Long-Term Turtle Declines: Protected Is a Verb, Not an Outcome

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Long-term studies on wildlife populations are necessary to track population abundance and shifts in demography over time, yet such studies are difficult to plan, fund, and conduct and are therefore rarely undertaken. Such studies are especially important for long-lived species that can persist for long periods of time with little to no reproductive output or recruitment. We conducted two population studies spanning a 30-year time frame on the globally endangered Spotted Turtle (*Clemmys guttata*) on protected land in the center of their range. Spotted Turtles are endangered in Canada, listed as globally endangered on the IUCN red list, and declining throughout their range. However, there has only been one previous long-term study tracking their long-term population trajectory. Here, we use mark–recapture data collected over a 30-year time frame and report that the estimated population size of Spotted Turtles has decreased by 49% at our study site despite the habitat residing with a protected area. This decline was concurrent with a significant increase in the proportion of larger individuals within the population, indicating a lack of recruitment into the sub-adult stage class. These results highlight the value of long-term studies in monitoring population changes of long-lived species, the importance of active management within protected areas, and the ability of long-lived species to persist for long periods of time despite having little recruitment and a declining population trajectory.

O NE of the most widely used management strategies for declining species is the protection of critical habitat from future habitat loss and fragmentation. This approach to conservation assumes that protecting intact habitat will be enough to mitigate population declines caused by human activities. However, several studies and reviews have challenged the effectiveness of a simple protected areas approach to endangered species management (Dudley and Stolton, 1999; Bruner et al., 2001; Joppa and Pfaff, 2010; Mora and Sale, 2011; Geldmann et al., 2013). A review by Geldmann et al. (2013) concluded that there is little evidence that simply conserving protected areas is enough to maintain the population size of declining species. While these protected areas are no longer subject to ongoing habitat loss and fragmentation, a host of other threats (e.g., road mortality, human disturbance, non-point source pollution, increased mesopredator abundance, climate change, disease, and habitat succession) may still cause habitat degradation and cause subsequent population declines. For example, researchers have detected declines of amphibians (Fellers and Drost, 1993; Knapp and Matthews, 2000; Bosch et al., 2001), squamate reptiles (Weatherhead et al., 2002), mammals (Rosenblatt et al., 2014), fishes (Bradshaw et al., 2008), insects (Schlicht et al., 2009), and birds (Suárez et al., 1993) all within protected habitat. Unsurprisingly, multiple studies have also demonstrated that turtle populations may follow this trend and may continue to decline or even become extirpated within protected areas (Lovich, 1989; Garber and Burger, 1995; Klemens and Moll, 1995; McCoy et al., 2006; Browne and Hecnar, 2007; Enneson and Litzgus, 2009; Lovich et al., 2014; Loehr, 2017).

Long-lived organisms generally have long-generation times and delayed sexual maturity, low fecundity, slow growth rate, and high sensitivity to losses from additive adult mortality (Congdon et al., 1993, 1994; Heppell, 1998; Musick, 1999; Sukumar, 2003; Heppell et al., 2005; Enneson and Litzgus, 2008, 2009). These traits can mask slow long-term population declines that may easily be overlooked and prevent rapid recovery following population losses (Congdon et al., 1994; Heppell, 1998; Musick, 1999; Gibbs and Amato, 2000; Wheeler et al., 2003; Heppell et al., 2005). For example, Crouse et al. (1987) estimated that Loggerhead (*Caretta caretta*) populations may not respond to conservation actions for as long as 70 years after initiation. Consequently, studies attempting to monitor population trajectories or effectiveness of management strategies need to occur over long time frames (Gibbons et al., 2000; Enneson and Litzgus, 2009).

Turtle populations are in a state of global decline and are one of the most threatened vertebrate clades, with 52% of all identified species threatened with extinction and 20% listed as critically endangered (Klemens, 2000; Rhodin et al., 2018), and 61% of turtle species listed as Threatened by the IUCN (Rhodin et al., 2018). Like other long-lived organisms, turtles rely on high adult survivorship to compensate for high rates of hatchling and juvenile mortality (Type III survivorship curve; Congdon and Gibbons, 1990; Heppell, 1998). Turtle populations are threatened by habitat loss and degradation, poaching, introduced diseases, increased meso-predator abundance, and other anthropogenic sources of additive adult mortality (Klemens, 2000; Lovich et al., 2018). Sometimes these declines are directly attributable to habitat loss, road mortality, predation from corvids, or poaching (Garber and Burger, 1995; Dorcas et al., 2006; Walker and Rafeliarisoa, 2012; Loehr, 2017); however, many times the declines are due to synergistic effects from multiple factors that are much more difficult to identify and manage (Lovich, 1989; McCoy et al., 2006; Browne and Hecnar, 2007; Erb et al., 2015; Lovich et al., 2018).

