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Freshwater turtle populations as bioindicators following an oil spill: Delayed demographic changes reveal long-term impacts

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ABSTRACT

Chronic, long-term impacts of oil spill disasters on wildlife often exceed short-term, highly visible mass mortalities and widespread oiling of individuals. Species with long lifespans, late maturation, and low recruitment rates are particularly vulnerable to long-term population-level impacts but can be useful as indicator species for ecosystem recovery. In 2010, one of the largest freshwater oil spills in the U.S. occurred in the Kalamazoo River, MI, when 3.2 million L of spilled oil impacted 56 km of river and associated wildlife. During cleanup and restoration efforts in 2010–2011, thousands of northern map turtles (*Graptemys geographica*) were captured, cleaned, and released. During 2019–2020 northern map turtles were captured to evaluate changes in the population size, demography, and size classes nine to ten years later.

Population demography shifts occurred in the first few years after the spill in a species that otherwise exhibits minimal demographic fluctuation under "normal" conditions. In comparing demographic parameters at the time of the oil spill to values ~ 10 years post-spill, a nearly 30% reduction in population size was detected, distribution of body size shifted to smaller males and females, and there was a shift in the population sex ratio between 2011 and 2019. There were also signals of failed recruitment in cohorts that would have hatched during the years immediately before and after the oil spill. These data suggest that beyond the direct mortality caused by the spill, declines in the estimated population size and shifts in the size distribution of northern map turtles are likely indicative of negative demographic impacts incurred following the 2010 oil spill and resulting cleanup.

1. Introduction

The adverse effects of oil spill disasters on wildlife populations are highly visible and well documented, often including mass mortalities and widespread oiling of large numbers of individuals (Bourne et al. 1967; Dunnet 1982; Barron et al. 2020). Studies regarding the impacts of oil spills on vertebrate species generally focus on acute effects (i.e., short-term impacts, typically due to initial oil exposure) rather than monitoring potential chronic effects (i.e., long-term impacts that persist after initial oil exposure that may be due to lingering toxins in the environment; Helm et al. 2015). Importantly, however, chronic effects can extend years beyond the oil spill itself and subsequent environmental cleanup operations, may impact entire populations, and may exceed the cumulative impacts of acute effects (Iverson and Esler 2010; Monson et al. 2011).

Chronic effects of oil spills on animal populations can be difficult to quantify, as individual-level data such as duration of oiling or mechanism of exposure (e.g., inhalation, absorption, ingestion) are often unknown. The costs of monitoring and assessing for chronic effects are substantial, and often outside the initial scope of cleanup. The differences in acute and chronic effects may be further exacerbated in situations with varying concentrations of pollutants, differing toxicity of oil types, variation in wildlife responses to chemical exposure, and the length of time and exposure necessary to induce lethal responses (Schwarzenbach et al. 2006; Rowe 2008). Baseline pre-spill population data often do not exist, as it is nearly impossible to predict spills.

Emergency spill response and cleanup operations may have additional impacts on wildlife populations. For example, mitigation activities such as hydraulic sediment flushing, aquatic vegetation harvesting, dispersant use, oil vacuuming, and dredging of sediments can create physical disturbances that impact wildlife (Vandermeulen and Ross 1995; Bejarano 2018). Such physical disturbances may alter local trophic structures, which could subsequently cause individuals to spend more time foraging, change their diet, or leave the area entirely.

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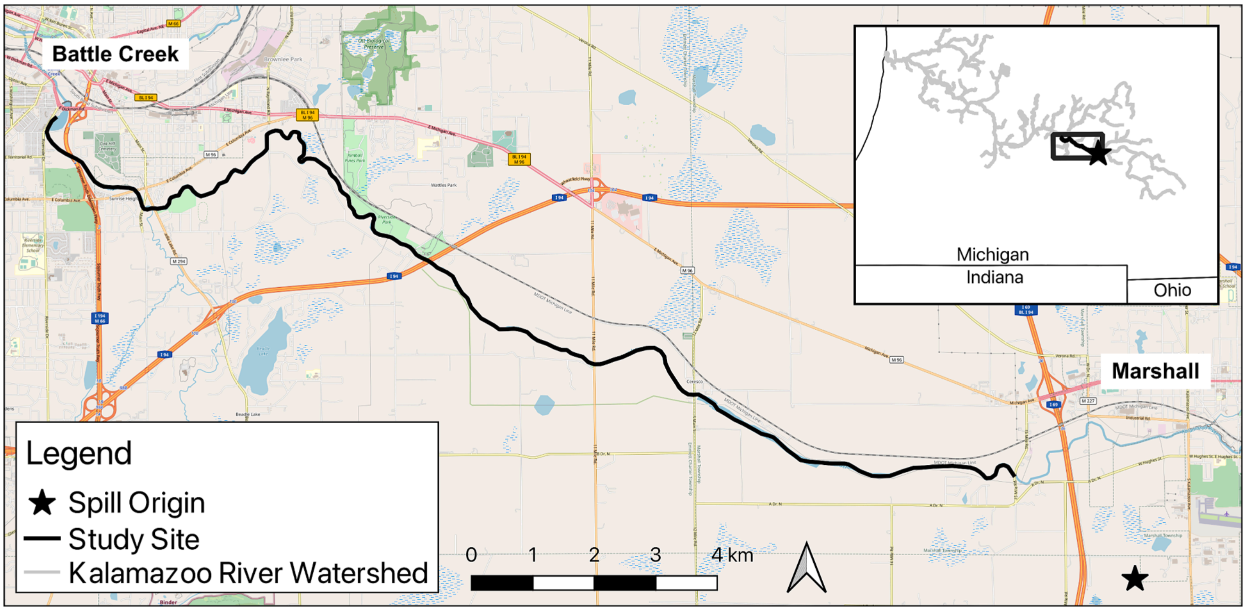

Fig. 1. The Kalamazoo River Study Site that was surveyed for northern map turtles (*Graptemys geographica*) during 2010, 2011, 2019, and 2020 following the July 2010 oil spill. The Study Site was 20.2 km stretch of the Kalamazoo River that was impacted by nearly 4.5 million L of spilled diluted bitumen oil in Calhoun County, MI, USA.

