A Dissertation

entitled

Long-term Impacts of a Freshwater Oil Spill on an Aquatic Turtle Species

by

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Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in

Biology

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An Abstract of

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The adverse effects of oil spill disasters on wildlife populations often include mass mortalities and widespread oiling of large numbers of individuals. While these incidents are highly visible and well documented, chronic, long-term impacts on vertebrate species may often persist after the initial oil exposure due to lingering toxins in the environment. These chronic effects may often exceed the short-term impacts caused by initial oil exposure. Additionally, emergency spill response, cleanup operations, and mitigation measures may have additional impacts on populations exposed to oil spills. Species that have long lifespans, late age maturation, and low recruitment rates are particularly vulnerable to population-level impacts if oil spills, and subsequent cleanup operation cause an increase in mortality.

Regarding the effects of oils spills in freshwater ecosystems, very little is known in comparison to marine ecosystems. In particular, almost nothing is known about the impacts on freshwater organisms' exposure to diluted bitumen (dilbit) oil. 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 United States history, the Kalamazoo River oil spill, which spilled between 3 and 4.5 million L of dilbit in Calhoun and

Kalamazoo counties, Michigan, impacting 56 km of the Kalamazoo River and the species within. Of the vertebrate species known to have been oiled during the Kalamazoo River oil spill, northern map turtles (*Graptemys geographica*) were the most observed oiled animal.

As a result of the Kalamazoo River oil spill, extensive effort occurred in 2010 and 2011 to clean and restore the freshwater ecosystem impacted by the spill. During 2010, this included the capture, cleaning, rehabilitating, and releasing of more than 2,000 northern map turtles. In 2010, we documented a nearly 6% direct mortality rate (i.e., individuals captured dead, died in care, or transferred to a permanent rehabilitation center as a result of injuries suffered) of sexable northern map turtles. During 2019 and 2020 we captured turtles within the Kalamazoo River to evaluate changes in the estimated number of individuals in the population, demographics, and size classes nine to ten years after the spill. I found that the estimated number of male northern map turtles decreased by over 30% between 2010 and 2011, while the number of females decreased by nearly 40% between 2011 and 2019. A decrease in the mean size of northern map turtle males and females occurred between 2011 and 2019, due in part to increased recruitment and capture of individuals less than 5 years of age in 2019. Fewer 8–12-year-old females were captured in 2019 and 2020, a result of potentially losing a large portion of a generation during the 2010 oil spill. This was evident in that 2% of captures in 2010 were less than 2 years of age, while in subsequent years of survey these age classes made up of over 20% of individuals captured. These data suggest that beyond the direct mortality of the spill, shifts in the estimated number of northern map turtles and size class distribution

are likely indicative of negative impacts incurred following the 2010 oil spill and resulting cleanup.

During cleanup efforts following the 2010 Kalamazoo River oil spill, over 1,000 individuals spent at least one night in a rehabilitation facility to have any oil removed. Rehabilitation is often used to mitigate the adverse effects of oil spills on wildlife; however, limited post-release monitoring studies have been conducted to quantify survival of rehabilitated animals. Utilizing mark-recapture data collected from northern map turtles in 2010, 2011, and 2018-2021 I evaluated the effectiveness of turtle rehabilitation following the Kalamazoo River oil spill. To do this I compared monthly survival rates of turtles that were either “non-rehab” (i.e., turtles captured in the field with no oil or <2% body oil, that were cleaned, marked, and released at the point of capture), “rehab” (i.e., any individual that spent at least one night in the rehabilitation facility, marked, and released within 1 km of its original capture location in 2010), or “overwintered” (i.e., turtle that were still requiring cleaning or medical assistance in mid-October 2010, so they could not be safely released during 2010, and were kept during the winter at normal summer temps, and released spring 2011 at their point of capture) during 2010. I compared monthly survival rates for the three rehab types for the period of time 1-14 months after the spill and 8-11 years after the spill. I found that rehabilitated or overwintered turtles had a higher probability of survival 1-14 months post-spill than non-rehabilitated turtles; however, 8-11 years post-spill the among-group differences in monthly survival probability had become negligible.

Finally, as an emergency mitigation strategy because of the oil spill, nearly 700 marked northern map turtles were translocated to similar habitat connected via river or creek channel. These were turtles that had gone through the rehabilitation process and were healthy, free of oil, and cleared by a licensed veterinarian for release. Because oil remained or cleanup work was occurring at or near their original capture locations, these individuals were translocated to potentially unfamiliar locations. To determine the distance of translocation which would be considered potentially unfamiliar locations, I conducted a home range study in 2019 on male and female northern map turtles within the Kalamazoo River. We found no difference in 95% and 50% kernel density estimated home ranges among turtles from an area of the Kalamazoo River that had been heavily oiled in 2010, an area of the Kalamazoo River that had been lightly oiled, and a tributary that had never been oiled. I did find that the stream home range of female northern map turtles in the heavily oiled area of the river were significantly smaller than those from the non-oiled site, potentially a result of females traveling further to find suitable nest locations. Finally, as part of this study, I determined the mean stream home range of male northern map turtles within the Kalamazoo River to be 2.4 km long while females were 4.6 km.

Based on stream home calculations, 686 northern map turtles were captured from oil-impacted stretches of the Kalamazoo River, cleaned, rehabilitated, and translocated 2.5–84.3 km from their original capture location. The goal of the translocation was to release turtles within the same watershed, but away from ongoing operation so that individuals could potentially return to their original home range after it had been cleaned of oil and restored. I evaluated the success of translocation as an emergency mitigation strategy for freshwater turtles by quantifying recapture probability and homing by northern map turtles translocated varying distances away from their home ranges. During subsequent years of survey up to 10 years post-spill, 230 of the translocated turtles were recaptured, of which 104 exhibited homing by returning to their original home ranges. Turtles translocated to sites nearest their original capture location had a higher probability of recapture and homing than those translocated further away. Females had a higher probability of returning to original home ranges than males when translocate greater distances. In addition, four females and one male are known to have traveled >50 km between capture locations, which to my knowledge is the greatest travel distance recorded for any freshwater turtle species in the U.S. My results demonstrate that riverine turtles have considerable homing ability when displaced long distances, which has important implications for design and success of translocation projects. Overall, these studies provide details on changes a population of northern map turtles underwent 10 years after a catastrophic diluted bitumen oil spill, specifically evaluating emergency response mitigation strategies such as rehabilitation and translocation.

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Chapter 1

Ten Years After a Major Riverine Oil Spill: Effects on a Freshwater Turtle Population

1.1 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 on 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 chronic effects (i.e., long-term impacts that persist after initial oil exposure and 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 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), as well as pre-spill, baseline population data, are often unknown for specific populations impacted by oil spills.

Emergency spill response and cleanup operations may have additional impacts on populations exposed to oil spills. For example, mitigation activities such as hydraulic sediment flushing, aquatic vegetation harvesting, oil vacuuming, and dredging of sediments can create physical disturbances that impact wildlife (Vandermeulen and Ross 1995; Bejarano 2018). Such physical disturbances to habitat may alter local trophic structures, which could subsequently cause individuals to spend more time foraging, change their diet, or leave the area entirely. 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. Vertebrate species characterized by long lifespans, late age at maturation, and low recruitment rates are particularly vulnerable to population-level impacts if oil spills, and subsequent cleanup operations cause an increase in mortality (McCann and Shuter 1997; Musick 1999; Norse et al. 2012). 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).

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 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. In addition to impacts resulting from physical exposure to spilled oil, 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 these 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 had declined in density, while 21% (3 species) had increased (Esler et al. 2018). 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 had 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 very little regarding effects of oil spills in freshwater ecosystems. In particular, almost nothing is known about the impacts on freshwater organisms to exposure of diluted bitumen (dilbit). Dilbit is pure bitumen oil that is mixed with natural gas condensates for easier transport, and which has 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 (834,444 gallons) of dilbit were reportedly released after a pipeline rupture (NTSB 2012). The U.S. Environmental Protection Agency (EPA) estimated that 4.5 million L (1,181,599 gallons) were subsequently recovered during cleanup operations (EPA 2016). The spilled dilbit initially pooled in a marshy area near the ruptured pipeline, before flowing 213 m 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; Figure 1-1). The Kalamazoo River oil spill provided an opportunity to study the potential population-level effects of oil exposure on freshwater organisms in a wild system.

Of the vertebrate species known to have been oiled during the 2010 Kalamazoo River oil spill, northern map turtles (*Graptemys geographica*) were the most commonly observed oiled animal (EPA 2016). Cleanup, rescue, and rehabilitation efforts in 2010–2011 captured >2,100 individual northern map turtles exhibiting varying degrees of oiling. 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. In this study, our goal was to determine whether the Kalamazoo River northern map turtle population differed in total population size, sex ratio, or body size class structure among years following the 2010 oil spill.

1.2 Methods

1.2.1 Study Site

Our Study Site was a 20.2 km stretch of the Kalamazoo River from Talmadge Creek to E. Dickman Road in Calhoun County, Michigan, USA, 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 (Figure 1-1). The Kalamazoo River within the Study Site ranges from 9.0–40.0 m wide and 0.2–3.5 m deep.

1.2.2 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, which makes them susceptible to oiling during a spill event. 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 1–2 years of age using secondary sex characteristics (e.g., longer and thicker tail, and cloacal placement relative to shell margins; Lindeman 2013).

1.2.3 Initial Turtle Capture and Rehabilitation

In 2010, in immediate emergency response to the oil spill, volunteers and paid contractors, including J.O., and overseen by the U.S. Fish and Wildlife Service (USFWS; EPA 2016), captured oiled turtles in the Kalamazoo River from 30 July - 24 October. Most turtles were captured by hand (as in Lager 1943) from boats, although some turtles were also captured in hoop or basking traps; therefore, capture effort was calculated in terms of boats per day (boat-day). One boat actively capturing turtles was considered one boat-day, rounded to the nearest ½ day. 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 implanted transponder (PIT) tag (Avid Identification Systems, Inc.). Individuals <100 g were marked with a unique set of notches filed along the marginal scutes (as in Cagle 1939). Turtles exhibiting any signs of oiling were taken into captivity for rehabilitation as described in Chapter 2. Turtles with no visible signs of oiling were processed as described above and then released at the point of capture. From 31 July - 6 October 2010, turtles captured at the Study Site were translocated to tributaries, or locations upstream and downstream from the oiled stretch of river, to protect them from additional oiling and ongoing disturbance from cleanup operations. Because translocated turtles were released >5 km from the Study Site, we excluded them from demographic analysis.

1.2.4 Recapture Surveys

In April - October 2011, field crews used the same methods as described for 2010 to both continue capturing oiled turtles, and to recapture previously rehabilitated and released turtles to assess survival rates. In 2019–2020, researchers from the University of Toledo returned to the Study Site to recapture turtles that had been captured, rehabilitated, and released in 2010–2011. During weekly surveys from April 2019 - October 2020, we captured turtles using dipnets from a boat or kayak (as in Lager 1943), baited hoop traps, basking traps, by hand while snorkeling (Marchand 1945), and by hand when females were found traveling overland to nest. 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 along marginal scutes (Cagle 1939). We recorded all capture locations and turtle morphology data as described above for 2010, and all turtles were released at the point of capture within 24 hours.

1.2.5 Data Analysis

We used northern map turtle capture records in 2010, 2011, 2019, and 2020 to calculate: 1) survey effort and captures per unit effort (CPUE) each year, 2) yearly population sizes by sex, 3) yearly population sex ratio, and 4) yearly population body size distribution by sex. For analysis of 2010 data, we excluded all turtles that were translocated, died during rehabilitation, or were too severely injured to release following rehabilitation. All estimates of population size, sex ratio, and body size distribution excluded turtles <5.0 cm SCL to eliminate potential bias due to many nests being protected against predation in separate studies in 2019–2020. We used R 3.6.3. (R Core Team 2020) to complete all statistical analysis.

1.2.5.1 Turtle Capture and Survey Effort

For each year, we included all captures of northern map turtles, including those that were released unmarked, translocated, or too young to identify to sex, to determine the total number of northern map turtles captured, the average number captured per day, and the total days spent surveying. To calculate CPUE for a given year, we divided the total number of captures by the total number of boat days for that year.

1.2.5.2 Population Size

We calculated the total number of new captures and recaptures each year for both sexes. 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. We compared the mean number of captures and recaptures between sexes using a two-sample t-test.

We estimated the number of males and females in each year of the study using mark-recapture methods with compiled weekly capture histories. An individual's captures were counted no more than once per week, regardless of the number of times it was captured, to reduce bias from "trap happy" turtles. We used the Schnabel method for estimating population size, as this method is best for studies with small sample sizes for individual sampling efforts, which is typical of many turtle studies (Graham 1979; Lindeman 1990). Schnabel estimates were calculated for each sex and year independently as described in Tanner (1978). To meet the assumptions of the Schnabel method, a variety of capture methods were used so no bias was placed on the capture of a particular size or sex, and only individuals with clearly identifiable marks were included in analysis (Otis et al. 1978). Because we captured turtles over an entire active season, the assumption of population closure was probably violated; however, monthly survival rates for this population are high (Chapter 2), and therefore mortality rates between sampling events were presumably low. In addition, only turtles >5.0 cm SCL were included, which excluded "births" between sampling events. Finally, turtles in this population show site fidelity by three years of age, which likely decreases potential immigration or emigration (Chapter 4). Turtles from 2010 that were translocated, died, or could not be released due to severe injuries were not included in 2010 estimates. Survey periods were divided into weekly intervals with Mondays used as the constant for the beginning of a week, regardless of number of boat-days completed.

1.2.5.3 Sex Ratios

We estimated sex ratio in each survey year using the estimated number of individuals of each sex calculated from the population size estimates. We used generalized linear models 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 (R Core Team).

1.2.5.4 Body Size Distribution

We examined changes in the relative distribution of body sizes in males and females separately. Because adult turtles are difficult to accurately age (Ross 1989), we used body size distribution measured as SCL, rather than age distribution, as an indicator of population demographic structure, and assumed that turtles grow larger as they age (Zweifel 1989). Therefore, a higher proportion of large-bodied turtles would indicate a higher proportion of older individuals in the population. We used analysis of variance (ANOVA) to test for differences in SCL among years for males and females separately. For all significant ANOVA results ($p<0.05$), we used a Tukey's HSD post-hoc test to identify which years differed from one another. In addition, we used the Kolmogrov-Smirnov two-sample test to compare both the shape and central tendency of SCL distributions between subsequent years. Due to turtle longevity and indeterminate growth, we would expect size distributions 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 increased survival occurred in younger individuals we would expect size distributions to skew towards smaller size classes.

1.3 Results

1.3.1 Turtle Capture and Survey Effort

We recorded 6,653 captures of 2,918 individual northern map turtles over 305 days during the four years of survey (Table 1.1). Of the 2,918 unique individuals captured, 683 (381 males, 241 females, and 61 of unknown sex) were translocated out of the Study Site in 2010, and an addition 67 individuals (27 males and 40 females) died during rehabilitation or were unable to be released due to injuries (Chapter 2). These 750 individuals were excluded from all subsequent analyses. Over the four years of survey, individual turtles were captured an average of 2.1 times (±1.8 SD; range 1–21 times); 45.1% of individuals (1,316) were recaptured at least twice. Thirty-three individuals were captured in all four years of survey, and 115 individuals were captured in three years of surveys. Within year recapture rates varied, 14.3% of individual females first captured in 2010 were subsequently recaptured that year, while 43.1% of females captured in 2020 were recaptured at least once more that year. Male recapture rates ranged from 30.6% of individual males recaptured in 2010 to 55.6% of individual males captured in 2011 were subsequently recaptured that year. Mean number of new captures and recaptures over the four years of study were similar for each sex (new capture x^2=0.39, df=3, p=0.71; recapture x^2=0.78, df=3, p=0.47). Overall CPUE was 12.4 northern map turtles, ranging from 7.0 in 2010 to 30.7 in 2020 (Table 1.1).

1.3.2 Population Size

We found a significant difference in the total population size (which included only turtles identifiable to sex) of northern map turtles at the Study Site among years (Table 1.2; Figure 1-2). The highest estimated population size was 1,304 in 2010 and included 446 (95% CI 411–488) males and 858 (95% CI 726–1050) females. The population size was similar in 2011 with an estimated total of 1,172 individuals; however, compared to 2010, the number of males decreased by about 30% to 316 [95% CI 296–338]), while the number of females was similar to 2010 (856 [95%CI 808–910]). In 2019, the estimated number of turtles in the population had decreased from 1,172 individuals in 2011 to 886 total individuals, with females decreasing by about 40% to 489 (95% CI 446–541) and males increasing by about 20% to 397 (95% CI 350–457). Estimates for the total number of males and females were similar for 2019 and 2020 (Table 1.2; Figure 1-2).

1.3.3 Sex Ratios

We determined the sex of 1,796 individuals. Estimated population sex ratios, based on the estimated numbers of males and females in each year as described above, differed significantly among years ($F_{3,1,792}$=74.50, p = <0.01; Table 1.2; Figure 1-3), demonstrating a female bias in all years, ranging from 0.37 males: 1 female in 2011 to 0.81 males: 1 female in 2019.

1.3.4 Body Size Distribution

A total of 720 males and 1,052 females were used in analysis of body size during the four survey years. Mean SCL for both males and females differed significantly among years (males $F_{3,987}$=23.2, P=<0.01; females $F_{3,1503}$=4.4, P=<0.01; Table 1.2; Figure 1-4). Mean male SCL was similar in 2010 and 2011 (p=0.47), before decreasing by nearly 10% in 2019 (p<0.01). Males in 2020 were 5% larger than those in 2019 (p<0.01; Table 1.2; Figure 1-4). Mean female SCL was also similar between 2010 and 2011 (p=0.56), but then decreased by nearly 6% between 2011 and 2019 (p=0.01; Table 1.2; Figure 1-5). The shape of the SCL distributions for males differed among years, except 2010 and 2011 (D=0.11, p=0.11; Figure 1-5). Males exhibited a shift towards smaller size classes from 2010–2011 to 2019–2020 (Figure 1-5). Female SCL distribution shape was similar in 2010 and 2011 (D=0.07, p=0.33), and in 2019 and 2020 (D=0.07, p=0.33; Figure 1-6). A lower proportion of females 13.0–19.0 cm SCL were captured in 2019 and 2020 compared to 2010 and 2011. Finally, a higher proportion of individuals <9.0 cm SCL were captured in 2019 and 2020 compared to 2010 and 2011 (Figure 1-6).

1.4 Discussion

Aquatic turtles may serve as bioindicators of environmental pollutants, such that declines in the health of turtle populations likely indicate a decline in environmental quality (Aguirre and Lutz 2004). Studies on the impacts of conventional crude oil spills on freshwater turtle populations have found that polluted sites support fewer individuals and lower species diversity compared to unpolluted sites (Luiselli and Akani 2003; Luiselli et al. 2005). Moreover, turtle species in heavily polluted areas can have altered diets (Luiselli et al. 2005), and increased hatchling deformities, particularly near heavily oiled habitat (Bell et al. 2006). The impacts of dilbit contamination on freshwater turtle populations are likely similar to those of conventional crude oil but have not previously been investigated.

Our study revealed changes in the northern map turtle population one to ten years after the 2010 Kalamazoo River oil spill. We found that one year after the spill of dilbit oil, the estimated number of male northern map turtles in the population had decreased by nearly 30%. The estimated number of females in the population also declined by about 40%, but in contrast to males, the decline for females occurred between one and nine years post-spill. It is important to note that these declines were in addition to the 750 individuals that either died or were translocated out of the population in 2010 as a direct result of the oil spill. Although a large population of northern map turtles currently exists at the Study Site ten years post-spill, our study revealed additional shifts in the population's sex ratio and size class distribution, which are likely indicative of negative impacts incurred by this population following the 2010 oil spill beyond initially observed direct mortality.

Population Size Estimate – We documented a mortality rate of 5.7% in the northern map turtles captured following the 2010 oil spill, which included individuals that either died during rehabilitation or were too severely injured to be released (Chapter 2). The majority of individuals that died were under veterinarian care during rehabilitation and died an average of 57.6 (±61.4 SD) days after capture. The cause of death for turtles that died despite undergoing rehabilitation efforts in captivity for many weeks may be latent physiological effects that took weeks or months to develop. However, during the first three days of survey in April - May 2011, 9 of 25 previously unmarked turtles captured at the Study Site died within 9.3 days of capture (2 were collected dead and 7 died in captivity). These nine turtles were all females that appeared to have been oiled in 2010, which suggests that while some turtles survived for weeks or months after being oiled, substantial mortality may have occurred shortly after turtles emerged from hibernation in early spring 2011. While only limited surveys were conducted for turtles in early spring 2011, areas where some turtles were known to hibernate (Otten personal observation) were also locations where dilbit had settled into the sediment (EPA 2016). Therefore, it is plausible that turtles exposed to high concentrations of dilbit during hibernation had low survival rates after emergence the following spring.

