Mass Mortality in a Community of Headstarted (Emydoidea blandingii) and Naturally Occurring (Chrysemys picta marginata) Freshwater Turtles in Protected Urban Wetlands

Authors: Wijewardena, Tharusha, Drader, Christine A., Gasbarrini, Donnell M. L., Litzgus, Jacqueline D., Kerr, Kevin C. R., et al.

Source: Ichthyology & Herpetology, 113(1) : 75-83

Published By: The American Society of Ichthyologists and Herpetologists

URL: https://doi.org/10.1643/h2024045

Ichthyology & Herpetology 113, No. 1, 2025, 75–83

Mass Mortality in a Community of Headstarted (*Emydoidea blandingii*) and Naturally Occurring (*Chrysemys picta marginata*) Freshwater Turtles in Protected Urban Wetlands

Tharusha Wijewardena[1,2], Christine A. Drader[3], Donnell M. L. Gasbarrini[3], Jacqueline D. Litzgus[1], Kevin C. R. Kerr[3,4,5], and Nicholas E. Mandrak[2]

Turtles experience numerous threats and high mortality in urban areas. Mass-mortality events (MMEs) are localized, sudden events resulting in a catastrophic increase in mortality rate. Turtles are susceptible to MMEs because their long generation times do not permit density-dependent compensation. We retrospectively investigated an MME in 2020 that affected two species, headstarted Blanding's Turtles (*Emydoidea blandingii*) and naturally occurring Midland Painted Turtles (*Chrysemys picta marginata*) in Rouge National Urban Park (RNUP) in Ontario, Canada, where we monitored the freshwater turtle community from 2014 to 2022. In 2020, 48 juvenile headstarted Blanding's Turtle and 57 Midland Painted Turtle carcasses were found, most (75%) of which were females. The turtles were likely depredated by Raccoons (*Procyon lotor*) or American Minks (*Neogale vison*). Documented mortalities were highest in release sites for headstarted turtles, possibly due to increased monitoring efforts at those sites. Conservation initiatives such as headstarting are useful to recover turtle populations, but MMEs may disrupt and delay population stabilization, especially in areas where other threats (e.g., subsidized predators, road mortality) are prevalent. We emphasize the importance of long-term monitoring of turtle populations, especially after conservation interventions, to detect challenges that affect their persistence.

TURTLES have persisted on Earth for hundreds of millions of years, and yet they are among the most imperiled taxonomic groups at both local and global scales (Gibbons et al., 2000; Lovich et al., 2018; Stanford et al., 2020). Of the eight native freshwater turtle species found in Ontario, Canada, seven are recognized as species-at-risk (SAR) by the provincial government (COSSARO, 2023), and all eight are listed as SAR by the federal government (Government of Canada, 2023). The greatest threats to turtles are shared across Ontario and are especially prevalent in urban settings: habitat loss and fragmentation; road mortality; subsidized predators; emerging diseases; and poaching (Browne and Hecnar, 2007; Beaudry et al., 2008; Millar and Blouin-Demers, 2011; Bennett and Litzgus, 2014). Considering the life-history traits shared among turtle species (e.g., low recruitment, long generation times), it is no surprise that these threats can, both individually and in combination, have devastating, long-lasting impacts on turtle populations, even when small in magnitude (Keevil et al., 2018).

Mass-mortality events (MMEs) are defined as localized, rapidly occurring events that result in a catastrophic increase in mortality rate (Fey et al., 2015). MMEs can affect multiple life stages simultaneously and can be caused by one or more contributing factors (Lande, 1993; Reed et al., 2003; Ameca y Juárez et al., 2012). On a global scale, the frequency and severity of MMEs in recent years may be linked to extreme climate events such as droughts, floods, and wildfires (Fey et al., 2015; Barton et al., 2023). An animal population's ability to recover following an MME is greatly dependent on species-specific life-history traits. For example, sea urchins have a fast life-history strategy, and some populations can recover within months (Gizzi et al., 2020), whereas turtles may not recover even decades after an MME despite survival rates returning to pre-catastrophe levels (Keevil et al., 2018). As such, turtle populations may not show immediate signs of population collapse but may be on a trajectory toward extirpation. The susceptibility of turtles to lasting impacts of MMEs is due to their lack of density-dependent responses to reduced population size, such as increased survival of young, earlier age of maturity, or greater reproductive output (Brooks et al., 1991).

Over the past 20 years, the Toronto Zoo and its partners have led a turtle conservation program in what is now Rouge National Urban Park (RNUP), Ontario, Canada. Their projects have included habitat restoration, installation of artificial nesting areas for turtles, establishment of wildlife corridors to facilitate movement between fragmented habitats, Blanding's Turtle (*Emydoidea blandingii*) headstarting, and mark–recapture and radio-telemetry research. While collecting data for conservation, a concentrated pattern of mortality was documented in recent years, consistent with the criteria of an acute MME.

Using opportunistic observations, our goal was to characterize the MME and offer potential hypotheses with supporting evidence for the cause(s) of mortality. We report the species affected and the demographics of the carcasses, and we discuss the repercussions that this MME may have on

[1] School of Natural Sciences, Laurentian University, 935 Ramsey Lake Road, Sudbury, Ontario, P3E 2C6, Canada.
[2] Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, Ontario, M1C 1A4, Canada; ORCID: (TW) 0000-0003-4167-5031; Email: (TW) tharusha.wijewardena@gmail.com. Send correspondence to TW.
[3] Toronto Zoo, 361A Old Finch Avenue, Toronto, Ontario, M1B 5K7, Canada.
[4] Centre for Biodiversity Genomics, University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada.
[5] Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada.
Submitted: 17 May 2024. Accepted: 17 January 2025. Associate Editor: J. M. Davenport.
 DOI: 10.1643/h2024045 Published online: 5 March 2025

the viability of the remaining turtle community in the RNUP. The observations detailed here add to a growing body of literature on MMEs in freshwater turtles in Ontario (Karson et al., 2018; Keevil et al., 2018; Gasbarrini et al., 2021) but are unique for involving a headstarted population (i.e., whereby eggs are collected from the wild, artificially incubated, and the young raised in human care before being released into the wild; Wijewardena et al., 2023). Long-term conservation initiatives, such as headstarting, nest protection, and translocations, have been documented in turtle species around the world to mitigate threats and accelerate population recovery (García et al., 2003; Field et al., 2007; Burke, 2015). Despite ongoing conservation efforts, MMEs can impede progress and delay or reverse species recovery.

MATERIALS AND METHODS

Study sites.—Three sites within the RNUP, totaling 33.8 ha of wetland habitat, were monitored as part of ongoing turtle headstarting and monitoring initiatives. Actual site names, locations, and associated details are omitted to protect the SAR involved, including Blanding's, Midland Painted (hereafter Painted Turtle; *Chrysemys picta marginata*), and Common Snapping (hereafter Snapping Turtle; *Chelydra serpentina*) turtles.