Similarly, if physical disturbances have disproportionate impacts on particular size or age classes, the relative number of individuals from different life stages may be altered, which could impact demographic parameters. In vertebrate species with typically stable populations, populations are generally thought to compensate for low recruitment rates and late age maturation with long lifespans and high adult survival rates (Congdon et al. 1983; Congdon et al. 1993). Such species may be particularly vulnerable to population-level impacts of oil spills, often causing an increase in mortality, which may take months to emerge and years from which to recover (McCann and Shuter 1997; Musick 1999; Norse et al. 2012). Additional population-level impacts may include reduced recruitment, increased replacement rate of breeding adults, or decreased mate encounter rates, all of which can further exacerbate population-level declines (Stearns 1992), highlighting the necessity of population monitoring and assessment years after an ecological disaster.

Massive oil spill disasters such as the *Exxon Valdez* (1989) and the Deepwater Horizon (2010) resulted in extensive literature on the population-level impacts of conventional crude oil spills on marine wildlife (Barron et al. 2020). The most visible impacts of crude oil, or liquid oil pumped from underground deposits, include both acute and sublethal effects in exposed wildlife via physical mechanisms such as coating of fish gills, feathers, or permeable skin surfaces. Wildlife can also be affected through exposure or ingestion of toxins present in the oil itself, specifically polycyclic aromatic hydrocarbons (PAHs; Peterson et al. 2003; Barron 2012; Esler et al. 2018; Barron et al. 2020). Exposure to PAHs can cause cardiotoxicity, behavioral changes, immunotoxicity, and decreases in reproductive success, all of which may cause population declines over time by increasing mortality or decreasing reproductive rates (Barron 2012; Honda and Suzuki 2020).

The impacts of crude oil spills on wildlife populations have been equivocal. In marine systems, for example, 64% (9 species) of bird species surveyed nine years after the *Exxon Valdez* oil spill declined in density following the spill event, while 21% (3 species) increased (Esler et al. 2018). One population of sea otters (*Enhydra lutris*) in the same area nearly quadrupled seven years after the spill; however, other sea otter populations that experienced severe oiling and mortality immediately following the oil spill did not similarly increase, which was attributed to poor survival or emigration (Esler et al. 2018; Barron et al. 2020). In the five years following the Deepwater Horizon spill, bottlenose dolphins (*Tursiops truncates*) in Bartaria Bay exhibited decreased calving and adult survival rates compared to previously reported rates in a reference population (Lane et al. 2015). Mitchelmore et al. (2017) estimated that ~ 30% of all minimally oiled sea turtles in the region affected by the Deepwater Horizon spill likely died later from oil ingestion. Moreover, although observed declines in nesting sea turtles following the Deepwater Horizon spill cannot be causally linked to the spill, they may be due to reduced prey availability for nesting females (Lauritsen et al. 2017).

In comparison to marine ecosystems, we know little regarding the effects of oil spills in freshwater ecosystems. In particular, almost nothing is known about the impacts of free-ranging freshwater organisms exposed to diluted bitumen (dilbit) in natural ecosystems. Dilbit is pure bitumen oil that is mixed with natural gas condensates for easier transport, having a similar chemistry but higher density, viscosity, and adhesion than conventional crude oil (Dew et al. 2015). To date, most data on the effects of dilbit on free-ranging freshwater organisms were collected in relation to one of the largest inland oil spills in U.S. history, the Kalamazoo River oil spill, which occurred near Marshall, Michigan, USA. On 25–26 July 2010, 3.2 million L of dilbit were reportedly released after a pipeline rupture (NTSB, 2012). The U.S. Environmental Protection Agency (EPA) oversaw cleanup operations through November 2014, estimating that 4.5 million L were recovered during cleanup operations (EPA, 2016). Oversight was transferred in November 2014 to the Michigan Department of Environmental Quality (MDEQ) for continued cleanup and restoration efforts consistent with state law. MDEQ also conducted an Environmental Assessment and Final Damage Assessment and Restoration Plan for restoration to pre-spill baseline conditions (USFWS, 2015).

The spilled dilbit initially pooled in a marshy area near the ruptured pipeline, before flowing into Talmadge Creek, and then into the Kalamazoo River, where it impacted nearly 56 km of river channel, including mixing with sediment (Crosby et al. 2013; EPA, 2016; Fig. 1). Due to the spill occurring within riverine habitat, no oil dispersants were used during cleanup, but a variety of mechanical processes were used to clean the river, including physical removal of oiled sediment, vegetation, and woody debris (EPA, 2016). The Kalamazoo River oil spill provided an opportunity to assess the potential population-level effects of oil exposure on freshwater organisms in a natural freshwater ecosystem through monitoring changes to a population of long-lived vertebrate species in the short-term (1-year post spill) and long-term (10-years post spill).

Northern map turtles (*Graptemys geographica*) were the most commonly observed and captured oiled vertebrate during the 2010 Kalamazoo River oil spill (EPA, 2016). Freshwater turtles can serve as excellent bioindicators due to their strong site fidelity, stable populations, longevity, varied diet, and ability to accumulate pollutants without lethal effects (Bonin et al. 1995; Aguirre and Lutz 2004; Adams et al. 2016). Cleanup, rescue, and rehabilitation efforts in 2010–2011 captured > 2,100 individual northern map turtles exhibiting varying degrees of oiling (EPA, 2016). During rehabilitation efforts, short-term turtle survival rates were generally high, although some mortalities occurred 2–12 months post spill while under veterinarian care (Otten et al. 2022). Thus, the Kalamazoo River oil spill provided a natural experiment to test the demographic impacts of a freshwater oil spill 1–10 years later using a common wildlife species as a model testing for lingering negative effects to species directly impacted. Here, we monitored the northern map turtle population from the Kalamazoo River during 2010, 2011, 2019, and 2020 to determine whether the population differed in total size, sex ratio, or body size structure following the 2010 oil spill.