We estimated that the number of both male and female northern map turtles decreased significantly following the 2010 Kalamazoo River oil spill. However, the decrease for each sex occurred over different time scales, with male numbers decreasing within one year of the spill, and female decline occurring sometime between one and nine years post-spill. In turtles, longevity and iteroparity are thought to buffer turtle populations against high mortality of vulnerable egg and hatchling stages (Congdon et al. 1983), with populations compensating for high mortality rates in early life stages through increased fecundity during favorable years, which effectively smooths inter-annual changes in population growth rate (Litzgus 2006). Our population size estimates included only individuals at least two years of age, which should have dampened population fluctuations due to inter-annual variability in egg and hatchling survival. However, potentially higher rates of hatchling mortality induced by the oil spill, as well as the translocation of 241 females out of the population, may have had delayed population-level impacts by decreasing recruitment, which would have been exacerbated by an increase in adult female mortality. Overall, our population size estimates of Kalamazoo River northern map turtles were similar for 2019 and 2020, which is expected in long-lived species with high adult survival, relatively low recruitment, and low survival of early life stages (Congdon et al. 2003). This may indicate that the Kalamazoo River ecosystem has since recovered since the 2010 oil spill.

Sex Ratio – In addition to the overall decrease in northern map turtle population size, we also observed shifts in sex ratios from 2010 to 2020. We estimated a female-biased sex ratio in every year of the study, but the female bias was greatest from 2010 to 2011, followed by a smaller female bias from 2011 to 2019. The smaller magnitude in female bias in 2019 compared to 2011 is likely due to an increase in young males in the population in 2019, combined with the overall decrease in estimated number of females from 2011 to 2019.

In a stable population with similar survival rates between the sexes, we would expect to see little variation in adult sex ratio between years. Deviations from an estimated 1:1 sex ratio could be explained by sex bias in capture methods, climatic differences among years altering hatchling sex ratios in species with temperature-dependent sex determination (including *Graptemys* species), or female-biased mortality (i.e., road collisions due to nesting forays; Gibbons et al. 1990, Ream and Ream 1996). In our study, sex ratio bias due to capture methods was likely minimal, as we used multiple capture methods and trap types that allowed for all sizes and sexes of northern map turtles to be detected and captured. The fact that the estimated population sex ratios were similar between 2010 and 2020 suggests that while the population size was lower overall following the 2010 oil spill, population dynamics were recovering enough that the estimate sex ratio in 2020 had returned to pre-spill levels. Future research is needed to determine whether the observed female bias in sex ratio is due to sex-specific responses to oil pollution, female bias in hatchling production, or higher mortality rates in males of later life stages.

Body Size Distribution – We found a significant shift towards smaller body sizes in male and female northern map turtles from 2010–2011 to 2019–2020. Assuming that capturing young turtles <6.0 cm SCL is indicative of successful recruitment, the decrease in population mean body size from 2011 to 2019 was likely due to increased recruitment of small individuals in later years of this study. This assumption is supported by the greater number of turtles <6.0 cm captured in 2011 and 2019, compared to 2010. Specifically, only 6 of the 505 individuals captured in 2010 (1.2%) were <5.0 cm SCL, corresponding to ages of <2 years old (Iverson 1988). An additional nine individuals (1.8%) <6.0 cm SCL, or <3 years old, were captured in 2010. Capture rates of these small size classes increased substantially in 2011: of all 2011 captures, 235 individuals (21.3%) were <6.0 cm SCL. In 2019 and 2020, capture rates of juvenile size classes were even higher than in 2011: 50.9% and 29.2% of all captures in 2019 and 2020, respectively, were juveniles, which is likely indicative of successful oil spill cleanup efforts in 2010–2011 resulting in increased recruitment in subsequent years. Importantly, the high proportion of juveniles captured in 2019 and 2020 suggests that turtles <3 years old were either missing from the population or were undetected in 2010. The survey methods used during all four years of this study 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. The hypothesis that turtles under the age of 3 were "missing" from the population in 2010 and 2011 is further supported in 2019 and 2020 surveys, in which females in the size range of 13.0–18.0 cm SCL were captured in the lowest abundance of any size class. Turtles within this size range would be approximately 8–12 years old, and therefore would likely represent individuals that hatched in 2007–2012. Similarly, males >12.0 cm SCL (or >10 years old) were captured in the lowest abundance during 2019 and 2020 compared to other years. It seems likely, therefore, that the 2010 oil spill caused disproportionately high mortality of juvenile size classes in 2010 and 2011. While recruitment rates may have returned to normal ~10 years after the spill, gaps in the population's body size class distribution corresponding to individuals that would have been small juveniles at the time of the 2010 oil spill are still evident 10 years post-spill.

Surveys immediately following the 2010 Kalamazoo River oil spill recorded direct mortality rates of 5.7% in northern map turtles exposed to dilbit. Our results also revealed a variety of impacts on population demography 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, we detected a reduction in population size, shifts in the distribution of body size classes, and dramatic shifts in the population sex ratio. We also observed signals of failed recruitment in cohorts that would have hatched during the years just before through just after the oil spill. However, encouragingly, our results also suggest that 10 years after a catastrophic oil spill and extensive cleanup and restoration work, the population of northern map turtles within the Kalamazoo River appears to be on its way to recovery.

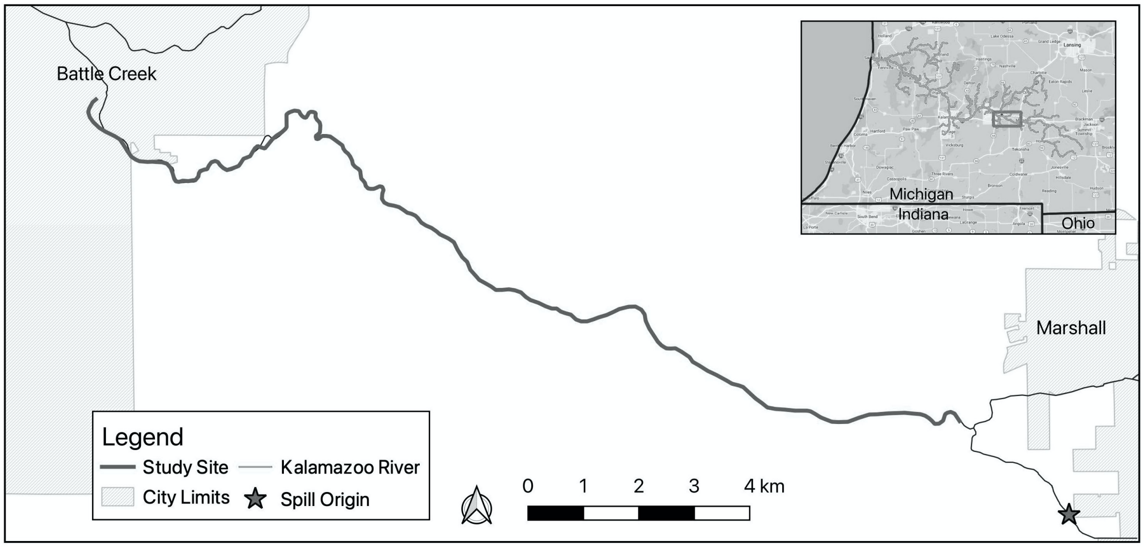

Figure 1-1. The Kalamazoo River Study Site that was surveyed for northern map turtles (*Graptemys geographica*) during 2010, 2011, 2019, and 2020 following the July 2010 oil spill. The Study Site was 20.2 km of the Kalamazoo River that was impacted by diluted bitumen oil in Calhoun County, MI, USA.

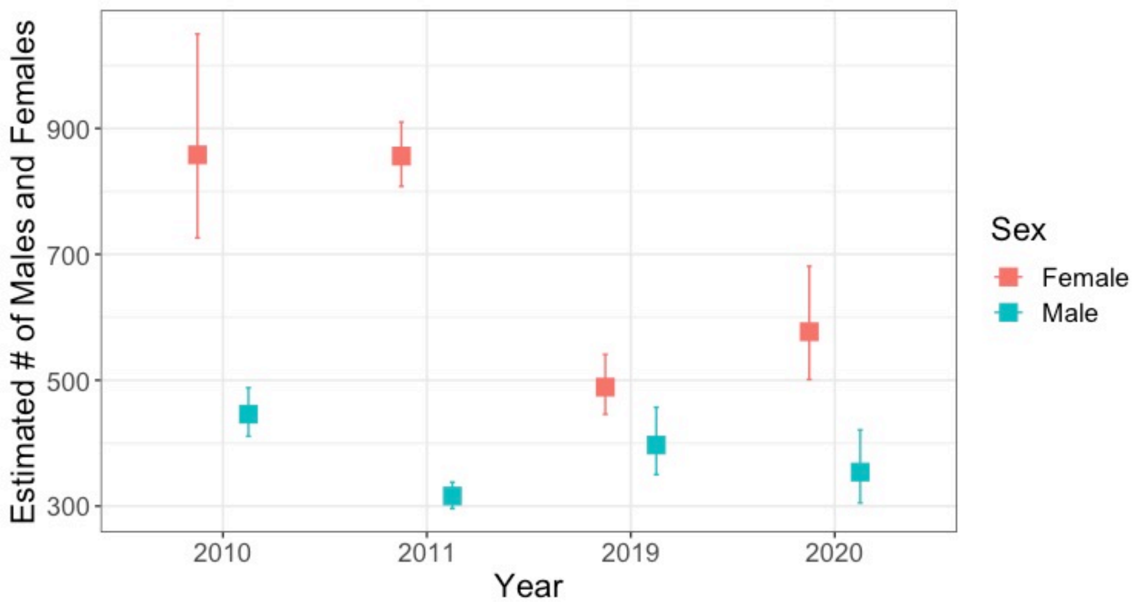

Figure 1-2. Yearly estimated number of male (blue) and female (pink) northern map turtles (*Graptemys geographica*) and 95% confidence intervals following the 2010 Kalamazoo River oil spill. Yearly estimates were derived independently using the Schnabel mark-recapture method. Data were collected from weekly surveys conducted on a 20.2 km stretch of the Kalamazoo River in Calhoun County, MI, USA.

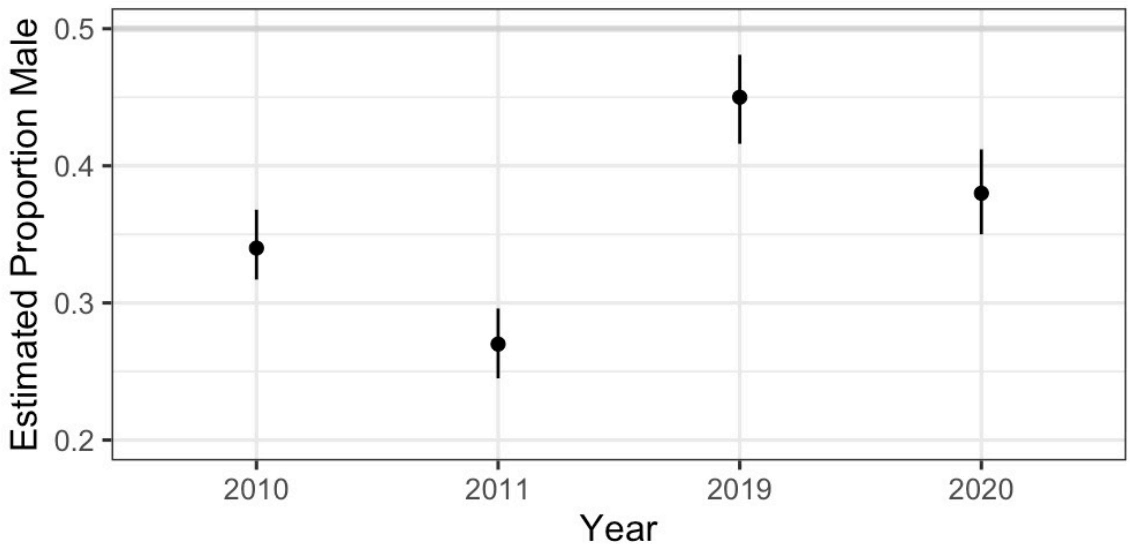

Figure 1-3. Estimated sex ratios (presented as proportion male) of northern map turtles (*Graptemys geographica*) and 95% confidence intervals estimated from weekly mark-recapture population estimates from the Kalamazoo River, Calhoun County, MI, USA during 2010, 2011, 2019, and 2020 surveys.

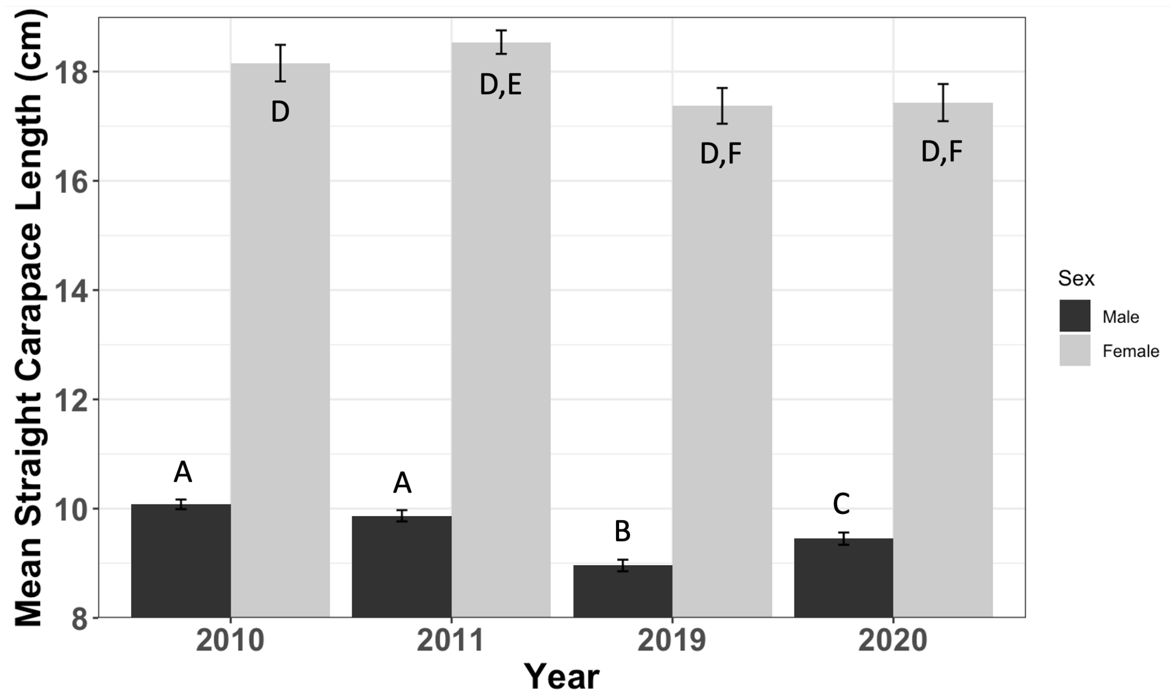

Figure 1-4. Mean straight carapace length (SCL) in cm of male (black) and female (gray) northern map turtles (*Graptemys geographica*) captured during surveys in 2010, 2011, 2019, and 2020 following the 2010 Kalamazoo River oil spill (Calhoun County, MI, USA). For turtles that were captured multiple times in a year, only the first SCL measurement was included in analyses for that year. Statistically different pairwise comparisons are denoted by capital letters.

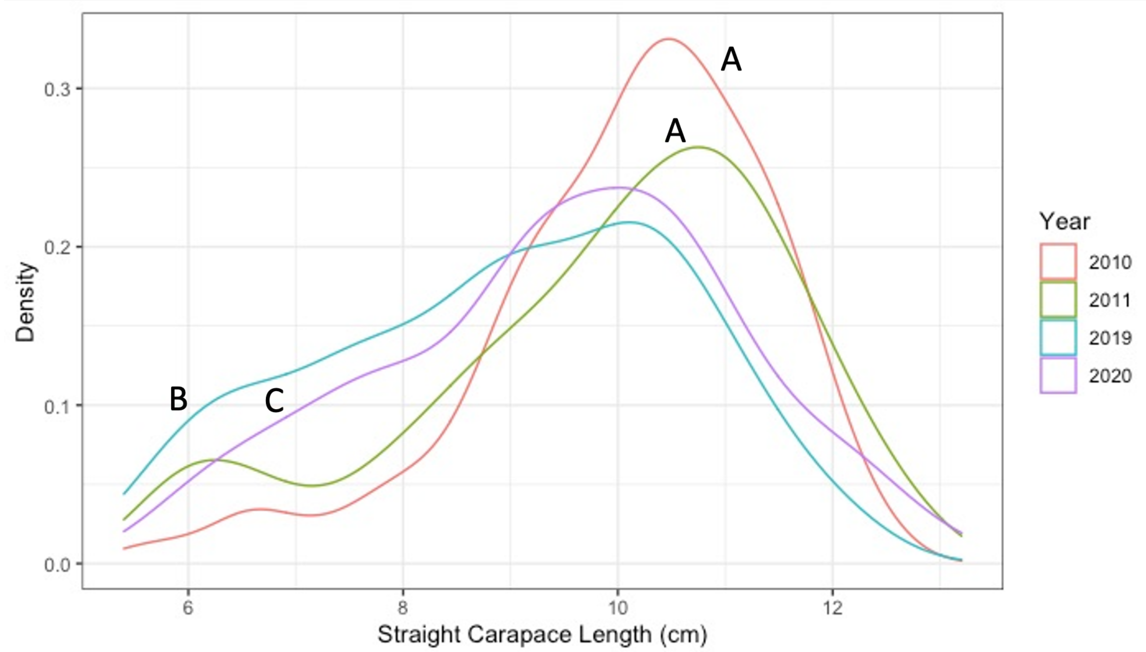

Figure 1-5. Density plots of straight carapace lengths (SCL) from male northern map turtles (*Graptemys geographica*) captured during surveys in the Kalamazoo River in Michigan, Calhoun County, MI, USA. For males that were captured multiple times in a year, only the first SCL measurement was included in analyses for that year. Statistically different pairwise comparisons are denoted by capital letters.

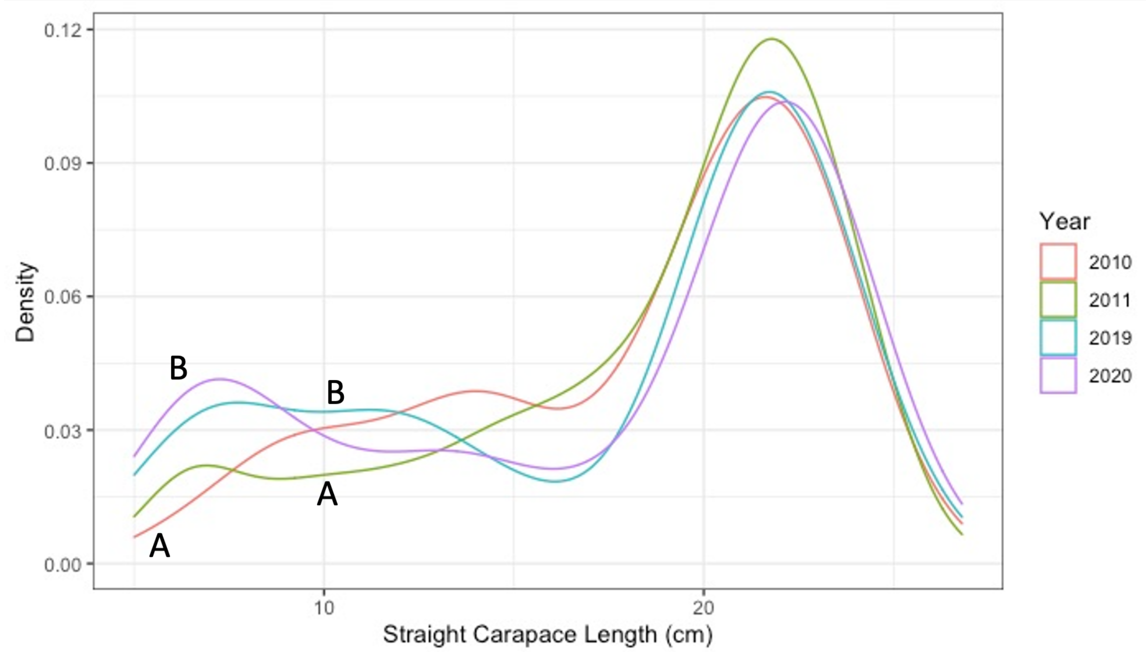

Figure 1-6. Density plots of straight carapace lengths (SCL) from female northern map turtles (*Graptemys geographica*) captured during surveys in the Kalamazoo River in Michigan, Calhoun County, MI, USA. For males that were captured multiple times in a year, only the first SCL measurement was included in analyses for that year. Statistically different pairwise comparisons are denoted by capital letters.