Site A is composed of a complex of shallow ponds, meadow marsh, thicket swamp, and successional woodland. Its prominent aquatic vegetation species include Canadian Pondweed (*Elodea canadensis*), cattails (*Typha* spp.), Common Bladderwort (*Utricularia vulgaris*), Common Reed (*Phragmites australis*), Coontail (*Ceratophyllum demersum*), muskgrasses (*Chara* spp.), and water lilies (*Nymphaea* spp.). Site B is composed of shallow ponds and marsh surrounded by mineral deciduous swamp and successional woodland. Its prominent aquatic vegetation includes cattails, Common Duckweed (*Lemna minor*), Greater Duckweed (*Spirodela polyrhiza*), Purple Loosestrife (*Lythrum salicari*), and Sago Pondweed (*Stuckenia pectinata*). Site C is a cedar-grove wetland complex composed of a central shallow pond with nearby smaller waterbodies. Its prominent aquatic vegetation includes cattails, Common Duckweed, Common Reed, Coontail, and water lilies.

Blanding's Turtle headstarting project.—The Blanding's Turtle headstarting project began with egg collection in 2012, and individuals were first released in 2014. This headstarting project aims to augment a historic population of Blanding's Turtles that had decreased to fewer than ten individuals and was deemed functionally extinct following extensive surveys conducted in the 1990s and early 2000s (Wijewardena et al., 2023). The causes of the original population decline are unknown, but the effects of urbanization (e.g., habitat loss and fragmentation, road mortality) likely played a role. Populations at sites A (2014–2020) and B (2021–present) have been annually augmented with ~22-month-old headstarted and 2–14-day-old hatchling Blanding's Turtles. From 2014 to 2020, a total of 270 headstarted turtles and 184 hatchlings were released into site A, and from 2021 to 2022, 104 headstarted turtles and 34 hatchlings were released into site B. Headstarted individuals were marked with notches on their marginal scutes (Cagle, 1939) upon hatching, then prior to release, passive integrated transponder (PIT) tags were injected into their left hind-leg pocket and photos were taken of their plastron. Additionally, a randomly selected portion of headstarted turtles were fitted with an external glue-on radio transmitter. Notches, PIT tags, plastron photos, and transmitter frequencies (where possible) were used to verify the identity of dead Blanding's Turtles. See Wijewardena et al. (2023) for additional details about the headstarting project.

Survey protocol.—The Toronto Zoo conducted surveys from 2014 to 2022 using radio-telemetry of headstarted Blanding's Turtles, live-trapping, opportunistic captures, nest site surveys, and road surveys for all freshwater turtle species at sites A–C (Table S1; see Data Accessibility). Due to the COVID-19 pandemic, sites were minimally surveyed in 2020. Radio-telemetry surveys were done more frequently during the active season of turtles (4–17 days/month; May–September) than during the non-active season (1–6 days/month; October–April) each year at site A (2014–2022) and sites B and C (2021–2022). A subset of released headstarted turtles and several adult Blanding's Turtles were tracked using R4000 or R410 Receivers (Advanced Telemetry Systems, Inc., MN). Turtles were captured, processed, and released at the site of capture approximately monthly from May to October. Turtles of any species opportunistically captured by hand were also processed.

Live-trapping was conducted from 2017–2021, during the active season only, at site A (2017–2021), site B (2017–2021), and site C (2018–2019, 2021). We deployed 5–10 hoop traps, baited with cat food or canned sardines, and three basking traps (2018 only). Traps were checked daily, and captured turtles were photographed, measured (straight-line carapace length, carapace width, plastron length, plastron width, and shell height), unmarked individuals were notched and PIT tagged, and all turtles were released at the site of capture.

Nesting surveys were conducted during the nesting season (late May–early July 2017–2022) in the afternoon and evening to identify nesting turtles and depredated nests. Data were collected by walking 700–2,500 m transects, scanning for presence of nesting female turtles, other turtle nesting activity, and depredated nests and turtles. Road surveys were conducted at sites B and C during the active season (2017, 2018, 2021); in 2018 and 2021, road surveys were extended until October. Data were collected by walking each side of the road scanning for live and dead on- and beside-road wildlife from the center median line toward the shoulder. Mortalities of all animal species were recorded, but here we report only the mortalities of turtles.

In summary, survey effort generally increased from 2014 until 2019, and Toronto Zoo staff conducted rigorous surveys during summer across sites A–C. However, in 2020, only three survey types (radio-telemetry, live-trapping, and water chemistry sampling) were conducted in two sites (A and B) with reduced effort across all survey types, as a result of the COVID-19 pandemic (Table S1; see Data Accessibility).

Upon discovery of a carcass, the location, species, identifiers (notch, PIT tag number, plastron pattern, transmitter frequency), sex (male, female, juvenile, unknown), injuries, shell measurements, and probable cause of death (predation, road mortality, winterkill, unknown) of the turtle were recorded. Probable cause(s) of mortality were determined based on the condition of the carcass (Fig. 1). Predation was cued by obvious signs of injuries and damage to, or removal of, the head and limbs, which is characteristic of species known to prey on freshwater turtles (Lanszki et al., 2006). In contrast, road mortality was the documented cause of death