2. Materials and methods

2.1. Study site

The Study Site was a 20.2 km stretch of the Kalamazoo River from Talmadge Creek to E. Dickman Road in Calhoun County, Michigan, USA (Fig. 1), where the majority of wildlife recovery and rescue work occurred following the 2010 oil spill, and which was 3.5–23.7 river km from the spill origin (EPA, 2016; Fig. 1). The Kalamazoo River within the Study Site ranges from 9.0 to 40.0 m wide and 0.2–3.5 m deep. Ample basking structures in the form of woody debris and exposed banks are found throughout the Study Site.

2.2. Study species

Northern map turtles are primarily aquatic and live in rivers and large lakes throughout their geographic range. During the active season, they leave the water to bask daily on woody debris, rocks, or banks. This species exhibits pronounced sexual dimorphism, with adult females growing to nearly twice the length of males (18.0–27.3 cm straight carapace length [SCL] vs 9.0–15.9 cm SCL, respectively). Males reach sexual maturity at 3–5 years of age (Iverson 1988) while females mature after at least 10 years (Lindeman 2013; Nagle and Congdon 2016). Sex can typically be identified between 1 and 2 years of age using secondary sex characteristics (e.g., tail length and width, and cloacal placement relative to shell margins; Lindeman 2013). It is estimated that wild map turtles can live between 30 and 50 years (Lindeman 2013), and individuals of both sexes at the Study Site have been estimated to be 40 + years of age (Otten 2022). Annual survival rates are slightly higher in females (87–94%) compared to males (81–83%; Bulté and Blouin-Demers 2009; Bulté et al. 2009). Natural predators of adult map turtles include river otters (*Lontra canadensis*); however, no otters were observed during surveys (Otten unpubl. data). Raccoon (*Procyon lotor*), coyote (*Canis latrans*), and skunks (*Mephitis mephitis*) were all observed during the course of this study and are known to depredate turtle nests and hatchlings (Otten, unpubl. data).

Northern map turtles may serve as excellent bioindicators following an oil spill due to their exposure to oil via several potential pathways. Oiling of the skin and shell primarily occurs during turtles' movement throughout the water column, breaking the water surface to breathe, and while basking on materials to which oil adheres. Inspiration and ingestion of toxic volatiles may have occurred as the species operates at the air/water interface to breathe and may have fed on oiled food sources. In addition, dilbit sank and mixed with sediment in backwater areas where some turtles hibernated, providing another potential point of exposure (EPA, 2016; Otten 2022).

2.3. Initial turtle capture and rehabilitation

Beginning immediately following the 2010 oil spill, turtle capture and rehabilitation occurred from 30 July to 24 October 2010, was conducted by numerous volunteers and paid contractors, including J.O., and was overseen by L.W. and the U.S. Fish and Wildlife Service (USFWS; EPA, 2016). Over 93% of the 1,385 northern map turtle captures in 2010 were made by hand or with extendable hand nets from boats (Lager 1943), with the remainder captured in hoop traps. Hand nets were typically 2.3 m long with mesh ranging from 1 to 2.3 cm, allowing for capture of all age classes of turtles, including hatchlings. Field crews recorded capture location of each turtle using a handheld GPS unit (Garmin International Inc.; <3m accuracy), identified sex when possible, measured straight carapace length (SCL) along the midline to the nearest mm, and mass to the nearest 0.1 g. Upon initial capture, field crews marked each individual > 100 g with a passive integrated transponder (PIT) tag (Avid Identification Systems, Inc.) injected in the body cavity anterior to either rear leg. Due to the limited number of PIT tags available, size of injection needle, and scope and oversight of the cleanup operations, individuals < 100 g were marked with a unique set of notches filed along the marginal scutes when possible (Cagle 1939), instead of being PIT-tagged. Turtles exhibiting any signs of oiling were taken into captivity for rehabilitation as described in Otten et al. (2022). Turtles with no visible signs of oiling were processed as described above and released at the point of capture. From 31 July – 5 October 2010, turtles captured at the Study Site, cleaned of oil, and cleared by veterinarians for release were translocated to tributaries, or locations upstream and downstream from the oiled stretch of river, thus protecting them from additional oiling and ongoing disturbance from cleanup operations. We excluded translocated turtles that were released > 8 km from the Study Site location from demographic analysis.

2.4. Recapture surveys

From 29 April to 1 October 2011, field crews used the same methods as described for 2010 to continue capturing oiled turtles for cleanup and rehabilitation, and to recapture previously rehabilitated and released turtles. Over 87% of 1,960 northern map turtle captures in 2011 were made via hand netting as described above, with the remainder captured in hoop traps. In 2019–2020, researchers from the University of Toledo returned to the Study Site to capture turtles to determine if they had been captured, rehabilitated, and released in 2010–2011, or were new captures. From 2 April 2019 to 9 October 2020, turtles were captured using the same methods as 2010, with the addition of capturing turtles via hand nets from a kayak (Lager 1943) and by hand while snorkeling (Marchand 1945). Over 96% of captures made during 2019–2020 were made via hand nets, the same type of nets that were used in previous surveys. All captured turtles were checked for PIT tags or shell notches and were measured as described above. Unmarked turtles were individually marked with a unique combination of notches filed or clipped along marginal scutes (Cagle 1939). All capture locations and turtle morphology data were recorded as described above for 2010, and all turtles were released at the point of capture within 24 h.

2.5. Data analysis

For each year of survey (i.e., 2010, 2011, 2019, and 2020), northern map turtle captures were used to calculate: 1) survey effort and captures per unit effort (CPUE), 2) population sizes by sex, 3) population sex ratio, and 4) population body size distribution by sex.

All estimates of population size, sex ratio, and body size distribution included only turtles identifiable to sex that were > 6.0 cm SCL to

Table 1
Total number of survey days, individual northern map turtles (*Graptemys geographica*) captured, and total captures made during surveys in the Kalamazoo River, Calhoun County, MI, USA. Individuals included all captures regardless of sex or age. Catch per unit effort (CPUE) was calculated by dividing survey days by the total number of captures made for each year.

	Survey Days	Individuals	Total Captures	CPUE
2010	73	1,220[1]	1,385[1]	19.0
2011	96	1,071	1,960	20.4
2019	98	1,118	1,952	19.9
2020	54	831	1,258	23.3
TOTAL	321	3,128	6,555	20.4

[1] Includes captures of turtles that were translocated, died, and transferred.