Table 1.1. Total number of survey days and boat-days, total number of individual turtles captured, and total captures made in 2010, 2011, 2019, and 2020 on the Kalamazoo River, Calhoun County, MI, USA. One survey day was defined as a day in which at least one boat conducted surveys, while boat-days were calculated as the number of boats surveying on a particular day regardless of number of people on the boat. Individuals (Ind.) included all captured northern map turtles regardless of sex or age; Total Captures included all captures of northern map turtles made that year. Catch per unit effort (CPUE) was calculated by dividing boat-days by the total number of captures.

	# Days	Boat-Days	Individuals	Total Captures	CPUE
2010	79	201	505	1,417[1]	7.0
2011	86	174	1,102	1,992	11.4
2019	99	121.5	1,141	1,986	16.3
2020	41	41	830	1,258	30.7
TOTAL	305	537.5	2,918	6,653	12.4

[1]Includes captures of turtles that were translocated, died, or were deemed unfit for release.

Table 1.2. Summary of captures, estimated number of individuals, and mean straight carapace length (SCL) of male and female northern map turtles (*Graptemys geographica*) captured in the Kalamazoo River Study Site (Calhoun County, MI, USA) during each year of survey. Captures are presented as number of new and recaptured turtles each year. The estimated number (Est. Number) of each sex and 95% confidence intervals (parentheses) were calculated independently for each year using the Schnabel mark-recapture method, based on weekly surveys. Only individuals identifiable to sex were included in Mean SCL calculations. For individuals that were captured multiple times in a year, only the first SCL measurement was included in analyses for that year. Statistically different pairwise comparisons of Mean SCL are denoted by superscript.

	Males			
	New Capture	**Recapture**	**Est. Number**	**Mean SCL (n)**
2010	249	89	446 (411-488)	10.1^{a} (229)
2011	133	529	316 (296-338)	9.9^{a} (282)
2019	209	223	397 (350-457)	9.0^{b} (258)
2020	58	287	354 (305-421)	9.5^{c} (222)
TOTAL	738	1,128	NA	NA

	Females			
	New Capture	**Recapture**	**Est. Number**	**Mean SCL (n)**
2010	246	33	858 (726-1,050)	18.2^{d} (233)
2011	424	574	856 (808-910)	$18.5^{d,e}$ (576)
2019	174	487	489 (446-541)	$17.4^{d,f}$ (348)
2020	91	470	577 (501-681)	$17.4^{d,f}$ (350)
TOTAL	974	1,564	NA	NA

Chapter 2

Survival Outcomes of Rehabilitated Riverine Turtles Following a Freshwater Oil Spill

2.1 Introduction

The adverse effects of oil spills on wildlife populations are highly visible and well documented, from the oiling of large numbers of individuals to direct oil exposure mortalities (Dunnet 1982, Barron et al. 2020; King et al. 2020). The emergency response to oil spills generally includes rescue of oiled wildlife in the first days to weeks following a spill, focusing on the rehabilitation of oil-exposed animals, or collection of individuals that died (Jessup 1998). Studies documenting the effects of oil spills on wildlife generally focus on acute (i.e., short-term; typically, a result of initial oiling) rather than chronic effects (i.e., long-term; persisting after the initial oiling or resulting from persistent environmental pollution; Helm et al. 2015). Despite being less well-studied, chronic effects can extend for months or years after the spill and cleanup, and often exceed the magnitude of acute effects and mortalities (Iverson and Esler 2010; Monson et al. 2011).

While rescue and rehabilitation of oiled wildlife has become routine over the past 50 years (Newman et al. 2003; Wolfaardt et al. 2008; De la Cruz et al. 2013), there is on-going debate about the effectiveness and conservation value of rehabilitating oiled wildlife. Questions remain regarding whether extensive effort and financial resources should be spent on rehabilitating individual animals, particularly if there is uncertainty over their survival after release (Moore et al. 2007; Baker et al. 2015; Henkel and Ziccardi 2018). Critics argue that funds spent on rehabilitation would be better spent on other conservation efforts such as restoring and conserving natural habitat (Henkel and Ziccardi 2018). There is also no clear consensus on how to evaluate the success of a rehabilitation effort: that is, should rehabilitation success be measured as the survival rate of oiled animals during the rehabilitation process (Mignucci-Giannoni 1999; Newman et al. 2003; Stacy 2015), or is it instead necessary to assess post-release survival rates of rehabilitated animals (Seivwright et al. 2019), and if so, for how long? Relatively few post-release monitoring studies have been conducted to quantify survival of rehabilitated animals despite their importance for assessing the effectiveness and conservation value of expensive rehabilitation efforts. Furthermore, with the exception of one freshwater turtle study (Saba and Spotilla 2003), most research on rehabilitation of oiled vertebrates has focused on birds and marine animals and has generally found lower post-release survival rates of rehabilitated individuals compared to control groups (Seivwright et al. 2019). While freshwater oil spills are usually smaller in scale than spills in marine systems, freshwater spills may have a greater relative impact on oiled wildlife because the oil cannot be diluted and degraded by large volumes of water, as can occur with marine oil spills (Lee et al. 2015).

Environmental catastrophes like the Exxon Valdez (1989) and the Deepwater Horizon (2010) oil spills have led to a large body of research on the toxicity of conventional crude oil on wildlife. The toxic effects of such spills are mainly attributed to polycyclic aromatic hydrocarbons (PAHs; Peterson et al. 2003; Barron 2012; Esler et al.

2018; Barron et al. 2020). Exposure to PAHs, whether acute or chronic, can lead to cardiotoxicity, behavioral changes, immunotoxicity, and decreases in reproductive success in a variety of aquatic invertebrates, fish, seabirds, and marine mammals (e.g., Barron 2012; Wilkin et al. 2017; Honda and Suzuki 2020). Due to recent, increased demand for crude oil and oil-related products, the use of alternatives to conventional crude oil have also increased. One such alternative is bitumen oil, production of which in the Canadian Oil Sands nearly quadrupled from 2000 to 2017 (Heyes et al. 2018). Pure bitumen oil is too viscous to be transported via pipelines directly, so it is mixed with natural gas condensates for ease of transport, which creates a product known as diluted bitumen ("dilbit" hereafter; Dew et al. 2015).

Although many of the chemical compounds in dilbit are also found in other crude oils, their relative proportions may differ. As a result, dilbit has higher density, viscosity, and adhesion than conventional crudes. It is also known to weather more rapidly than conventional crudes such that its low-molecular-weight components will evaporate quickly upon exposure to wind and wave action. Weathering of dilbit leaves a mixture of high-molecular-weight compounds that may become denser than water, especially freshwater, and sinks through the water column to settle on the sediment (Dew et al. 2015; Hua et al. 2018). Laboratory studies suggest that dilbit can have similar morphological and physiological effects on wildlife as conventional crude (Dew et al. 2015; Madison et al. 2015; Philibert et al. 2021); specifically, toxicity of dilbit to fish (Alderman et al. 2016; Robidoux et al. 2018; Timlick et al. 2020; Philibert et al. 2021), invertebrates (Robidoux et al. 2018; Barron et al. 2018; Barron et al. 2021) and birds (Ruberg et al. 2022) is similar to that of conventional crude. Importantly, to our knowledge, there are no published studies on the effects of dilbit on reptiles, marine mammals, or any other free-ranging animals.

We currently know very little regarding the fate of dilbit in natural ecosystems and its effects on aquatic species. To date, most data describing the effects of dilbit on free-ranging freshwater organisms were collected in relation to the Kalamazoo River oil spill, which occurred near Marshall, Michigan, USA. On 25-26 July 2010, 3.2 million L (834,444 gallons) of material were reportedly released after a pipeline carrying dilbit ruptured (NTSB 2012). The U.S. Environmental Protection Agency (EPA) estimated that 4.5 million L (1,181,599 gallons) of dilbit were recovered, which would make the Kalamazoo River oil spill one of the largest inland oil spills in U.S. history, and the largest dilbit spill to date (EPA 2016). The dilbit initially pooled in a marshy area near the ruptured pipeline before flowing 213 m into Talmadge Creek, and then into the Kalamazoo River where it impacted nearly 56 km of river channel (EPA 2016; Figure 2-1). The presence of submerged and sunken oil deposits reported by responders within days following the spill suggests that weathering may have occurred quickly as dilbit flowed from the pipeline rupture into the creek and river, which were in flood stage and presumably carrying large amounts of suspended solids. Ultimately, 10-20% of recovered dilbit was found to be mixed with sediment (Crosby et al. 2013). The volume of weathered and unweathered dilbit removed from the Kalamazoo River and river sediment provided an opportunity to study the potential acute and chronic effects of dilbit in a natural environment on survival of freshwater turtles, the most commonly captured animal during the Kalamazoo River oil spill cleanup.

Seven species of aquatic turtles were known to have been oiled during the Kalamazoo River oil spill, with northern map turtles (*Graptemys geographica*) being the most commonly observed and captured oiled turtle (EPA 2016). In 2010 and 2011, >2,100 northern map turtles with varying degrees of oiling were captured, cleaned, rehabilitated, and released back in the Kalamazoo River. Here, we estimate monthly survival rates of northern map turtles exposed to this freshwater spill of dilbit, 1-14 months post-spill and then again for 8-11 years post-spill. We also modeled whether rehabilitation type affected monthly survival probability of male and female northern map turtles.

2.2 Methods

2.2.1 Study Site

Our Study Site was ~20.2 km of channel from Talmadge Creek to E. Dickman Road in Calhoun County, MI, USA, as this was where the majority of wildlife survey work occurred in 2010 (Figure 2-1). Within the Study Site, the Kalamazoo River ranges from 9.0–40.0 m wide and 0.2–3.5 m deep.

2.2.2 Study Species

Northern map turtles exhibit pronounced sexual dimorphism, with adult females growing nearly twice the length of males (18.0–27.3 cm straight carapace length [SCL], 9.0–15.9 cm SCL, respectively). Males reach sexual maturity at 3–5 years of age (Iverson 1988), while females mature after 10 years (Lindeman 2013). Sex can typically be identified between 1–2 years of age using secondary sex characteristics (e.g., longer thicker tail, cloacal placement; Lindeman 2013). Both sexes are primarily aquatic but leave the water to bask daily on deadfall, rocks, or banks. Turtles at the Study Site are typically active from April to October and enter a state of brumation when air and water temperatures decrease, during which they are entirely aquatic, either buried in sediment, wedged between rocks and branches, or under banks (Otten personal observation).

2.2.3 Turtle Capture and Rehabilitation

Following the 2010 oil spill, turtle rescue and rehabilitation began on 29 July 2010, was conducted by numerous volunteers and paid contractors, including J.O., and was overseen by the U.S. Fish and Wildlife Service (USFWS; EPA 2016). In 2010, turtle rescue efforts concluded on 24 October due to changes in weather conditions that made it difficult to capture additional turtles as they entered brumation. Level of effort differed daily, with one to five boats surveying the Study Site each day. A survey day constituted an 8-hr day in which at least one boat actively captured turtles within the Study Site, and we used boats per day (boat-day) to calculate survey effort. One boat actively capturing turtles was considered one boat-day regardless of the number of people on the boat, rounded to the nearest ½ day. For example, if five boats surveyed the Study Site on a particular day, this would be considered five boat-days.

Turtles were captured using dipnets from a boat (as in Lager 1943), baited hoop traps, and basking traps. Field crews recorded capture location of each turtle with a handheld GPS (Garmin International Inc.; <3m accuracy), identified sex when possible, measured shell length (SCL) along the midline to the nearest mm, mass to the nearest 0.1 g, and marked turtles >100 g with passive implanted transponder (PIT) tags (Avid Identification Systems, Inc.). Individuals <100 g were marked with a unique set of notches filed into the marginal scutes when possible (Cagle 1939).

Upon capture, turtles exhibiting any visible oiling were retained for rehabilitation. Turtles that were not visibly oiled were processed as described above, and then released at the point of capture. The USFWS and Michigan Department of Natural Resources (MDNR) worked with Enbridge Inc., the operator of the ruptured pipeline, and their contractors (primarily Focus Wildlife and Stantec) to establish a temporary oil decontamination and wildlife rehabilitation facility in Marshall, MI (EPA 2016) where oiled turtles were photographed, physically examined by licensed veterinarians (veterinary staff was overseen by Dr. Chris Tabaka, DVM), and stabilized in individual housing until healthy enough to be cleaned.

During rehabilitation, each turtle was housed alone in a Rubbermaid™ tub (~53 to 208 L) or stock tank (~189 to 568 L), filled approximately half full of tap water and containing a basking structure (e.g., rock, large log, etc.) large enough for the turtle to pull itself entirely out of the water. Basking lights for heat, and a UVA/UVB light were placed ~30 cm over the basking structure and kept on for ~12 hours a day. Turtles were fed a daily mix of pellets, crickets, and worms, and tanks were cleaned and re-filled with fresh water after feeding. Licensed veterinarians administered medications daily when necessary.

To remove oil, each turtle was washed for ~30–60 min in warm water (~40°C) with dissolved detergent (Dawn™ Dish Soap) or mayonnaise, using pads, brushes, and cotton swabs. If turtles could not be completely cleaned in 60 min, they were allowed to rest and recover in their stabilization tanks between cleaning sessions when multiple cleaning sessions were required, which sometimes occurred over multiple days. After turtles were cleaned, they were monitored by veterinarians before being cleared for release. After 6 October, any newly captured turtles, any turtles still requiring cleaning, and any needing continued monitoring were retained over the winter in the rehabilitation facility. Overwintered turtles were kept communally (typically 1 adult male and 4 adult females) in 1,136 L stock tanks with artificial plants, submerged logs, rocks for shelter, and a curtain blocking the tank to decrease human habituation and minimize disturbance. Each tank was filtered with a sand filter (Intex, 3000 GPH) and had at least 50% water changes weekly. Each tank contained large (30+ cm width, 1.5 m length) logs for turtle to climb entirely out of the water, and three basking bulbs (as described above), programmed for 12-hr on/off cycles. Feeding followed similar methods described above, with leftover food removed 30 min after feeding. Air and water temperatures were maintained at those found in the Kalamazoo River during the months of May and June.

Overwintered turtles were released at their point of capture in spring 2011, once activity by wild turtles within the Study Site was observed. Alternatively, if overwintered turtles were deemed unfit for release due to injuries sustained because of the spill (e.g., buoyancy issues where animal could not swim below the water surface), they were instead transferred to licensed wildlife rehabilitators for permanent captive care. The average captive duration of turtles rehabilitated in 2010 was 27.2 days (range: 1–294 days).

2.2.4 Turtle Release and Translocation

Release of rehabilitated turtles was complicated by the conflicting goals of releasing individuals back to their capture location as soon as they were cleared by veterinarians, while also endeavoring to protect them from additional oiling and ongoing disturbance from cleanup operations at their original capture locations. Initially, if oil precluded the release of turtles at their original capture location, the USFWS and MDNR coordinated translocation and release of rehabilitated turtles to other areas within the Kalamazoo River watershed (e.g., upstream or downstream of the spill, or within tributaries; Chapter 4). On 22 September 2010, the EPA cleared impacted stretches of the Kalamazoo River for turtle release, following which turtles were released as near to their initial capture location as possible. Releases ceased on 6 October 2010, when air and water temperatures dropped to levels that stimulated winter brumation. Turtles captured after 6 October, or those still requiring cleaning and medical assistance, were overwintered and released at their point of capture between 26 April and 19 May 2011.

2.2.5 Surveys

We surveyed the Study Site in 2011 to both continue capturing oiled turtles, and to recapture previously rehabilitated and released turtles to assess survival. In 2018–2021, researchers from the University of Toledo (led by J.O.) attempted to recapture turtles that had been captured, rehabilitated, and released in 2010–2011 to assess survival rates. Surveys and turtle capture efforts used the same methods as described above for 2010. Level of effort and number of survey days varied by month and year, with the majority of effort from April to September. We checked for PIT tags or shell notches on captured turtles and measured each as described above. Unmarked turtles were marked with a unique combination of notches along marginal scutes (Cagle 1939). We recorded all capture locations and turtle morphology data as described above, and all turtles were released at the point of capture within 24 hours.

2.2.6 Data Analysis

We used northern map turtle survey data collected over six years (2010–2011 and 2018–2021) to calculate: 1) total survey effort and total number of turtle captures each year, 2) mortality rates of turtles captured in 2010 following the oil spill, 3) the monthly survival and recapture probabilities for overwintered, rehabilitated, and non-rehabilitated turtles *1-14 months post-spill*, and 4) monthly survival and recapture probabilities for overwintered, rehabilitated, and non-rehabilitated turtles *8-11 years post-spill*. Estimates of mortality rates, monthly survival, and recapture probabilities included only turtles that were identifiable to sex.

2.2.6.1 Turtle Capture and Survey Effort

For each survey year, we used all captures of northern map turtles, including individuals that were either released unmarked or were too young to identify to sex, to determine the total number of northern map turtles captured, and the average number captured per day. To calculate catch per unit effort (CPUE), we divided the total number of captures by the total number of boat-days.

2.2.6.2 Mortality Rates Following the Oil Spill

When determining the mortality rates of turtles in the months following the oil spill, we included only turtles captured before 1 November 2010. We defined mortality as any individual that was collected dead, died during rehabilitation or overwintering, or sustained injuries that did not allow for release back into the wild (e.g., turtles that could no longer fully submerge and therefore were transferred permanently to wildlife rehabilitators). We used a chi-square proportion test to compare the proportion of mortalities between the sexes.

2.2.6.3 Monthly Survival and Recapture Probabilities

In our calculations of monthly survival and probability of recapture, we included only turtles that were captured, marked, and released within 1 km of their original 2010 capture location. We excluded any turtles that were considered mortality events as described above and any originally captured within the Study Site but were translocated and released elsewhere during cleanup operations. All turtles included in this analysis were categorized as “rehabilitated,” “overwintered,” or “non-rehabilitated.” We considered any turtle that spent at least one night in captivity but was released in 2010 as “rehabilitated.” Turtles that were overwintered in the rehabilitation facility during the winter of 2010–2011 and released in spring 2011 were categorized as “overwintered.” Finally, turtles captured in 2010 or 2011 that did not go through any rehabilitation or overwintering were categorized as “non-rehabilitated.” These were individuals with either no visible oiling, or light spotty oiling covering <5% of their body which could be easily removed with a brush. These individuals were cleaned immediately in the field and released at their point of capture.

To estimate monthly survival, and recapture probabilities of rehabilitated, overwintered, and non-rehabilitated turtles, we constructed the capture history of each individual based on its capture or non-capture during a particular sampling event. The two time periods (i.e., 2010–2011 and 2018–2021) were analyzed separately. To calculate 1-14 months post-spill monthly survival and recapture probability, we used seven sampling events: September and October 2010 combined; and monthly from May to September 2011. To calculate 8-11 years post-spill monthly survival and recapture probability, we used 19 sampling events: 3 in 2018 (May to July), 6 in 2019 (April to

September), 6 in 2020 (April to September), and 4 in 2021 (April to June, and August). Only the first capture of each individual during each sampling event was included in models. Survey effort was calculated for each sampling event by totaling the number of boat-days.

We used the Cormack-Jolly-Seber (CJS) mark-recapture method (Cormack 1964; Jolly 1965; Seber 1965) in program MARK (R software; White and Burnham 1999) to estimate survival and recapture probabilities. To explain the mark-recapture data for each time period with respect to survival and recapture probabilities, we constructed 16 biologically plausible candidate models. Models estimating survival included all combinations of sex and rehabilitation category (i.e., rehabilitated, overwintered, or non-rehabilitated in 2010-11). Models of recapture probability also included the effect of time between surveys (*t*) and number of boat-days (survey effort; Table 2.1). We also evaluated a model with constant survival and recapture probabilities.

We conducted a goodness-of-fit (GOF) test prior to model selection to verify whether data met the assumptions of the CJS model that every animal present in the population at time *t* has the same recapture probability, and that every animal in the population immediately after time *t* has the same survival to time *t+1* (Arnason-Schwarz Model, Pradel et al. 2003). We performed the GOF test using the R2ucare package in R (Choquet et al. 2009). We tested for overdispersion of the global model (Survival $_{\text{sex * rehab type}}$ Recapture $_{\text{effort * t}}$) using the median c-hat method in MARK (R software; White and Burnham 1999), which assumes that a c-hat estimate near 1 indicates the model has reasonable fit to the data, whereas c-hat estimates >3 indicate structural deficiencies in the global model (Gonzalez-Tokman et al. 2012). Because our models were slightly over-dispersed, $QAIC_c$ (Quasi Akaike's Information Criterion corrected for bias and overdispersion) was used to compare the 16 models for survival, both 1-14 months post-spill, and 8-11 years post-spill. If the $QAIC_c$ was <2, we assumed no difference between alternative models.