Spotted Turtles are a small aquatic emydid freshwater turtle distributed throughout the eastern United States and Canada (Ernst and Lovich, 2009). They are currently listed as an endangered species in Canada (COSEWIC, 2015), listed on the International Union for Conservation of Nature (IUCN) Red List as globally endangered (Van Dijk, 2016), and listed

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on Appendix II of the Convention on International Trade in Endangered Species (CITES). Their populations suffer from road mortality, predation by subsidized predators, habitat loss and fragmentation, poaching, and pollution (Ernst and Lovich, 2009; COSEWIC, 2015). While Spotted Turtles are a relatively well-studied species, there has only been one ongoing study that has tracked estimated population size for more than a single generation (i.e., roughly 25 years; Litzgus, 2006; Enneson and Litzgus, 2008, 2009; Van Dijk, 2016).

While the one-time purchase of land for imperiled species is cheaper and easier than indefinite ecosystem management, habitat protection may not ensure long-term persistence of endangered species or populations. Here we report the population decline of the globally endangered Spotted Turtle within protected habitat in Central Maryland, USA over a 30-year time frame using mark–recapture data from two sampling periods (historical and contemporary), a stage distribution analysis, and a population viability analysis model validation.

MATERIALS AND METHODS

Study site.—The study site is located in Central Maryland (exact location withheld due to poaching concerns) on the floodplain of the Chesapeake Bay and is bisected by a two-lane road that serves both residential and commercial traffic. A daily average of 2087 vehicles used the road in 2017, with an AM peak of 128 per hour and a PM peak of 169 per hour (Maryland Department of Public Works; Division of Highways, pers. comm.). There are numerous vernal pools and permanent emergent wetlands within the study site that contain Spotted Turtles; however, only the eight largest wetlands were adequately sampled during both mark–recapture studies to obtain accurate population size estimates.

The protected area is roughly 80 ha in total. To the north of the road there are four woodland vernal pools, totaling 2.28 ha, that we termed the North Wetland Complex (NWC). To the south there are three permanent wetlands and one ephemeral vernal pool, totaling 1.35 ha, that will be referred to as the South Wetland Complex (SWC). Wetland sizes were estimated by collecting GPS points from around each wetland's perimeter in April 2014 and by the polygon function in Google Earth. Historically, the same wetlands within both complexes were roughly equal in size (data obtained from 1994 U.S. Geological Survey imagery). For all analyses, these wetland complexes were treated as two separate populations and not as a metapopulation. Despite years of intensive mark–recapture study, we recorded no movement between complexes during 2014–2017 and extremely rare movement ($n = 3$) during 1987–1992. The surrounding upland forest was an oak and hickory (*Quercus* and *Carya* spp.) dominated forest before a Gypsy Moth (*Lymantria dispar dispar*) invasion in 1989–1991. Following the severe infestation and consequent mass die-off of the oaks, the forest returned to a Sweetgum (*Liquidambar styraciflua*) and Tulip Poplar (*Liriodendron tulipifera*) dominated forest. The forest is currently transitioning back from shade-intolerant Tulip Poplars, Sweetgums, and Red Maples (*Acer rubrum*) to an oak and hickory dominated hardwood forest (R. Legere, pers. obs.).

Since 1987, human development near the site and active recreation on the site has increased, three of the wetlands have been invaded by the invasive haplotype of the common

reed *Phragmites*, and known poaching occurred on the site in 1992 (R. Legere, pers. obs.). The invasion by *Phragmites* in all three of the invaded permanent wetlands is quite substantial and has led to a dramatic decline in suitable habitat within those wetlands. The protected area is bordered on either side by continuously expanding residential housing. The wetlands themselves have had little habitat management, resulting in encroachment of woody vegetation. In 1994, the NWC was purchased by a land conservation organization and set aside without any form of active management except for deer hunting. The same group purchased the SWC in 2013, and there was no development of the SWC prior to its conservation. Active recreation (e.g., hiking, nature watching, etc.) is permitted across the site, and the surrounding human community has created a trail crossing the SWC that is frequently used by hikers and outdoor enthusiasts.