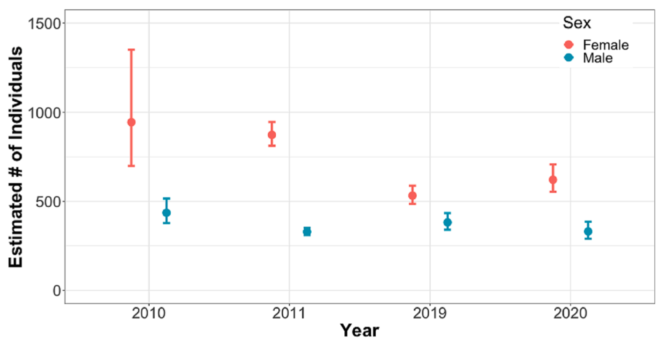

Fig. 2. Yearly estimated number and 95% confidence intervals of male (teal) and female (red) northern map turtles (*Graptemys geographica*) following the 2010 Kalamazoo River oil spill. Yearly population estimates were derived independently using RCapture in R from data collected during weekly surveys conducted on a 20.2 km stretch of the Kalamazoo River in Calhoun County, MI, USA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

eliminate potential bias due to many nests being protected against predation in separate studies in 2019–2020. Northern map turtles < 6.0 cm SCL are typically those<3 years old (Iverson 1988). For individuals captured multiple times in a year, only the first measurement was included in analysis for that year, and for individuals captured in multiple years, only the first measurement from each year was included. All statistical analysis were completed using R 3.6.3. (R Core Team 2021).

2.5.1. Turtle capture and survey effort

For each survey year, all captures of northern map turtles, including individuals that were released unmarked, translocated, or too young to identify to sex, were included to determine the total number of northern map turtles captured. Survey effort was consolidated to the total number of calendar days spent actively surveying for turtles (i.e., hand netting). For CPUE calculations, the total number of northern map turtle captures each year was divided by the total number of days spent surveying.

The total number of new captures and recaptures in each year for both sexes were calculated, excluding turtles that were translocated in 2010. An individual was considered a new capture the first time it was captured during each survey year, regardless of whether it had been captured in a previous year.

2.5.2. Population size

The number of males and females in each year of the study were estimated using mark-recapture capture histories with closed-population loglinear models (Otis et al. 1978; Rivest and Lévesque, 2001) in the "RCapture" package in R (version 1.4–4; Baillargeon and Rivest 2007). Only individuals with clearly identifiable marks were included in the analysis (Otis et al. 1978). To create an individual capture history, only one capture of each individual was counted per week (i.e., survey period), regardless of the number of times it was captured,

Table 2
Summary of male and female northern map turtle (*Graptemys geographica*) captures and recaptures (Recaps), estimated population size with 95% confidence intervals (Est. Number [95% CI]), and mean straight carapace lengths (SCL) during surveys conducted in 2010, 2011, 2019, and 2020 on the Kalamazoo River, Calhoun County, MI, USA. Only turtles identifiable to sex and with SCL $\geq$ 6.0 cm were included. Individuals new to the study were those that had not been previously captured in earlier years of survey. Yearly population size estimates were calculated independently for each year using RCapture in R derived from weekly survey events. Analysis of variance (ANOVA) was used to test for differences in mean SCL among years for males and females, with statistically different pairwise comparisons denoted by superscript.

	Individuals Captured (New to study)	Recaps	Total Captures	Est. Number (95% CI)	Mean SCL (SD; n)
Males					
2010	254 (254)	124	378	436 (378–516)	10.1 (1.3; 234)[a]
2011	276 (118)	366	642	329 (310–351)	9.9 (1.7; 276)[a]
2019	243 (181)	150	393	382 (341–434)	9.0 (1.6; 234)[b]
2020	216 (55)	116	332	331 (290–386)	9.4 (1.6; 210)[c]
TOTAL	989 (608)	756	1,745	NA	NA
Females					
2010	255 (255)	40	295	944 (699–1,351)	18.4 (4.9; 242)
2011	575 (406)	403	978	873 (812–945)	18.6 (5.1; 572)
2019	352 (153)	286	638	532 (486–588)	17.6 (5.9; 337)
2020	361 (88)	194	555	621 (553–707)	17.5 (6.3; 345)
TOTAL	1,543 (902)	923	2,466	NA	NA

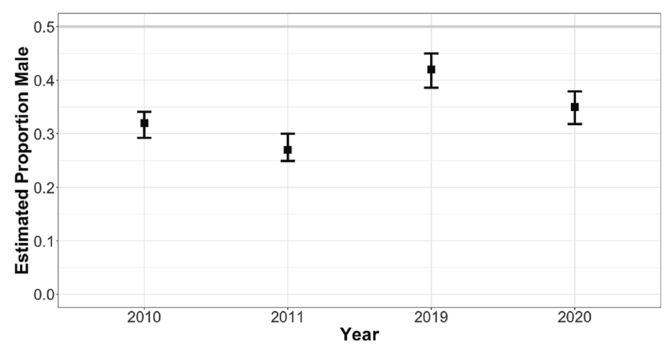

Fig. 3. Estimated sex ratios (presented as proportion male) and 95% confidence intervals of northern map turtles (*Graptemys geographica*) from weekly mark-recapture population estimates from the Kalamazoo River, Calhoun County, MI, USA during 2010, 2011, 2019, and 2020 surveys. Generalized linear models and post-hoc Tukey HSD tests were used to calculate linear contrasts to compare the population sex ratio among years, with sex ratio differing significantly among years, but 2010 and 2011, and 2010 and 2020, did not differ from each other.

to reduce bias from "trap happy" turtles. Survey periods were divided into weekly intervals with Mondays used as the constant for the beginning of a week, regardless of number of survey days. Two survey periods (i.e., weeks) were pooled together to create capture histories of each individual using the "periodhist" function. This function is used to analyze datasets with a large number of capture occasions but a limited number of captures. To account for uneven survey periods, week 41 was not included in 2020 analysis. We considered models that accounted for different capture probabilities between survey periods (M_t), varying capture probabilities between individuals (M_h), behavioral changes