2.3 Results

2.3.1 Turtle Capture and Survey Effort

A similar number of boat-days occurred in 2010–2011 compared to 2018–2021, with an average of 1.86 boat-days per survey day compared to 1.23, respectively. The overall CPUE for 2010–2011 was 12.6 northern map turtles per day compared to 16.8 northern map turtles per day during 2018–2021; however, the CPUE per day among years varied from 6.2 in 2018 to 32.3 in 2020, likely due to researchers' increased experience during this time period. During 2010–2011, we made 3,114 total captures of 2,015 individual northern map turtles over 133 survey days, while in 2018–2021, we made 3,976 captures of 1,845 individuals over 192 survey days (Table 2.1).

2.3.2 Mortality Rates Following the Oil Spill

We observed more mortalities of female northern map turtles immediately following the spill (up to 1 November 2010) compared to males (7.6% vs 4.1%; x^2= 5.83 p=0.02). Two individuals were found dead during surveys (1 female and 1 male), 50 turtles died in captivity during rehabilitation (31 females and 19 males), and 15 were deemed unfit for release and were transferred into permanent captivity (8 females and 7 males). On average, turtles that died during rehabilitation did so 57.6 (±61.4 SD) days after capture.

2.3.3 Monthly Survival and Recapture Probabilities

From 2010 to 2011, we made 2,414 captures of 1,166 unique individuals (704 females and 462 males; Table 2.1), with individuals recaptured 2–12 times. A total of 322 rehabilitated (128 females and 194 males), 285 overwintered (164 females and 121 males), and 559 non-rehabilitated turtles (412 females and 147 males) that were originally captured in 2010 or 2011 were included in monthly survival analyses (Table 2.1). Nearly 25% of these individuals were recaptured at least once between 2018–2021 (228 of 1,166), with the highest proportion of recaptures being turtles that were overwintered (31.6%; 90 of 285).

2.3.3.1 1-14 Months Post-Spill Monthly Survival (2010-2011)

The GOF test of the global 1-14 months post-spill monthly survival model indicated that the model was slightly over-dispersed but still had a reasonable fit to the data (c-hat=1.63). Of the candidate models considered, the best-supported model (i.e., lowest $QAIC_c$) was the model including rehabilitation type for survival and time between surveys for recapture probability (φ (Rehab) ρ (t); Table 2.2). Under this model, 1-14 months post-spill survival probability was affected by rehabilitation type (i.e., non-rehabilitation, rehabilitation, or overwintered) that occurred in 2010, but was otherwise unaffected by sex of the turtle, in contrast to the sex difference in mortality that was observed only immediately following the spill.

For both sexes, the estimated 1-14 months post-spill monthly survival probability of turtles that had been overwintered (females n=164, 0.983 ± 0.006 [SE; 95% CI= 0.964–0.992]; males n=121, 0.988 ± 0.005 [SE; 95% CI= 0.975–0.994]) was significantly higher than that of turtles that had been rehabilitated but not overwintered (females n=128, 0.910 ± 0.012 [SE; 95% CI= 0.883–0.931]; males n=194, 0.909 ± 0.010 [SE; 95% CI= 0.888–0.926]). Female turtles that were neither rehabilitated nor overwintered had the lowest 1-14 months post-spill monthly survival probability (n=412, 0.799 ± 0.037 [SE; 95% CI= 0.716–0.862]), while the 1-14 months post-spill monthly survival probability of males that were neither rehabilitated nor overwintered was similar to that of males that had undergone rehabilitation but not overwintering (n=147, 0.916 ± 0.036 [SE; 95% CI= 0.812–0.965] (Figure 2-2). Post hoc analysis indicated the 1-14 months post-spill monthly survival rates of rehabilitated, overwintered, and non-rehabilitated turtles to be similar for both sexes.

Under the *ρ* (*t*) model, recapture probabilities during this time period differed among survey periods, ranging from 0.036 ± 0.009 (95% CI= 0.022–0.059) in October 2010 to 0.414 ± 0.027 (95% CI= 0.362–0.468) in September 2011, with a mean of 0.251 ± 0.022 (95% CI= 0.211–0.295).

2.3.3.2 8-11 Years Post-Spill Monthly Survival (2018-2021)

The GOF test of the global 8-11 years post-spill monthly survival model indicated that the model was slightly over-dispersed but still had a reasonable fit to the data (c-hat=1.25). Of the candidate models considered, the best-supported model (lowest $QAIC_c$) was the model including sex for survival, and time between surveys for recapture probability (φ (Sex) ρ (*t*); Table 2.2). Under this model, the monthly survival probability for turtles that were alive 8-11 years post-spill was affected by sex but was otherwise unaffected by rehabilitation type. The estimated 8-11 years post-spill monthly survival probabilities of females that had been rehabilitated or overwintered were nearly identical to non-rehabilitated females (Figure 2-2; rehabilitated n=32, 0.998 ± 0.002 [SE; 95% CI=

0.987–0.999]; overwintered n=58, 0.996 ± 0.002 [SE; 95% CI= 0.961–0.999]; non-rehabilitated n=128, 0.991 ± 0.004 [SE; 95% CI= 0.977–0.996]). While females had significantly higher 8-11 years post-spill monthly survival rates than males, males that had been rehabilitated (n=22, 0.977 ± 0.014 [SE; 95% CI= 0.928–0.993]) or overwintered (n=32, 0.971 ± 0.014 [SE; 95% CI= 0.926–0.989])) were similar to those of males that were non-rehabilitated (Figure 2-2; n=15, 0.912 ± 0.035 [SE; 95% CI= 0.816–0.960]).

Under the ρ (*t*) model, recapture probabilities differed between among survey periods 8-11 years post-spill, ranging from 0.029 ± 0.012 (95% CI= 0.013–0.064) in April 2021 to 0.303 ± 0.040 (95% CI= 0.231–0.386) in June 2019, with a mean of 0.130 ± 0.028 (95% CI= 0.084–0.197).

2.4 Discussion

Determining the broad population- and community-level consequences of oil spills is necessary to establish the ecological impacts of pollution (Hinton et al. 2005). Here we provide an evaluation of the effectiveness of rehabilitation efforts on survival of a freshwater turtle population following exposure to a dilbit oil spill in the Kalamazoo River. We compared 1-14 months post-spill and 8-11 years post-spill monthly survival probabilities of oiled turtles that were either rehabilitated and released in 2010 or overwintered and released in 2011 following the 2010 oil spill, to those that had not been rehabilitated or overwintered. We found that both rehabilitated and overwintered turtles had a higher probability of survival 1-14 months post-spill than non-rehabilitated turtles; however, for those turtles surviving to 8-11 years post-spill, the among-group differences in survival probability by that time were similar. To our knowledge, this is the first study on impacts of a dilbit oil spill on long-term survival in a vertebrate species.

While an extensive effort was spent capturing and rehabilitating northern map turtles during 2010 cleanup activities, only 52 mortalities were directly observed. An additional 15 individuals were too severely injured for release and were instead transferred to permanent captivity; these turtles should be considered functional mortalities from a demographic perspective. Overall mortality (including un-releasable turtles) was 67 of 1,181 (5.7%) northern map turtles recovered after the oil spill, 66 of which had external oiling and one that was injured by a boat or other equipment. This apparent mortality rate was very similar to that of the only other freshwater crude oil spill that included turtle rehabilitation and reported rehabilitation mortality rates, wherein 5.3% of 19 oiled individuals died during rehabilitation (Saba and Spotila 2003). Our observed mortality rate was nearly three times that reported during offshore sea turtle recovery in the Gulf of Mexico during the Deepwater Horizon spill of 2010, during which 328 sea turtles were rehabilitated, 7 of which later died (2.1%; Stacy 2015; Stacy 2017). Our observed mortality rate from the 2010 spill is likely an underestimate because detectability and recovery of oiled turtle carcasses was complicated by the difficulty of visually detecting them in the heavily oiled river and floodplain; the probability that heavily oiled carcasses were inadvertently removed along with oil, oiled vegetation, debris, and sediment; the possibility that some carcasses could have been scavenged; and safety constraints on timing and coverage of searches over a large geographic area..

The similarity of our estimated mortality rate compared to those from other crude oil spills suggests that dilbit is similarly toxic to freshwater organisms as conventional crude oil. Importantly, the morphological and physiological effects of dilbit on freshwater organisms are still poorly understood. Toxicity of dilbit in some species of fish may be caused by PAHs binding to and activating aryl hydrocarbon (AhR) receptors (Hodson 2017; Madison et al. 2017; Alsaadi et al. 2018), but Everitt et al. (2021) suggested both AhR-dependent and -independent mechanisms as causes of toxicities of weathered dilbit in zebrafish. Although toxicity of dilbit in turtles has not been studied, turtles are known to accumulate heavy metals (Yu et al. 2011; Hopkins et al. 2013), coal fly ash (Nagle et al. 2001; Steen et al. 2015), and PAHs (Camacho et al. 2012; Ylitalo et al. 2017) in their tissues. Dilbit weathers and degrades faster than conventional crude oil (King et al. 2014); however, acute toxicity of unweathered and weathered dilbit is similar in fish and invertebrates (Barron et al. 2018; Robidoux et al. 2018). In particular, concentrations as low as 3.5 µg/L can induce a liver biomarker of PAH exposure, while concentrations of 16.4 µg/L can induce a PAH biomarker in the heart (Alderman et al. 2016). Because our study was conducted opportunistically following an unexpected oil spill in wild habitat, data on dilbit concentrations, degree of weathering, or the duration of individual turtles' exposure to dilbit were not recorded. Moreover, to our knowledge, no toxicological post-mortem necropsies were conducted that would have provided such data. We did, however, find that most observed turtle mortalities occurred during rehabilitation, after removal of surficial oil and while turtles were under the daily care of veterinarians. These mortalities occurred an average of 57.6 days after capture, suggesting that latent deleterious physiological effects may have occurred, and which may have taken weeks or months to develop. Determining the precise effects of dilbit on the health of exposed wildlife should be a research priority, particularly considering the trend to increase transport of dilbit as an alternative to traditional crude oil.

While rehabilitation efforts similar to those used here typically occur with emergency wildlife rescue efforts following oil spills, surprisingly little is known about the long-term effectiveness of the rehabilitation process on individuals after release, or on population demographics following the spill event (Murphy et al. 2016). Studies comparing individual survival rates after rehabilitation found lower survival in rehabilitated sea otters (*Enhydra lutris*) and sea birds compared to control animals (Hartung 1995; Rebar et al. 1995; Seivwright et al. 2019). In contrast, our results show that rehabilitated oiled turtles released back into the wild had higher monthly survival probabilities 1-14 months after the 2010 Kalamazoo River oil spill compared to turtles that had not been rehabilitated. Although non-rehabilitated turtles were not a true control population and experienced the same environmental conditions as rehabilitated turtles following their release, non-rehabilitated turtles had either no or very minor surficial oil that was easily cleaned in the field. Moreover, rehabilitation efforts that included overwintering oiled turtles in captivity further increased these survival rates in both sexes: turtles that were overwintered in captivity had a monthly survival probability almost 8% higher than turtles that had been rehabilitated but not overwintered in 2010, and 13% higher than non-rehabilitated turtles. This difference in survival could equate to nearly 50% fewer individuals in the population at the end of 2011 if no turtles had been overwintered in captivity as part of rehabilitation efforts, which could have severe consequences for population demographics (Chapter 1). In late-maturing, long-lived species such as turtles, even a slight decrease in adult survival could result in a substantial decrease in recruitment and population growth rates, a trend which could take many years to reverse (Congdon et al. 2003) and which would be particularly detrimental in threatened or endangered species.

We found that both temporarily maintaining turtles that had been oiled during rehabilitation and overwintering them in captivity until spring increased their probability of survival compared to turtles that had not been housed in captivity. On average, rehabilitated turtles were kept 6.2 days in captivity, while overwintered turtles were kept 210.2 days. Importantly, any time spent in captivity served not only to clean and rehabilitate individuals after exposure to oil, but also decreased their contact with residual oil in the environment and with human disturbance during subsequent cleanup operations. The increase in survival probability for overwintered turtles may have been a result of constant veterinarian supervision and feeding during a 6-month period in which they are usually dormant, which allowed turtles to gain additional mass and energetic resources necessary for survival. Cleanup operations observed that dilbit settled in the sediment in low-flow backwater areas of the river (EPA 2016), which are sometimes used for brumation by freshwater turtles. Sediment contaminated with dilbit would have led to additional exposure to weathered dilbit for several months during turtles' brumation period. In addition, lower flow depositional areas of the river were disturbed by oil recovery efforts including sediment agitation and dredging in 2010 and 2011.

We found that, while non-rehabilitated turtles had higher mortality than rehabilitated or overwintered turtles during the first year following the spill, 8+ years later the differences in monthly survival probabilities among survivors in the different rehabilitation categories were indistinguishable. Our monthly survival estimates (which included juveniles and subadults) were higher than the annual adult survival rates from a six-year study of an intact reference population of northern map turtles in Canada (94% for females and 81% for males; Bulte et al. 2009). Although the survival rates in our study population cannot be directly compared to those of the Bulte et al. (2009) study due to differences in the age classes included in the estimates, our results suggest that the map turtle population at our Study Site has returned to a "natural" mortality rate ~10 years after the 2010 oil spill. In comparison to long-lived species such as the northern map turtle, taxa with shorter generation times can likely recover more quickly following environmental disasters such as oil spills. For example, invertebrates in oil-impacted areas of the Kalamazoo River decreased in density and species diversity during 2010 and 2011 but appear to have recovered and stabilized within five years of the spill (Matousek 2018).

Our study 11 years after the 2010 Kalamazoo River oil spill suggests that dilbit exposure combined with other stressors from spill response and habitat restoration actions, may cause mortality to freshwater turtle species similar to that resulting from spills of conventional crude oil. Rehabilitation of oil-exposed northern map turtles significantly increased survival within 14 months of the spill, which emphasizes the importance and effectiveness of rehabilitation efforts for species such as freshwater turtles. While the same increase in survival probability was no longer apparent 8-11 years post-spill, nearly 25% of rehabilitated turtles were nonetheless recaptured during this time period, which is an impressive survival rate in a population that was severely impacted by a massive oil spill. With the predicted increase in dilbit production and transport in the near future, research should concentrate on determining specific pathways of dilbit toxicology in turtles and other wildlife, its residence time in tissues and potential for biomagnification at higher trophic levels, and the effects of long-term exposure to individuals and populations. It is also important to determine the potential impacts of physical emergency response and habitat restoration actions such as sediment agitation on habitat quality and long-term population recovery. Finally, determining the specific rehabilitation activities that are most effective at increasing survival of oiled animals is the next logical step. Our results demonstrated that overwintering turtles in captivity resulted in increased survival rates; therefore, future research should endeavor to compare the efficacy of different overwintering strategies, such as keeping turtles fed, warm, and awake throughout the winter vs. inducing them to hibernate in captivity. Empirically testing the effectiveness of specific wildlife rehabilitation strategies, emergency spill responses, and habitat restoration protocols is critical for developing best management practices in order to ensure the survival of long-lived wildlife species, such as turtles, following large-scale spill events.

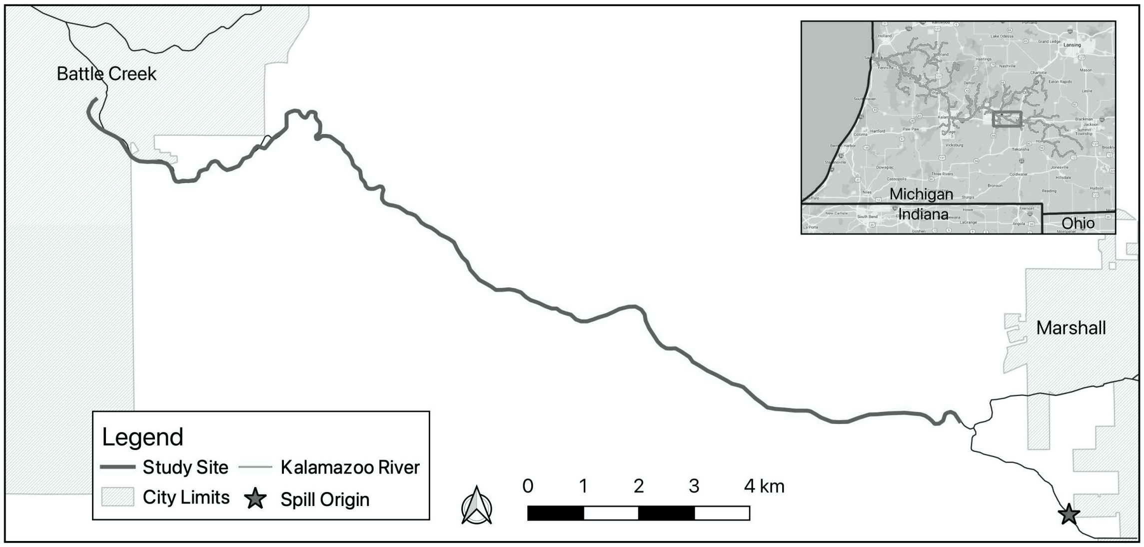

Figure 2-1. Area surveyed to compare survival rates based on rehabilitation type on northern map turtles (*Graptemys geographica*) in 2010-2011 and 2018-2021 following the 2010 Kalamazoo River oil spill. This Study Site was 20.2 km of the Kalamazoo River in Calhoun County, MI, USA.

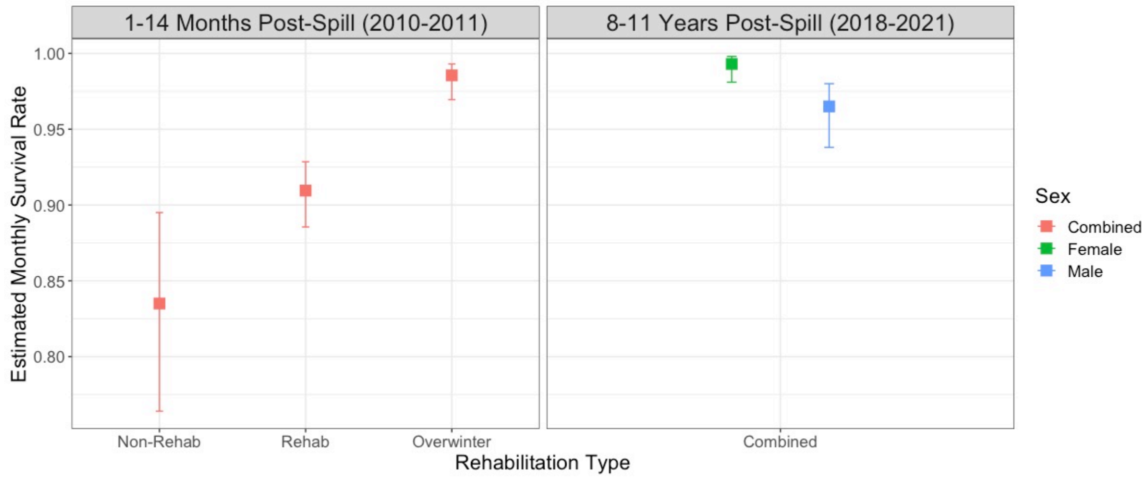

Figure 2-2. Estimated monthly survival probability for northern map turtles (*Graptemys geographica*) captured following the 2010 Kalamazoo River oil spill, 1-14 months post-spill (left) and 8-11 years post-spill (right). Turtles that spent at least one night in the rehabilitation facility and were released in 2010 were categorized as "Rehab," those that spent the winter of 2010–2011 in the facility were categorized as "Overwinter," and those that were neither rehabilitated nor overwintered were categorized as "Non-Rehab." Monthly survival estimates 1-14 months post-spill were calculated from September 2010 to October 2011, while estimates 8-11 years post-spill were calculated from May 2018 to Aug 2021.

Table 2.1. Summary of capture efforts and results for northern map turtles (*Graptemys geographica*) in the Kalamazoo River, MI during 2010–2011 and 2018–2021 survival surveys. A boat-day was determined by the number of boats actively surveying the Study Site. The total and mean number of captures were based on all individuals captured, regardless of recapture status, size, or sex. Catch per unit effort (CPUE) was calculated by dividing the total number of captures by the number of boat-days during a survey period. Total number of marked 2010/2011 turtles include only individuals marked in 2010 or 2011 and identifiable to sex. The number of individuals and number of times individually marked turtles were captured in subsequent years of survey (2018–2021) are divided by sex and rehabilitation category, i.e., whether an individual spent at least one night in the rehabilitation facility but was released in 2010 (rehab), was overwintered during the winter of 2010–2011 (overwintered), or was captured and either had no oil or was field cleaned (non-rehab).