Data collection.—Data were collected over two sampling periods: 1987–1992 (historic) and 2014–2017 (contemporary). During 1987–1992, Spotted Turtles were collected by visual encounter surveys within all wetlands and by trapping in deep wetlands where visual encounter surveys were more difficult. Visual encounter surveys consisted of first examining each wetland with a pair of binoculars from a distance of approximately 10 m and counting all observed *C. guttata*, then capturing them by hand. Cylindrical traps were constructed of stainless steel hardware cloth with 1.25 cm mesh or heavy gauge chicken wire. Traps were 0.9 m in length with a 15 cm diameter funnel at each end and were unbaited. To prevent drowning of turtles due to sudden increases in water levels, traps were open at the top and staked down. Both sampling types occurred between 2–4x per week throughout the active period of the Spotted Turtle (February–June). The road separating the wetland complexes was searched for dead Spotted Turtles each time the NWC was sampled (at least two times per week during the active season).

During the contemporary sampling period, from February to June in each of four years (2014, 2015, 2016, and 2017), Spotted Turtles were collected by visual encounter surveys, and trapping in all wetlands used a slightly different trap type than during the historic sampling period. Visual encounter surveys followed the methodology originally used during 1987–1992. Collapsible PROMAR minnow traps were baited with sardines, staked to prevent movement, and had floats placed inside to prevent drowning (Howell et al., 2016). Both sampling types occurred twice weekly during the full duration of the main active season (February–June). Both sides of the road were walked at least three times weekly during the active season to scan for dead turtles.

During both sampling periods, the sex of all captured turtles was determined based on tail length and concavity of the plastron (Ernst, 1976). Midline carapace length (CL) was measured using calipers accurate to the nearest 1 mm, and turtles were marked following procedures outlined in Ernst (1976). During 2014–2017, body mass was measured using a 300 g Pesola scale (accurate to 1 g) clipped directly to the posterior marginal scutes of the carapace. For a more detailed overview of the study site (i.e., wetland size, history, etc.), marking protocols, and trapping procedures see Howell et al. (2016).

We used body size measurements as a proxy for delineating age classes. Scute ring counts did not provide an accurate estimate of age in Spotted Turtles in a Canadian population (Litzgus and Brooks, 1998) or in our population (Howell and

Seigel, 2018; see Wilson et al., 2003 for a review). Therefore, we used midline plastron length (PL) as our delineating feature for separating individuals into juveniles (PL < 80 mm), sub-adults (81–100 mm), and adults (>100 mm) instead of attempting to obtain an exact age for each individual. Estimated size at maturity for Spotted Turtles is 80 mm PL (Ernst, 1970; Ernst and Zug, 1994), and at around 100 mm PL, there is a decline in annual growth rate (Ernst and Lovich, 2009). For statistical analysis, we compared size classes (<80 mm, 81–100 mm, >100 mm) between sampling periods using a contingency table analysis in R (Version 3.3.2; RStudio Team, 2016) based on the most recent data point for each individual turtle.

Population modeling.—Population size was estimated using a POPAN formulation of the Jolly-Seber model run in Program MARK (White and Burnham, 1999). Capture histories from each year of the historic study were binned into a single binary record for that year (i.e., a 1 for a capture and a 0 for no capture). For example, an individual captured in 1987, not captured in 1989 or 1990, and recaptured in 1991 and 1992 would have an encounter history of 10011. For the 2014–2017 sampling period, data binning was not possible due to the shorter time frame of the study and the inability of MARK to disentangle the survivorship and recapture parameter estimates during the final time interval. Therefore, we used all encounters to create the encounter histories. We constructed and evaluated four models that varied in their assumptions: a full time and encounter probability dependent model ($\Phi_t p_t$), a constant survival and encounter probability time dependent model ($\Phi.p_t$), a time dependent survival and constant encounter probability model ($\Phi_t p.$), and a constant survival and encounter probability model ($\Phi.p.$). If a time dependent survival model (Φ_t) was the most parsimonious, we used the second to last survivorship estimate (since final estimates are confounded with the recapture parameter). We used separate models to estimate the number of males and females within each population to prevent differences in capture rate from biasing population size estimates (McKnight and Ligon, 2017). The most parsimonious model was chosen based on minimizing corrected Akaike Information Criterion (AICc) values (Table 1). We used Program RELEASE within Program MARK to test the fit of our data to the assumptions of the Jolly-Seber models; these goodness-of-fit tests showed no violations of the assumptions. There were sufficient numbers of juveniles captured during 1987–1992 to produce population size estimates for this age class; however, during 2014–2017 we only found two juveniles, so this age class was removed from all further analyses for both sampling periods.

Population viability analysis.—A previous study on this population produced a population viability analysis (PVA) with Program VORTEX using demographic parameters estimated from the contemporary population (2014–2017) and collected from the literature (see table 1 in Howell and Seigel, 2019). Here we test the predictive accuracy of that PVA model by using the same set of values from the PVA model and the estimated historic population size, and then projecting the model 150 years into the future. We then compared the rates of estimated decline from the PVA model (Howell and Seigel, 2019) with the observed rates of decline over the 30 years between sampling periods.