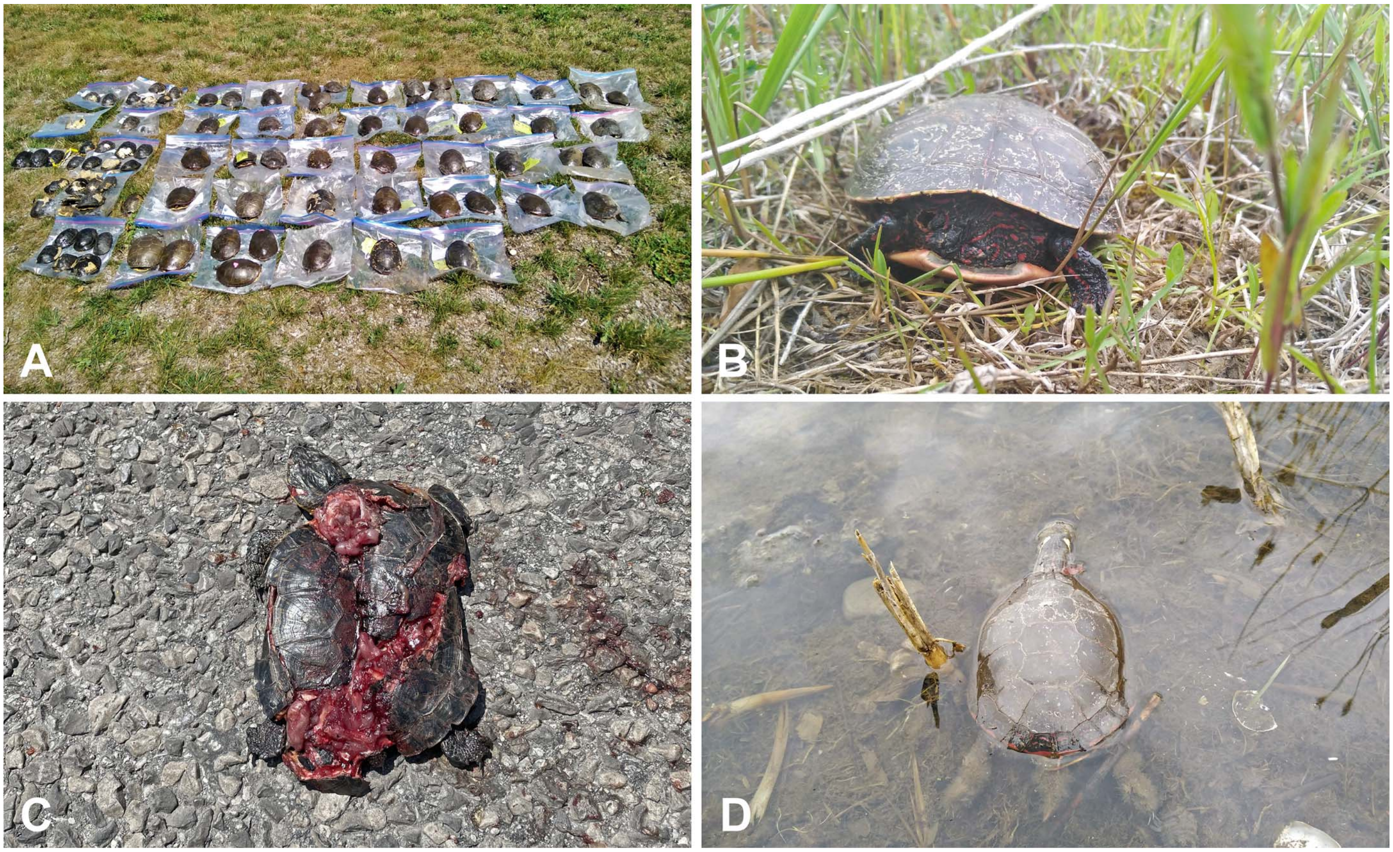

Fig. 1. Turtle mortality observed from 2020 to 2022 in Rouge National Urban Park, Ontario, Canada. (A) Carcasses of Midland Painted Turtles (*Chrysemys picta marginata*) and headstarted Blanding's Turtles (*Emydoidea blandingii*). (B) Close-up image of a depredated Midland Painted Turtle with decapitation. (C) Blanding's Turtle mortality as a result of vehicular collision. (D) Midland Painted Turtle mortality from winter-kill. Photos: (A, B, D) Tharusha Wijewardena; (C) copyright Casey Howard.

when there was excessive bodily damage (e.g., cracked carapace and/or plastron) to carcasses located on or near a road. Winterkill was the documented cause of death when carcasses with no obvious signs of injuries or damage were observed in the winter or spring. Unknown cause of death was documented when there were no obvious signs of injury or damage on carcasses found outside of winter or spring, when destruction and/or decomposition of the carcass was too advanced to determine any signs of injury or damage, or as a result of missing data. Carcasses collected/identified by Parks Canada staff, who have regular presence in the RNUP, were also included in our summaries and analyses.

Demography of carcasses.—Sex was determined using secondary sex characteristics when present (Ernst and Lovich, 2009). Turtles were categorized into three age/sex classes based on relevant size for each species: female (adult), male (adult), and juvenile (unknown sex; Table 1). Shell measurements were recorded to the nearest 0.1 mm using ZEAST Vernier calipers (Table 2). Measurements were taken either *in situ* or at the Toronto Zoo. Carcass demography was recorded as "unknown" when characteristics required to determine sex or age class were not discernible and other identification methods (i.e., notch, PIT tag number, or transmitter frequency) could not be verified. The carcasses were brought to the Wildlife Health Centre at the Toronto Zoo to be temporarily stored in a freezer. Carcasses that had evidence of predation, or for which the cause of death was unknown and had enough soft tissue remaining, were sent to the Canadian Wildlife Health Cooperative (CWHC; Guelph, ON) for necropsy. Samples with tissue that were severely autolyzed only received gross examination. Where able, histopathology was used to rule out underlying disease. All other carcasses were sent to Parks Canada to be shared with partners where feasible, stored for future use, or respectfully buried.

Ethics approval.—The use of animals in this study was approved by the Toronto Zoo (Ref. No. 2010-01-01, 2014-03-01, 2017-03-01, 2020-02-01), Laurentian University (protocols AUP 2017-02-01 and 6020983), Parks Canada, and the Ministry of Natural Resources (MNR) Animal Care Committees, based on guidelines of the Canadian Council on Animal Care. All work was authorized by the provincial Endangered Species Act permits (GU-B-014-13, AU-B-010-14, AU-B-008-15, AU-B-011-16, AU-B-008-17, AU-B-009-18, and PS-B-004-18, CN-B-006-20), Fish and Wildlife Conservation Act Authorization to Keep Wildlife (1094480/Local Ref. No. AU20191892, 1095663/Local Ref. No. AU2020-2321, 1095690/Local Ref. No. AU2020-2325, 1097716/Local Ref. No. AU2021-00077, and 1100047/Local Ref. No. AU2022-00100), Wildlife Scientific Collector's Authorizations (1077386, 1080550, 1083631, 1086727, 1090044, 1089107/Local Ref. No. AU2018-0533, 1092254/Local Ref. No. 053, 1092095/Local Ref. No. AU2019-1299, 1095664/Local Ref. No. AU2020-2308, 1095693/Local Ref. No. PS2020-0839, 1095690/Local Ref. No. AU2020-2325, 1096136/Local Ref. No. AU2020-2501, 1096490/

Table 1. Number of mortalities by survey location, turtle species, and age class at the Rouge National Urban Park in Ontario, Canada from 2014 to 2022. Blanding's Turtles include naturally occurring and head-started individuals while all other turtles are naturally occurring individuals.

Survey location	Species	Age class	Number of mortalities
Site A	Blanding's	Adult (male)	1
		Juvenile	93
	Painted	Adult (male)	2
		Adult (female)	48
		Juvenile	10
		Unknown**	34
	Snapping	Juvenile	2
		Total	190
Site B	Blanding's	Juvenile	28
	Painted	Adult (male)	3
		Juvenile	14
		Unknown**	16
	Snapping	Juvenile	17
		Unknown**	1
		Total	79
Site C	Blanding's (naturally occurring)	Juvenile	1
		Unknown**	1
	Painted	Adult (female)	1
		Juvenile	1
		Unknown**	19
	Snapping	Juvenile	4
		Unknown**	3
	Undetermined*	Unknown**	2
		Total	32
Other	Painted	Adult (male)	1
		Juvenile	1
		Unknown**	10
		Total	12

* Turtle species not identified due to the condition of the carcass.
** Age class data were not available because of the condition of the carcass or because data were not recorded.

