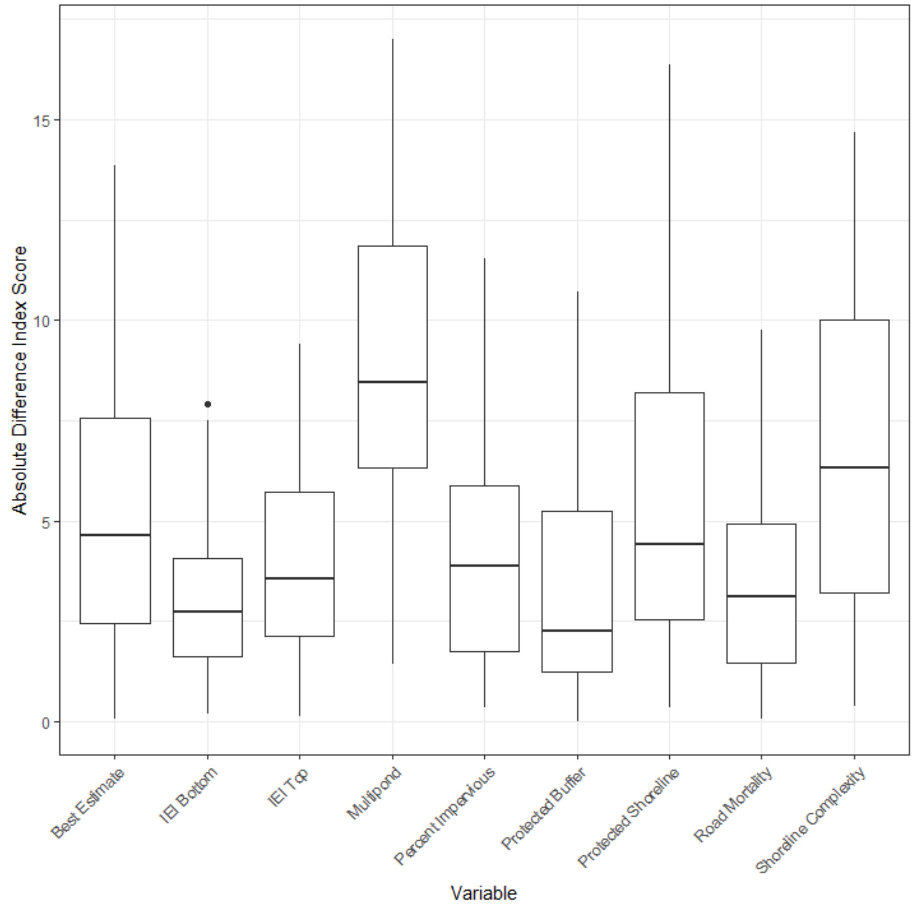

Figure A2. Boxplots depicting the absolute difference in index values over the range of weights tested per variable per analysis unit.

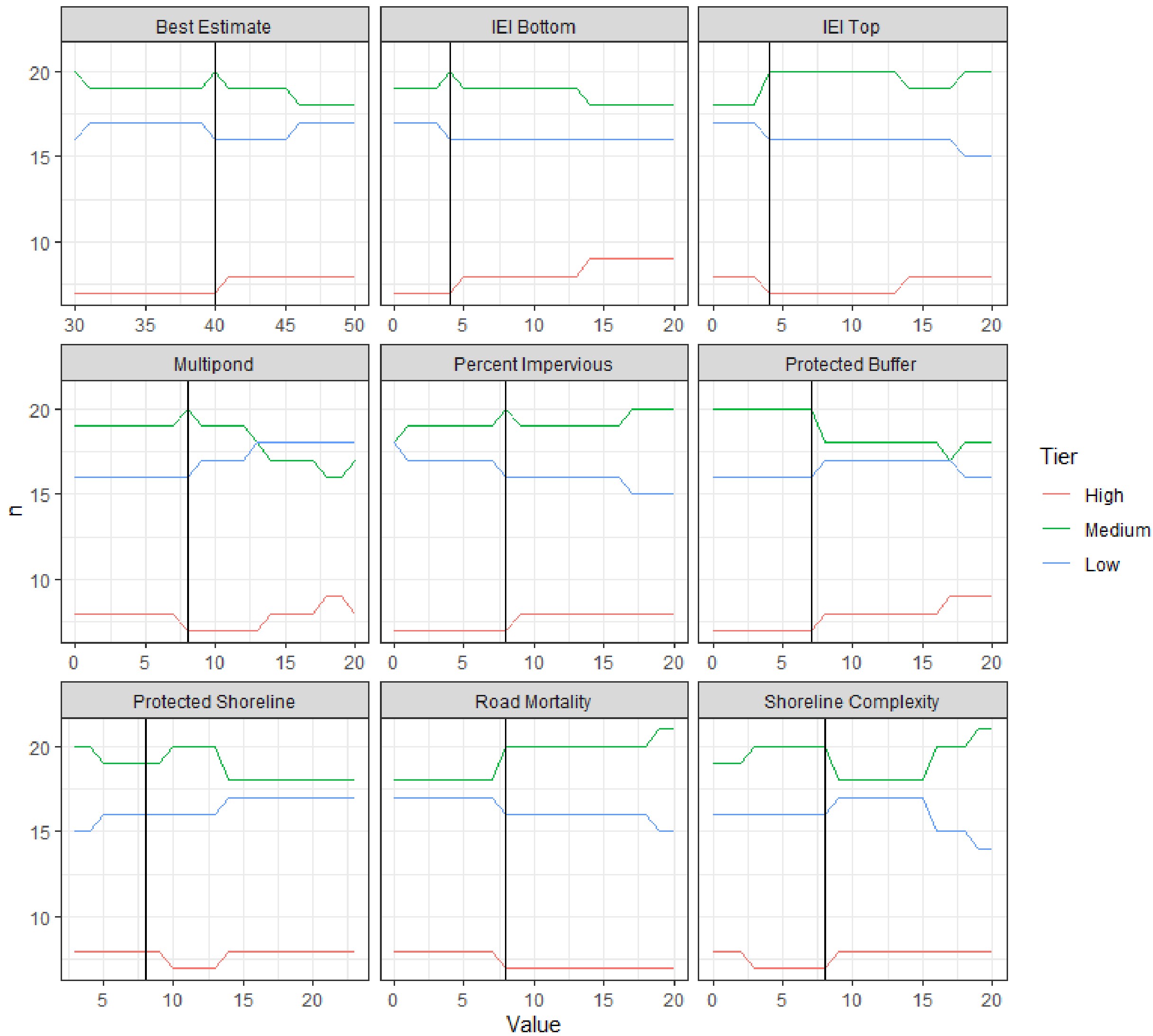

Figure A3. Line graph depicting the number of analysis units in high, medium, and low tiers over the range of weights tested per variable. Vertical lines indicate the selected current condition model variable weight.