Unless noted, all summary statistics are from R, Program VORTEX, or Program MARK (White and Burnham, 1999;

Table 1. Model selection table identifying the most parsimonious POPAN formulation of the Jolly-Seber model for historic (1987–1992) and contemporary (2014–2017) Spotted Turtle populations. We modeled survivorship (Φ) and recapture rate (p) as either constant (.) or as a function of time (t). The most parsimonious models were those with $\Delta AIC = 0$, and are marked with an *.

Population	Model	AICc	ΔAIC
NWC Historic Females	$\Phi.p_t$	236.045	11.08
	*$\Phi_t p.$	224.964	0
	$\Phi.p.$	233.739	8.77
	$\Phi_t p_t$	227.895	2.93
NWC Historic Males	*$\Phi.p_t$	251.4	0
	$\Phi_t p.$	263.16	11.76
	$\Phi.p.$	265.54	14.14
	$\Phi_t p_t$	263.16	11.76
NWC Contemporary Females	$\Phi.p_t$	581.202	21.342
	*$\Phi_t p.$	559.861	0
	$\Phi.p.$	889.789	329.92
	$\Phi_t p_t$	574.628	14.768
NWC Contemporary Males	*$\Phi.p_t$	533.7495	0
	$\Phi_t p.$	572.295	38.455
	$\Phi.p.$	579.205	45.45
	$\Phi_t p_t$	536.7677	3.018
SWC Historic Females	$\Phi.p_t$	228.976	2.27
	$\Phi_t p.$	231.765	5.06
	$\Phi.p.$	233.793	7.09
	*$\Phi_t p_t$	226.697	0
SWC Historic Males	*$\Phi.p_t$	217.481	0
	$\Phi_t p.$	224.942	7.46
	$\Phi.p.$	226.467	8.98
	$\Phi_t p_t$	220.327	2.84
SWC Contemporary Females	$\Phi.p_t$	349.5	16.0682
	$\Phi_t p.$	343.69	10.258
	*$\Phi.p.$	333.431	0
	$\Phi_t p_t$	351.276	17.844
SWC Contemporary Males	*$\Phi.p_t$	452.914	0
	$\Phi_t p.$	465.964	13.05
	$\Phi.p.$	467.072	14.16
	$\Phi_t p_t$	456.868	3.95

Lacy and Kreeger, 2012; R Core Team, 2016). Means are followed by ± one SE or SD, as indicated.

RESULTS

In the historical dataset (1987–1992), we recorded a total of 225 captured individual turtles. In the final year of the historical dataset (1992), we found a 98.4% recapture rate (124 recaptures of 126 individuals) in the NWC and a 98.3% recapture rate (119 recaptures of 121 individuals) in the SWC. In the contemporary dataset (2014–2017), we recorded a total of 104 captured individual turtles with a 96.5% recapture rate (82 recaptures of 85 individuals) in the NWC and an 88.9% recapture rate (64 recaptures of 72 individuals) in the SWC during the final year of the study (2017). Our high recapture rates indicates that we sampled the population thoroughly during both sampling periods. We recaptured 39 individuals in the 2014–2017 study period that were originally marked as at least sub-adults (i.e., plastron length was >80 mm) in the 1987–1992 study period. Overall, we captured 121 fewer individuals during the 2014–2017 study than were previously observed during 1987–1992, while maintaining similar recapture rates.
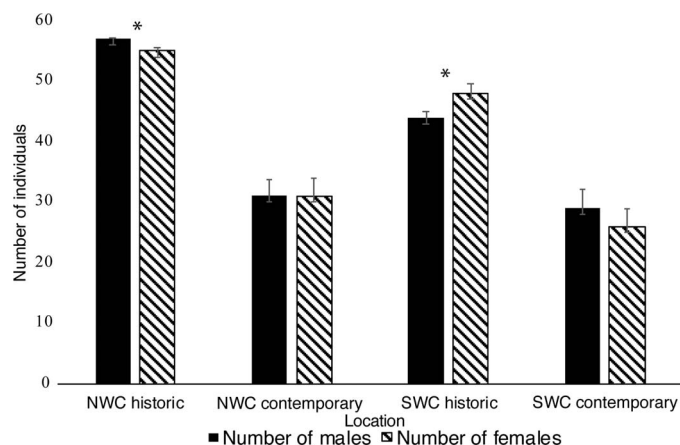
Fig. 1. Estimated population size of Spotted Turtles over time (historic population sizes are from 1987–1992 and contemporary population sizes are from 2014–2017). The estimated mean number of individuals shown separately for males (solid black bars) and females (diagonally striped bars), and the North Wetland Complex (NWC) and the South Wetland Complex (SWC). Error bars are standard error estimates of population size from Program MARK. Significant differences between the estimated mean number of males and females are denoted with an *.