Local Ref. No. AU2020-2550, 1097514/Local Ref. No. PS2021-1039, 1097470/Local Ref. No. AU2021-00104, 1100042/Local Ref. No. PS2022FW0014, and 1100043/Local Ref. No. AU2022-00099), Parks Canada Research and Collection (RNUP-2020-35017), and federal Species At Risk Act permits (RNUP-2020-35017).

RESULTS

Observed mortalities prior to mass-mortality event.—From 2014 to 2019, while conducting radio-telemetry, nesting, live-trapping, and road surveys (typically from mid-April to early September; Table S1; see Data Accessibility), we observed 42 Blanding's (41 were headstarted juveniles) and six Painted Turtle carcasses at site A, 26 Painted and 14 Snapping Turtle carcasses at site B, and two naturally occurring Blanding's, 18 Painted, five Snapping Turtle carcasses, and one turtle carcass of an unknown species at site C. An additional 11 Painted Turtle carcasses were observed in areas outside sites A–C. During this period, roadkill resulted in 76 mortalities, predation resulted in 34 mortalities, winterkill resulted in six mortalities, and unknown causes resulted in nine mortalities, for a total of 125 mortalities (Fig. 2).

Mass-mortality event.—While conducting only live-trapping and radio-telemetry surveys in 2020 (Table S1; see Data Accessibility), 48 juvenile headstarted Blanding's and 57 Painted Turtle carcasses were observed at site A, none of which were associated with roads or showed evidence of vehicle strikes. Carcasses of juvenile headstarted turtles were found mostly on land in Common Reed or grass patches. Of the 57 Painted Turtle mortalities documented in 2020, 43 were adult female Painted Turtles suspected to be depredated while nesting on land, while 14 were of unknown sex. The number of turtle mortalities observed for Blanding's and Painted Turtles in 2020 was 105, while the number of observed mortalities in the previous six years combined was 125 (Fig. 2). Various degrees of damage to hard and soft tissues were observed in Painted and Blanding's Turtles. Of the 83 turtles that were suspected to be depredated, field notes indicated 36 were decapitated, seven were missing head and at least one limb, four were decomposed, and 36 turtles lacked specific details of carcass condition. We could not determine the cause of death for some carcasses ($n = 22$) because of advanced decomposition, resulting in recovery of only shell fragments, intact shells without flesh, or carcasses in the later stages of autolysis. We acknowledge that disease is a potential cause of mortality and cannot be ruled out, especially where carcasses showed no obvious signs of predation; however, no evidence of disease was detected during necropsies ($n = 25$ carcasses).

Post-mass-mortality event.—While conducting a combination of three to five survey types in 2021 and 2022 (radio-telemetry, nesting, road, live-trapping, water-chemistry sampling;

Table 2. Mean straight-line carapace length (CL) and standard deviation of turtle carcasses* based on species and age class at the Rouge National Urban Park in Ontario, Canada.

Species	Age class	Number of individuals	Mean CL (mm) ± standard deviation	Min CL (mm)	Max CL (mm)
Blanding's	Juvenile	52	99.18 ± 9.35	82.50	134.20
Painted	Adult (male)	2	106.75 ± 9.55	100.00	113.50
	Adult (female)	48	138.59 ± 11.27	104.00	171.30
	Juvenile	14	56.26 ± 31.01	23.00	118.70
	Unknown**	6	130.67 ± 17.64	108.00	150.00
Snapping	Juvenile	6	78.26 ± 36.45	40.00	129.60

* We were unable to report measurements for 72 Blanding's, 90 Painted, 21 Snapping, and 2 unidentifiable turtles because of the destruction and/or decomposition of the carcass or as a result of missing data.
** An unknown status was assigned because of the condition of the carcass or because data pertaining to age class or sex were not recorded.