		2010-2011	**2018-2021**	**Total**
Number of Survey Days		133	192	**325**
Number of Boat-Days		247	236	**483**
Total Turtle Captures		3,114	3,976	**7,090**
Avg. Turtle Captures/Day		23.4	20.7	**21.8**
CPUE		12.6	16.8	**14.7**
Total Sexable Captures		2,623	2,645	**5,268**
Total Captures of Marked 2010/2011 Turtles		2,414	784	**3,198**
Individual Females	Non-Rehab	412	128	**-**
	Rehab	128	32	**-**
	Overwintered	164	58	**-**
Individual Males	Non-Rehab	147	15	**-**
	Rehab	194	22	**-**
	Overwintered	121	32	**-**

Table 2.2. Model types describing 1-14 months post-spill and 8-11 years post-spill monthly φ (survival) and *ρ* (recapture) probabilities of northern map turtles (*Graptemys geographica*) in the Kalamazoo River, MI following the 2010 oil spill. Only individuals identifiable to sex were included in analysis. 1-14 months post-spill models include data collected in 2010 and 2011, while 8-11 years post-spill models include data collected from 2018–2021. For each model we included model rank, variables included (model), number of parameters (K), difference of QAICc value from top model (ΔQAICc), Akaike weight (*w*), and Qdeviance. Model variables included in φ probabilities included sex, rehabilitation category (rehab; i.e., "rehabilitation", "overwinter", or "no rehabilitation"), and interactions. Model variables included in *ρ* probabilities included time between surveys (*t*), level of effort for survey period (effort), and the interactions. Both φ and *ρ* also included constant probabilities (.; null model). Only the top five and null model results are included. Top model in bold.

Type	Rank	Model	K	QAICc	Δ QAICc	*w*	QDeviance
1-14 months post-spill	**1**	**φ (Rehab) ρ (*t*)**	**9**	**279.59**	**0.00**	**0.50**	**426.68**
	2	φ (Sex * Rehab) ρ (*t*)	12	281.84	2.25	0.50	420.57
	3	φ (Rehab) ρ (effort * *t*)	15	291.59	12.00	0.00	426.68
	4	φ (Sex * Rehab) ρ (effort * *t*)	18	293.84	14.25	0.00	420.57
	5	φ (Rehab) ρ (effort)	5	331.50	51.91	0.00	524.41
	15	φ (.) ρ (.)	2	486.20	206.61	0.00	786.53
8-11 years post-spill	**1**	**φ (Sex) ρ (*t*)**	**20**	**1136.05**	**0.00**	**0.79**	**1371.15**
	2	φ (Sex * Rehab) ρ (*t*)	24	1139.37	3.32	0.20	1365.31
	3	φ (.) ρ (*t*)	19	1142.96	6.91	0.01	1382.31
	4	φ (Rehab) ρ (*t*)	21	1146.50	10.45	0.01	1371.15
	5	φ (Sex) ρ (effort * *t*)	38	1172.05	36.00	0.00	1365.31
	15	φ (.) ρ (.)	2	1276.77	140.72	0.00	1592.24

Chapter 3

Long-term Effects of an Oil Spill on the Spatial Ecology of a Riverine Turtle Species, the Northern Map Turtle (*Graptemys geographica*)

3.1 Introduction

Oil contamination of freshwater ecosystems can affect aquatic wildlife in three overarching ways: by changing the structure and size of populations, by decreasing reproduction and recruitment, and by altering habitats (Wiens 1995; Day et al. 1997). Oiled habitats in freshwater ecosystems are visually striking at the time of the spill (e.g., oil slicks on water, oil on banks and deadfall); however, the effects of oil contamination following a spill on habitat quality and use by wildlife are poorly understood (Chapman 1984; Day et al. 1997; Wiens et al. 2004; Luiselli et al. 2006). Oil spills may alter and degrade habitat directly by contaminating substrates or changing vegetation structure (Jackson et al. 1989; Duke et al. 1997; Mendelssohn et al. 2012), and indirectly by altering trophic cascades or increasing algae production (Peterson 2001; Fleeger et al. 2003). Additionally, cleanup and restoration work following an oil spill can alter habitat through substrate compaction by heavy machinery, use of high-pressure cleaning techniques that can displace rocks and invertebrates, or removal of large volumes of oiled sediment or banks and associated habitat structures (Peterson 2001; Fleeger et al. 2003; Silliman et al. 2012).

The design and scope of most studies on the effects of oil spills are necessarily limited, as spills cannot be predicted or replicated; therefore, most such studies are conducted immediately following an oil spill during the time when acute effects and direct mortalities in wildlife are observed (Peterson et al. 2003; Bell et al. 2006; Van Meter et al. 2006). However, chronic effects of oil spills can extend for months, years, or even decades, with mortalities attributable to chronic effects sometimes exceeding the magnitude of mortalities observed immediately following the oil spill (Iverson and Esler 2010; Monson et al. 2011). Chronic effects may result from indirect oil spill-induced changes to prey availability, predator abundance, and habitat quality (Peterson et al. 2003; Luiselli et al. 2006). It is important to understand the effects of oil spills and subsequent restoration efforts on habitat quality and use by affected species to improve effectiveness of future cleanup operations and restoration efforts.

Suitable habitat is necessary to meet the basic ecological requirements of a species and is critical if a population is to recover from a large-scale disturbance such as an oil spill (Morrison 1986). General wildlife response to changes in habitat suitability may be gauged by distribution and abundance of species, while space use by specific taxa may indicate habitat quality (Bjorneraas et al. 2012; Belgrad and Griffen 2020). In particular, assessing home range size may help determine the distribution and spatial dynamics of populations, while also identifying critical habitat and dispersal patterns (Bowler and Benton 2005). An animal’s home range is the area within which it travels to meet its ecological needs (e.g., food, shelter, and mates), and a home range may be maintained at some or all life stages (Burt 1943; Borger et al. 2006; Laver and Kelly 2008). Once a home range is established, its geographical extent is typically determined by a combination of resource availability, energy expenditure, inter- and intra-species competition, and predator-prey interactions (Rincon-Diaz et al. 2011; Barraquand and Murrell 2012). Intra-species differences and changes in home range size can offer insight into habitat quality, as well as the distribution and predictability of resources on the landscape (Switzer 1993; Edwards et al. 2009). Within a population, larger home ranges may be a result of lower habitat quality, lower resource availability, or habitat avoidance compared to smaller home ranges (Gibbons et al. 1990; Saïd et al. 2009; Kapfer et al. 2009). For example, gopher tortoises (*Gopherus polyphemus*) abandon burrows and extend their range as habitat degrades (Aresco and Guyer 1999). Similarly, home ranges of eastern box turtles (*Terrapene carolina*) from higher-quality fire-maintained sites were half the size of home ranges compared to individuals from sites not maintained by burning regimens (Roe et al. 2019). In addition, sexes may differ in home range sizes, movement patterns, and resource requirements (Gaulin and Fitzgerald 1988; Steinmann et al. 2005), and such differences should be considered in developing conservation or restoration plans. In particular, understanding the general space use of a species, and how it may be altered as a result of oil contamination, is critical to maximizing restoration efforts following an oil spill.

In this study, we quantified home range size of a riverine turtle species nine years after a major freshwater oil spill as an indirect measure of the effects of the oil spill and subsequent restoration efforts on aquatic turtle habitat quality. Riverine turtle species require habitat within lotic habitat (e.g., woody debris for basking) and immediately adjacent to it (e.g., banks for nesting) to meet their ecological needs, making them highly vulnerable to oil spills that occur within rivers (Moll 1980; Moll and Moll 2004). While it can be difficult to quantify change in habitat quality following an oil spill because data on pre-spill habitat are rarely available for a specific site, comparisons of space use and home range size at sites that varied in magnitude of oil contamination and restoration efforts may provide insight into the potential long-term effects of the spill itself, or of the resulting restoration work. For example, if oil contamination affects critical resources (e.g., food, shelter, mates, and nesting habitat), turtles at impacted sites may increase the size of their home ranges to procure those resources elsewhere. Alternatively, if long-term impacts of oil contamination on aquatic turtle habitat have become negligible due to successful restoration efforts, then turtles are more likely to remain in areas that were directly impacted by the oil spill, and therefore their home range sizes should be similar to conspecifics in adjacent habitat unaffected by the oil spill.

Our primary objective was to test for long-term, combined effects of a freshwater oil spill, immediate cleanup operations, and two to three subsequent years of restoration work on space use of northern map turtles (*Graptemys geographica*), nine years after the Kalamazoo River oil spill. We predicted that turtles occupying habitat that was more heavily impacted by the oil spill (i.e., closer to the spill origin) would have altered space use, measured as larger home ranges, than turtles in habitat either farther from the spill site, or in areas unaffected by the oil spill. We also compared home range sizes between sexes and determined the extent to which size, sex, or number of radio telemetry locations predict home range size.

3.2 Methods

3.2.1 Background on the Kalamazoo River Oil Spill

On 25-26 July 2010, at least 843,444 gallons of crude oil (diluted bitumen, or dilbit) spilled from a ruptured pipeline operated by Enbridge Inc. (Enbridge) near Marshall, MI (NTSB 2012). Enbridge reported an estimate of the spilled volume at 843,444 gallons, but the U.S. Environmental Protection Agency later estimated that response operations recovered 1,181,559 gallons (EPA 2016), and the volume spilled would have necessarily been greater than the amount recovered. Dilbit flowed into Talmadge Creek and from there into the Kalamazoo River, where it impacted nearly 56 km of river channel and 40 km of riverbanks on both sides of the river (EPA 2016; Figure 3-1). At the time of the oil spill the river was at flood stage, which resulted in direct oiling of aquatic vegetation, floodplain wetlands, terrestrial vegetation, and woody debris (EPA 2016). In addition, the dilbit initially floated on the water surface, but over time sank and mixed within the water column before settling on the substrate (NTSB 2012).

Oil cleanup and habitat restoration efforts began on 28 July 2010 and continued intensively until June 2012, with additional work at targeted sites through 2014 (EPA 2016). This work included sediment disturbance and flushing; removal of oiled vegetation, woody debris, and sediment; altering the river flow and depth through removal of small, vegetated islands; and mechanical oil removal by heavy machinery (EPA 2016). Oiled riverbank vegetation and sediment was removed, but the resulting additional boat traffic resulted in increased bank erosion. Human disturbance occurred daily along riverbanks and within the channel and included crews performing manual labor, noise from machinery and vehicles, and boat traffic (e.g., motor and air).

Additional targeted cleanup in 2012–2014 focused in a few slow-moving areas where oil-contaminated sediment was removed (EPA 2016).

3.2.2 Study Sites

Throughout 2019 and 2020, we studied northern map turtles at three sites. Two sites were within the Kalamazoo River (Calhoun and Kalamazoo counties, MI, USA; Figure 3-1), and both were directly impacted by the oil spill, cleanup, and restoration work. Of these two sites, the upstream site, hereafter the Heavy Oil site, was closer to the origin of the oil spill (3.1–23.9 river km from the spill origin) and was therefore more heavily oiled than the downstream site (hereafter the Light Oil site, 29.4–48.4 river km from the spill origin). Although the magnitude of oil contamination was not measured at the time of the oil spill, as the oil flowed downstream from the Heavy Oil site to the Light Oil site, the oil adhered to vegetation and riverbanks, settled to the substrate, and was slowed by spillways and backwater areas, which resulted in fewer direct impacts to the Light Oil site compared to the Heavy Oil site. Our third study site, hereafter the No Oil site, was Battle Creek, a third-order lotic tributary that drains into the Kalamazoo River between the Heavy Oil and Light Oil sites (Barry and Calhoun counties, MI, USA; Figure 3-1). Habitat at the No Oil site was similar to that of the Kalamazoo River but was not contaminated during the oil spill. All three study sites were connected via river channel and were within 5.5–9.4 river km of each other, with channels 9.0–65.0 m wide and 0.2–3.5 m deep. The No Oil site commonly floods during rain events, whereas the Kalamazoo River typically remains within the main channel banks at the Heavy Oil and Light Oil sites. Two retired hydroelectric dams (3.7 and 4.0 m tall), one 1.2 m spillway, and a 1.4 km long manmade concrete channel potentially limited turtle movement among study sites (Fongers 2008; Figure 3-1). Riparian zone habitat was dominated by upland and floodplain woodlands.

3.2.3 Study Species

The northern map turtle is the most common turtle species in the Kalamazoo River. It inhabits medium to fast flowing rivers and streams, impoundments, lakes, and backwaters (Ernst and Lovich 2009). Map turtles rarely leave the water except to nest, bask on banks and woody debris, or locate suitable habitat when wetlands dry. Nesting occurs from late May to early July, with females sometimes traveling large distances to suitable nest habitat (Lindeman 2013). In the Kalamazoo River population, individuals were found to move nearly 5.0 km upriver or downriver to nest at a communal site (Otten unpublished data). Northern map turtles exhibit pronounced sexual dimorphism, with adult females growing nearly twice the length of males. Males of this population reach sexual maturity at 4–5 years of age while females reach sexual maturity at about 13–14 years (Otten personal observation).

3.2.4 Data Collection

3.2.4.1 Turtle Capture

We captured turtles throughout each of the three study sites using dipnets from a boat (Lager 1943), or by hand while snorkeling (Marchand 1945), during 2018 and 2019. Turtles captured in 2018 were used only for nesting surveys in late May and early June, and home range studies on these individuals did not begin until 2019. We measured each individual's straight carapace length (SCL) and plastron length (PL) along the midline with calipers to the nearest mm, and mass using a digital scale to the nearest 0.1 g. Each turtle was individually marked by filing a unique combination of notches in the marginal scutes for future identification (Cagle 1939). We included only sexually mature adult turtles (at least 6.6 cm PL for males; and at least 16.9 cm PL for females; Lindeman 2013) in this study.

3.2.4.2 Radio Telemetry

We affixed radio transmitters to adult females (ATS model RI-2C2; [15 g]) from all three study sites, and to adult males from the Heavy Oil site (ATS model R1-2B; 5g) . Transmitters were affixed with marine epoxy to the posterior two costal scutes where they would not impede feeding, swimming, mating, or nesting movements. Transmitters and epoxy never exceeded 5.0% of a turtle's body mass. We held turtles overnight following transmitter attachment to allow epoxy to cure, and we released turtles the following day at their point of capture.

We radio-tracked turtles periodically beginning in January 2019 and weekly beginning in mid-March. We conducted telemetry from a single-person kayak using a R410 telemetry receiver (ATS Isanti, MN) and a Yagi 3-element collapsible antenna, with all turtles tracked at least once per week during the active season (late March to mid-October) of 2019 and 2020. Additional telemetry occurred twice during December 2019 and February 2020 to determine overwintering locations. We tracked each individual for approximately one full year of activity, either from January 2019 to January 2020, or from the date of first capture in 2019 to the same week in 2020 (e.g., June 1, 2019, to first week of June in 2020). A radio-location was recorded for an individual when it was directly observed (e.g., basking or visible in water); when an individual was not directly observed, we estimated its location by triangulation, typically from above a turtle when it was underwater. At either the observed or triangulated location, we recorded coordinates with a handheld GPS unit (Garmin International Inc.) with an accuracy of <3 m.

3.2.5 Data Analysis

3.2.5.1 Home Range

We calculated home range sizes using all locations collected during one full year of activity. This included only one overwintering location per season; that is, if a turtle was tracked to its overwintering site during January 2019 and December 2019, both locations were included in home range analysis. Three different home range estimators were calculated: linear stream home range length (SHR), and two types of kernel density estimators (KDE), 95% KDE (i.e., statistical home range), and 50% KDE (i.e., core home range). The SHR estimate is most useful in species that primarily occupy lotic habitats (Doody et al. 2002; Riedle et al. 2006; Chen and Lue 2008; Sterrett et al. 2015) and estimates home range length by calculating the shortest straight-line distance within the river channel between the furthest upstream and downstream locations recorded for an individual (Plummer et al. 1997). While SHR does not quantify patterns of habitat use, it does provide a metric of space use in small riverine ecosystems. To calculate SHR we used the Riverdist package (Tyers 2017) in R 3.6.3 (R Core Team).

The KDE method provides a non-parametric estimate of the likelihood of finding an individual at particular locations within its home range, assuming that no barriers exist to prevent the individual from moving in any direction across the landscape (Worton 1989). In situations where barriers exist or individuals are restricted to irregular wetted channels, KDE home ranges can be clipped to the channel boundary to exclude areas of dry land between stream meanderings (Blundell et al. 2001; Vokoun 2003; Ross et al.

2019). We calculated both the 95 and 50% KDE home range with the AdehabitatHR package (Calenge 2006) in R 3.6.3 (R Core Team) using the kernelUD and getverticesHR functions. The kernel smoothing parameter for both KDE calculations was the least squares cross-validation based on each individual (Horne and Garton 2006). The 95% KDE home range size for an individual was calculated from 95% of the radio-locations for that individual, assuming that the remaining 5%, as determined by the software, were outliers that represented excursions. The 50% KDE for an individual was calculated using the 50% of radio-locations for that individual that were within the closest proximity of each other. The 50% KDE is also defined as the core use area, which is used disproportionately more than other areas (Samuel et al. 1985). Both the 95 and 50% KDE home range contours were clipped to the river boundary in QGIS 3.16. Only individuals with at least 20 active season radio-locations were used for calculating each of the three home range estimators, as <20 locations can bias estimates of kernel densities (Seaman et al. 1999).

3.2.6 Statistical Analysis

We used analysis of variance (ANOVA) to test for differences among females from the three study sites in each of the three home range estimators. For all significant ANOVA results ($p<0.05$), a Tukey's HSD post-hoc test was used to identify the specific groups that differed from each other. We used three t-tests, one for each home range estimator, to compare male and female home range size at the Heavy Oil site.

To determine which factors best predicted home range sizes for each of the 3 estimators, we created 10 candidate linear models. We used study site, sex, SCL, and number of radio-locations as predictors. We examined the residual plot and histogram of the global model and found residuals to be normally distributed. The null model included only the intercept, whereas the global model included all variables. We considered $\alpha \leq 0.05$ to be statistically significant, and models were ranked and chosen using Akaike's Information Criterion adjusted for small sample sizes (ΔAICc; Burnham and Anderson 2002). A parameter was assumed to be uninformative if a model including that parameter was within two AICc of the highest-ranking model (Arnold 2010).

3.3 Results

From January 2019 to June 2020, we recorded an average of 33.52 (±5.80 SD) radio-locations for 61 adult northern map turtles (51 females, 10 males). Adult females appeared to be in lower abundance and were more difficult to capture at both the Light Oil and No Oil sites, so our analyses included 40 turtles (30 females and 10 males) from the Heavy Oil site, 10 females from the Light Oil site, and 11 females from the No Oil site (Table 3.1). Individuals were radio-tracked over a mean period of 369.8 ± 18.9 days. We recorded a mean of 36.3 ± 4.6 radio-locations for females and 35.4 ± 4.8 radio-locations for males (Table 3.1). The home ranges for a representative female at the Heavy Oil site calculated from the three different home range estimators are shown in Figure 3-2.

The mean SHR length for all turtles was 5.0 ± 4.9 km (range 1.1–27.7 km for females and 0.5–6.4 km for males). Female SHR length differed among sites ($F_{1,2} = 5.13$, $p<0.01$), with mean SHR length of females from the No Oil site of 9.2 ± 8.2 km and 3.9 ± 2.9 km of females from the Heavy Oil site (Tukey HSD $p=0.01$). There was no difference in SHR length between the sexes at the Heavy Oil site (2.4 ± 2.0 km for males and 3.9 ± 2.9 km for females; $t_{38} = 1.53$, $p=0.14$).

The 95% KDE home range sizes varied substantially among individuals, with a mean for all radio-tracked turtles of 6.2 ± 6.0 ha (range 1.1–27.7 ha for females and 0.5–6.4 ha for males). However, there was no difference in 95% KDE among sites ($F_{1,2}$ = 1.24, p=0.30) or between the sexes (4.1 ± 5.5 ha for males; 5.6 ± 4.9 ha for females; t_{38} = 2.102, p=0.45; Table 3.1). Of the three home range estimators, the 50% KDE home ranges varied the least among individuals and sites (Table 3.1). The mean 50% KDE for all radio-tracked turtles was 0.5 ± 0.5 ha (range 0.002–2.8 ha for females and 0.002–1.0 ha for males). Female 50% KDE home range did not differ among sites ($F_{1,2}$ = 0.81, p=0.45), and there was no difference between sexes at the Heavy Oil site (t_{38} = 1.43, p=0.16).

For SHR length, the model including body size and number of radio-locations as predictors had the lowest AICc. Estimated home range size was negatively correlated with number of radio-locations, and positively correlated with individual body size. For 95% KDE, four models were within 2 AICc of the top model (Null, SCL, Sex, and SCL + Locations), with the model containing only individual size having the strongest effect on 95% KDE home range size. Again, estimated 95% KDE home range size was positively correlated with body size. For 50% KDE, three models had ΔAICc <2 (Null, SCL, and Sex), with the null model having the lowest value.