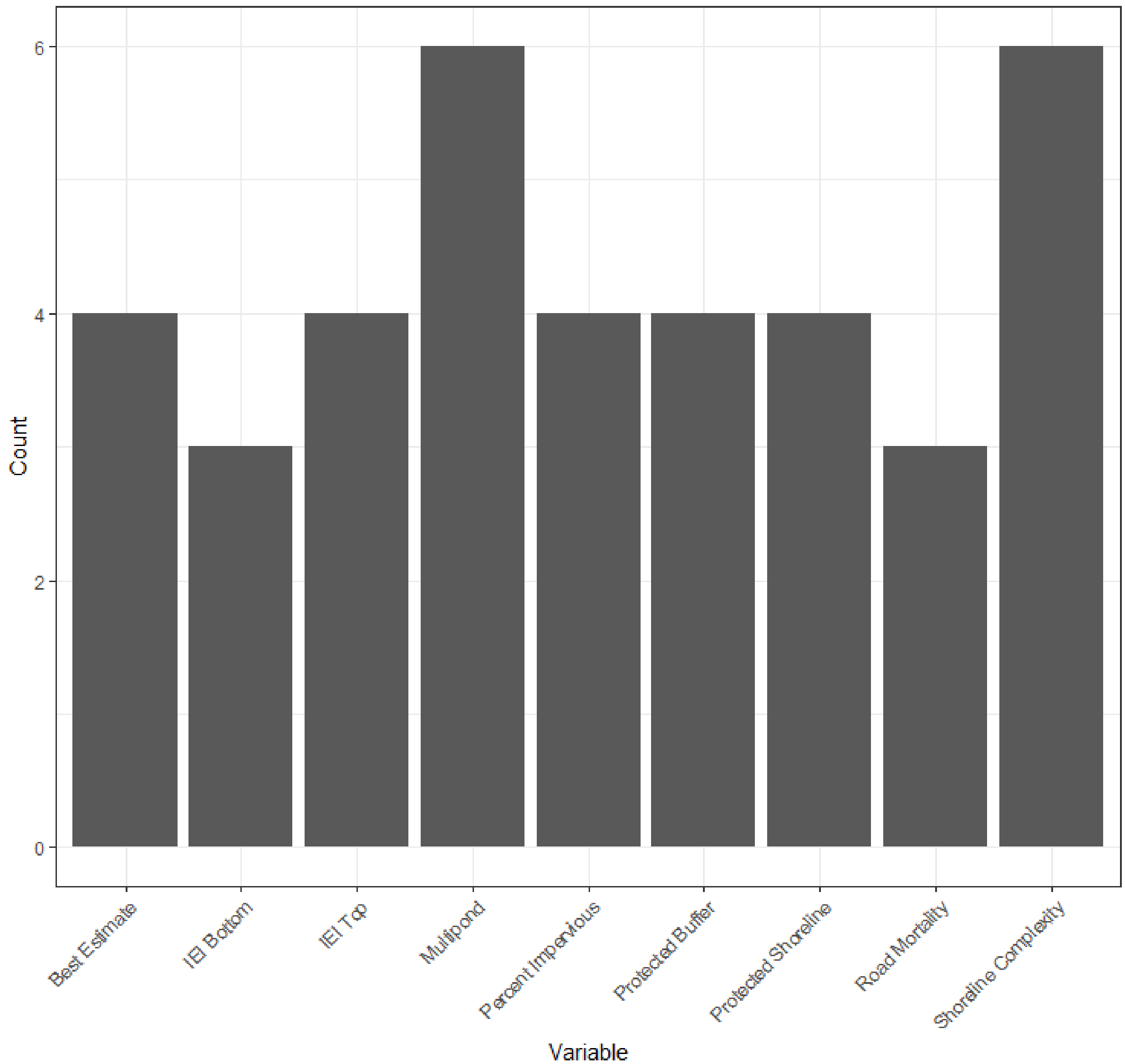

Figure A4. Bar graph depicting the number of analysis units that changed a tier over the range of weights tested per variable.

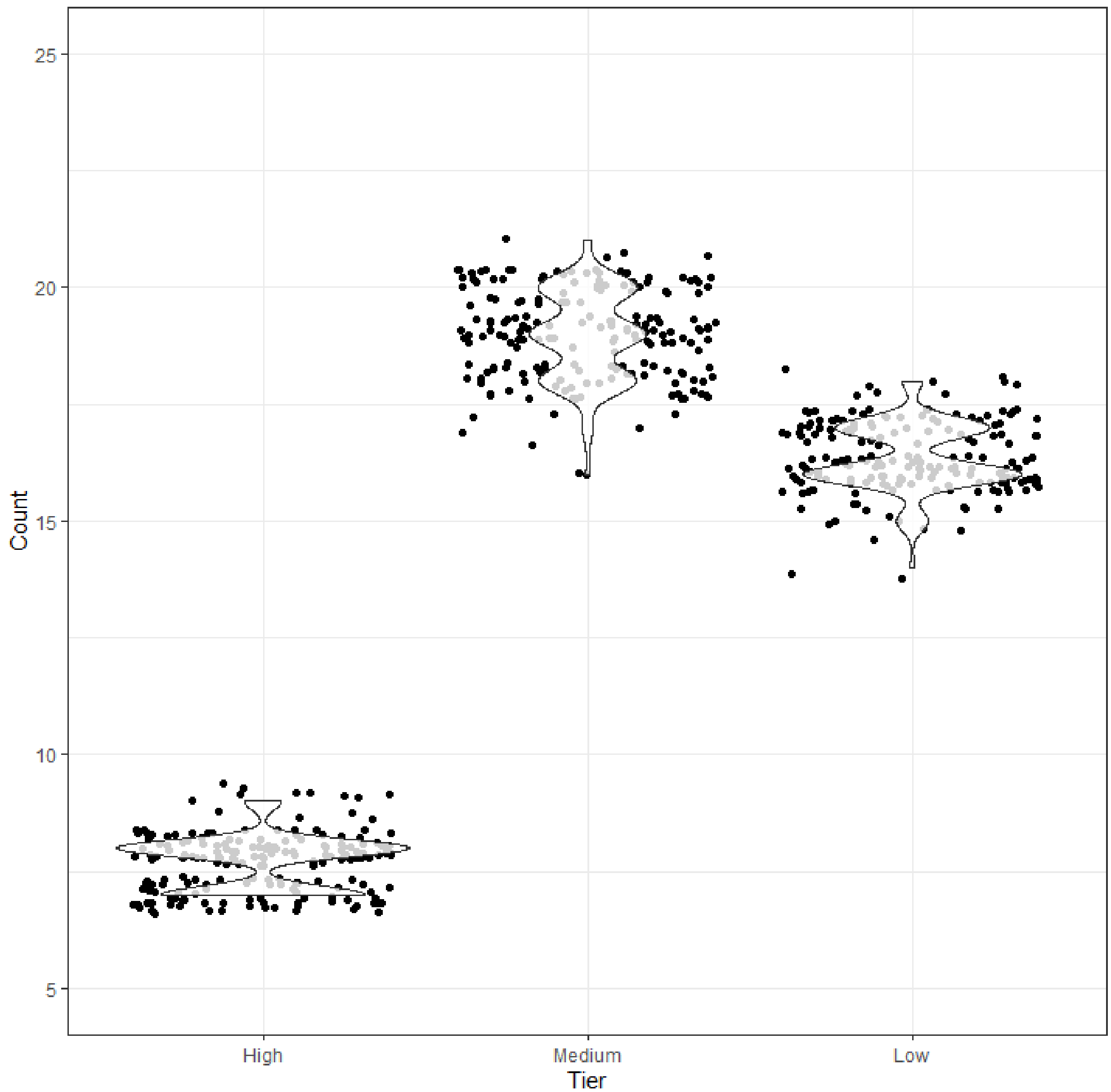

Figure A5. Violin plots and jittered point values depicting the distribution of the number of analysis units in high, medium (moderate), and low tiers for all of the weight scenarios tested.

A.5. Total Population Estimate

To add to our overall assessment of current condition, we used the best available information that was gathered for our AU resiliency condition assessment to determine a total population estimate for the Massachusetts northern red-bellied cooter population. Of the 43 AUs used in our resiliency condition analysis, a total of 23 AUs had sufficient information that could be used to calculate AU population estimates. We did not estimate populations sizes for AUs that only had limited information available in the form of occupancy or EO data. When available, we used existing AU population estimates from Regosin et al. (2017, table 5, p. 62). For AUs where no population estimate existed but headstart release data was available, we calculated population estimates by applying an annual survivorship rate of 0.91 to headstart release data from 1985-2019 (MADFW, unpubl. data). To provide a conservative estimate, we assumed no recruitment had occurred. We selected a survivorship rate of 0.91 because it represents the most conservative estimated survivorship of headstarted turtles from a headstart-only pond complex (Regosin et al. 2017, table 5, p. 62). By summing AU population estimates, we estimated a total population size of 1950.73 individuals throughout the current range of the Massachusetts population (table A7).

Table A7. Total population estimate for the northern red-bellied cooter in Massachusetts. Existing population estimates from Regosin et al. (2017 table 5, p. 62) were used when available, all other AU population estimates were calculated from headstart release data (MADFW, unpubl. data).

Analysis Unit (AUID)	Population Estimate
1	245.20
2	49.90
3	25.80
4	54.00
5	11.68
6	8.46
7	132.10
8	3.06
9	118.70
10	9.50
14	290.60
15	103.80
16	2.58
17	377.54
18	13.36
23	1.02
34	0.47
36	6.45
37	5.53
38	0.14
39	2.33
40	67.80
42	420.70
Total Population Estimate	1950.73

APPENDIX B Future Condition Analysis Methodology

B.1. Units of Analysis

The same AU used for the current condition analysis were also assessed in the future condition analysis.

B.2. Time Step

A time step of approximately 60 years (2020-2080) was chosen to assess the future condition of each AU. This time step was chosen due to availability of modeled data at the 2080 time step for a selection of the habitat variables assessed in the current condition analysis. Sixty years also represents approximately one or two northern red-bellied cooter generation lengths for this species of turtle, although exact generation length is unknown.