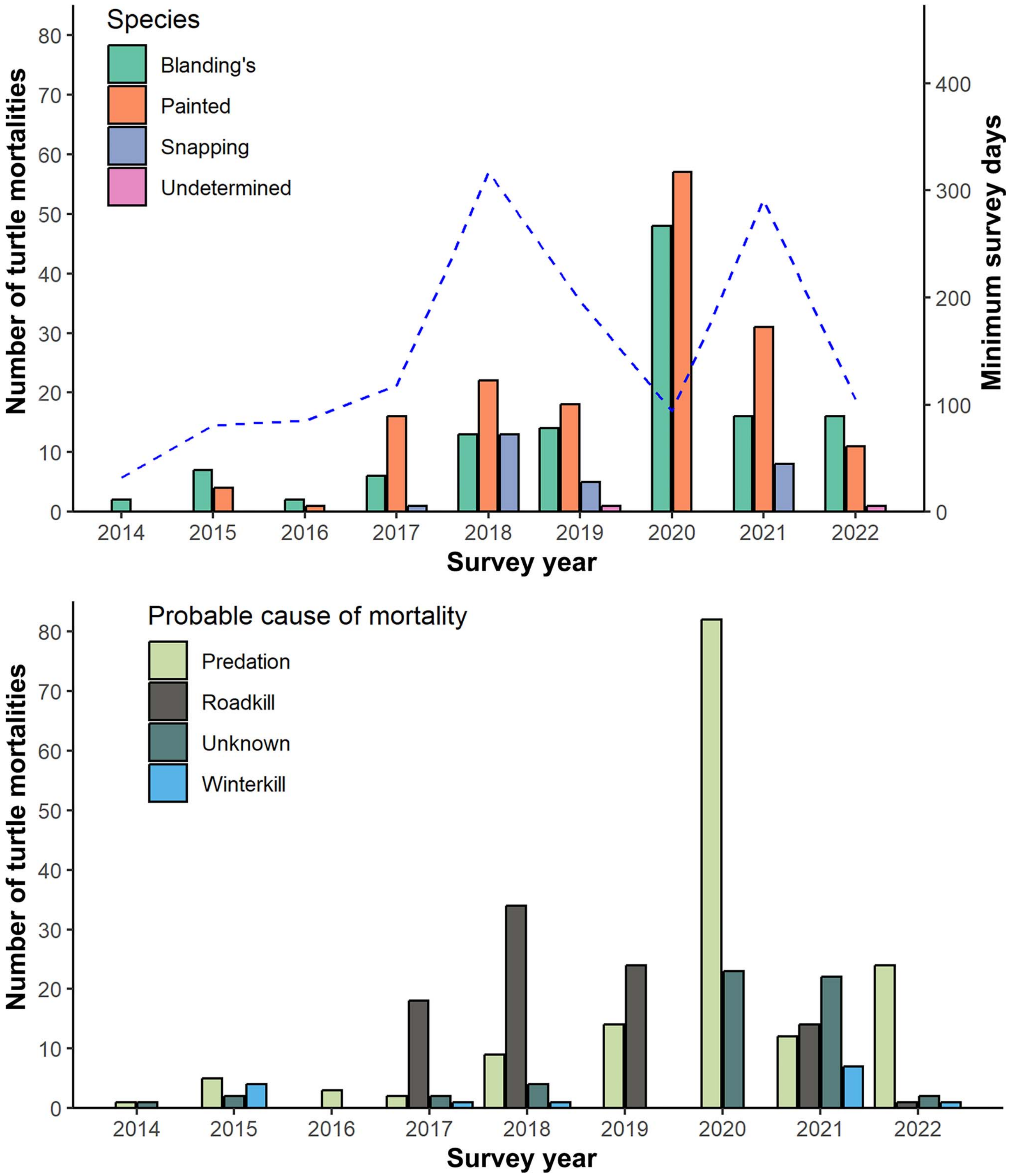

Fig. 2. Turtle mortalities based on species and minimum number of survey days (upper panel) and probable cause of mortality (lower panel) observed from 2014 to 2022, in Rouge National Urban Park, Ontario, Canada. The minimum number of survey days (dashed blue line) was obtained by multiplying the survey type and the unique number of survey days and summing the survey days for each year. An 'Undetermined' status was assigned to two turtles whose species could not be identified because of carcass decomposition. An 'Unknown' status was assigned when the cause of mortality was unclear.

Table S1; see Data Accessibility), we observed four headstarted Blanding's, 31 Painted, and two Snapping Turtle carcasses at site A; 28 headstarted Blanding's, seven Painted, and four Snapping Turtle carcasses at site B; and three Painted and two Snapping Turtle carcasses and one carcass of an unknown turtle species at site C. One Painted Turtle carcass was also found outside of these three study sites.

Mortality trends and demography by site.—Overall, across all years, the majority of the turtle mortalities occurred at site A ($n = 190$), followed by site B ($n = 79$), and finally site C ($n = 32$). In sites A and B, headstarted Blanding's and Painted Turtle mortalities were commonly documented ($n = 94$ of each), whereas in site C, where no headstart Blanding's Turtles have previously been released, only Painted Turtle mortalities ($n = 21$) were documented (Table 1). In sites A and B, documented Blanding's Turtle mortalities consisted of headstarted juveniles (except for one male). Among Painted Turtle carcasses, adult females were common in site A ($n = 48$), and juveniles were common in site B ($n = 14$). Snapping Turtle carcasses were found in relatively lower abundance across all three sites ($n = 27$), and most Snapping Turtle carcasses were juveniles ($n = 23$). Twelve additional carcasses were documented in areas not routinely surveyed outside of sites A–C (e.g., opportunistically found when surveyors traveled between sites and/or when conducting other monitoring activities). Of these carcasses, an adult male and a juvenile Painted Turtle were identified, while the age classes of the rest were unknown. At sites A–C, the sex or age class was determined for only 225 of 301 carcasses due to the condition of the carcass or because of missing data (Table 1).

DISCUSSION

Observed mortalities prior to mass-mortality event.—Turtle mortalities have been observed at three sites in the RNUP since monitoring began for the headstarting program in 2014. In earlier years (2014–2016), observed mortalities were relatively low, and the carcasses were primarily of headstarted Blanding's Turtles. Survey efforts increased in subsequent years (2017 onward), with a concomitant increase in observed mortalities. However, increased survey effort is likely just one factor that contributed to the increase in observed mortalities (see next section). A greater number of Painted Turtle carcasses were found than those of Blanding's Turtle. This observation is not surprising given the higher abundance of Painted Turtles compared to Blanding's Turtles across all surveyed sites. Painted Turtle mortalities were commonly associated with either predation or vehicular collisions, whereas the majority of headstarted Blanding's Turtle mortalities were due only to predation. As the headstarted Blanding's Turtles reach maturity, we expect individuals to exhibit larger home-range sizes (Edge et al., 2010), as observed in adults at our study sites (Toronto Zoo, unpubl. data). Given the highly fragmented nature of the RNUP and the extensive road network (Frost-Wicks, 2019), such movements will likely increase risk of road mortality for turtles. The major causes for pre-MME deaths (e.g., road mortality and predation) are typical of turtle populations inhabiting urban habitats (Marchand et al., 2002; Steen and Gibbs, 2004; Urbanek et al., 2016; Piczak et al., 2019). To a lesser degree, winterkill has also been documented in both urban and non-urban habitats (Christiansen and Bickham, 1989; Seburn et al., 2021).

Mass-mortality event.—In 2020, a large number of headstarted Blanding's and female Painted Turtles were depredated at site A. Despite relatively lower survey duration and survey area (i.e., site A only had radio-telemetry and trapping surveys for two months, site B was only surveyed using trapping for a week, and site C was not surveyed; Table S1; see Data Accessibility), mortalities were markedly higher in 2020 compared to previous years (2014–2019). Thus, we conclude the observed trends are not simply a result of changes in survey efforts.

A preliminary study based on a four-year mark–recapture survey at the three sites indicated a population size estimate of 306 (CI = 170–600) female and 142 (CI = 76–291) male Painted Turtles at site A in 2020 (Wijewardena et al., in press). As such, the observed mortalities resulted in ~13% population loss at site A. Similarly, in 2020, we estimated the population size of headstarted Blanding's Turtles to be 183 individuals (Wijewardena et al., 2023); thus, a loss of 48 individuals equates to a ~26% population decrease in Blanding's Turtles at site A. Different age classes of turtles are preyed upon by a variety mammals (Congdon et al., 1987; Cypher et al., 2018), such as American Mink (*Neogale vison*), Raccoon (*Procyon lotor*), Coyote (*Canis latrans*), and birds (Baxter-Gilbert et al., 2013), such as American Crow (*Corvus brachyrhynchos*). There are unique cases where one or a few individual predators caused MMEs of freshwater turtles (Brooks et al., 1991; Karson et al., 2018; Keevil et al., 2018; Jones et al., 2021; Bulté et al., 2024), and we suspect this to be the case in the RNUP as well. We suspect that Raccoons (Seigel, 1980) or mustelids (Stacy et al., 2014) were the likely cause of the mortality increase observed in 2020 due to similar damage to carcasses, which could have resulted from the dexterity of these predators. Coyote, Raccoon, River Otter (*Lontra canadensis*), and Mink have been observed at our study sites (Parks Canada, unpubl. data), and historical surveys (2005–2006) in the study areas indicated that Raccoons and other wildlife were fed by park visitors and residents in the area (Toronto Zoo, unpubl. data). We were unable to determine exactly when the mortalities at site A occurred because the decomposition rates of depredated turtles have not been examined at our study site. A study in Misery Bay Provincial Park in Ontario, Canada, reported that turtle soft tissue takes about three weeks to decompose under normal weather conditions, and decomposition rates can vary depending on whether the carcass was on land or submerged in water (Gasbarrini et al., 2021). We are unaware whether similar mortalities occurred in other sites, but a week-long survey at site B in 2020 and surveys at site C in subsequent years did not produce similar numbers of depredated turtles as we observed at site A. Drought conditions observed during 2020 and 2021 may have played a role in observed high mortalities (Table S2; see Data Accessibility). Droughts and intentional water drawdowns are associated with higher predation of turtles elsewhere, in part because turtles become concentrated into high densities in the limited remaining aquatic habitats, increasing their vulnerability (Gibbons et al., 1983; Lindeman and Rabe, 1990; Hall and Cuthbert, 2000; Anthonysamy et al., 2013; Gasbarrini et al., 2021; Beard and Powell, 2023). Freshwater turtles may also die due to diseases (Fey et al., 2015; Agha et al., 2017; Allender et al., 2018). In the subset of carcasses that were necropsied in our study, disease was not identified as a potential cause of mortality by CWHC. However, not all carcasses were fresh and, as a result, we cannot rule out disease alone or in combination with other factors.