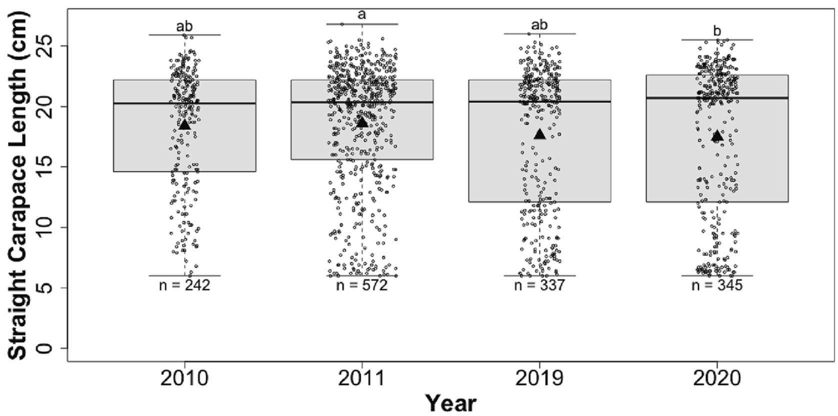

Fig. 4. Variation in straight carapace length (SCL; cm) of male northern map turtles (*Graptemys geographica*) with a SCL ≥ 6.0 cm captured during surveys in the Kalamazoo River in Calhoun County, MI, USA. Each box plot shows the yearly median (dark bar), mean (triangle), limits of the 2nd and 3rd quartiles (solid box), range (brackets), and outliers (large open dots). Analysis of variance (ANOVA) was used to test for differences in SCL among years with a Tukey's HSD post-hoc test used to identify which years differed significantly, denoted by lowercase letters above upper brackets.

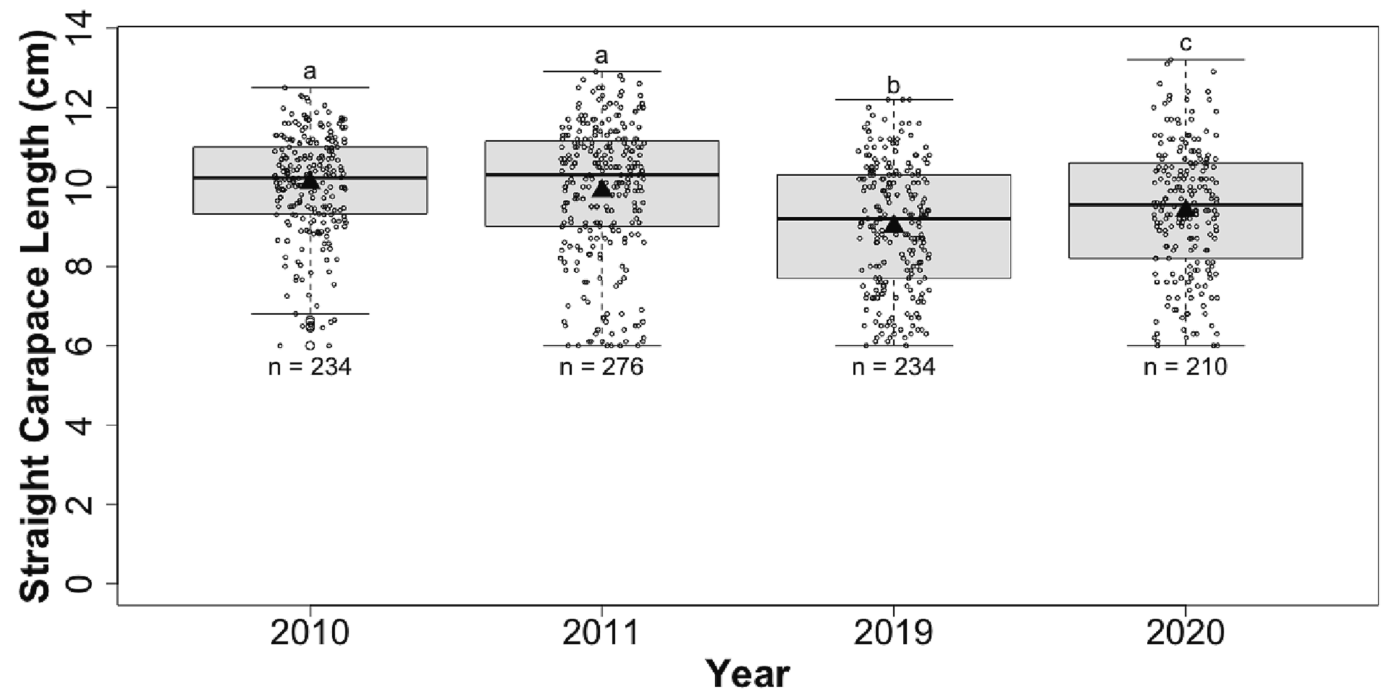

Fig. 5. Variation in straight carapace length (SCL; cm) of female northern map turtles (*Graptemys geographica*) with a SCL ≥ 6.0 cm captured during surveys in the Kalamazoo River in Calhoun County, MI, USA. Each box plot shows the yearly median (dark bar), mean (triangle), limits of the 2nd and 3rd quartiles (solid box), range (brackets), and outliers (large open dots). Analysis of variance (ANOVA) was used to test for differences in SCL among years with a Tukey's HSD post-hoc test used to identify which years differed significantly, denoted by lowercase letters above upper brackets.

resulting from initial capture (M_{bh}), and equal capture probability across survey period and individuals (M_0) for each sex and survey year independently. The AIC value was used to assess model fitness and selection (Burnham and Anderson 2002).

The closed-population assumption was potentially violated, as turtles were captured over an entire active season; however, monthly and annual survival rates for this population are high (monthly > 0.97 for males, >0.99 for females; annual > 0.81 for males, >0.94 for females [Otten et al. 2022]), and therefore mortality rates between weekly sampling events were presumably low. In addition, only turtles > 6.0 cm SCL were included in analyses, which would exclude "births" between sampling events. Finally, turtles in this population show site fidelity by three years of age, which likely decreases potential immigration or emigration (Otten et al. 2023).