B.3. Metrics

The metrics assessed in the future condition analysis consisted of the same environmental variables examined in the current condition analysis. For the following metrics, the values used for the current condition analysis were also used for the future condition analysis: Water Body Shape Complexity, Multi-pond Complex, Percent Shoreline Protected, and Percent Protected Land (see appendix A for more details about these metrics). Modeled data outputs were available at the 2080 time step for a selection of variables including ecological integrity, impervious surface, and likelihood of road mortality, through the UMass DSL project (McGarigal et al. 2017b, entire). The metrics updated to the 2080 time step are described in more detail below.

Habitat Integrity Metrics

The Index of Ecological Integrity (IEI) dataset was used to generate a metric indicating the degree of high habitat integrity of the AU and its surrounding landscape in 2080 (NALCC 2017, entire; McGarigal et al. 2017b, entire). IEI includes a multitude of ecological metrics that pertain to two main categories of landscape metrics: 1) intactness, or freedom from human influences and 2) resiliency, or the capacity to recover from disturbance (McGarigal et al. 2017a, entire). The index ranges from 0 to 1 and was reclassified into three categories, low (0-0.33) medium (0.33-0.66) and high (0.66-1) ecological integrity. The 2080 Percent High IEI habitat integrity metric was derived by calculating the percentage of area categorized as high ecological integrity in 2080 within a 400 m (1312 ft) buffer of the water bodies in each AU, including the water bodies themselves.

Habitat Degradation Metrics

An impervious surface metric was included in the future condition analysis to characterize the predicted level of threat of human influence (roads, buildings, etc.) on the landscape surrounding each AU in 2080. The 2080 imperviousness index ranges from 0 to 100 and measures the percentage of the ground surface area that is impervious to water infiltration

(MassGIS 2019, entire; McGarigal et al. 2017b, entire). The 2080 Percent Impervious Surface metric was derived by calculating the percentage of the total land area within a 400 m (1312 ft) buffer of the water bodies in each AU, excluding the water bodies themselves, that is predicted to be at least 50%. In a few cases, when percent imperviousness in 2080 was lower than the current imperviousness, we assumed that localized decreases in impervious surface will be very unlikely, and set the percent imperviousness in 2080 equal to the current imperviousness.

Threat from roads in 2080 was also incorporated as a metric, using a traffic model which ranges between 0 and 1, representing the probability that an animal will be killed crossing the road (McGarigal et al 2017b, entire). The 2080 Average Likelihood of Road Mortality metric was calculated over the land area within a 400 m (1312 ft) buffer of the water bodies within each AU, excluding the water bodies themselves using the traffic model.

The Index of Ecological Integrity was again used to generate a metric indicating the degree of low habitat integrity of the AU and its surrounding landscape in 2080. See 'Habitat Integrity Metrics' section for further details. The Percent Low IEI habitat degradation metric was derived by calculating the percentage of area categorized as low ecological integrity in 2080 within a 400 m (1312 ft) buffer of the water bodies in each AU, including the water bodies themselves.

B.4. Future Scenarios

Six different future condition scenarios were considered to examine the effect of varying two major variables on the population Best Estimate metric and overall outcomes for each AU (see appendix A for more information on the Best Estimate metric). The two variables used include: 1) population support via the headstart program and 2) population growth rates to approximate population stability or decline in the future.

<u>Headstart Scenarios</u>

1. No headstarts: no individuals released in the future
2. Rule-based headstarts: individuals released in select AUs based on a set of rules:
    a. Rules:
        i. If an AU includes ponds designated as 'Original' according to Regosin et al. 2017, it will receive headstarts in the future.
        ii. AU 14, AU 15, and AU 9 will not receive headstarts in the future because they are designated study ponds.
        iii. If an AU ranks in Tier 1 or Tier 2 only considering future habitat variables (without Best Estimate), it will receive headstarts in the future.
    b. The global average of individual turtles released per year across AUs was calculated using the headstart release table (MADFW unpubl. data).
    The global average of 127 turtles was divided evenly among the 29 AUs that were designated to receive headstarts according to the above rules. This meant approximately 4 turtles on average would be released in each of the 29 selected AUs each year, for a total of 263 turtles added by 2080.
    c. For each of the 29 selected AUs, 263 turtles were added to the current Best Estimate to derive the 2080 Best Estimate.

3. Historical headstarts: individuals released in the AUs that have historically received headstarts
    a. Using the headstart release table (USFWS), site-specific release averages per year were calculated for each AU that has received headstarts since 1985. That average per year was multiplied by 60 years to derive the total number of individuals that would be released by 2080, assuming similar rates of future headstart releases compared to historical releases.
    b. For each of the 23 AUs that have received headstarts in the past, the total number of individuals projected to be released by 2080 was added to the current Best Estimate to derive the 2080 Best Estimate.

Population Growth Rate Values

1. Optimistic: Best Estimate will remain the same in 2080. $\lambda = 1.0$
2. Pessimistic: populations in all AUs will experience an overall rate of decline of 66% in Best Estimate. $\lambda = 0.98167$
    a. We selected a population growth rate from Federal Pond because out of the three ponds where we had enough demographic information to estimate a population growth rate, Federal Pond was the most pessimistic. This population growth rate value was calculated based on population estimates for Federal Pond from 1981 and 2015. The population estimate from 1981, 158 individuals, was selected because it was the largest population estimate prior to the introduction of any headstarts to the water body (Regosin et al. 2017, p. 62). The most recent population estimate available was from 2015 (132.1 individuals), and we corrected that estimate to exclude headstarts and arrived at a total of 84.25 nonheadstarted individuals for our 2015 headstart-corrected population estimate. To exclude headstarts, we calculated the estimated surviving number of headstarts, 47.85 individuals, by applying an annual survivorship rate (0.95) which was calculated by averaging survivorship estimates from three pond complexes (Regosin et al. 2017, p. 62) to annual headstart release data (MADFW unpubl. data).

Future Condition Scenarios

Six scenarios were developed to explore the range of effects of varying between two population growth rates and three potential headstart scenarios. These six scenarios represent every combination of the two variables.

Weights

The same weights were used as in current condition analysis.

Scoring

For each of the AUs, the raw values of each of the final metrics were converted to a percentile rank based on the distribution of the current condition resiliency values across all AUs. If any of the future condition metrics fell above or below the current condition resiliency value range, they were automatically designated as 100 or 0, respectively.

For the multi-pond complex binary variable, multi-pond complexes were scored as 100 and single pond complexes were scored as 0. For the three metrics under the habitat quality-degradation category, the percentile ranks were inverted to indicate the inverse relationship (higher raw value → lower score for resiliency condition).

Tier Designation

The natural breaks in the distribution of current condition analysis resiliency condition scores were used to designate two thresholds to divide the AUs into three resiliency condition tiers: 1-High, 2-Moderate, 3-Low. These same tiers were used for the future condition analysis. A fourth tier (4-Extirpated) was added for the future condition analysis in some scenarios where populations were modeled to be 0 at the 2080 time step.

APPENDIX C Additional Tables and Figures

C.1. Current Condition Results

Table C1. Northern red-bellied cooter AUs and their corresponding population and habitat parameters, ranked by current resiliency condition scores. Resiliency condition tiers are as follows: Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low. Best Estimate Methods are described in detail in appendix A (1 = total population estimate, 2 = headstart or occupancy assessment, 3 = max observation recorded).

Rank	AUID	Best Estimate (individuals)	Best Estimate Method	Percent Shoreline Protected (%)	Percent Protected Land (%)	Water Body Shape Complexity	Multi-pond Complex (0/1- No/Yes)	Percent High IEI (%)	Percent Impervious Surface (%)	Average Likelihood of Road Mortality (0-1)	Percent Low IEI (%)	Current Condition Score (0-100)	Tier (1-3)
1	42	737.06	2	88.62	60.28	1.44	0	55.27	1.90	0.01	22.01	82.15	1
2	1	245.2	1	49.55	50.34	1.28	1	52.36	2.37	0.00	16.88	81.81	1
3	7	132.1	1	37.09	50.84	1.20	1	41.67	2.51	0.00	26.53	78.59	1
4	8	10.22	2	100.00	100.00	1.30	1	70.38	3.34	0.00	14.02	75.78	1
5	17	997.73	2	59.94	40.65	1.26	1	34.04	6.49	0.03	26.91	75.04	1
6	14	290.6	1	68.20	74.58	1.28	0	35.37	3.98	0.00	25.92	73.87	1
7	40	101.38	2	94.33	20.23	1.58	0	2.46	0.77	0.01	67.37	72.84	1
8	9	118.7	1	32.70	35.37	1.36	1	7.75	4.50	0.02	48.88	72.51	1
9	39	7.49	2	100.00	100.00	1.41	0	69.29	4.77	0.00	14.96	67.03	1
10	37	20.62	2	100.00	43.27	1.44	0	2.85	2.72	0.03	36.18	66.03	1
11	4	54	1	36.15	18.85	1.31	1	3.09	9.89	0.02	74.40	63.78	1
12	6	23.74	2	0.00	26.32	1.40	0	65.68	2.41	0.00	16.27	58.77	2
13	15	103.8	1	1.46	1.58	1.49	0	0.10	5.52	0.03	56.53	56.76	2
14	5	49.74	2	0.00	0.00	1.22	1	0.13	2.06	0.01	52.46	54.84	2
15	3	25.8	1	16.16	12.30	1.34	1	1.04	9.66	0.04	83.47	54.80	2
16	2	49.9	1	19.86	19.46	1.27	1	0.00	11.92	0.03	94.92	54.39	2
17	36	16	2	41.85	25.13	1.45	0	5.00	6.40	0.09	37.32	53.75	2