Populations sizes decreased from the historic (1987–1992) to the contemporary (2014–2017) sampling periods. The constant survivorship and time dependent recapture model ($\Phi.p_t$) was the most parsimonious for all four of the male models (SWC contemporary and historic and NWC contemporary and historic). In contrast, the time dependent survivorship and constant recapture parameter ($\Phi_t p.$) was the most parsimonious model for both the contemporary and historic NWC female models. The SWC historic female model was best fit by a time dependent survivorship and recapture model ($\Phi_t p_t$), and the SWC contemporary female model was best fit by a constant survivorship and recapture model ($\Phi.p.$). For a summary of AICc values see Table 1.

These models estimated population size at 135.5 and 62.0 individuals in the NWC during the historic and contemporary sampling periods, respectively (Fig. 1). For the SWC population, the most parsimonious models estimated a population size of 102.5 and 55.0 individuals for the historic and contemporary sampling periods, respectively (Fig. 1). Our population size estimates declined from a total of 238 individuals during 1987–1992 to 117 individuals during 2014–2017, representing a 49% decline in estimated total population size within 30 years (Fig. 1).

From 1992 to 2017, both the SWC and NWC populations experienced a significant shift toward having a higher proportion of larger (and putatively older) individuals (SWC $\chi^2 = 10.77$, df = 1, $P = 0.0045$; NWC $\chi^2 = 10.76$, df =1, $P = 0.0046$; chi-square contingency table analysis; Fig. 2). During 1987–1992, the density at NWC was ~18 turtles/ha and the density at SWC was ~16 turtles/ha. During 2014–2017, the density of both the NWC and SWC had declined to ~8 Spotted Turtles per hectare. We did not observe any significant changes in estimated survivorship rates between sampling periods (Fig. 3). The observed sex ratio (M:F) for the historic NWC was 1.05:1 (57M: 54F), the historic SWC was 0.89:1 (44M: 48F), the contemporary NWC was 1:1(27M: 27F), and the contemporary SWC was 1.2:1 (27M: 23F). We used chi-square tests to determine if the sex ratios differed from the expected 1:1 ratio. We found no differences that
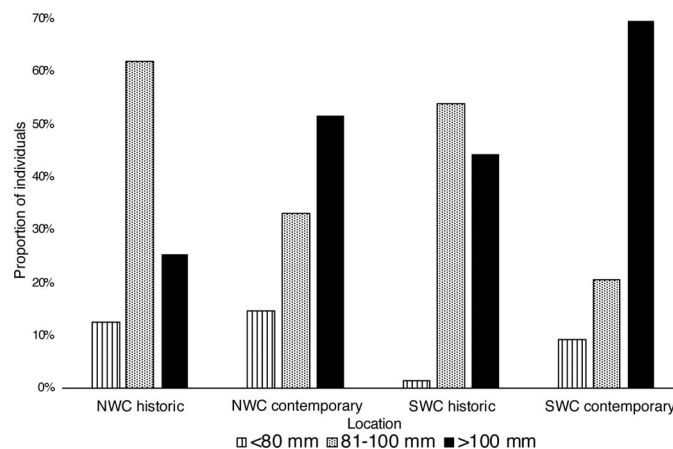

Fig. 2. Size class (straight line carapace length, CL) distribution of collected individuals during the historic (1978–1992) and contemporary (2014–2017) sampling periods. Estimates are shown separately for the North Wetland Complex (NWC) and the South Wetland Complex (SWC).

would suggest that this is the case; χ^2 values ranged from 0–0.09 (all with 1 df) and *P*-values ranging from 0.77–1. Additionally, we used a chi-square test to determine if the sex ratios within populations had shifted between sampling periods. Again, we found no differences that would suggest that this had occurred; χ^2 values ranged from 0.03–0.49 (all with 1 df) and *P*-values ranging from 0.48–0.87.