Post-mass-mortality event trends.—Blanding's Turtle mortality rates returned to pre-MME (2014–2019) levels post-2020, whereas Painted Turtle mortalities remained elevated in 2021, returning to pre-MME levels in 2022. Suspected predation was the most common cause of mortality for Blanding's and Painted Turtles; however, it is plausible that some deaths occurred in 2020 but were discovered in subsequent years based on carcass deterioration. Road surveys in the area of these sites also typically resulted in more observations of Painted Turtle mortalities than other species (Leermakers, 2020). These observations are typical for our study area given the relative abundance of each species, i.e., Painted Turtles have the highest abundance, followed by Blanding's Turtles (due to headstarting), and Snapping Turtles.

Mortality trends and demography by site.—Blanding's Turtle mortalities were observed at sites A and B because they are release sites for the headstarted population that are routinely surveyed. Given that a greater number of headstarted turtles have been released at site A compared to site B, it is not surprising that the majority of Blanding's Turtle mortalities were observed at site A. In contrast, at site C, Painted Turtle mortalities were common because of their higher abundance (Toronto Zoo, unpubl. data). Despite the proximity of the surveyed sites to each other (within <2 km), we have not observed juvenile headstarted Blanding's Turtles at site C, indicating that headstarted turtles remain in the release sites. We have occasionally observed Snapping or naturally occurring Blanding's Turtles at site C and believe both species are likely transients that occupy permanent wetlands elsewhere. Roadkill is a major threat to turtles at sites B and C because the wetlands are close to roads. Since female turtles use road shoulders to nest (Aresco, 2005), high female mortality is often associated with roads (Steen and Gibbs, 2004). However, juveniles are also affected by roads and may be killed more frequently than adults (Keevil et al., 2023). We observed several ($n = 36$) juvenile deaths during road surveys between 2017 and 2022. Ultimately, roads pose a threat to both sexes and all life stages of turtles (Carstairs et al., 2018; Keevil et al., 2023).

Conservation implications.—Achieving and maintaining high survival is a challenge for turtle populations in fragmented habitats, such as the case in the RNUP. This is true for Blanding's Turtles, which have been the target of conservation management for over ten years. Based on our findings from 2014 to 2022, at least 124 of the 374 (33%) headstarted Blanding's Turtles released have been lost to predation, winter-kill, wildlife–vehicular collisions, and unknown causes. A population viability analysis of headstarted Blanding's Turtles that incorporates the effect of catastrophes is currently being undertaken by the Toronto Zoo to determine management implications. We have yet to determine the extent of the long-term consequences that will follow the acute mortality of female Painted Turtles in 2020. Demographic models highlight the need for high adult (Enneson and Litzgus, 2008; Shoemaker et al., 2013) and juvenile survival for population stability in turtles (Congdon et al., 1993). If observed mortality trends continue, the Painted and Blanding's Turtle community at the three sites is likely at risk of extirpation; as a result, they will require longer population recovery times and more intense human-mediated conservation interventions. Conservation initiatives focusing on endangered species already face multiple challenges in terms of limited funding, lack of long-term monitoring, and controversy regarding the most effective method to recover SAR (Dodd and Seigel, 1991; Heppell et al., 1996; Martin et al., 2018). MMEs can result in unanticipated strain on limited resources, which will delay recovery of populations with slow life histories. Additionally, due to stochasticity in natural ecosystems, catastrophes such as MMEs are perhaps the least understood in terms of frequency of occurrence and magnitude of impact. Our study is a cautionary tale for headstarting projects and highlights how MMEs could limit population recovery despite ongoing conservation efforts. Similarly, MMEs can impact naturally occurring populations (Keevil et al., 2018) that are perceived as robust given their relative abundance, as observed in our study for Painted Turtles. Continued monitoring of an endangered species provided us with a unique opportunity to monitor a Painted Turtle population that typically receives less conservation focus. Our study further highlights the importance of monitoring common species that may undergo catastrophes and face extirpation before we recognize the urgency of their conservation needs.

We conclude that long-term monitoring is necessary to understand population trends, especially in species with delayed sexual maturity and long generation times. MMEs, paired with ongoing challenges to survival (e.g., nest predation, road mortality), pose serious risks to the persistence of species at risk populations, especially in urban areas where multiple threats are simultaneously exacerbated (e.g., habitat fragmentation, degradation). Freshwater turtle populations, even in protected areas, can suffer from sudden, chronic, or synergistic sources of mortality due to natural or anthropogenic threats (Browne and Hecnar, 2007; Jones et al., 2021). Incorporation of catastrophes into projection tools for naturally occurring and augmented populations, such as population viability analyses, is needed to better understand the combined factors that affect individual- and population-level mortality, and inversely, survival. Ultimately, we strongly recommend that adaptive management (e.g., additional release sites) and multi-species approaches (e.g., subsidized predator control, nest protection, road mortality mitigation) be taken when pursuing conservation action specific to turtles.

DATA ACCESSIBILITY

Supplemental material is available at https://www.ichthyologyandherpetology.org/h2024045.

AI STATEMENT

The authors declare that they used ChatGPT to troubleshoot a coding error in creating Figure 2 and Grammarly (free version) to check for grammar and punctuation errors.