Rank	AUID	Best Estimate (individuals)	Best Estimate Method	Percent Shoreline Protected (%)	Percent Protected Land (%)	Water Body Shape Complexity	Multi-pond Complex (0/1-No/Yes)	Percent High IEI (%)	Percent Impervious Surface (%)	Average Likelihood of Road Mortality (0-1)	Percent Low IEI (%)	Current Condition Score (0-100)	Tier (1-3)
18	11	2	3	76.27	51.17	1.28	1	13.58	7.14	0.01	35.01	53.11	2
19	18	51.33	2	35.88	9.49	0.76	0	6.71	5.29	0.03	71.91	50.66	2
20	16	13.45	2	100.00	3.29	1.38	0	1.23	11.18	0.04	93.46	50.26	2
21	26	2	3	92.53	39.41	1.37	0	4.07	3.89	0.00	31.50	50.04	2
22	35	2	3	100.00	59.88	1.33	0	7.31	5.66	0.02	55.71	48.80	2
23	28	1	3	100.00	92.22	1.43	0	22.46	3.49	0.00	27.75	47.16	2
24	29	1	3	100.00	99.06	1.45	0	35.42	8.63	0.00	29.83	45.14	2
25	41	1	3	100.00	77.53	1.39	0	16.23	3.64	0.01	56.08	42.72	2
26	23	4.65	2	0.00	0.00	1.32	0	13.72	3.67	0.02	57.44	38.91	2
27	10	9.5	1	0.00	1.09	1.42	0	0.00	8.22	0.04	99.81	34.93	3
28	24	2	3	100.00	3.89	1.29	0	0.00	10.38	0.05	90.19	34.71	3
29	27	1	3	6.17	32.56	1.34	0	31.04	1.93	0.00	22.30	33.87	3
30	19	2	3	25.74	9.56	0.71	0	4.50	4.90	0.04	66.09	32.59	3
31	20	2	2	0.00	0.00	1.44	0	0.00	2.98	0.02	95.93	32.18	3
32	21	2	3	0.00	0.00	1.43	0	6.92	8.73	0.04	44.23	29.60	3
33	45	1	2	62.75	10.88	1.38	0	4.49	9.99	0.01	62.79	29.29	3
34	12	1	3	1.72	12.81	1.46	0	0.24	3.93	0.02	81.06	27.75	3
35	22	1	3	9.67	13.63	1.34	0	0.00	3.77	0.02	76.72	24.20	3
36	38	0.48	2	7.24	11.74	1.48	0	0.79	11.32	0.03	63.12	21.78	3
37	25	1	3	15.95	10.65	1.37	0	0.00	4.77	0.10	69.56	20.35	3
38	44	1	3	9.43	9.88	0.83	0	4.99	13.97	0.03	80.73	15.67	3
39	43	1	3	34.31	13.02	0.82	0	0.00	40.87	0.11	100.00	12.09	3
40	31	1	3	0.00	0.23	1.34	0	0.00	10.35	0.10	99.09	9.32	3
41	30	1	3	0.00	0.00	1.37	0	0.00	15.01	0.05	100.00	8.15	3
42	32	1	3	0.00	0.00	1.34	0	0.00	14.28	0.06	100.00	7.02	3
43	34	0.93	2	0.00	0.00	1.32	0	0.64	12.37	0.07	97.78	7.01	3

Table C2. The analysis units with standardized percentile rank (PR) scores on each metric, current resiliency condition scores, and final tier designations are summarized below, sorted by current resiliency condition score from highest to lowest. Resiliency condition tiers are as follows: Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low.

Rank	AUID	Best Estimate PR	Percent Shoreline Protected PR	Percent Protected Land PR	Water Body Shape Complexity PR	Multi-pond Complex PR	Percent High IEI PR	Percent Impervious Surface PR	Average Likelihood of Road Mortality PR	Percent Low IEI PR	Current Condition Score (0-100)	Tier (1-3)
1	42	97.6	73.8	85.7	80.9	0	92.8	97.6	73.8	90.4	82.15	1
2	1	92.8	61.9	76.1	23.8	100	90.4	90.4	85.7	92.8	81.81	1
3	7	90.4	57.1	78.5	9.5	100	88	85.7	88	83.3	78.59	1
4	8	59.5	100	100	28.5	100	100	78.5	92.8	100	75.78	1
5	17	100	64.2	71.4	14.2	100	80.9	40.4	35.7	80.9	75.04	1
6	14	95.2	69	88	21.4	0	83.3	61.9	90.4	85.7	73.87	1
7	40	83.3	78.5	57.1	100	0	40.4	100	76.1	40.4	72.84	1
8	9	88	47.6	66.6	52.3	100	66.6	59.5	54.7	61.9	72.51	1
9	39	54.7	100	97.6	71.4	0	97.6	54.7	92.8	97.6	67.03	1
10	37	66.6	100	73.8	83.3	0	42.8	83.3	42.8	69	66.03	1
11	4	80.9	54.7	52.3	30.9	100	45.2	26.1	61.9	33.3	63.78	1
12	6	69	0	61.9	69	0	95.2	88	83.3	95.2	58.77	2
13	15	85.7	23.8	21.4	97.6	0	23.8	47.6	40.4	52.3	56.76	2
14	5	73.8	0	0	11.9	100	26.1	92.8	69	59.5	54.84	2
15	3	71.4	40.4	42.8	42.8	100	35.7	28.5	23.8	23.8	54.80	2
16	2	76.1	42.8	54.7	16.6	100	0	11.9	45.2	16.6	54.39	2
17	36	64.2	59.5	59.5	90.4	0	57.1	42.8	7.1	66.6	53.75	2
18	11	35.7	71.4	80.9	19	100	69	38	71.4	71.4	53.11	2
19	18	78.5	52.3	28.5	2.3	0	59.5	50	30.9	35.7	50.66	2
20	16	61.9	100	23.8	64.2	0	38	16.6	26.1	19	50.26	2
21	26	35.7	76.1	69	54.7	0	47.6	66.6	80.9	73.8	50.04	2
22	35	35.7	100	83.3	38	0	64.2	45.2	52.3	57.1	48.80	2
23	28	4.7	100	92.8	76.1	0	76.1	76.1	92.8	78.5	47.16	2

Rank	AUID	Best Estimate PR	Percent Shoreline Protected PR	Percent Protected Land PR	Water Body Shape Complexity PR	Multi-pond Complex PR	Percent High IEI PR	Percent Impervious Surface PR	Average Likelihood of Road Mortality PR	Percent Low IEI PR	Current Condition Score (0-100)	Tier (1-3)
24	29	4.7	100	95.2	88	0	85.7	33.3	92.8	76.1	45.14	2
25	41	4.7	100	90.4	66.6	0	73.8	73.8	64.2	54.7	42.72	2
26	23	52.3	0	0	33.3	0	71.4	71.4	59.5	50	38.91	2
27	10	57.1	0	19	73.8	0	0	35.7	21.4	7.1	34.93	3
28	24	35.7	100	26.1	26.1	0	0	19	14.2	21.4	34.71	3
29	27	4.7	28.5	64.2	40.4	0	78.5	95.2	78.5	88	33.87	3
30	19	35.7	45.2	30.9	0	0	52.3	52.3	28.5	42.8	32.59	3
31	20	35.7	0	0	85.7	0	0	80.9	50	14.2	32.18	3
32	21	35.7	0	0	78.5	0	61.9	30.9	19	64.2	29.60	3
33	45	4.7	66.6	38	61.9	0	50	23.8	66.6	47.6	29.29	3
34	12	4.7	26.1	45.2	92.8	0	28.5	64.2	57.1	26.1	27.75	3
35	22	4.7	35.7	50	45.2	0	0	69	47.6	30.9	24.20	3
36	38	0	30.9	40.4	95.2	0	33.3	14.2	38	45.2	21.78	3
37	25	4.7	38	35.7	57.1	0	0	57.1	4.7	38	20.35	3
38	44	4.7	33.3	33.3	7.1	0	54.7	7.1	33.3	28.5	15.67	3
39	43	4.7	50	47.6	4.7	0	0	0	0	0	12.09	3
40	31	4.7	0	16.6	50	0	0	21.4	2.3	9.5	9.32	3
41	30	4.7	0	0	59.5	0	0	2.3	16.6	0	8.15	3
42	32	4.7	0	0	47.6	0	0	4.7	11.9	0	7.02	3
43	34	2.3	0	0	35.7	0	30.9	9.5	9.5	11.9	7.01	3