A previous study undertook a population viability analysis (PVA) using only data collected from 2014–2017 (Howell and Seigel, 2019). Using the same set of parameters and number of iterations from that PVA and the population size of Spotted Turtles estimated by Program MARK during 1987–1992, we analyzed the historic data set and found that the magnitude of the real-world decline was similar to that of the projected decline. The PVA projected that within 30 years the NWC population would have declined from 135.5 individuals to a mean population size of 69.83 (SD ± 25.15; Fig. 4A). Similarly, the PVA projected that within 30 years
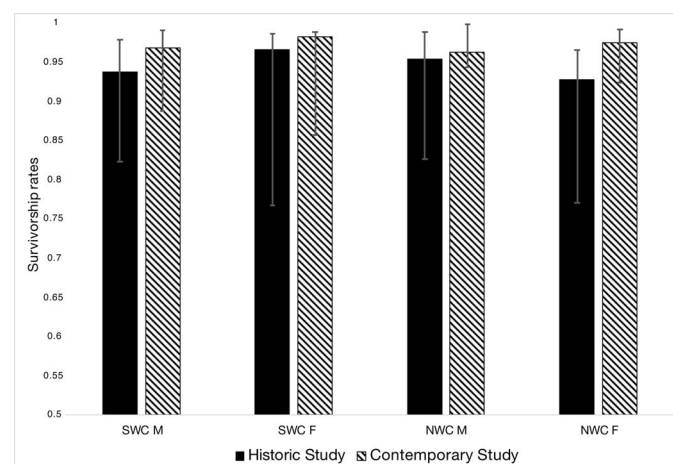

Fig. 3. Estimates of annual survivorship rates for Spotted Turtles from the historic (1987–1992) and contemporary (2014–2017) sampling periods. Estimates are broken down by population (NWC, North Wetland Complex; SWC, South Wetland Complex) and sex (M, males; F, females). There were no significant changes in survivorship rates between sampling periods within any population. Error bars are 95% confidence intervals of survivorship estimates generated in Program MARK.

the SWC population would have declined from 102.5 individuals to a mean population size of 52.63 (SD ± 20.48) individuals (Fig. 4B). Our population models from 2014–2017 estimated that the mean population size of the NWC was 62 individuals and the SWC was 55 individuals (Howell and Seigel, 2019). In both cases, the observed declines were within ten individuals of the mean population size projected by the PVAs.

DISCUSSION

We recorded a long-term decline of a globally endangered freshwater turtle within a protected area. The estimated number of individuals decreased by ∼50% over 30 years, and there was a concomitant increase in the proportion of larger individuals in the population. During 1992–2017, the overall estimated population size dropped by 117 individuals (73.5 individuals in NWC and 47.5 individuals in SWC) or roughly a loss of 4 individuals per year. The 30-year time frame (1992–2017) is roughly equal to the estimated generation time of the Spotted Turtle (25 years; Davy and Murphy, 2014; COSEWIC, 2015) and so may serve as a proxy for the estimated rate of decline per generation at this site. For our population, the Spotted Turtle's current IUCN classification of Endangered seems to underestimate the potential risk for decline. Three generations would be roughly 75 years from 1992, at which point the population will most likely reach a level closer to the Critically Endangered threshold of an 80% decline within three generations.

We recorded low capture rates of juveniles, especially very small juveniles. It is possible that juveniles exist at a higher abundance than we recorded but that our collection method was biased towards adults, or that juveniles are rare in the population. Collection methods are often biased toward adult reptiles and most likely miss a large proportion of juveniles that may be using different habitat, may not be as active, or may not be attracted to traps (Tesche and Hodges, 2015). We had a lower recapture rate of sub-adults in the final year of the 2014–2017 study (76%) compared to adults (98%); however, this may be due to juveniles entering the sub-adult size class and may not reflect differential capture rates.

We found a significant increase in the proportion of larger, and therefore putatively older, individuals in the population between the two sampling periods (Fig. 2). This suggests a lack of recruitment into the population, causing its mean age to increase, mirroring the results of Browne and Hecnar (2007), who recorded significantly increased age classes for declining populations of Blanding's (*Emydoidea blandingii*) and Common Snapping Turtles (*Chelydra serpentina*) within a protected area in Ontario. While the proportion of juveniles collected in our study is quite low (<15%), other studies examining Spotted Turtle populations have recorded juvenile occurrence rates of 5% (Litzgus and Brooks, 1998), 12% (Chippindale, 1984), 18% (Graham, 1995), and 32% (Ernst, 1976). While it is unclear if these rates are accurate or represent collection bias, if there were a large number of juveniles that we failed to sample and that survived to adulthood, our analysis of age classes over time should not have shown such a drastic decrease in the number of sub-adults, which are collected at statistically similar frequencies as adults (Howell et al., 2016).