2.5.3. *Sex ratios*

Sex ratios were calculated in each survey year using the estimated number of individuals of each sex from the population size estimates. Generalized linear models were used to calculate linear contrasts to compare the population sex ratio among years. Each linear contrast was back-transformed from the log-scale to provide the odds ratio with 95% confidence intervals. For the linear contrast analyses and post-hoc comparisons using Tukey HSD tests, we used the 'emmeans' package (Lenth 2020).

2.5.4. *Body size distribution*

Differences in the relative distribution of body sizes in male and female northern map turtles in this population were examined among survey years. Only the first measurement of SCL for each individual was used for analysis in each year that it was captured. Because turtle body size can be partially influenced by age, and body size can substantially influence reproductive output (e.g., clutch size, hatchling size; Congdon and van Loben Sels 1991; Zuffi et al. 1999; Congdon et al. 2001) body size (SCL) distribution, rather than age distribution, was used as an indicator of population demographic structure. Analysis of variance (ANOVA) was used to test for differences in SCL among years for males

Table 1
Top four models describing capture probabilities of male northern map turtles (*Graptemys geographica*) during each of the four years of survey (2010, 2011, 2019, and 2020) on the Kalamazoo River, MI, following the 2010 oil spill. We considered models that accounted for different capture probabilities between survey periods (M_t), varying capture probabilities between individuals (M_h), behavioral changes resulting from initial capture (M_{bh}), and equal capture probability across survey period and individuals (M_0). The AIC value was used to assess model fitness and selection.

Sex	Year	Rank	Model	K	AIC	Δ AIC
Male	2010	1	Mt	10	76.83	0.00
		2	Mbh	11	99.84	23.01
		3	Mb	12	210.64	133.81
		4	M0	13	211.06	134.23
	2011	1	Mt	509	410.20	0.00
		2	Mbh	508	451.26	41.06
		3	Mb	507	455.67	45.47
		4	M0	509	713.62	303.42
	2019	1	Mt	16,368	537.79	0.00
		2	Mbh	16,379	578.69	40.90
		3	M0	16,380	594.75	56.96
		4	Mb	16,381	595.58	57.79
	2020	1	Mt	4082	405.99	0.00
		2	Mbh	4091	450.13	44.14
		3	Mb	4092	467.70	61.71
		4	M0	4093	497.65	91.66

Table 2
Top four models describing capture probabilities of female northern map turtles (*Graptemys geographica*) during each of the four years of survey (2010, 2011, 2019, and 2020) on the Kalamazoo River, MI, following the 2010 oil spill. We considered models that accounted for different capture probabilities between survey periods (M_t), varying capture probabilities between individuals (M_h), behavioral changes resulting from initial capture (M_{bh}), and equal capture probability across survey period and individuals (M_0). The AIC value was used to assess model fitness and selection.

Sex	Year	Rank	Model[a]	K[b]	AIC	Δ AIC
Female	2010	1	Mt	29	58.38	0.00
		2	Mbh	27	154.49	96.11
		3	Mb	28	177.12	118.74
		4	M0	29	317.77	259.39
	2011	1	Mt	509	494.59	0.00
		2	Mbh	507	548.14	53.55
		3	Mb	508	587.22	92.63
		4	M0	509	660.16	165.57
	2019	1	Mt	16,368	671.10	0.00
		2	Mbh	16,379	858.89	187.79
		3	Mb	16,380	924.71	254.61
		4	M0	16,381	929.29	258.19
	2020	1	Mt	4082	462.17	0.00
		2	Mb	4092	596.84	134.67
		3	Mbh	4091	598.49	136.32
		4	M0	4093	600.45	138.28

and females separately. For all significant ANOVA results ($p < 0.05$), a Tukey's HSD post-hoc test was used to identify which years differed. Due to turtle longevity and high adult survival rates, size distributions are expected to skew towards larger size classes in populations where recruitment of young, small individuals is limited. In populations where large numbers of individuals were born, or survival rates were higher for younger age classes compared to older cohorts, size distributions would be expected to skew towards smaller size classes.

3. Results

3.1. Turtle capture and survey effort

A total of 6,555 captures of northern map turtles were made over 328 days of surveys (Table 1). Of the 3,316 unique individuals captured, 1,083 were identified as males, 1,295 were females, and 938 were of unknown sex. There were 682 individuals (376 males, 252 females, and 54 of unknown sex) translocated out of the Study Site in 2010 and thus excluded from all population and demographic analyses presented here. An additional 67 individuals (27 males and 40 females) died during rehabilitation or were unable to be released due to injuries suffered as a result of the oil spill (Otten et al. 2022). Over four years of survey, individual turtles were captured an average of 2.0 times (±1.7 SD; range 1–21 times); 39.4% of individuals (1,307) were recaptured at least twice. Thirty-three individuals were captured in all four years of surveys, and 115 individuals were captured in three years of surveys. Within-year recapture rates varied, such that 10.9% of individual females first captured in 2010 were subsequently recaptured that year, while 43.0% of females captured in 2019 were recaptured at least once more that year. Male recapture rates ranged from 28.3% in 2010 to 61.4% in 2011 that were subsequently recaptured the same year. Overall CPUE was 20.4 northern map turtles per day, ranging from 19.4 in 2010 to 23.3 in 2020 (Table 1).

3.2. Population size

The M(t) model was the best fit for all years and both sexes (Appendix A; Tables 1 and 2); therefore, we selected it for our population estimates. A significant difference in the total population size of northern map turtles identifiable to sex and > 6.0 cm SCL was found at the Study Site among years (Table 2; Fig. 2). The highest estimated population size was 1,380 (95% CI 1,077–1,867) in 2010 and included 436 (95% CI 378–516) males and 944 (95% CI 699–1,351) females. The population size was similar in 2011 with an estimated total of 1,202 (95% CI 1,122–1,296) individuals; however, compared to 2010, the number of males in 2011 decreased by about 25% to 329 (95% CI 310–351), while the number of females remained similar to 2010 (873 [95 %CI 812–945]). In 2019, the estimated population had decreased to 914 (95% CI 827–966) total individuals, with females decreasing by 40% to 532 (95% CI 486–532) and males increasing by 14% to 382 (95% CI 341–434). Estimates for the total number of males and females were similar in both 2019 and 2020 (Table 2; Fig. 2).