C.2. Future Condition Results

Table C3. Future resiliency condition results, Scenario Opt-NoHS, with raw values for habitat and demographic metrics, sorted based on Opt-NoHS Score (resiliency condition score) high to low. Resiliency condition tiers are as follows: Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low, Tier 4 = Extirpated. Best Estimate Methods are described in detail in appendix A (1 = total population estimate, 2 = headstart or occupancy assessment, 3 = max observation recorded).

Rank	AUID	Best Estimate (individuals)	Best Estimate Method*	Percent Shoreline Protected (%)	Percent Protected Land (%)	Water Body Shape Complexity	Multi-pond Complex (0/1-No/Yes)	Percent High IEI (%)	Percent Impervious Surface (%)	Average Likelihood of Road Mortality (0-1)	Percent Low IEI (%)	Opt-NoHS Score (0-100)	Opt-NoHS Tier (1-3)
1	8	10.22	2	100.00	100.00	1.30	1	29.49	3.34	0.00	20.18	74.48	1
2	14	290.6	1	68.20	74.58	1.28	0	8.28	3.98	0.00	27.56	72.73	1
3	42	737.06	2	88.62	60.28	1.44	0	0.00	5.09	0.01	71.38	72.27	1
4	40	101.38	2	94.33	20.23	1.58	0	2.46	2.03	0.01	61.55	72.01	1
5	7	132.1	1	37.09	50.84	1.20	1	7.75	11.00	0.00	42.43	71.01	1
6	1	245.2	1	49.55	50.34	1.28	1	14.48	16.44	0.01	70.30	70.69	1
7	17	997.73	2	59.94	40.65	1.26	1	1.11	19.63	0.04	81.19	66.58	1
8	39	7.49	2	100.00	100.00	1.41	0	39.21	4.77	0.00	21.50	66.26	1
9	9	118.7	1	32.70	35.37	1.36	1	0.93	31.46	0.04	85.55	62.52	1
10	4	54	1	36.15	18.85	1.31	1	0.00	46.31	0.04	99.30	56.30	2
11	37	20.62	2	100.00	43.27	1.44	0	0.00	23.63	0.04	100.00	53.09	2
12	2	49.9	1	19.86	19.46	1.27	1	0.03	35.14	0.04	99.79	51.87	2
13	3	25.8	1	16.16	12.30	1.34	1	0.00	41.13	0.05	99.51	49.91	2
14	15	103.8	1	1.46	1.58	1.49	0	0.00	27.80	0.05	99.76	48.28	2
15	28	1	3	100.00	92.22	1.43	0	21.96	3.49	0.00	43.84	46.48	2
16	5	49.74	2	0.00	0.00	1.22	1	0.03	9.74	0.02	99.08	46.38	2
17	6	23.74	2	0.00	26.32	1.40	0	0.74	15.77	0.01	64.64	46.21	2
18	16	13.45	2	100.00	3.29	1.38	0	0.00	38.79	0.05	100.00	45.71	2
19	36	16	2	41.85	25.13	1.45	0	0.00	27.79	0.10	98.27	45.64	2

Rank	AUID	Best Estimate (individuals)	Best Estimate Method*	Percent Shoreline Protected (%)	Percent Protected Land (%)	Water Body Shape Complexity	Multi-pond Complex (0/1-No/Yes)	Percent High IEI (%)	Percent Impervious Surface (%)	Average Likelihood of Road Mortality (0-1)	Percent Low IEI (%)	Opt-NoHS Score (0-100)	Opt-NoHS Tier (1-3)
20	11	2	3	76.27	51.17	1.28	1	0.07	17.71	0.02	85.08	44.57	2
21	29	1	3	100.00	99.06	1.45	0	4.73	8.63	0.00	32.39	43.57	2
22	18	51.33	2	35.88	9.49	0.76	0	0.02	17.80	0.04	96.50	43.45	2
23	35	2	3	100.00	59.88	1.33	0	0.56	19.42	0.03	63.31	42.67	2
24	26	2	3	92.53	39.41	1.37	0	0.00	10.26	0.01	88.22	41.84	2
25	41	1	3	100.00	77.53	1.39	0	0.09	7.63	0.02	84.48	35.08	2
26	24	2	3	100.00	3.89	1.29	0	0.00	32.23	0.07	100.00	31.92	3
27	10	9.5	1	0.00	1.09	1.42	0	0.00	29.04	0.06	100.00	31.11	3
28	23	4.65	2	0.00	0.00	1.32	0	0.00	17.31	0.04	98.07	26.49	3
29	19	2	3	25.74	9.56	0.71	0	0.02	15.19	0.04	97.79	25.35	3
30	20	2	2	0.00	0.00	1.44	0	0.00	24.80	0.04	99.48	23.88	3
31	21	2	3	0.00	0.00	1.43	0	0.00	14.80	0.04	97.52	22.78	3
32	45	1	2	62.75	10.88	1.38	0	0.00	24.92	0.02	99.84	22.21	3
33	27	1	3	6.17	32.56	1.34	0	0.06	16.86	0.01	88.40	20.78	3
34	12	1	3	1.72	12.81	1.46	0	0.06	23.90	0.03	99.18	20.15	3
35	22	1	3	9.67	13.63	1.34	0	0.00	45.09	0.03	100.00	16.91	3
36	38	0.48	2	7.24	11.74	1.48	0	0.00	37.01	0.05	99.21	16.23	3
37	25	1	3	15.95	10.65	1.37	0	0.00	20.44	0.10	100.00	14.42	3
38	44	1	3	9.43	9.88	0.83	0	0.03	32.07	0.04	95.51	12.18	3
39	43	1	3	34.31	13.02	0.82	0	0.00	67.06	0.12	100.00	12.09	3
40	30	1	3	0.00	0.00	1.37	0	0.00	33.45	0.08	100.00	7.30	3
41	31	1	3	0.00	0.23	1.34	0	0.00	41.21	0.12	99.90	7.28	3
42	32	1	3	0.00	0.00	1.34	0	0.00	35.70	0.08	100.00	6.38	3
43	34	0.93	2	0.00	0.00	1.32	0	0.00	49.72	0.08	100.00	4.38	3

Table C4. Standardized percentile rank (PR) scores for each metric, Scenario Opt-NoHS scores, and final tier designations are summarized below, sorted by Opt-NoHS Score (resiliency condition score) from highest to lowest. Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low, Tier 4 = Extirpated.