The results from the previous PVA showed that both populations (NWC and SWC) are declining (Howell and Seigel, 2019). This PVA projected a 93% and a 94%

probability of quasi-extinction (fewer than eight adults) in the NWC and SWC, respectively, within the next 150 years (Howell and Seigel, 2019). The PVA output closely mirrored the observed declines at our study sites (Fig. 4), suggesting that the PVA model is an accurate representation of the actual population trajectory. Obviously, population vital rates are subject to both stochastic and deterministic changes over the next 150 years, and it is possible that the population may respond to changes in population size with density-dependent shifts in vital rates (although this is rare in turtle populations; Brooks et al., 1991; Keevil et al., 2018), and so this projected population decline should be treated as one potential outcome if vital rates remain constant. Our results add to the small but growing body of literature on the accuracy of PVA models (see Brook et al., 2000; Spencer, 2018), but like all PVA models, ours will require extensive field verification to determine if the long-term population trajectory matches the model's projection.

While during both sampling periods we used a combination of visual encounter surveys and trapping to collect Spotted Turtles, there were slight differences in trap type and collection intensity. It is possible that different researchers were more efficient at spotting and catching individuals and that one of the trap types was better at collecting either a larger number of individuals or a specific stage of individuals. Our Cormack-Jolly-Seber models assumed equal catchability between sampling methodologies, so changes in sampling protocol may introduce bias into the comparison between sampling periods. However, we feel that our very high recapture rates, the accuracy and predictive capacity of our PVA model (Howell and Seigel, 2019), and the concurrent recorded increases in larger individuals, all point towards a long-term population decline and not an artifact of minor changes in sampling protocol.

This decline in population size within a protected area should serve as a warning to conservation organizations seeking to mitigate declines by simply setting aside habitat for imperiled species. Despite being protected from direct mortality from habitat loss and fragmentation, populations within protected areas are still subject to other anthropogenic factors that may affect survivorship or reproductive output. We categorized the site as a protected area since it has been placed under a perpetual conservation easement and is not at risk for future habitat loss, but this does not imply that the area is immune to habitat degradation from a host of external factors. At our study site, Spotted Turtles are still being impacted by road mortality, mesopredators, invasive *Phragmites australis*, habitat succession, and poaching (Howell and Seigel, 2019). Our results mirror those of Garber and Burger (1995), who recorded the complete extirpation of two Wood Turtle (*Glyptemys insculpta*) populations within one decade within a protected area in Connecticut, those of Lovich (1989), who recorded declines of Spotted Turtles within a protected habitat in Ohio, and those of Browne and Hecnar (2007), who recorded extirpation of Spotted Turtles and declines in Blanding's Turtles within a protected area in Ontario.

One hypothesized cause for the decline and the putative lack of recruitment in our populations is the increased abundance of subsidized mesopredators (e.g., raccoons, *Procyon lotor*). The impacts of raccoons on turtle populations are well studied and devastating (Stancyk, 1982; Ernst et al., 1994; Mitchell and Klemens, 2000). While we did not actively attempt to measure rates of nest predation, we did find several predated Spotted Turtle nests and witnessed
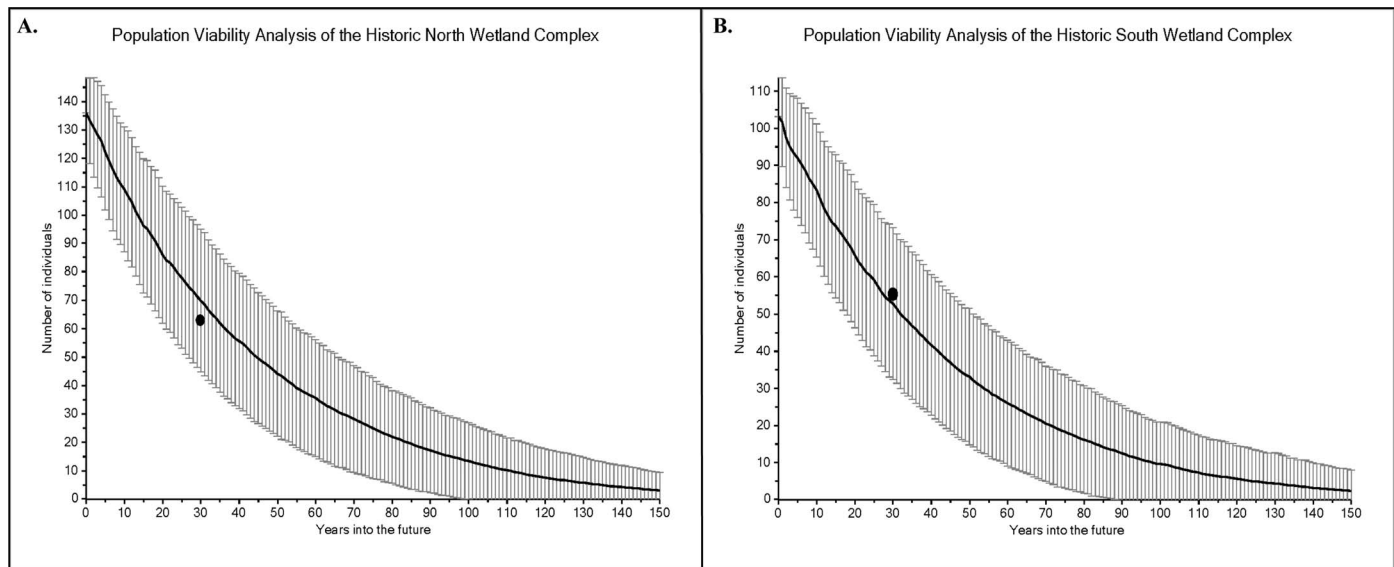
Fig. 4. (A) Population Viability Analysis for Spotted Turtles for the historic North Wetland Complex (NWC) site. (B) Population Viability Analysis for Spotted Turtles for the historic South Wetland Complex (SWC) site. In both graphs, the line represents the average number of individuals starting with the estimated population size from 1987–1992 and projected into the future using Program VORTEX (error bars are SDs). The black circle represents the estimated mean population size from Program MARK for 2014–2017. In both cases, the estimated population size projected from VORTEX was within ten individuals from the estimated population size from MARK during 2014–2017.