3.4 Discussion

The 2010 Kalamazoo River oil spill had substantial acute effects on both biotic and abiotic components of the riverine and adjacent terrestrial systems, including extensive oiling of riverbanks, woody debris, and aquatic vegetation, as well as high concentrations of oil in the sediment and water column (EPA 2016). All of these habitat features are used extensively by northern map turtles for foraging, basking, avoiding predators, and overwintering. During cleanup efforts, these oiled habitat features were physically removed from the river, and subsequent restoration projects stabilized banks and replaced woody debris that had been removed due to extensive oiling. Thus, in addition to direct oil contamination from the spill itself, the river was extensively disturbed during restoration efforts, and the combination of these impacts likely had profound effects on the quality of northern map turtle habitat in the Kalamazoo River. Here, we determined whether home ranges of river turtles differ among sites along a disturbance gradient nine years post-oil spill, with the assumption that among-site differences in spatial ecology could be used as indicators of habitat quality, particularly as a result of the extensive disturbance caused by the combination of the oil spill and subsequent restoration efforts.

The unpredictable nature of oil spills often means that we lack baseline, pre-spill data for sites at which oil spills have occurred. Therefore, studies on potential effects of oil spills on aquatic communities are necessarily correlational. Indeed, in our study we cannot infer direct causation between the 2010 oil spill and/or restoration efforts, and any subsequent differences among sites in species' basic ecology. However, we can test the hypothesis that home range sizes are larger in areas that experienced a higher degree of oiling compared to nearby, ecologically similar sites unaffected by the oil spill. Our prediction of larger home range sizes in areas more extensively disturbed by direct oiling and cleanup operations assumes that turtles in more-disturbed sites need to travel farther to acquire required resources than turtles in less-disturbed sites. Contrary to our predictions, we found that only SHR length differed between the Heavy Oil site and the No Oil site, with SHR length of females in the Heavy Oil site about half the SHR length of females from the No Oil site. There were no differences between sexes for any of the three home range estimators, and there were no differences among sites in female home range sizes for either 95 or 50% KDE.

Our results contrast those of freshwater turtles in oil-polluted areas of the Niger River delta in southern Nigeria; turtles (*Pelusios castaneus, Pelusios niger, Pelomedusa subrufa, and Trionyx triunguis*) from oil-polluted areas had larger home ranges, altered habitat use, had fewer total individuals based on capture records, and lower species diversity of aquatic turtles compared to adjacent unpolluted habitats (Luiselli and Akani 2003; Luiselli et al. 2005; Luiselli et al. 2006). However, in a separate study, oiled freshwater turtles (*Chelydra serpentina, Chrysemys picta, Psuedemys rubiventris*, and *Trachemys scripta*) that were rehabilitated and released behaved similarly, and had similar home range sizes, to turtles from the same location that were unexposed to oil (Saba and Spotila 2003).

The results from our study suggest that habitat quality as it relates to northern map turtles either has not been permanently degraded, or post-spill cleanup and restoration efforts successfully mitigated negative impacts of the spill nine years later. We found that females from the Heavy Oil site had smaller home ranges than those from the No Oil site; indeed, 82% (23 of 28) of females with the shortest SHR length were from the Heavy Oil site. The relatively small home range sizes of female northern map turtles at the Heavy Oil site suggest that map turtle habitat quality there may currently be higher than at the No Oil site. Notably, during wildlife rehabilitation in 2010 and 2011 following the oil spill, >2,000 northern map turtles were captured directly from oiled sections of the Kalamazoo River (EPA 2016), suggesting that map turtles did not alter habitat use or avoid oiled habitat immediately after an oil spill, unlike some bird and mammal species that leave oil-impacted areas (Day et al. 1997; Bowyer et al. 2003).

The length of SHR in northern map turtles may be driven more by the location of nesting habitat used by females than by the quality of aquatic habitat, which is supported by our observations that some females from the Heavy Oil site traveled nearly 5.0 km to suitable nesting habitat. The No Oil site flooded more severely and frequently than the Heavy Oil site, which could force females at the No Oil site to travel further to nest, thereby resulting in a longer SHR than that of females from other sites located closer to suitable nesting habitat. SHR length has previously been reported for other populations of northern map turtles (Bennett et al. 2010; Richards-Dimitrie 2010; Ouellette and Cardille 2011), and our mean SHR length of female turtles within oiled areas of the Kalamazoo River (n = 40; 4.6 ± 3.6 km) falls within the range of SHR lengths for other populations. Although pre-spill home range data do not exist for Kalamazoo River northern map turtles, SHR lengths from other studies, as well as our control, No Oil site are generally similar to the SHR lengths from the Heavy Oil and Light Oil sites, suggesting that SHR lengths of turtles from our oil-impacted study sites are likely similar today to what they would have been at the same sites pre-spill.

Our models indicate that turtle size and number of radio-locations were the most important predictors of SHR length in northern map turtles. In both males and females, SHR length increased with turtle size, which may be due to larger turtles maneuvering more efficiently against strong river currents. The model indicated that as the number of radio-locations increased, the SHR length actually decreased, which may reflect that individuals that remained near their original capture location were easier to relocate, whereas turtles that moved greater distances were more difficult to relocate, thereby resulting in fewer overall radio-locations, but longer SHR lengths.

If oil contamination from the Kalamazoo River oil spill has had chronic effects on the northern map turtle population, these effects do not appear to have negatively impacted spatial ecology, as measured by individuals' home range sizes, nearly 10 years after the combined oil spill, cleanup, and restoration work. Additional studies should investigate potential chronic effects of the oil spill on other aspects of the northern map turtle population such as demography, individual health, survival, and recruitment. Our study has important implications for future freshwater cleanup and habitat restoration efforts following a major disturbance such as an oil spill. While the majority of studies on oil spill effects focus on short term results, our research suggests that extensive cleanup of a riverine environment combined with restoring habitat to its pre-spill state may minimize potential long-term impacts on habitat quality for vertebrate species, at least in terms of individuals' spatial ecology. Such restoration efforts may be especially critical for turtles, which are long-lived, slow-growing, late-maturing species with high degrees of site fidelity.

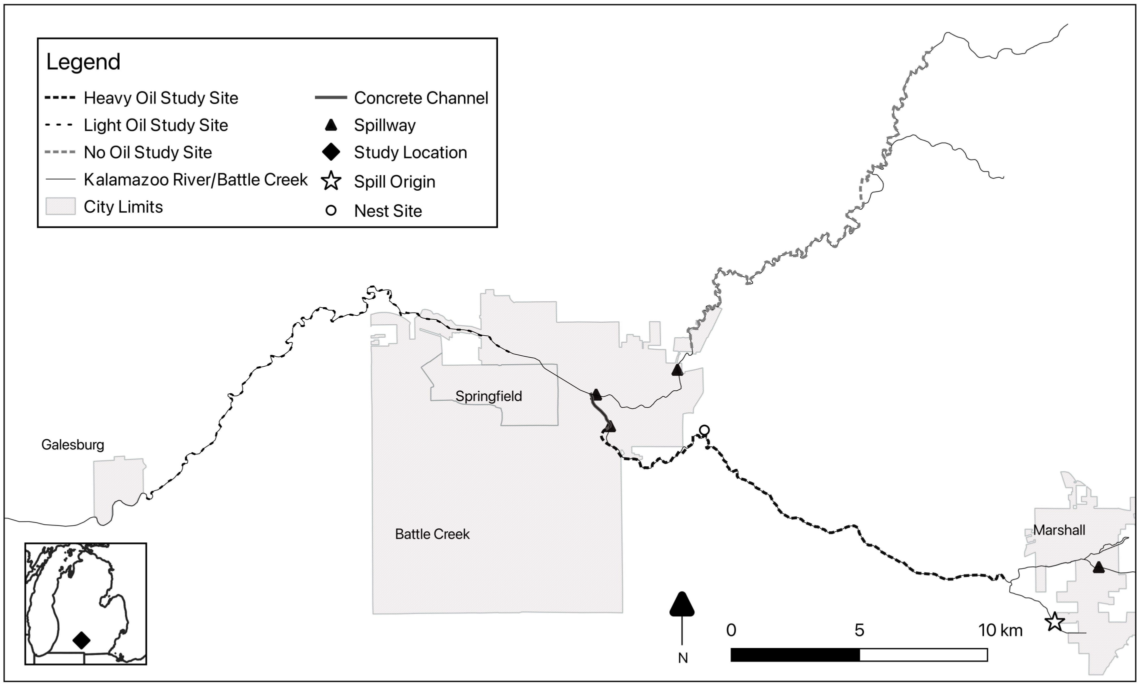

Figure 3-1. The three study sites used for home range studies of northern map turtles (*Graptemys geographica*) in Calhoun and Kalamazoo Counties, MI, USA. The Heavy Oil and Light Oil sites were stretches of the Kalamazoo River, while the No Oil control site was within Battle Creek, which drains into the Kalamazoo River and was not contaminated during the 2010 oil spill. The concrete spillways ranged from 1.2–4.0 meters high.

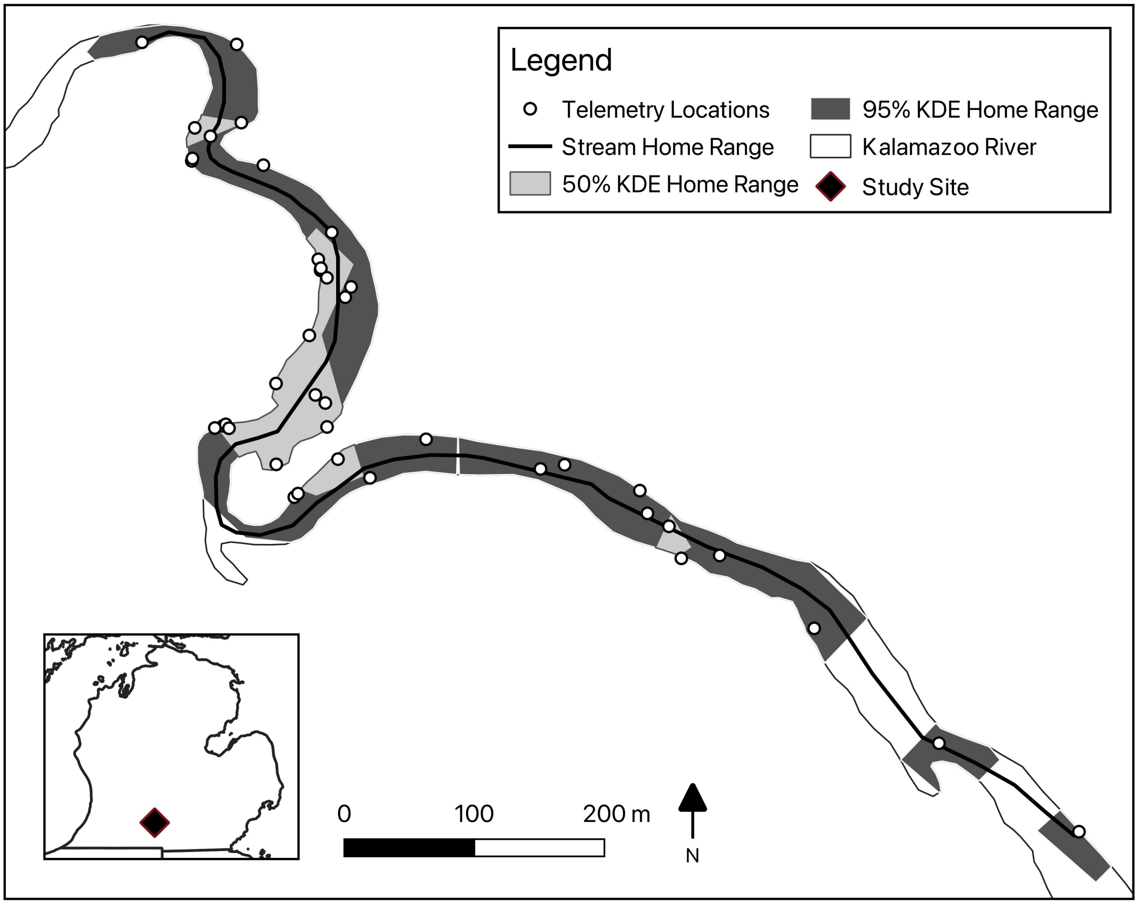

Figure 3-2. Representative stream home range (SHR), 95% kernel density estimator (KDE), and 50% KDE home range of an adult female northern map turtle (*Graptemys geographica*) from the Heavy Oil site in the Kalamazoo River in Calhoun County, MI, USA. All telemetry locations occurred from 2019 and 2020, representing one full active season.

Table 3.1. Summary of radio-tracked northern map turtles (*Graptemys geographica*) from the three study sites in the Kalamazoo River and Battle Creek, MI in 2019-2020. Mean number of locations, stream home range length (SHR), and kernel density estimation (KDE) home ranges (with standard deviations) are based on locations recorded for one active season for each individual between January 2019 and June 2020. Home range estimators that were significantly different from one another ($p<0.05$) are shown in bold.

Site	n	Mean # locations	SHR length (km)	50% KDE (ha)	95% KDE (ha)
Heavy oil (Female)	30	36.3 ± 4.6	**3.90 ± 2.85**	0.60 ± 0.69	5.55 ± 4.94
Heavy oil (Male)	10	35.4 ± 4.8	2.41 ± 1.97	0.28 ± 0.27	4.12 ± 5.50
Light oil	10	30.1 ± 6.1	6.59 ± 4.79	0.55 ± 0.32	8.86 ± 7.18
No oil	11	27.5 ± 2.7	**9.17 ± 8.16**	0.35 ± 0.29	7.33 ± 7.54
Total	61	33.5 ± 5.8	5.04 ± 4.94	0.50 ± 0.54	6.18 ± 5.99

Chapter 4

Homeward Bound: Long-distance Homing in Translocated Turtles

4.1 Introduction

Wildlife translocation is becoming an increasingly common conservation and mitigation practice to reduce the impacts of anthropogenic activities across taxa. Conservation-driven translocations often aim to augment, re-establish, or re-introduce a population to areas from which they have been extirpated or are in decline, while mitigation-driven translocations try to reduce wildlife mortality directly caused by human activities (e.g., development, pollution) by relocating individuals or populations away from an area that is or will become uninhabitable (Craven 1998; Germano et al. 2015). There have been notable translocation success stories, such as successful re-establishment of a black bear (*Ursus americanus*) population in Arkansas (Smith and Clark 1994), but there have also been translocations that failed to achieve their goals. For example, thousands of kangaroo rats were translocated in various parts of California, but no individuals appear to have survived 1-year post-release (Shier and Swaisgood 2012). Failure of translocations most often result from improper planning and management, unsuitable habitat at the release site, or disease transfer (e.g., case studies; Soorae 2018;

Soorae 2021). Therefore, substantial planning, pilot studies, and use of best practices are critical to maximize the likelihood of a translocation effort's success.

Mitigation translocations are often regarded by the public as a humane and effective solution to human-wildlife conflict, leading to them becoming even more commonplace than conservation-driven translocations (Massei et al. 2010; Bradley et al. 2020). If mitigation translocation strategies are to be successful conservation tools, it is critical that we find species-specific methods to maximize benefits relative to cost. In particular, the goals of a specific translocation effort should be established *a priori* to inform the post-release monitoring strategy in determining if those goals were met. Inadequate post-release monitoring or metrics of success can erroneously lead to labeling an effort as "successful" when in fact it was unsuccessful, potentially leading to replicated failures (Wolf et al. 1998; Fischer and Lindenmayer 2000). Mitigation translocations often have poorly documented outcomes due to lack of monitoring or publicly accessible results (Taylor et al. 2017; Silcock et al. 2019; Nash et al. 2020). Mitigation translocations with documented outcomes often have high failure rates (Sullivan et al. 2015), especially in reptile and amphibian species where translocations of all types have resulted in successful outcomes only 41% of the time (Germano and Bishop 2009). Typically, data for conservation translocations involving reptile species are available in primary literature, while the data for mitigation-based reptile translocations are often inaccessible, non-existent, or lack measurable objectives (Armstrong and Seddon 2008; Germano et al. 2015; Taylor et al. 2017). Moreover, mitigation translocation projects typically include insufficient monitoring to ascertain their long-term success, especially in long-lived species such as many reptiles (Sullivan et al. 2015).

Insufficient monitoring coupled with reluctance to report failed translocation efforts has likely led to a high frequency of failure in mitigation translocation projects for reptiles (Germano et al. 2015).

Translocated reptiles appear to suffer high mortality rates relative to resident individuals due to increased stress, susceptibility to disease, and the fact that many reptiles exhibit strong site fidelity and homing ability (Cornelis et al. 2021). Site fidelity and homing ability can lead to aberrant movement patterns in translocated reptiles, which can increase negative human-wildlife interactions and decrease survival if individuals are unable to find critical resources, such as hibernacula, in their new environment (Brown et al. 2008; Harvey et al. 2014; Sullivan et al. 2015). Species with strong homing ability and a high degree of site fidelity may also be poor candidates for translocation because individuals may attempt to return to their original home area, which may have become uninhabitable (Dodd and Seigel 1991; Germano and Bishop 2009; Sosa and Perry 2013). In turtles, translocated individuals of species that exhibit strong site fidelity have been found to "wander" more, have larger home ranges, and have increased mortality compared to resident turtles, presumably as a result of translocated individuals trying to return to their original home range (Cook 2004; Rittenhouse et al. 2007; Hinderle et al. 2015). The high failure rate of many translocation efforts, particularly those involving reptiles, has led to the suggestion that regulation of translocation efforts should be changed to match conservation outcomes (Germano et al. 2015). However, regulation of mitigation translocations can be difficult, as such translocations may be conducted as emergency responses to large-scale disturbances such as chemical spills. In such situations translocations are generally a last resort because the risks associated with moving individuals are less than the risk of losing the entire population if no action is taken. Although emergency translocations admittedly have very limited time available for decisions on experimental design, all such efforts should include post-release monitoring, which can serve as a learning opportunity to improve success and regulation of future translocation efforts in similar situations.

One such learning opportunity arose from the 2010 Kalamazoo River oil spill (Michigan, USA), during which emergency translocation efforts were undertaken for nearly 700 oiled northern map turtles (*Graptemys geographica*). On 25-26 July 2010, 3.2 million L (834,444 gallons) of diluted bitumen (dilbit) crude oil were reportedly released after a pipeline rupture (NTSB 2012). The U.S. Environmental Protection Agency (EPA) later estimated that 4.5 million L (1,181,599 gallons) were recovered, which made the Kalamazoo River spill one of the largest inland oil spills in U.S. history (EPA 2016). Emergency cleanup and habitat restoration efforts began on 28 July 2010 and continued until June 2012, with additional targeted work continuing through 2014 (EPA 2016). As part of cleanup activities, approximately 5,000 freshwater turtles, predominately northern map turtles, were captured, rehabilitated, and released (EPA 2016). Release of rehabilitated turtles was complicated by the conflicting goals of releasing animals back to their capture location as soon as they were cleared by veterinarians, while also endeavoring to protect them from additional oiling and ongoing disturbance from cleanup operations at their original capture locations. To avoid releasing rehabilitated turtles back into habitat where they may become re-oiled, but also to allow these individuals to potentially return “home,” the U.S. Fish and Wildlife Service (USFWS) and Michigan Department of Natural Resources (MDNR) translocated rehabilitated turtles to other areas within the Kalamazoo River watershed while remaining oil precluded release of turtles at their original capture locations.

The emergency mitigation translocation of nearly 700 northern map turtles following the 2010 Kalamazoo River oil spill provided a unique opportunity to assess 1) the success of a mitigation translocation of freshwater turtles following a large-scale oil spill, using northern map turtles as a model species, and 2) the homing ability of northern map turtles when translocated varying distances from their original home ranges. It is important to note that, in the case of the Kalamazoo River oil spill, turtles only needed to be temporarily removed from their home area while oil was removed from the river, at which point it was again habitable for northern map turtles. The objectives of the present study were to use recapture records up to 10 years post-spill to assess the success of translocation to mitigate the effects of an oil spill on northern map turtles, and to quantify homing in northern map turtles that had been moved known distances from uninhabitable home areas. Our study provides novel insight into the effectiveness of translocation for mitigating the effects of an environmental disaster on a riverine turtle species.

4.2 Methods

4.2.1 Study Site

Our Study Site was ~50 km of Kalamazoo River channel impacted by the 2010 oil spill, from the confluence of Talmadge Creek to Morrow Lake (Calhoun and Kalamazoo counties, Michigan, USA; Figure 4-1). Two retired hydroelectric dams (spillways; 3.7 and 4.6 m-tall) and a 1.4 km-long concrete channel within the Study Site could potentially limit movement of turtles (Fongers 2008; Figure 4-1). An additional 4.3 m-tall active hydroelectric dam and five smaller spillways are between the Study Site and translocation sites (two within tributaries, three upstream, and one downstream from translocation sites; Figure 4-1).

4.2.2 Study Species

Riverine turtles are vulnerable to floating dilbit when they surface to breathe and as they leave the water to bask. In river habitat, a portion of the spilled dilbit mixture can sink over time (Dew et al. 2015), so turtles can also be exposed to oil when submerged. Turtle rehabilitation efforts following the 2010 oil spill included all species found in the river, although northern map turtles were the most abundant in the Kalamazoo River and are the focus of this study (EPA 2016). Northern map turtles exhibit 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; Ernst and Lovich 2009). Males of this population reach sexual maturity at 4–5 years, while females become mature at 12–14 years (Otten personal observation).