3.3. Sex ratio

The sex of 1,510 unique individuals was determined during the study (Table 2). Estimated population sex ratios, based on the estimated numbers of males and females in each year as described above, differed significantly among years ($X^2 = 51.05$, df = 3, p = <0.01; Table 2; Fig. 3), and demonstrated a female bias in all years, ranging from 0.38 males: 1 female in 2011 to 0.72 males: 1 female in 2019. Pairwise post hoc comparisons revealed that 2010 and 2011, and 2010 and 2020, did not differ from each other.

3.4. Body size distribution

A total of 608 males and 902 females were used in analysis of body size distribution during the four survey years. Mean SCL for both males and females differed significantly among years (males $F_{3,950} = 21.7$, $P < 0.01$; females $F_{3,1492} = 3.5$, $P = 0.01$; Table 2; Figs. 4 and 5). Mean male SCL was similar in 2010 and 2011 ($p = 0.81$), decreased by nearly 10% in 2019 ($p < 0.01$), before increasing by 4% in 2020 ($p = 0.04$; Table 2; Fig. 4). Mean female SCL was similar between all years except 2011 and 2020, in which females in 2011 were approximately 6% larger than in 2020 ($p = 0.03$; Table 2; Fig. 5). Males exhibited a shift towards smaller size classes from 2010–2011 to 2019–2020 (Fig. 4). Fewer females in the 13.0–19.0 cm SCL size class, and more females < 9.0 cm SCL, were captured in 2019–2020 compared to 2010–2011 (Fig. 5).

4. Discussion

Aquatic turtles may serve as bioindicators of environmental pollutants, such that declines in the health of individuals or size of populations likely indicate a decline in environmental quality (Aguirre and Lutz 2004). To our knowledge, this is the first study to investigate the long-term impacts of dilbit contamination on any free-ranging vertebrate species. We specifically used broad population-level responses of an aquatic turtle species directly impacted by the Kalamazoo River dilbit oil spill to establish long-term ecological impacts of dilbit pollution. Although impacts of dilbit contamination on freshwater turtles have not previously been investigated, they are likely similar to those of conventional crude, such that crude oil-polluted sites support fewer individuals, have lower species diversity (Luiselli and Akani 2003; Luiselli et al. 2005), exhibited altered diets of impacted individuals (Luiselli et al. 2006), and increased rates of hatchling deformities (Bell et al. 2006).

Overall, numerous changes occurred in the northern map turtle population one to ten years after the 2010 Kalamazoo River oil spill. The estimated number of male and female northern map turtles decreased by nearly 25% and 40%, respectively, with decreases in males occurring in the year following the spill, and females declining between one and nine years post-spill. These declines were partially due to direct mortality following the spill; nearly 10.6% ($n = 27$) of males and 15.6% of females ($n = 40$) died during rehabilitation from injuries sustained from the spill (Otten et al. 2022), with deaths occurring on average 57.6 (±61.4 SD) days after capture, suggesting that the cause of death may be due to latent physiological effects of dilbit exposure that took weeks or months to develop. The potential latent effects of dilbit exposure were further evident through captures made in early 2011, wherein 9 of 25 turtles captured in April and early May died an average 9.3 days after capture, and while under veterinarian care. All nine were females that appeared to have been oiled in 2010, suggesting that while some turtles survived for months after being oiled, substantial mortality may have occurred shortly after turtles emerged from hibernation in early spring 2011. It is possible that females can survive initial oiling longer than males before succumbing, which may explain the delayed decline in the population of females. In further evidence of potential delays in female mortality, of the 20 cases of observed mortality (i.e., dead on arrival, died in care, or transferred to permanent captivity due to injuries) in 2011, 19 were adult females and 1 was an adult male (Otten 2022).

Although the global total volume of oil spilled annually is generally declining, catastrophic releases continue to occur and are impossible to predict (Eckle et al., 2012). The uncertainty of such environmental disasters, the scope of oil spill response activities (i.e., rescue and rehabilitation, physical removal of oiled habitat features), and lack of pre-spill data on affected populations makes it difficult to establish true reference sites for comparisons of long-term ecological impacts of oil spills. For example, while Canada has increased oil production and transport of bitumen from Alberta's oil sands (CAPP, 2019), there have been relatively few studies of dilbit impacts in natural, non-controlled ecosystems (Dew et al. 2015; Ruberg et al. 2021), and specifically how dilbit may impact freshwater systems, invertebrate, and vertebrate populations (Black et al. 2021; Utting et al. 2022). The majority of dilbit exposure studies have evaluated its effects in simulated controlled mesocosms (Dew et al. 2015; Bérubé et al. 2022; Zhong et al 2022), compared organisms in clean areas to areas with naturally occurring bitumen (Dew et al. 2015; Patterson et al. 2022), or compared reference areas to those that are actively being mined for bitumen (Tetreault et al. 2003). Although the effects of dilbit exposure observed under these conditions are predicted to also occur following a dilbit oil spill in a natural aquatic ecosystem, in natural systems, the degree of oiling, and length and type of exposure to wildlife are nearly impossible to predict and identify; therefore, events such as the Kalamazoo River oil spill provide important insight into dilbit impacts on wildlife in uncontrolled settings.

To date, the majority of data describing the effects of dilbit on free-ranging freshwater organisms were collected in relation to the Kalamazoo River oil spill of 2010. Benthic macroinvertebrate surveys showed initial impacts on invertebrate communities, with recovery reported from 2011 to 2014. Sediment from dilbit-affected areas significantly increased mortality in *Chironomus dilutus* and *Hyalella azteca* (Fitzpatrick 2012; USFWS, 2015). Mortality of unionid mussels occurred post-spill, and fewer species were found alive two years after the oil spill in dilbit-impacted areas compared to reference sites (Winter 2013; USFWS, 2015). Few fish were collected dead following the oil spill in 2010 ($n = 42$), and fish species diversity and individual growth at impacted sites varied from 2011 to 2013. However, compared to a reference site, fish collected from oiled sites three weeks after the spill showed a pattern of biomarker modulation seen in laboratory exposure of fish to PAH's (Papoulias et al. 2014). Although scope and length of these surveys was limited, all were conducted on species that are possible food sources for northern map turtles, which may have cascading indirect effects on the map turtle population. Finally, no long-term effects on either physiological stress or innate immune functioning were observed in northern map turtles a decade after the spill (Refsnider et al. 2023).