raccoons searching grass tussocks and root masses within the wetlands both in person and using camera traps within wetlands during 2014 and 2017 (Howell, unpubl. data). Raccoons exist at population levels above a natural carrying capacity in many areas (Prange et al., 2003) and have been found to be a main contributor to the extirpation of Spotted Turtles at other sites (Browne and Hecnar, 2007).

Direct disturbance by humans can have multiple negative impacts on wildlife populations (Gill et al., 2001; Beale and Monaghan, 2004). However, for species threatened by a high risk of poaching (e.g., Spotted Turtles, Wood Turtles, and Bog Turtles [*Glyptemys muhlenbergii*]), human disturbance may take the more nefarious form of illegal collection. Between 1989 and 1994, an estimated 4,692 specimens of Bog, Spotted, and Wood turtles were shipped to international markets from the United States (HSUS, 1994). In 1992, traps were found at our study site that were used for illegal harvesting of Spotted Turtles, causing us to stop our mark–recapture study at that time. While it may be impractical to exclude humans from protected areas, the conservation benefits of a protected area likely decrease when recreation is allowed on the site (Garber and Burger, 1995).

Because Spotted Turtles are also sensitive to habitat succession, it is imperative that any protected area plan details the proposed protocol for maintaining a wetland either within the early stages of succession, or for providing open, sunny areas for nesting and thermoregulation (Burke et al., 2000). Without open sunny areas for thermoregulation of both adults and nests, Spotted Turtle reproductive output and survivorship will likely decline. It is critical that protected areas have a concurrent conservation action plan that clearly lays out plans to mitigate or eliminate anthropomorphic impacts, manage habitat, and collect necessary data to assess the efficacy of the conservation practices. Because there is a significant lack of information regarding the causal links between the establishment of protected areas and the subsequent change in population size, researchers need to develop long-term studies with pre-selected counter-

factuals that either examine changes using a before vs. after framework or a control vs. intervention framework. Such studies can provide data on the effectiveness of protected areas and can identify important causal links demonstrating the effectiveness of different management strategies (Geldman et al., 2013).

Undertaking long-term population studies to monitor changes in population size are difficult and costly. Conversely, occupancy analyses are more cost effective and easier to perform but may miss gradual declines. Therefore, monitoring programs should attempt to develop a robust sampling design that incorporates both intensive population studies at a selected subset of sites to gauge population growth rates and include occupancy analysis at a larger number of sites to determine the number of populations and track changes in the species range. This type of two-tiered management system has been effectively implemented for the two closest relatives of the Spotted Turtle, the Bog Turtle (Klemens, 1993; USFWS, 1997; Smith, 2001) and the Wood Turtle (Jones et al., 2015; Brown et al., 2017). It is clear from the decline in estimated abundance and through the use of a PVA that the population at our site is likely to reach quasi-extinction unless some sort of active management strategy is implemented to reduce mortality rates (Howell and Seigel, 2019). The decline at a site that has seen little direct habitat loss between sampling periods is concerning and questions the efficacy of unmanaged protected areas to slow declines of imperiled species. Our study throws into sharp relief the necessity of active management within protected areas, and the importance of long-term studies with robust sampling methods in detecting declines in long-lived organisms.

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