ACKNOWLEDGMENTS

Funding for this work was provided by Parks Canada, Natural Sciences and Engineering Research Council of Canada (including Discovery Grants to NEM and JDL, and CREATE ReNewZoo), and Ruffed Grouse Society Wildlife Conservation Bursary. We thank collaborators at (in alphabetical order): City of Toronto; Friends of the Rouge Watershed; Georgian Bay Biosphere Reserve; Laurentian University; Magnetawan First Nation; Ministry of the Environment, Conservation, and Parks; Ministry of Natural Resources and Forestry; Parks Canada; Shawanaga First Nation; Toronto and Region Conservation Authority (TRCA); and University of Toronto. We thank all individuals who worked on this project in different capacities, including graduate students (Amy Mui, Nicole Richards, Jethro Valido, Tisha Tan, Shannon Ritchie, Courtney Leermakers); field technicians; past Adopt-A-Pond, Toronto Zoo, and Parks Canada staff; and volunteers at the Toronto Zoo and Parks Canada. We are also especially thankful for the efforts of Rachelle Fortier from the Toronto Zoo; Julia Phillips, Juliana Skuza, Jory Mullen, and Richard De Paulsen from Parks Canada; and Karen McDonald from the TRCA for their insightful contributions.

LITERATURE CITED

Agha, M., S. Price, A. Nowakowski, B. Augustine, and B. D. Todd. 2017. Mass mortality of Eastern Box Turtles with upper respiratory disease following atypical cold weather. Diseases of Aquatic Organisms 124:91–100.

Allender, M. C., A. C. Barthel, J. M. Rayl, and K. A. Terio. 2018. Experimental transmission of frog virus 3-like ranavirus in juvenile chelonians at two temperatures. Journal of Wildlife Diseases 54:716–725.

Ameca y Juárez, E. I., G. M. Mace, G. Cowlishaw, and N. Pettorelli. 2012. Natural population die-offs: causes and consequences for terrestrial mammals. Trends in Ecology & Evolution 27:272–277.

Anthonysamy, W. J. B., M. J. Dreslik, and C. A. Phillips. 2013. Disruptive influences of drought on the activity of a freshwater turtle. The American Midland Naturalist 169:322–335.

Aresco, M. J. 2005. The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. Biological Conservation 123:37–44.

Barton, P. S., A. Reboldi, S. Bonat, P. Mateo-Tomás, and T. M. Newsome. 2023. Climate-driven animal mass mortality events: is there a role for scavengers? Environmental Conservation 50:1–6.

Baxter-Gilbert, J., J. L. Riley, and J. D. Litzgus. 2013. *Chrysemys picta marginata* (Midland Painted Turtle). Avian predation. Herpetological Reviews 44:302–303.

Beard, A. N., and L. A. Powell. 2023. Drought influences annual survival of Painted Turtles in western Nebraska. Diversity 15:597.

Beaudry, F., P. G. deMaynadier, and M. L. Hunter. 2008. Identifying road mortality threat at multiple spatial scales for semi-aquatic turtles. Biological Conservation 141:2550–2563.

Bennett, A. M., and J. D. Litzgus. 2014. Injury rates of freshwater turtles on a recreational waterway in Ontario, Canada. Journal of Herpetology 48:262–266.

Brooks, R. J., G. P. Brown, and D. A. Galbraith. 1991. Effects of a sudden increase in natural mortality of adults on a population of the Common Snapping Turtle (*Chelydra serpentina*). Canadian Journal of Zoology 69:1314–1320.

Browne, C. L., and S. J. Hecnar. 2007. Species loss and shifting population structure of freshwater turtles despite habitat protection. Biological Conservation 138:421–429.

Bulté, G., J. A. Robichaud, E. J. Shadlock, S. J. Cooke, and G. Blouin-Demers. 2024. Overwintering site fidelity and communal hibernation predispose Northern Map Turtles to mass mortality events. Canadian Journal of Zoology 102:166–174.

Burke, R. L. 2015. Head-starting turtles: learning from experience. Herpetological Conservation and Biology 10(Symposium):299–309.

Cagle, F. R. 1939. A system of marking turtles for future identification. Copeia 1939:170–173.

Carstairs, S., M. Dupuis-Desormeaux, and C. M. Davy. 2018. Revisiting the hypothesis of sex-biased turtle road mortality. The Canadian Field-Naturalist 132:289–295.

Christiansen, J. L., and J. W. Bickham. 1989. Possible historic effects of pond drying and winterkill on the behavior of *Kinosternon flavescens* and *Chrysemys picta*. Journal of Herpetology 23:91–94.

Congdon, J. D., G. L. Breitenbach, R. C. van Loben Sels, and D. W. Tinnkle. 1987. Reproduction and nesting ecology of Snapping Turtles (*Chelydra serpentina*) in southeastern Michigan. Herpetologica 43:39–54.

Congdon, J. D., A. E. Dunham, and R. C. Van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's Turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. Conservation Biology 7:826–833.

COSSARO. 2023. Committee on the Status of Species at Risk in Ontario. Available from http://cossaroagency.ca/species/ (accessed 24 January 2023).

Cypher, B. L., E. C. Kelly, T. L. Westall, and C. L. Van Horn Job. 2018. Coyote diet patterns in the Mojave Desert: implications for threatened desert tortoises. Pacific Conservation Biology 24:44–54.

Dodd, C. K., and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica 47:336–350.

Edge, C. B., B. D. Steinberg, R. J. Brooks, and J. D. Litzgus. 2010. Habitat selection by Blanding's Turtles (*Emydoidea blandingii*) in a relatively pristine landscape. Ecoscience 17:90–99.

Enneson, J. J., and J. D. Litzgus. 2008. Using long-term data and a stage-classified matrix to assess conservation strategies for an endangered turtle (*Clemmys guttata*). Biological Conservation 141:1560–1568.

Ernst, C. H., and J. E. Lovich. 2009. Turtles of the United States and Canada. Second edition. Johns Hopkins University Press, Baltimore, Maryland.

Fey, S. B., A. M. Siepielski, S. Nusslé, K. Cervantes-Yoshida, J. L. Hwan, E. R. Huber, M. J. Fey, A. Catenazzi, and S. M. Carlson. 2015. Recent shifts in the occurrence, cause, and magnitude of animal mass mortality events. Proceedings of the National Academy of Sciences of the United States of America 112:1083–1088.

Field, K. J., C. R. Tracy, P. A. Medica, R. W. Marlow, and P. S. Corn. 2007. Return to the wild: translocation as a tool in conservation of the Desert Tortoise (*Gopherus agassizii*). Biological Conservation 136:232–245.

Frost-Wicks, A. 2019. Modeling habitat connectivity and movement of Blanding's Turtles (*Emydoidea blandingii*) across a fragmented landscape in Rouge National Urban Park, Ontario, Canada. Environmental Science Undergraduate Honours Thesis, Dalhousie University, Halifax, NS, Canada.

García, A., G. Ceballos, and R. Adaya. 2003. Intensive beach management as an improved sea turtle conservation strategy in Mexico. Biological Conservation 111:253–261.