Rank	AUID	Best Estimate PR	Percent Shoreline Protected PR	Percent Protected Land PR	Water Body Shape Complexity PR	Multi-pond Complex PR	Percent High IEI PR	Percent Impervious Surface PR	Average Likelihood of Road Mortality PR	Percent Low IEI PR	Opt-NoHS Score (0-100)	Opt-NoHS Tier (1-3)
1	8	59.5	100.0	100.0	28.5	100.0	78.1	78.5	91.8	91.3	74.48	1
2	14	95.2	69.0	88.0	21.4	0.0	66.8	61.9	87.8	79.1	72.73	1
3	42	97.6	73.8	85.7	80.9	0.0	0.0	51.2	70.2	36.2	72.27	1
4	40	83.3	78.5	57.1	100.0	0.0	40.4	93.4	68.4	48.1	72.01	1
5	7	90.4	57.1	78.5	9.5	100.0	66.6	17.2	81.7	64.9	71.01	1
6	1	92.8	61.9	76.1	23.8	100.0	72.1	2.2	71.8	37.3	70.69	1
7	17	100.0	64.2	71.4	14.2	100.0	36.5	1.9	18.1	26.0	66.58	1
8	39	54.7	100.0	97.6	71.4	0.0	87.1	54.7	91.8	90.7	66.26	1
9	9	88.0	47.6	66.6	52.3	100.0	34.6	0.8	24.0	23.0	62.52	1
10	4	80.9	54.7	52.3	30.9	100.0	0.0	0.0	29.3	8.8	56.30	2
11	37	66.6	100.0	73.8	83.3	0.0	0.0	1.5	18.7	0.0	53.09	2
12	2	76.1	42.8	54.7	16.6	100.0	22.0	0.5	18.8	7.2	51.87	2
13	3	71.4	40.4	42.8	42.8	100.0	0.0	0.0	16.9	8.1	49.91	2
14	15	85.7	23.8	21.4	97.6	0.0	0.0	1.2	15.2	7.3	48.28	2
15	28	4.7	100.0	92.8	76.1	0.0	75.9	76.1	91.5	64.4	46.48	2
16	5	73.8	0.0	0.0	11.9	100.0	22.0	27.7	55.4	9.5	46.38	2
17	6	69.0	0.0	61.9	69.0	0.0	32.4	2.3	68.9	44.0	46.21	2
18	16	61.9	100.0	23.8	64.2	0.0	0.0	0.1	14.2	0.0	45.71	2
19	36	64.2	59.5	59.5	90.4	0.0	0.0	1.2	3.6	11.0	45.64	2
20	11	35.7	71.4	80.9	19.0	100.0	23.1	2.1	47.5	23.2	44.57	2
21	29	4.7	100.0	95.2	88.0	0.0	53.5	33.3	90.7	73.2	43.57	2
22	18	78.5	52.3	28.5	2.3	0.0	21.8	2.1	18.6	13.5	43.45	2
23	35	35.7	100.0	83.3	38.0	0.0	30.4	1.9	41.9	45.0	42.67	2

Rank	AUID	Best Estimate PR	Percent Shoreline Protected PR	Percent Protected Land PR	Water Body Shape Complexity PR	Multi-pond Complex PR	Percent High IEI PR	Percent Impervious Surface PR	Average Likelihood of Road Mortality PR	Percent Low IEI PR	Opt-NoHS Score (0-100)	Opt-NoHS Tier (1-3)
24	26	35.7	76.1	69.0	54.7	0.0	0.0	22.0	72.7	22.1	41.84	2
25	41	4.7	100.0	90.4	66.6	0.0	23.6	37.0	46.3	23.4	35.08	2
26	24	35.7	100.0	26.1	26.1	0.0	0.0	0.7	8.4	0.0	31.92	3
27	10	57.1	0.0	19.0	73.8	0.0	0.0	1.0	11.9	0.0	31.11	3
28	23	52.3	0.0	0.0	33.3	0.0	0.0	2.1	28.6	11.3	26.49	3
29	19	35.7	45.2	30.9	0.0	0.0	21.9	2.3	18.7	11.8	25.35	3
30	20	35.7	0.0	0.0	85.7	0.0	0.0	1.4	28.8	8.2	23.88	3
31	21	35.7	0.0	0.0	78.5	0.0	0.0	3.0	18.7	12.2	22.78	3
32	45	4.7	66.6	38.0	61.9	0.0	0.0	1.4	46.0	6.7	22.21	3
33	27	4.7	28.5	64.2	40.4	0.0	22.9	2.2	68.7	22.0	20.78	3
34	12	4.7	26.1	45.2	92.8	0.0	22.8	1.5	36.1	9.2	20.15	3
35	22	4.7	35.7	50.0	45.2	0.0	0.0	0.0	40.9	0.0	16.91	3
36	38	0.0	30.9	40.4	95.2	0.0	0.0	0.3	17.2	9.1	16.23	3
37	25	4.7	38.0	35.7	57.1	0.0	0.0	1.8	4.8	0.0	14.42	3
38	44	4.7	33.3	33.3	7.1	0.0	22.0	0.8	19.0	15.2	12.18	3
39	43	4.7	50.0	47.6	4.7	0.0	0.0	0.0	0.0	0.0	12.09	3
40	30	4.7	0.0	0.0	59.5	0.0	0.0	0.6	7.6	0.0	7.30	3
41	31	4.7	0.0	16.6	50.0	0.0	0.0	0.0	0.0	6.0	7.28	3
42	32	4.7	0.0	0.0	47.6	0.0	0.0	0.4	8.3	0.0	6.38	3
43	34	2.3	0.0	0.0	35.7	0.0	0.0	0.0	7.6	0.0	4.38	3

Table C5. Resiliency condition scores for a rule-based headstart future scenario (Opt-RuleHS) sorted by Scenario Opt-RuleHS Score (resiliency condition score) highest to lowest. Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low, Tier 4 = Extirpated.

AUID	Population Designation (Regosin et al 2017)	Future Condition Score- Habitat Only	Future Tier- Habitat Only	Future headstarts (0/1-No/Yes)	Turtles added by 2080	Best Estimate 2080	Best Estimate 2080 PR	Opt-RuleHS Score (0-100)	Opt-RuleHS Tier (1-3)
8	introduced	50.68	1	1	263	273.69	94.3	88.40	1
28		44.60	1	1	263	264.47	93.8	82.12	1
39	introduced	44.38	1	1	263	270.96	94.2	82.06	1
29		41.69	1	1	263	264.47	93.8	79.21	1
40		38.69	1	1	263	364.85	95.6	76.93	1
14	introduced	34.65	1	1	263	554.07	96.6	73.29	1
42	introduced	33.23	1	1	263	1000.53	100	73.23	1
7	original	34.85	1	1	263	395.57	95.7	73.13	1
1	original	33.57	1	1	263	508.67	96.4	72.13	1
41		33.20	1	1	263	264.47	93.8	70.72	1
11		30.29	1	1	263	265.47	93.9	67.85	1
17		26.58	1	1	263	1261.20	100	66.58	1
35		28.39	1	1	263	265.47	93.9	65.95	1
9	introduced	27.32	1	1	263	382.17	95.7	65.60	1
26		27.56	1	1	263	265.47	93.9	65.12	1
37		26.45	1	1	263	284.09	94.8	64.37	1
4	original	23.94	2	1	263	317.47	95.3	62.06	1
2	original	21.43	2	1	263	313.37	95.3	59.55	1
3	original	21.35	2	1	263	289.27	95.1	59.39	1
16	introduced	20.95	2	1	263	276.92	94.5	58.75	2
45	no record	20.33	2	1	263	264.47	93.8	57.85	2
36	introduced	19.96	2	1	263	279.47	94.6	57.80	2
6	introduced	18.61	2	1	263	287.21	95	56.61	2
27		18.90	2	1	263	264.47	93.8	56.42	2
12		18.27	2	1	263	264.47	93.8	55.79	2

AUID	Population Designation (Regosin et al 2017)	Future Condition Score- Habitat Only	Future Tier-Habitat Only	Future headstarts (0/1-No/Yes)	Turtles added by 2080	Best Estimate 2080	Best Estimate 2080 PR	Opt-RuleHS Score (0-100)	Opt-RuleHS Tier (1-3)
24		17.64	2	1	263	265.47	93.9	55.20	2
5	introduced	16.86	2	1	263	313.21	95.3	54.98	2
38		16.23	2	1	263	263.95	93.8	53.75	2
22		15.03	2	1	263	264.47	93.8	52.55	2
15	introduced	14.00	2	1	263	367.27	95.6	52.24	2
18		12.05	3	0	0	51.33	78.5	43.45	2
10	original	8.27	3	0	0	9.50	57.1	31.11	3
23		5.57	3	0	0	4.65	52.3	26.49	3
19		11.07	3	0	0	2.00	35.7	25.35	3
20	original	9.60	3	0	0	2.00	35.7	23.88	3
21		8.50	3	0	0	2.00	35.7	22.78	3
25		12.54	3	0	0	1.00	4.7	14.42	3
44		10.30	3	0	0	1.00	4.7	12.18	3
43		10.21	3	0	0	1.00	4.7	12.09	3
30		5.42	3	0	0	1.00	4.7	7.30	3
31		5.40	3	0	0	1.00	4.7	7.28	3
32		4.50	3	0	0	1.00	4.7	6.38	3
34		3.46	3	0	0	0.93	2.3	4.38	3

Table C6. Resiliency condition scores for a historical headstart future scenario (Opt-HistoricalHS), sorted based on Opt-HistoricalHS Score (resiliency condition score) highest to lowest. Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low, Tier 4 = Extirpated.