Other significant effects of dilbit exposure to vertebrate species have been found to vary with age. In salmon species, early life stages exposed to dilbit exhibited increased mortality, reduced body condition, and developmental delays compared with unexposed fish of the same age (Perugini et al. 2022; Bérubé et al. 2023). While it is difficult to ascertain the immediate direct impact of the Kalamazoo River oil spill on young turtles, or to definitively determine whether the increased vulnerability of early life stages to dilbit in salmon also occurred in map turtles, we did detect a significant shift towards smaller body sizes in both male and female northern map turtles from 2010–2011 to 2019–2020. The decrease in mean body size from 2011 to 2019 was likely due to only a few individuals < 6.0 cm SCL captured immediately following the spill. Specifically, only 15 of the 505 individuals captured in 2010 (3.0%) were < 6.0 cm SCL, corresponding to ages of < 3 years old (Iverson 1988). We cannot definitely state whether individuals of these size classes were not present during 2010, or if younger size classes had a higher mortality rate due to dilbit exposure (as demonstrated for salmon, Perugini et al. 2022; Bérubé et al. 2023), but our capture rates of the smallest size classes increased substantially in subsequent years. In 2011, 17.0% of individuals captured were < 6.0 cm SCL, and 50.9% of 2019 captures and 29.2% of 2020 captures were < 6.0 cm SCL. Importantly, the survey methods used during all years of this study were nearly identical and were effective at capturing turtles < 6.0 cm SCL, suggesting that the lack of small turtles observed in 2010 was not simply an artifact of size-specific detection bias. It seems likely, therefore, that the 2010 oil spill caused disproportionately high mortality of juvenile size classes immediately following the spill.

In turtles, longevity and iteroparity are thought to buffer populations against high mortality of egg and hatchling stages (Congdon et al. 1983), with populations compensating for high mortality in early life stages through increased fecundity during favorable years and high adult survival, effectively smoothing inter-annual changes in populations (Litzgus 2006). In the Kalamazoo River, northern map turtle annual hatchling survival rates were nearly 35%, and stage-specific survival rates increased to ~ 80% in adult males and 95% in adult females, which if these rates remained constant over time, then the number of individuals in the population would decrease annually by about 7 (Otten and Refsnider, unpub. data).

Our population size estimates included only individuals > 2 years of age, which should have dampened the appearance of substantial population fluctuations that were actually due to low and inter-annual variability in egg, hatchling, and one-year old juvenile survival. However, potentially higher rates of hatchling mortality induced by the oil spill may have had delayed population-level impacts by decreasing recruitment, which would have been further exacerbated by an increase in adult female mortality. Overall, our estimates of the number of turtles in the Kalamazoo River were most similar between 2019 and 2020, which is expected between subsequent years of survey in long-lived species with high adult survival, relatively low recruitment, and low survival of early life stages (Congdon et al. 2003). The population decline we observed here following the oil spill has also been seen in other species with high adult survival, with such declines attributed to increased adult mortality caused by pollution (Luiselli and Akani 2003), anthropogenic disturbance (Aresco 2005; Steen and Gibbs 2004; Gibbs and Steen 2005), or poaching (Gong et al. 2017). Although the scope of our study did not include measures of change in anthropogenic disturbance or road mortality, no road construction or human development occurred within 20 m of the riverbank, and as northern map turtles remain either entirely within the banks or basking on banks, limited road mortality would be expected at our study site. Additionally, female map turtles leave the riverbanks annually to nest farther upslope, which may increase vulnerability to road mortality; however, during multiple years of radio-telemetry of 73 females, and over 200 nesting observations, no instance of female road mortality was observed (Otten 2022). In fact, only 2 mortality events of adult female map turtles were recorded from 2019 to 2022, representing $< 1\%$ of captures during this time. Thus, the 40% decline in number of females we observed from 2011 to 2019 would seem to be far outside the normal range for this species under natural conditions.

It is important to note that the declines in numbers of males and females described above did not include the additional 682 individuals that were translocated out of the population in 2010 to avoid further exposure to the oil spill. Although only 92 of translocated turtles were adult females that presumably contributed to annual egg production, the removal of these females may have resulted in the loss of approximately 1,000 eggs produced annually (Otten and Refsnider, unpub. data), which may cause cascading decreases in the number of individuals in future age-classes. A large population of northern map turtles exists at the Study Site ten years post-spill, but this study revealed additional shifts to population demographics, which are likely indicative of impacts incurred by this population following the 2010 oil spill, beyond the direct mortality of some individuals in the immediate aftermath of the oil spill.

5. Conclusion

Surveys in the three months following the 2010 Kalamazoo River oil spill recorded direct mortality in 5.5% of all known individual northern map turtles exposed to dilbit. While it is often difficult to document direct mortality as a result of oiling, or to ascertain the long-term impacts of an oil spill on a population, the results of our study revealed a variety of demographic changes that occurred in the first few years after the spill. In comparing demographic parameters at the time of the oil spill to values $\sim$ 10 years post-spill, we detected a reduction in population size, shifts in the distribution of body size towards smaller size classes, and shifts in the population sex ratio. Signals of failed recruitment were also observed in cohorts that would have hatched during the years just before and just after the oil spill. However, minimal change in these same demographic parameters have occurred between 9- and 10-years post spill, suggesting that any lingering negative impacts on this population are minimal. Because catastrophic spill events cannot be predicted, baseline data is often lacking for impacted populations, which makes it difficult to compare population parameters pre- and post-spill. Our study provides insight into the impacts that other species with similar life history strategies may incur as the result of a large, diluted bitumen oil spill.

CRediT authorship contribution statement

Joshua G. Otten: Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Writing – original draft, Visualization, Data curation. **Lisa Williams:** Writing – review & editing. **Jeanine M. Refsnider:** Writing – review & editing, Funding acquisition, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A

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