Gasbarrini, D. M. L., D. Lesbarrères, A. Sheppard, and J. D. Litzgus. 2021. An enigmatic mass mortality event of Blanding's Turtles (*Emydoidea blandingii*) in a protected area. Canadian Journal of Zoology 99:470–479.

Gibbons, J. W., J. L. Greene, and J. D. Congdon. 1983. Drought-related responses of aquatic turtle populations. Journal of Herpetology 17:242–246.

Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C. T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. BioScience 50:653–666.

Gizzi, F., J. Jiménez, S. Schäfer, N. Castro, S. Costa, S. Lourenço, R. José, J. Canning-Clode, and J. Monteiro. 2020. Before and after a disease outbreak: tracking a keystone species recovery from a mass mortality event. Marine Environmental Research 156:104905.

Government of Canada. 2023. Species at risk public registry. Government of Canada. Available from https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html (accessed 24 January 2023).

Hall, C. D., and F. J. Cuthbert. 2000. Impact of a controlled wetland drawdown on Blanding's Turtles in Minnesota. Chelonian Conservation and Biology 3:643–649.

Heppell, S. S., L. B. Crowder, and D. T. Crouse. 1996. Models to evaluate headstarting as a management tool for long-lived turtles. Ecological Applications 6:556–565.

Jones, M. T., L. L. Willey, J. D. Mays, and C. K. Dodd. 2021. Wildfire, depredation, and synergistic management challenges contribute to the decline of a significant population of Florida Box Turtles (*Terrapene bauri*). Chelonian Conservation and Biology 20:200–210.

Karson, A., S. Y. J. Angoh, and C. M. Davy. 2018. Depredation of gravid freshwater turtles by Raccoons (*Procyon lotor*). The Canadian Field-Naturalist 132:122–125.

Keevil, M. G., R. J. Brooks, and J. D. Litzgus. 2018. Post-catastrophe patterns of abundance and survival reveal no evidence of population recovery in a long-lived animal. Ecosphere 9:e02396.

Keevil, M. G., N. Noble, S. P. Boyle, D. Lesbarrères, R. J. Brooks, and J. D. Litzgus. 2023. Lost reproductive value reveals a high burden of juvenile road mortality in a long-lived species. Ecological Applications 33:e2789.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. The American Naturalist 142:911–927.

Lanszki, J., M. Molnár, and T. Molnár. 2006. Factors affecting the predation of otter (*Lutra lutra*) on European Pond Turtle (*Emys orbicularis*). Journal of Zoology 270:219–226.

Leermakers, C. G. 2020. Mitigating road mortality of wildlife in Rouge National Urban Park. Unpubl. M.S. thesis, University of Toronto, ON, Canada. Available from https://tspace.library.utoronto.ca/bitstream/1807/101089/1/Leermakers_Courtney_Grace_202006_MSc_thesis.pdf

Lindeman, P. V., and F. W. Rabe. 1990. Effect of drought on the Western Painted Turtle, *Chrysemys picta belli*, in a small wetland ecosystem. Journal of Freshwater Ecology 5:359–364.

Lovich, J. E., J. R. Ennen, M. Agha, and J. W. Gibbons. 2018. Where have all the turtles gone, and why does it matter? BioScience 68:771–781.

Marchand, M. N., J. A. Litvaitis, T. J. Maier, and R. M. DeGraaf. 2002. Use of artificial nests to investigate predation on freshwater turtle nests. Wildlife Society Bulletin 30:1092–1098.

Martin, T. G., L. Kehoe, C. Mantyka-Pringle, I. Chades, S. Wilson, R. G. Bloom, S. K. Davis, R. Fisher, J. Keith, K. Mehl, B. P. Diaz, M. E. Wayland, T. I. Wellicome, K. P. Zimmer, and P. A. Smith. 2018. Prioritizing recovery funding to maximize conservation of endangered species. Conservation Letters 11:e12604.

Millar, C. S., and G. Blouin-Demers. 2011. Spatial ecology and seasonal activity of Blanding's Turtles (*Emydoidea blandingii*) in Ontario, Canada. Journal of Herpetology 45:370–378.

Piczak, M. L., C. E. Markle, and P. Chow-Fraser. 2019. Decades of road mortality cause severe decline in a Common Snapping Turtle (*Chelydra serpentina*) population from an urbanized wetland. Chelonian Conservation and Biology 18:231–240.

Reed, D. H., J. J. O'Grady, J. D. Ballou, and R. Frankham. 2003. The frequency and severity of catastrophic die-offs in vertebrates. Animal Conservation 6:109–114.

Seburn, D. C., M. Burns, I. Akinrinola, S. Cecile, T. Farquharson, C. Hung, and P. McIntyre. 2021. Apparent winterkill of Painted Turtle (*Chrysemys picta*). The Canadian Field-Naturalist 135:383–386.

Seigel, R. A. 1980. Predation by raccoons on Diamondback Terrapins, *Malaclemys terrapin tequesta*. Journal of Herpetology 14:87–89.

Shoemaker, K. T., A. R. Breisch, J. W. Jaycox, and J. P. Gibbs. 2013. Reexamining the minimum viable population concept for long-lived species. Conservation Biology 27:542–551.

Stacy, B. A., D. A. Wolf, and J. F. X. Wellehan. 2014. Large-scale predation by River Otters (*Lontra canadensis*) on Florida Cooter (*Pseudemys floridana*) and Florida Softshell Turtles (*Apalone ferox*). Journal of Wildlife Diseases 50:906–910.

Stanford, C. B., J. B. Iverson, A. G. J. Rhodin, P. P van Dijk, R. A. Mittermeier, G. Kuchling, K. H. Berry, A. Bertolero, K. A. Bjordndal, T. E. G. Blanck, K. A. Buhlmann, R. L. Burke, J. D. Congdon, T. Diagne … A. D. Walde. 2020. Turtles and tortoises are in trouble. Current Biology 30:R721–R735.

Steen, D. A., and J. P. Gibbs. 2004. Effects of roads on the structure of freshwater turtle populations. Conservation Biology 18:1143–1148.

Urbanek, R. E., G. A. Glowacki, and C. K. Nielsen. 2016. Effect of Raccoon (*Procyon lotor*) reduction on Blanding's Turtle (*Emydoidea blandingii*) nest success. Journal of North American Herpetology 2016:39–44.

Wijewardena, T., M. G. Keevil, N. E. Mandrak, A. M. Lentini, and J. D. Litzgus. 2023. Evaluation of headstarting as a conservation tool to recover Blanding's Turtles (*Emydoidea blandingii*) in a highly fragmented urban landscape. PLoS ONE 18:e0279833.

Wijewardena, T., N. E. Mandrak, A. M. Lentini, and J. D. Litzgus. In press. Demographic assessment of a freshwater turtle assemblage in an urban protected area in the context of ongoing threats and a mass-mortality event. Chelonian Conservation and Biology.