AUID	Historical headstarts rate (individuals/year)	Turtles added by 2080	Best Estimate 2080	Best Estimate 2080 PR	Opt-HistoricalHS Score (0-100)	Opt-HistoricalHS Tier (1-3)
8	0.57	34.29	44.51	73.2	79.96	1
40	1.43	85.71	187.09	91.6	75.33	1
14	10.14	608.57	899.17	99	74.25	1
1	15.09	905.14	1150.34	100	73.57	1
42	19.94	1196.57	1933.63	100	73.23	1
7	4.51	270.86	402.96	95.8	73.17	1
39	0.40	24.00	31.49	71.9	73.14	1
17	43.46	2607.43	3605.16	100	66.58	1
9	4.91	294.86	413.56	95.8	65.64	1
37	1.31	78.86	99.48	83.2	59.73	1
3	4.91	294.86	320.66	95.3	59.47	1
4	0.86	51.43	105.43	85.9	58.30	2
2	1.03	61.71	111.61	86.9	56.19	2
5	3.69	221.14	270.88	94.2	54.54	2
16	1.26	75.43	88.88	82.7	54.03	2
6	1.31	78.86	102.60	84.5	52.41	2
15	3.14	188.57	292.37	95.2	52.08	2
36	0.57	34.29	50.29	76.8	50.68	2
18	4.00	240.00	291.33	95.2	50.13	2
28	0.00	0.00	1.00	4.7	46.48	2
11	0.00	0.00	2.00	35.7	44.57	2
29	0.00	0.00	1.00	4.7	43.57	2
10	1.74	104.57	114.07	87.3	43.19	2
35	0.00	0.00	2.00	35.7	42.67	2

AUID	Historical headstarts rate (individuals/year)	Turtles added by 2080	Best Estimate 2080	Best Estimate 2080 PR	Opt-HistoricalHS Score (0-100)	Opt-HistoricalHS Tier (1-3)
26	0.00	0.00	2.00	35.7	41.84	2
38	0.03	1.71	2.19	50.1	36.27	2
41	0.00	0.00	1.00	4.7	35.08	2
23	0.37	22.29	26.94	71.5	34.17	3
24	0.00	0.00	2.00	35.7	31.92	3
19	0.00	0.00	2.00	35.7	25.35	3
20	0.00	0.00	2.00	35.7	23.88	3
34	0.03	1.71	2.64	50.5	23.66	3
21	0.00	0.00	2.00	35.7	22.78	3
45	0.00	0.00	1.00	4.7	22.21	3
27	0.00	0.00	1.00	4.7	20.78	3
12	0.00	0.00	1.00	4.7	20.15	3
22	0.00	0.00	1.00	4.7	16.91	3
25	0.00	0.00	1.00	4.7	14.42	3
44	0.00	0.00	1.00	4.7	12.18	3
43	0.00	0.00	1.00	4.7	12.09	3
30	0.00	0.00	1.00	4.7	7.30	3
31	0.00	0.00	1.00	4.7	7.28	3
32	0.00	0.00	1.00	4.7	6.38	3

Table C7. Three headstart scenarios with pessimistic population growth rate applied (Pes-NoHS, Pes-RuleHS, and Pes-HistoricalHS), sorted by Pes-NoHS Score (resiliency condition score) highest to lowest. Resiliency condition tiers are as follows: Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low, Tier 4 = Extirpated.

AUID	Opt-NoHS Best Estimate	Opt-NoHS Best Estimate 66% decline	Opt-NoHS Best Estimate 66% decline PR	Pes-NoHS Score (0-100)	Pes-NoHS Tier (1-4)	Opt-RuleHS Best Estimate 2080	Opt-RuleHS Best Estimate 2080 66% decline	Opt-RuleHS Best Estimate 2080 66% decline PR	Pes-RuleHS Score (0-100)	Pes-RuleHS Tier (1-4)	Opt-HistoricalHS Best Estimate 2080	Opt-HistoricalHS Best Estimate 2080 66% decline	Opt-HistoricalHS Best Estimate 2080 66% decline PR	Pes-HistoricalHS Score (0-100)	Pes-HistoricalHS Tier (1-4)
8	10.22	3.37	51.2	71.16	1	273.69	90.32	82.7	83.76	1	44.51	14.69	63	75.88	1
42	737.06	243.23	92.8	70.35	1	1000.53	330.17	95.4	71.39	1	1933.63	638.10	97	72.03	1
14	290.6	95.90	83	67.85	1	290.60	95.90	83	71.25	1	899.17	296.73	95.2	72.73	1
40	101.38	33.46	72.1	67.53	1	364.85	120.40	88.3	74.01	1	187.09	61.74	81.3	71.21	1
1	245.2	80.92	82.3	66.49	1	508.67	167.86	91.2	70.05	1	1150.34	379.61	95.7	71.85	1
17	997.73	329.25	95.4	64.74	1	1261.20	416.20	95.9	64.94	1	3605.16	1189.70	100	66.58	1
39	7.49	2.47	50.4	64.54	1	270.96	89.42	82.7	77.46	1	31.49	10.39	59.6	68.22	1
7	132.1	43.59	73.1	64.09	1	395.57	130.54	90.1	70.89	1	402.96	132.98	90.4	71.01	1
9	118.7	39.17	72.7	56.40	2	118.70	39.17	72.7	63.08	1	413.56	136.47	90.5	63.52	1
4	54	17.82	65.2	50.02	2	317.47	104.76	85.8	58.26	2	105.43	34.79	72.3	52.86	2
37	20.62	6.80	54.1	48.09	2	284.09	93.75	82.9	59.61	1	99.48	32.83	72.1	55.29	2
2	49.9	16.47	64.5	47.23	2	313.37	103.41	85.3	55.55	2	111.61	36.83	72.5	50.43	2
28	1	0.33	0	44.60	4	264.47	87.27	82.6	77.64	1	1.00	0.33	0	44.60	4
3	25.8	8.51	55.9	43.71	2	289.27	95.46	83	54.55	2	320.66	105.82	86	55.75	2
15	103.8	34.25	72.2	42.88	2	103.80	34.25	72.2	49.40	2	292.37	96.48	83	47.20	2
5	49.74	16.41	64.4	42.62	2	313.21	103.36	85.2	50.94	2	270.88	89.39	82.7	49.94	2
16	13.45	4.44	52.1	41.79	2	276.92	91.38	82.8	54.07	2	88.88	29.33	71.7	49.63	2
29	1	0.33	0	41.69	4	264.47	87.27	82.6	74.73	1	1.00	0.33	0	41.69	4
36	16	5.28	52.9	41.12	2	279.47	92.22	82.8	53.08	2	50.29	16.59	64.5	45.76	2
6	23.74	7.83	55.1	40.65	2	287.21	94.78	83	51.81	2	102.60	33.86	72.2	47.49	2
18	51.33	16.94	64.7	37.93	2	51.33	16.94	64.7	37.93	2	291.33	96.14	83	45.25	2