4.2.3 Emergency Turtle Rescue and Translocation (2010–2011, 2013)

Immediately following the oil spill in July 2010, and extending into 2011, capture and translocation of northern map turtles in the Kalamazoo River was conducted by volunteers and paid contractors, including J.O., and was overseen by the USFWS (EPA 2016). Additional targeted surveys were conducted in 2013. Surveys focused on capturing oiled turtles for rehabilitation and individually identifying translocated turtles that had returned to the Study Site. During surveys in 2010–2011 and 2013, field crews captured turtles throughout the 50-km Study Site using dipnets from a boat (as in Lager 1943), hoop traps, and basking traps. Level of survey effort varied by day and year, with one to five boats surveying the Study Site each day. One survey day constituted a day in which at least one boat actively captured turtles within the Study Site. A total of 69 survey days occurred in 2010, 97 in 2011, and 60 in 2013. Field crews recorded capture locations of all turtles with a handheld GPS unit (Garmin International Inc.) with an accuracy of <3 m. They measured each individual's straight carapace length (SCL) along the midline to the nearest mm, and mass to the nearest 0.1 g. When possible, sex was determined using secondary sex characteristics (Ernst and Lovich 2009; Lindeman 2013). In 2010, field crews individually marked turtles >100 g with passive implanted transponder (PIT) tags (Avid Identification Systems, Inc.). Beginning on 22 September 2010 and continuing through 2013, instead of PIT tags, each newly captured individual was marked with a unique combination of notches filed along the marginal scutes (as in Cagle 1939).

Turtles captured and released between 29 July - 6 October 2010 were temporarily housed in a rehabilitation facility for 2–21 days for cleaning, rehabilitation, and health monitoring. Rehabilitated turtles were released following a final veterinarian health assessment and confirmation they appeared free of oil. After 6 October 2010, newly captured individuals, turtles requiring additional cleaning, and turtles requiring continued health monitoring were housed over the winter in the rehabilitation facility to be released in spring 2011.

Because the translocation effort described here was an emergency mitigation translocation event in response to an environmental disaster, the immediate goal was to return healthy turtles to the wild as quickly as possible following rescue, while also releasing them in locations that would minimize the potential for additional oiling and negative impacts from river channel cleanup operations. The secondary goal of this emergency mitigation effort was to translocate turtles to suitable habitat that was also connected via lotic habitat to the area of original capture. If northern map turtles exhibit homing ability similar to several terrestrial turtle species (Rittenhouse et al. 2007; Sosa and Perry 2013; Hinderle et al. 2015), releasing them at sites that were connected by river channel to their original home ranges should have allowed individuals to eventually return to their home ranges on their own. From 31 July - 6 October 2010, 601 northern map turtles (250 females and 351 males) were marked with PIT tags and translocated 2.5–84.3 km from their original capture location. Translocation sites were chosen by local agencies based on habitat suitability, the presence of local northern map turtles, distance from original capture site, and absence of oil and cleanup activities. Turtles were translocated between 31 July - 22 September to 21 locations divided into 3 groups: tributaries of the Kalamazoo River [hereafter, tributary], Kalamazoo River channel downstream of the Study Site [downstream], and Kalamazoo River channel upstream from the Study Site [upstream]). All translocation release sites were within the Kalamazoo River watershed and were inter-connected via lotic habitat (Figure 4-1). On 22 September 2010, the Study Site was cleared by the EPA for release of rehabilitated turtles, so all subsequent releases of rehabilitated turtles occurred in the Study Site as near to the turtles' original capture locations as possible (EPA 2016).

From April-June 2011, overwintered turtles were released at or near their original capture location. However, during this time, an additional 85 northern map turtles (42 females and 43 males) were translocated to sites within the Study Site due to continued cleanup work occurring at or near their original capture location. Translocation distances for turtles translocated in 2011 ranged from 2.5–25.7 km, with all such translocations occurring within the Study Site.

4.2.4 Post-spill Monitoring and Recapture Surveys (2018–2020)

In 2018–2020, we conducted surveys at the Study Site to recapture northern map turtles that had originally been marked following the 2010 oil spill. The objectives of these surveys were to determine how many translocated turtles had returned to the Study Site as a measure of the overall translocation effort's success, and to quantify homing ability of translocated turtles. Data collection in 2018–2020 followed the same methods as those used in 2010–2011. That is, we captured turtles throughout 47.0 km of the Study Site, from the confluence of Talmadge Creek to East Michigan Avenue, using dipnets from a boat or kayak (as in Lager 1943), hoop traps, basking traps, and by hand while snorkeling (as in Marchand 1945). Level of survey effort varied by day and year, with one to three boats surveying the Study Site on each survey day. A total of 62 survey days occurred in 2018, 117 in 2019, and 57 in 2020. We recorded capture location of each individual with a handheld GPS unit (Garmin International Inc.) with an accuracy of <3 m. We recorded the same morphological measurements as in 2010–2011, and we used the same sex characteristics to determine sex. We identified any previously marked individual by PIT tag or unique shell notches and recorded these individuals as recaptures.

4.2.5 Data Analysis

We used R 3.6.3. (R Core Team 2020) to conduct all statistical analyses. For all analyses, we used only individuals that were presumed to have been translocated to an unfamiliar location outside of their original home range. To determine whether an individual had been translocated outside its original home range, we used previously estimated mean stream home range lengths for this population of 2.4 km for males, and 4.6 km for females, based on radio-telemetry locations throughout an entire year (Chapter 3). In the present study, we considered *translocation distance* to be the distance between an individual's original capture location and its translocation release location (Figure 4-2). We calculated translocation distance with the Riverdist package (Tyers 2017) in R 3.6.3 (R Core Team 2020) by determining the shortest distance between points while staying entirely within the river channel. Therefore, any male turtle with a translocation distance >2.4 km and any female with a translocation distance >4.6 km was assumed to have been translocated to an unfamiliar area and was included in subsequent analyses.

4.2.5.1 Translocation Success

To evaluate the success of the mitigation translocation conducted as an emergency response to the 2010 Kalamazoo River oil spill, we determined the number of individuals translocated in 2010 or 2011 that were subsequently recaptured in the Study Site during each survey year. We pooled all recaptures regardless of year and used a chi-square proportion test to compare recapture rates between males and females. We modeled recapture probability using a generalized linear model with a binomial distribution and a logit link function, with translocation distance, sex, and translocation site (i.e., tributary, upstream, and downstream), and all two-way interactions as predictor variables (Neter et al. 1996). Recapture probability models were ranked, and the best-supported model was chosen using Akaike's Information Criterion adjusted for small sample sizes (AICc; Burnham and Anderson 2002). If ΔAICc <2, we assumed there was no difference between alternative models.

4.2.5.2 Homing

We calculated homing distance for each translocated turtle that was subsequently recaptured. We defined *homing distance* as the distance between an individual's original capture location and its subsequent recapture location; for individuals with multiple recaptures, we retained only the single, minimum distance for analysis. We used homing distance to determine whether an individual was recaptured within its potential home range (Figure 4-2): that is, if an individual's homing distance was less than the mean stream home range length for that sex, we categorized the individual as having homed.

We used a chi-square proportion test to compare homing rates between males and females, and to compare homing rates among the three translocation sites (i.e., tributary, upstream, and downstream). We modeled homing (i.e., whether or not a recaptured individual returned to its original home range following translocation) using a generalized linear model with a binomial distribution and a logit link function, modelled with translocation distance, sex, and translocation site, and all two-way interactions, as predictor variables (Neter et al. 1996). Homing models were ranked, and the best-supported model was chosen as described above.

4.2.5.3 Travel Distance

For each translocated individual that was subsequently recaptured, we calculated *travel distance,* which was defined as the distance between its translocation site and its subsequent recapture location (Figure 4-2). For individuals with multiple recaptures, we calculated travel distance for each recapture event and retained only the single, maximum distance for each individual in analysis, which was considered the maximum known distance the individual had traveled. We used a t-test to compare male and female travel distances. Finally, we used analysis of variance (ANOVA) to compare travel distances among individuals released at three translocation sites (i.e., tributary, upstream, and downstream). When ANOVA results were significant, we used Tukey's HSD test for pairwise comparisons between sites.

4.3 Results

4.3.1 Translocation Success

Overall, 686 northern map turtles were translocated to unfamiliar areas following the 2010 Kalamazoo River oil spill (601 in 2010 and 85 in spring 2011). We recaptured 230 (33.5%) of the 686 northern map turtles during subsequent surveys (Tables 4.1 and 4.2). Similar proportions of translocated males were recaptured (143 of 394; 36.3%) compared to females (87 of 292; 29.8%; $\chi^2=3.18$, df=1, p=0.07). Most recaptures of translocated turtles occurred in 2011 (159 of 230; 69.1%; Table 4.1). A total of 82 individuals were recaptured multiple times: 63 in two different years of this study, 13 in three years, 5 in four years, and 1 male in all five years.

The strongest predictors of an individual being recaptured were translocation site and translocation distance x sex interaction. Two additional models were also within 2 ΔAICc: the model including sex and translocation distance x site interaction, and the model including translocation distance and site (Table 4.3). The probability of recapture decreased with increasing translocation distance ($b=-0.05$, $SE=0.01$, $z=-5.76$, $p<0.01$; Figure 4-3), and recapture probability was highest from turtles translocated downstream of their original capture location (Figure 4-3).

4.3.2 Homing

Homing was confirmed for 104 (45.2%; 48 females and 56 males) of the 230 northern map turtles recaptured in this study (Tables 4.1 and 4.2). That is, these 104 individuals had been translocated outside their original home ranges following the oil spill but were subsequently recaptured within 2.4 km (for males) or 4.6 km (for females) of their original capture location. Overall, 15.2% of all translocated turtles were confirmed via recapture records to have homed, with 66% of these confirmations made in 2011 (i.e., within one year of the start of the spill response, and during ongoing habitat restoration efforts). We found that a higher proportion of recaptured females homed (55.2% of recaptured females and 16.4% of all translocated females) compared to males (39.2% of recaptured and 14.2% of all translocated males; χ^2=5.60, df=1, p<0.02; Table 4.2). Additionally, more recaptured turtles translocated upstream homed (69.8%) compared to turtles translocated to tributaries (56.5%) or downstream locations (37.2%; χ^2=15.91, df=2, p<0.01; Table 4.2).

The best-supported model predicting homing by translocated individuals included translocation distance x sex interaction. Two additional models were also within 2 ΔAICc: the model including translocation site and a translocation distance x sex interaction, and the model including translocation distance and sex (Table 4.3). The top three models predicted that probability of homing decreased as translocation distance increased (b=-0.04, SE=0.02, p=0.02 [top model]; Figures 4-4 and 4-5), while the top two models also predicted the probability of homing from greater translocation distances to be higher for females than for males (b=-0.04, SE=0.02, p=0.05 [top model]; Figures 4-4 and 4-5).

4.3.3 Travel Distance

Females traveled significantly farther (n=87, 16.2 ± 15.8 km) than males (n=143, 11.6 ± 12.2 km) after being translocated (t_{148}=2.31, p=0.02; Figure 4-6). In particular, two subadult females (8 and 10 years of age) traveled the farthest of any turtle in this study (65.9 and 72.4 km upriver, respectively), while the longest recorded travel distance by a male was 55.7 km upriver (Figure 4-6). We found differences among translocation sites in travel distance following translocation (f_2=6.49, p<0.01), wherein turtles translocated to tributaries moved significantly farther (n=23, 21.3 ± 8.4 km) than those translocated downstream (n=164, 13.5 ± 14.7 km) or upstream (n=23, 8.8 ± 10.4 km). In addition, we found that manmade obstacles posed little to no barrier to travel, as we observed that both sexes passed around or across spillways when traveling both upriver and downriver, as well as through the 1.4 km long concrete channel. Nearly equal numbers of both sexes traveled upriver (22 females and 20 males) and downriver (11 females and 10 males) around at least one spillway following translocation.

4.4 Discussion

Predicting the success of a translocation project is challenging, as site-specific characteristics and species-specific behaviors may interact in complex ways to influence the overall outcome. In environmental disasters such as the 2010 Kalamazoo River oil spill, crisis-driven decisions such as whether and how to conduct translocations of impacted species may be poorly informed if there are few published reports detailing what was and was not successful in the past. In particular, determining a species' ability to home and the factors that influence homing can increase the effectiveness of translocation projects. Here, we demonstrated that 33% of northern map turtles translocated following the 2010 Kalamazoo River oil spill survived to be recaptured in subsequent surveys up to ten years later. Moreover, 45% of these recaptured individuals homed back to their original capture site. While both sexes exhibited homing when translocated short distances from their capture location, homing probability decreased with increased translocation distances, although females were more likely to home from greater distances than were males. Overall, our results suggest that translocation projects for riverine turtles can result in high survival rates, as measured by our recaptures from 1-10 years post-translocation. An important consideration for future translocation efforts, however, is that the considerable distances over which northern map turtles traveled in this study, as well as their ability to return to their original home ranges, means that translocated individuals of both sexes are likely to attempt to return to the area from which they were moved. Homing may be beneficial in situations where habitat has been temporarily rendered unsuitable, but it could be detrimental to a translocated population if the original home area is no longer habitable, or impermeable travel barriers exist to individuals attempting to return home.

In turtles, homing has been documented in the context of natal philopatry (Valenzuela 2001; Bowen et al. 2004; Freedberg et al. 2005), nest site fidelity (Freedberg et al. 2005; Tucker and Lamer 2008; Moore et al. 2020), hibernaculum fidelity (Graham et al. 2000; Sweeten 2008), and experimental translocation (Attum et al. 2013; Otten and VanDeWalle 2014; Attum and Cutshall 2015; Roth and Krochmal 2015). Evidence from these studies generally supports substantial capacity for homing under natural conditions or when individuals are translocated short distances (i.e., <5 km). Our study expands the spatial scale at which homing in turtles has been assessed and demonstrates that turtles can home over substantially longer distances than previously reported (i.e., >25 km), and moreover can navigate manmade obstacles such as spillways. We confirmed that 15.2% of all translocated turtles subsequently returned home over a wide range of translocation distances, which is comparable to homing rates recorded in other turtle translocation studies. In particular, 11.8% of Alabama map turtle (*Graptemys pulchra*) translocated 24 km returned after 1-3 years (Shealy 1976), and 19.1% of desert tortoises translocated up to 5 km returned within 180 days (Hinderle et al. 2015).

In species with strong site fidelity, individuals attempting to return to their home areas after being translocated over longer distances would likely incur higher energetic costs and greater exposure to human threats both of which likely increase mortality rates, compared to individuals translocated over shorter distances (Dickens et al. 2010; Sullivan et al. 2015; Finn and Stephens 2017). Potential links to familiar feeding grounds, hibernacula, or mating opportunities may drive both sexes to travel long distances to return to their original home range. However, in our study, these resources were presumably readily available at all translocation release sites, as the observation of other northern map turtles was a pre-requisite for an area to be approved as a translocation site. Therefore, a lack of resources at the translocation sites was unlikely to drive homing in the translocated turtles. Instead, many translocated turtles that we later recaptured were likely attempting to return to familiar home ranges. The distances traveled by many translocated turtles, and the physical obstacles they overcame, were likely energetically expensive and may have increased turtles' exposure to anthropogenic threats.

Our results show that both sexes exhibit strong site fidelity after being translocated. In particular, three females and four males were recaptured <20 m from their original capture location after having been translocated >20 km following the oil spill. However, we observed differences in homing between the sexes based on translocation distance; namely, females were more likely to home from greater translocation distances than were males. Our results are consistent with other turtle translocation studies in that homing differs between sexes (Smar and Chambers 2005; Field et al. 2007; Nussear et al. 2012). Taken together, studies on homing in turtles suggest that translocation projects should consider differences in homing between sexes, particularly in species with pronounced sexual dimorphism such as *Graptemys* species. In addition, we found that female northern map turtles traveled significantly farther than males following translocation, including the longest recorded movement of any freshwater turtle species in the U.S., wherein a subadult female (15.6 cm SCL) traveled 72.4 km upriver and navigated a 4.3 m-tall active hydroelectric dam following translocation. A second subadult female (14.0 cm SCL) traveled 65.9 km upriver and around two spillways after being translocated. That both these long-distance homing movements occurred in subadults suggests that home range and ultimately homing ability to home develops in turtles before they reach sexual maturity. Similarly, the smallest male that successfully homed in this study was approximately one year old (5.9 cm SCL).

Although we observed no significant difference in the proportion of homing females and males that homed, any between-sex differences in homing and travel distances could result from females having strong fidelity to nesting sites. Females often travel long distances to nest in the same location from one year to the next (Freedberg et al. 2005; Freedberg 2020; Nagle and Russel 2020); in particular, female sea turtles migrate hundreds to thousands of kilometers among breeding, foraging, and nesting grounds, and exhibit natal philopatry to the beaches at which they hatched (Bowen et al. 1992; Plotkin 2003). Alternatively, the greater distances over which female northern map turtles homed in our study may be due to females increased physical ability to travel long distances compared to males, as females are substantially larger and likely stronger swimmers (Plutto and Bellis 1986; Jones 1996; Bodie and Semlitsch 2000). Our results are consistent with other northern map turtle studies in which females were found to travel greater distances than males (Plutto and Bellis 1988; Carriere et al. 2009; Chapter 3).

We were unable to determine how quickly individuals returned to their original home ranges because our study design depended on incidental recaptures of marked turtles, and we did not physically track translocated individuals following their release. In other turtle species, homing occurred almost immediately in individuals translocated <2 km (Smar and Chambers 2005; Hinderle et al. 2015). Based on incidental recapture data in 2010, we documented 17 individuals homing an average of 11.1 d after translocation. However, in a separate study, we radio-tracked female map turtles in this population to nesting sites and found that they regularly travel several kilometers in a single day, indicating that individuals may have the ability to return to the Study Site or home almost immediately if translocated near their original capture location (Chapter 3). Here, most turtles that successfully homed were recaptured within a year of being translocated, and while some individuals were not recaptured until the 2018–2020 study period (i.e., 8–10 years after translocation), we presume they were present near their original home range but were not detected in earlier years of the study. We recommend that during the design phase of translocation projects, managers should carefully consider where and when translocated individuals are to be released, particularly in the context of whether the goal of the project is to allow individuals to return to original capture locations, or to retain them permanently in the area to which they will be translocated.

Finally, as a caveat, we likely underestimated overall homing rate due to undetected mortalities or individual variation in home range size and detection rate. Female stream home range size ranged from 1.1–17.5 km, while that of males ranged from 0.5–6.4 km (Chapter 3). Therefore, it is possible that individuals with relatively large home ranges may have returned to their original home range following translocation, but if we recaptured them farther from their original capture location than the population mean home range length, we would have classified them as not having homed. We have previously estimated that annual detection rates of both adult females and males in this population are ~66%, and annual mortality rates are <5% for adult females and <10% for adult males (Chapter 2). Therefore, these detection rates likely mean that some translocated turtles that returned to their original home range were undetected, and therefore that our estimate of homing is conservative. Finally, any turtles that died after translocation would still have been included in our analyses as available for recapture, despite actually having been removed from the study population. Such undetected mortalities would have led us to underestimate the frequency of homing in this population.

Overall, our results demonstrate that if the goal of a mitigation-driven translocation project is for individuals to remain at the site to which they are translocated, the success of the effort may be impeded by individuals' homing behavior and their ability to move large distances out of a translocation site after release. Future research should determine the navigational mechanisms involved in homing, and whether hard vs. soft release strategies change the likelihood of individuals attempting to home. Additionally, it is important to reiterate the importance of post-translocation monitoring regimes, conducted at a temporal scale appropriate for the species, to accurately assess the long-term success of translocation efforts. In situations where translocation is used as an emergency mitigation measure, responsible parties should demonstrate the effectiveness of translocation as a tool to achieve conservation outcomes. This process should involve transparency, clear conservation-oriented goals, follow-up monitoring and surveys, and data made publicly available. This framework would ultimately provide future emergency or mitigation-driven translocations insight into potential success or failure.