AUID	Opt-NoHS Best Estimate	Opt-NoHS Best Estimate 66% decline	Opt-NoHS Best Estimate 66% decline PR	Pes-NoHS Score (0-100)	Pes-NoHS Tier (1-4)	Opt-RuleHS Best Estimate 2080	Opt-RuleHS Best Estimate 2080 66% decline	Opt-RuleHS Best Estimate 2080 66% decline PR	Pes-RuleHS Score (0-100)	Pes-RuleHS Tier (1-4)	Opt-HistoricalHS Best Estimate 2080	Opt-HistoricalHS Best Estimate 2080 66% decline	Opt-HistoricalHS Best Estimate 2080 66% decline PR	Pes-HistoricalHS Score (0-100)	Pes-HistoricalHS Tier (1-4)
41	1	0.33	0	33.20	4	264.47	87.27	82.6	66.24	1	1.00	0.33	0	33.20	4
11	2	0.66	0.9	30.65	3	265.47	87.60	82.6	63.33	1	2.00	0.66	0.9	30.65	3
35	2	0.66	0.9	28.75	3	265.47	87.60	82.6	61.43	1	2.00	0.66	0.9	28.75	3
10	9.5	3.14	51	28.67	3	272.97	90.08	82.7	28.67	3	114.07	37.64	72.6	37.31	2
26	2	0.66	0.9	27.92	3	265.47	87.60	82.6	60.60	1	2.00	0.66	0.9	27.92	3
45	1	0.33	0	20.33	4	264.47	87.27	82.6	53.37	2	1.00	0.33	0	20.33	4
23	4.65	1.53	34.6	19.41	3	4.65	1.53	34.6	19.41	3	26.94	8.89	56.4	28.13	3
27	1	0.33	0	18.90	4	264.47	87.27	82.6	51.94	2	1.00	0.33	0	18.90	4
12	1	0.33	0	18.27	4	264.47	87.27	82.6	51.31	2	1.00	0.33	0	18.27	4
24	2	0.66	0.9	18.00	3	2.00	0.66	0.9	50.68	2	2.00	0.66	0.9	18.00	3
38	0.48	0.16	0	16.23	4	263.95	87.10	82.6	49.27	2	2.19	0.72	1.2	16.71	3
22	1	0.33	0	15.03	4	264.47	87.27	82.6	48.07	2	1.00	0.33	0	15.03	4
25	1	0.33	0	12.54	4	264.47	87.27	82.6	12.54	3	1.00	0.33	0	12.54	4
19	2	0.66	0.9	11.43	3	2.00	0.66	0.9	11.43	3	2.00	0.66	0.9	11.43	3
44	1	0.33	0	10.30	4	1.00	0.33	0	10.30	4	1.00	0.33	0	10.30	4
43	1	0.33	0	10.21	4	1.00	0.33	0	10.21	4	1.00	0.33	0	10.21	4
20	2	0.66	0.9	9.96	3	265.47	87.60	82.6	9.96	3	2.00	0.66	0.9	9.96	3
21	2	0.66	0.9	8.86	3	2.00	0.66	0.9	8.86	3	2.00	0.66	0.9	8.86	3
30	1	0.33	0	5.42	4	1.00	0.33	0	5.42	4	1.00	0.33	0	5.42	4
31	1	0.33	0	5.40	4	1.00	0.33	0	5.40	4	1.00	0.33	0	5.40	4
32	1	0.33	0	4.50	4	1.00	0.33	0	4.50	4	1.00	0.33	0	4.50	4
34	0.93	0.31	0	3.46	4	0.93	0.31	0	3.46	4	2.64	0.87	2	4.26	3

Table C8. Resiliency condition scores and tiers for six future scenarios, sorted based on current condition resiliency score (CC Score) from highest to lowest. Resiliency condition tiers are as follows: Tier 1 = High, Tier 2 = Moderate, Tier 3 = Low, Tier 4 = Extirpated.

AUID	CC Score (0-100)	CC Tier (1-3)	Opt-NoHS Score (0-100)	Opt-NoHS Tier (1-3)	Opt-RuleHS Headstarts Added	Opt-RuleHS Score (0-100)	Opt-RuleHS Tier (1-3)	Opt-HistoricalHS Headstarts Added	Opt-HistoricalHS Score (0-100)	Opt-HistoricalHS Tier (1-3)	Pes-NoHS Score (0-100)	Pes-NoHS Tier (1-4)	Pes-RuleHS Score (0-100)	Pes-RuleHS Tier (1-4)	Pes-HistoricalHS Score (0-100)	Pes-HistoricalHS Tier (1-4)
42	82.15	1	72.27	1	263	73.23	1	1197	73.23	1	70.35	1	71.39	1	72.03	1
1	81.81	1	70.69	1	263	72.13	1	905	73.57	1	66.49	1	70.05	1	71.85	1
7	78.59	1	71.01	1	263	73.13	1	271	73.17	1	64.09	1	70.89	1	71.01	1
8	75.78	1	74.48	1	263	88.40	1	34	79.96	1	71.16	1	83.76	1	75.88	1
17	75.04	1	66.58	1	263	66.58	1	2607	66.58	1	64.74	1	64.94	1	66.58	1
14	73.87	1	72.73	1	263	73.29	1	609	74.25	1	67.85	1	71.25	1	72.73	1
40	72.84	1	72.01	1	263	76.93	1	86	75.33	1	67.53	1	74.01	1	71.21	1
9	72.51	1	62.52	1	263	65.60	1	295	65.64	1	56.40	2	63.08	1	63.52	1
39	67.03	1	66.26	1	263	82.06	1	24	73.14	1	64.54	1	77.46	1	68.22	1
37	66.03	1	53.09	2	263	64.37	1	79	59.73	1	48.09	2	59.61	1	55.29	2
4	63.78	1	56.30	2	263	62.06	1	51	58.30	2	50.02	2	58.26	2	52.86	2
6	58.77	2	46.21	2	263	56.61	2	79	52.41	2	40.65	2	51.81	2	47.49	2
15	56.76	2	48.28	2	263	52.24	2	189	52.08	2	42.88	2	49.40	2	47.20	2
5	54.84	2	46.38	2	263	54.98	2	221	54.54	2	42.62	2	50.94	2	49.94	2
3	54.80	2	49.91	2	263	59.39	1	295	59.47	1	43.71	2	54.55	2	55.75	2
2	54.39	2	51.87	2	263	59.55	1	62	56.19	2	47.23	2	55.55	2	50.43	2
36	53.75	2	45.64	2	263	57.80	2	34	50.68	2	41.12	2	53.08	2	45.76	2
11	53.11	2	44.57	2	263	67.85	1	0	44.57	2	30.65	3	63.33	1	30.65	3
18	50.66	2	43.45	2	0	43.45	2	240	50.13	2	37.93	2	37.93	2	45.25	2
16	50.26	2	45.71	2	263	58.75	2	75	54.03	2	41.79	2	54.07	2	49.63	2
26	50.04	2	41.84	2	263	65.12	1	0	41.84	2	27.92	3	60.60	1	27.92	3
35	48.80	2	42.67	2	263	65.95	1	0	42.67	2	28.75	3	61.43	1	28.75	3
28	47.16	2	46.48	2	263	82.12	1	0	46.48	2	44.60	4	77.64	1	44.60	4
29	45.14	2	43.57	2	263	79.21	1	0	43.57	2	41.69	4	74.73	1	41.69	4
41	42.72	2	35.08	2	263	70.72	1	0	35.08	2	33.20	4	66.24	1	33.20	4

AUID	CC Score (0-100)	CC Tier (1-3)	Opt-NoHS Score (0-100)	Opt-NoHS Tier (1-3)	Opt-RuleHS Headstarts Added	Opt-RuleHS Score (0-100)	Opt-RuleHS Tier (1-3)	Opt-HistoricalHS Headstarts Added	Opt-HistoricalHS Score (0-100)	Opt-HistoricalHS Tier (1-3)	Pes-NoHS Score (0-100)	Pes-NoHS Tier (1-4)	Pes-RuleHS Score (0-100)	Pes-RuleHS Tier (1-4)	Pes-HistoricalHS Score (0-100)	Pes-HistoricalHS Tier (1-4)
23	38.91	2	26.49	3	0	26.49	3	22	34.17	3	19.41	3	19.41	3	28.13	3
10	34.93	3	31.11	3	0	31.11	3	105	43.19	2	28.67	3	28.67	3	37.31	2
24	34.71	3	31.92	3	263	55.20	2	0	31.92	3	18.00	3	50.68	2	18.00	3
27	33.87	3	20.78	3	263	56.42	2	0	20.78	3	18.90	4	51.94	2	18.90	4
19	32.59	3	25.35	3	0	25.35	3	0	25.35	3	11.43	3	11.43	3	11.43	3
20	32.18	3	23.88	3	0	23.88	3	0	23.88	3	9.96	3	9.96	3	9.96	3
21	29.60	3	22.78	3	0	22.78	3	0	22.78	3	8.86	3	8.86	3	8.86	3
45	29.29	3	22.21	3	263	57.85	2	0	22.21	3	20.33	4	53.37	2	20.33	4
12	27.75	3	20.15	3	263	55.79	2	0	20.15	3	18.27	4	51.31	2	18.27	4
22	24.20	3	16.91	3	263	52.55	2	0	16.91	3	15.03	4	48.07	2	15.03	4
38	21.78	3	16.23	3	263	53.75	2	2	36.27	2	16.23	4	49.27	2	16.71	3
25	20.35	3	14.42	3	0	14.42	3	0	14.42	3	12.54	4	12.54	3	12.54	4
44	15.67	3	12.18	3	0	12.18	3	0	12.18	3	10.30	4	10.30	4	10.30	4
43	12.09	3	12.09	3	0	12.09	3	0	12.09	3	10.21	4	10.21	4	10.21	4
31	9.32	3	7.28	3	0	7.28	3	0	7.28	3	5.40	4	5.40	4	5.40	4
30	8.15	3	7.30	3	0	7.30	3	0	7.30	3	5.42	4	5.42	4	5.42	4
32	7.02	3	6.38	3	0	6.38	3	0	6.38	3	4.50	4	4.50	4	4.50	4
34	7.01	3	4.38	3	0	4.38	3	2	23.66	3	3.46	4	3.46	4	4.26	3