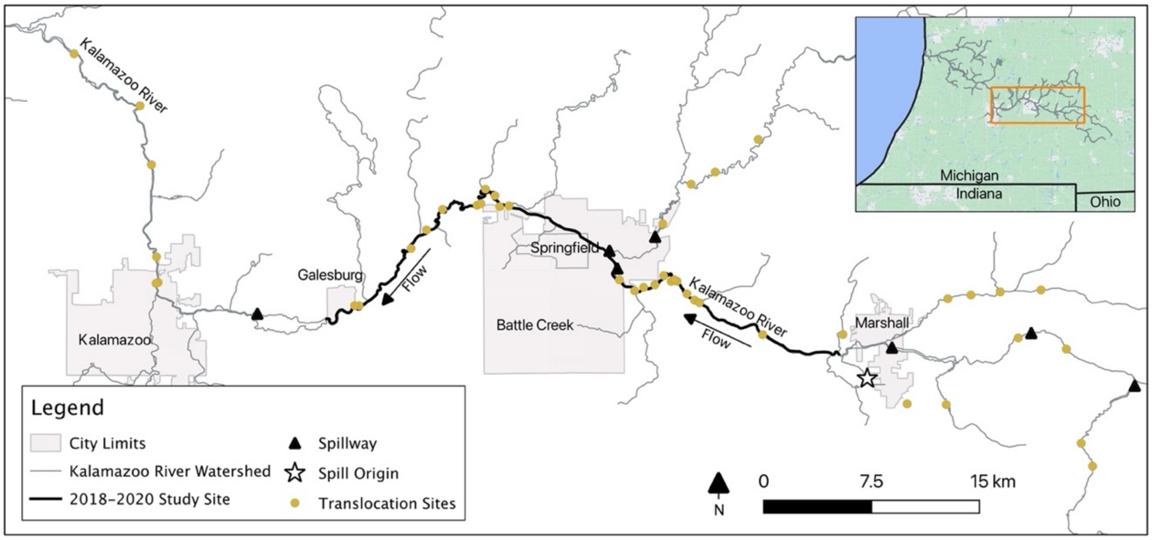

Figure 4-1. Study Site for the 2010-2011 translocations and subsequent recapture surveys for northern map turtles (*Graptemys geographica*) in the Kalamazoo River, Calhoun and Kalamazoo counties, Michigan following the Kalamazoo River oil spill on 25-26 July 2010. A total of 686 map turtles were translocated outside of presumed home ranges (based on mean stream home range lengths of each sex; Chapter 3) at various distances within tributaries, upstream, and downstream in the Kalamazoo River.

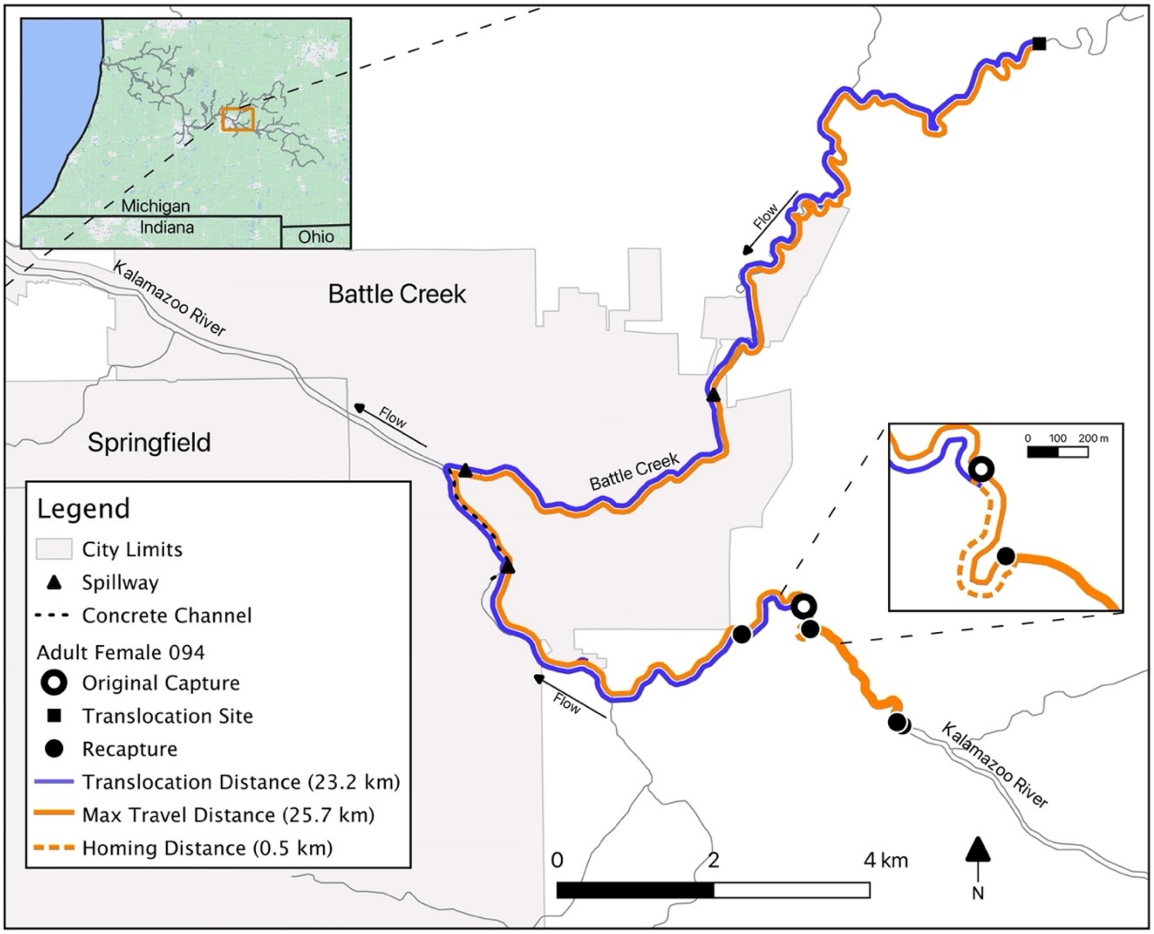

Figure 4-2. Translocation distance (i.e., distance between an individual's original capture location and its translocation site), travel distance (i.e., maximum distance between an individual's translocation site and subsequent recapture locations), and homing distance (i.e., minimum distance between an individual's original capture location and subsequent recapture locations) for a representative adult female northern map turtle (*Graptemys geographica*) translocated following the Kalamazoo River oil spill of 2010 in Calhoun County, Michigan. This individual traveled around multiple spillways and through a concrete channel, before being recaptured ~0.5 km from the original capture location.

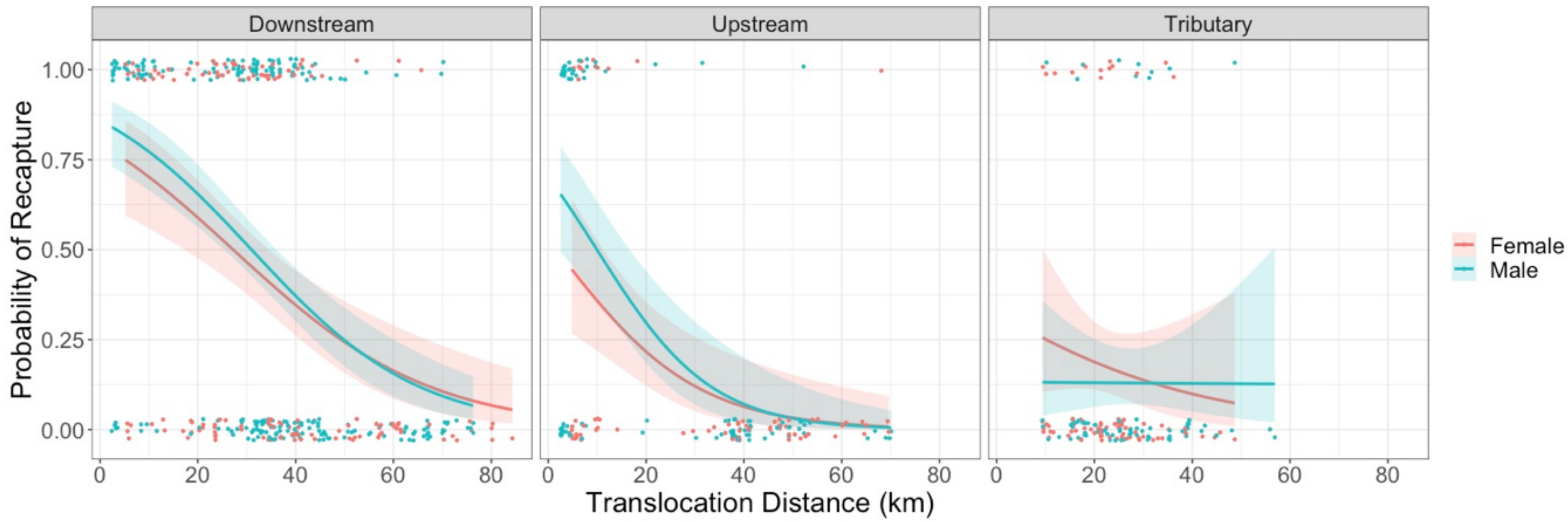

Figure 4-3. Probability of recapture by translocation distance in female (red) and male (blue) northern map turtles (*Graptemys geographica*) based on translocation site (i.e., downstream, upstream, and tributary) following the Kalamazoo River oil spill on 25-26 July 2010. Results are predicted by general linear models, with shading representing 95% confidence intervals. Individuals that were recaptured during subsequent surveys had a recapture probability of 1.0, while those not recaptured had a recapture probability of 0.0.

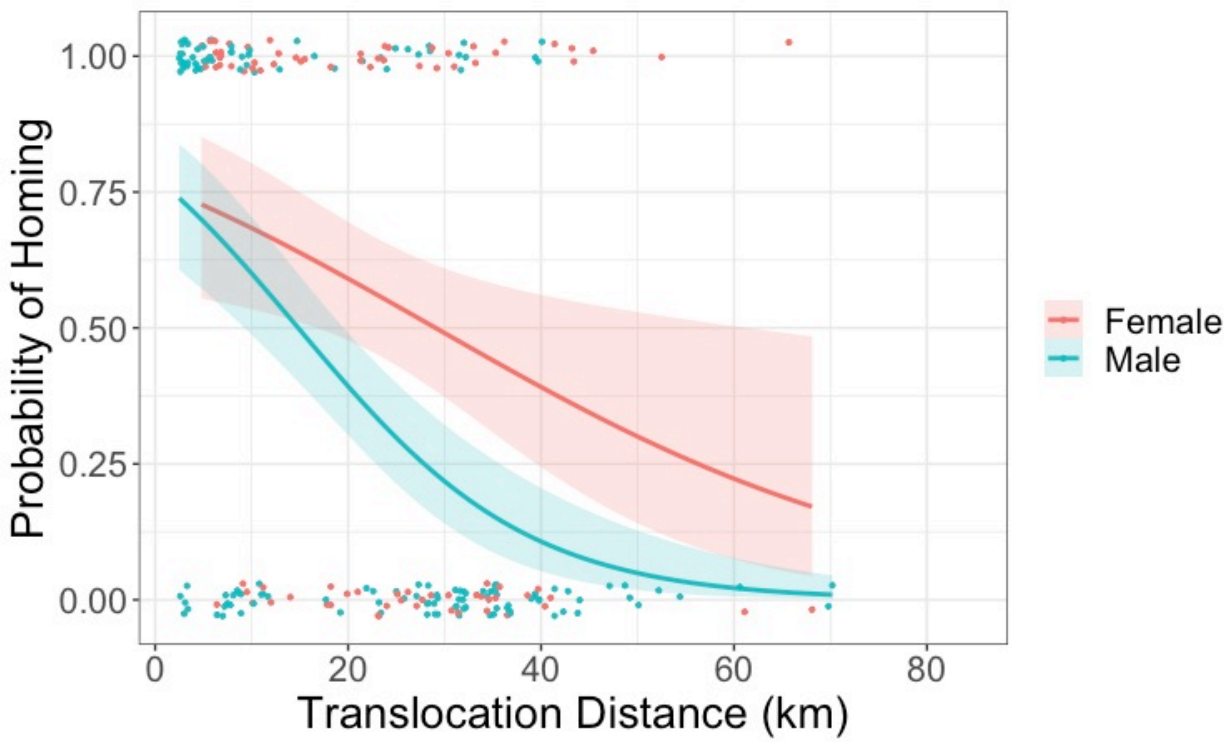

Figure 4-4. Probability of homing by translocation distance of female (red) and male (blue) northern map turtles (*Graptemys geographica*) following the Kalamazoo River oil spill on 25-26 July 2010. Results are predicted by the general linear models, with shading representing 95% confidence intervals. Individuals that were recaptured within 2.4 km of their original capture location for males and 4.6 km for females (Chapter 3) were defined as having homed, and therefore had a homing probability of 1.0, while those not recaptured within those distances had a homing probability of 0.0.

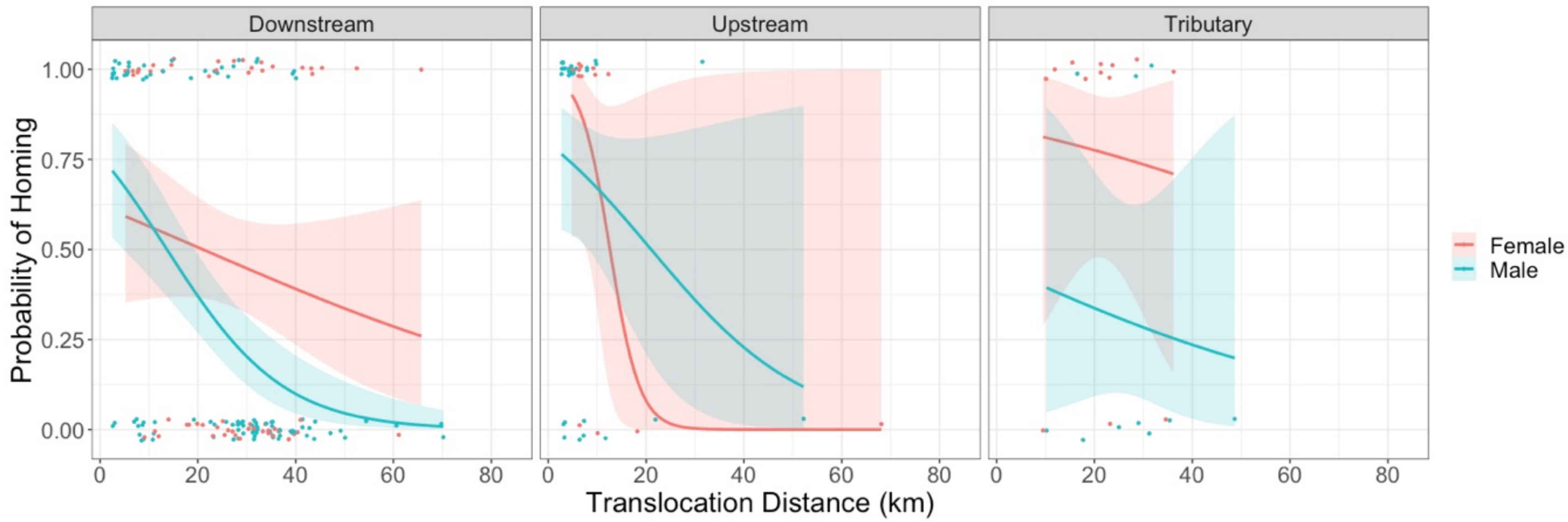

Figure 4-5. Probability of homing success by translocation distance in female (red) and male (blue) northern map turtles (*Graptemys geographica*) based on translocation site (i.e., downstream, upstream, and tributary) following the Kalamazoo River oil spill on 25-26 July 2010. Results are predicted by general linear models, with shading representing 95% confidence intervals. Individuals that were recaptured within 2.4 km of their original capture location for males and 4.6 km for females (Chapter 3) were defined as having homed, and therefore had a homing probability of 1.0, while those not recaptured within those distances had a homing probability of 0.0.

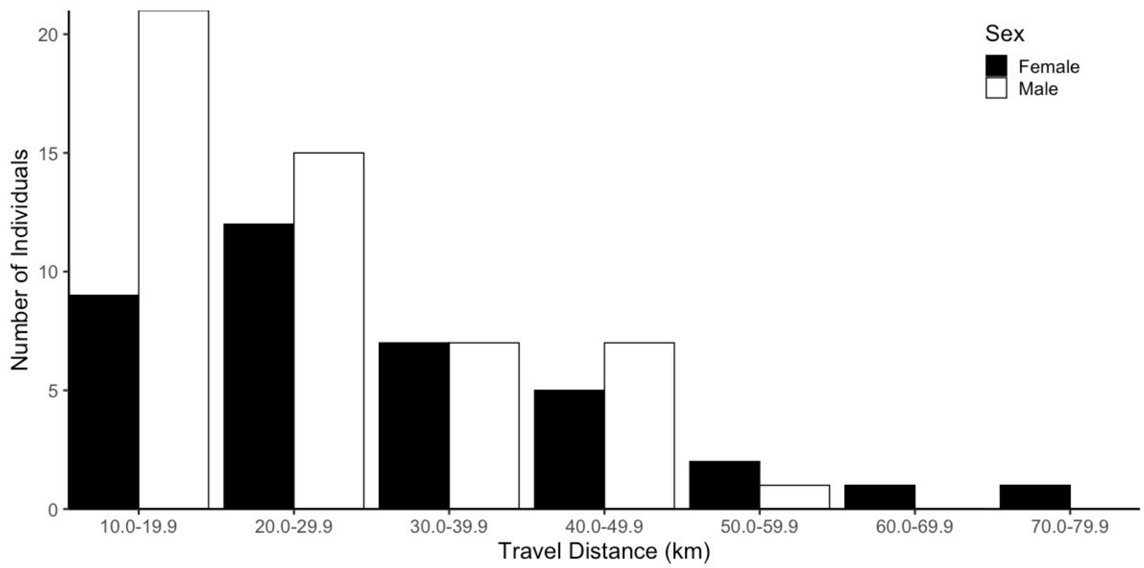

Figure 4-6. Maximum distances travelled by individual female and male northern map turtles (*Graptemys geographica*) following translocation due to the Kalamazoo River oil spill on 25-26 July 2010. Travel distance was calculated as the shortest distance between translocation site and any subsequent recapture location while staying entirely within the river channel.

Table 4.1. Summary of female and male northern map turtles (*Graptemys geographica*) translocated to potentially unfamiliar locations in 2010-2011 following the Kalamazoo River oil spill on 25-26 July 2010. “Recap” individuals represent the number of turtles of each sex that were recaptured during each survey year, while “Homed” individuals were recaptured within 2.4 km of their original capture location for males or 4.6 km for females (Chapter 3). The “% Total Homed” is the cumulative running percent of individuals that had homed after translocation.

	2010				2011				2013		
	Trans-located	Recap	Homed	% Total Homed	Trans-located	Recap	Homed	% Total Homed	Recap	Homed	% Total Homed
Female	250	29	2	0.008	42	58	31	10.1	6	2	11.3
Male	351	66	15	4.3	43	101	39	10.2	10	6	12.2
Total	601	95	17	2.8	85	159	70	10.1	16	8	11.8

	2018			2019			2020			Total Individuals	
	Recap	Homed	% Total Homed	Recap	Homed	% Total Homed	Recap	Homed	% Total Homed	Recap	Homed
Female	4	4	13.0	19	15	14.7	16	11	16.4	87	48
Male	7	3	12.4	13	7	13.2	9	5	14.2	143	56
Total	11	7	12.5	32	22	13.8	25	16	15.2	230	104

Table 4.2. Total number of individual female and male northern map turtles (*Graptemys geographica*) that were translocated and subsequently recaptured at their original home site from three different translocation sites following the Kalamazoo River oil spill on 25-26 July 2010. Translocations within tributaries included only tributaries that were directly connected via lotic habitat to the Kalamazoo River. Downstream and upstream translocations sites were within the Kalamazoo River channel and were relative to the original capture location.

		Tributary	**Upstream**	**Downstream**	**Total Individuals**
Female	Translocated	75	74	143	292
	Recaptured	13	14	60	87
	Homed	10	10	28	48
Male	Translocated	76	90	227	394
	Recaptured	10	29	104	143
	Homed	3	20	33	56

Table 4.3. Models describing probability of recapture and probability of homing for northern map turtles (*Graptemys geographica*) translocated after the Kalamazoo River oil spill on 25-26 July 2010. For each model we report model rank, predictor variables (Model), number of parameters (K), ΔAICc, Akaike weight (*w*), and log-likelihood. Translocation Km is the river-distance between original capture location and translocation site. Site categories included a tributary connected to the Kalamazoo River, or downstream or upstream of the original capture location within the Kalamazoo River.

Type	Rank	Model	K	Δ AICc	*w*	Log-likelihood
Probability of Recapture	1	Translocation Km * Sex + Site	6	0.00	0.40	-351.38
	2	Translocation Km * Site + Sex	7	1.28	0.21	-351.00
	3	Translocation Km + Site	4	1.36	0.20	-354.09
	4	Translocation Km + Sex + Site	5	2.27	0.13	-353.53
	5	Translocation Km * Site	6	3.8	0.06	-353.27
	12	null	2	162.27	0.00	-437.57
Probability of Homing	1	Release Km * Sex	4	0.00	0.44	-131.67
	2	Release Km * Sex + Site	6	1.25	0.23	-130.19
	3	Release Km + Sex	3	1.60	0.20	-133.50
	4	Release Km + Sex + Site	5	2.97	0.10	-132.11
	5	Release Km * Site + Sex	7	7.10	0.02	-131.77
	12	null	2	23.21	0.00	-158.37

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