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Remarkable Male Bias in a Population of Midland Painted Turtles (*Chrysemys picta marginata*) in Ontario, Canada

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***Abstract*.—We report on male sex bias in a population of Midland Painted Turtle (*Chrysemys picta marginata*) at a wetland near Brampton, Ontario, Canada. The wetland is bisected by a major arterial road and characterized by high traffic volume and substantial wildlife-vehicle collisions. Road mortality surveys conducted in 2011, 2013, and 2015 documented 217 dead turtles, of which 118 could positively be identified as Midland Painted Turtle. From 2014–2015, we conducted a mark-recapture survey and captured 34 individual Midland Painted Turtles. The male:female sex ratio of 21:4 is one of the most skewed sex ratios for this species on record. We attribute this skew to sex-specific road mortality amongst other possible factors such as predation and nesting conditions. This study adds to the general body of work that has found a male sex ratio bias in areas of high road mortality.**

***Résumé*.—Notre étude rapporte un biais sexuel masculin dans une population de Tortue Peinte du Centre (*Chrysemys picta marginata*) dans un complexe d'étangs à Brampton, Ontario, Canada. Une artère principale bissecte le complexe. Cette route se caractérise par son volume élevé de circulation véhiculaire et par les nombreuses collisions avec la faune. Les relevés de mortalité routière menés en 2001, 2013 et 2015 ont permis de documenter 217 tortues tuées, dont 118 étant des Tortues Peintes. En 2014 et en 2015, nous avons mené un recensement de la population de tortues en utilisant un protocole de marquage de la carapace. Nous avons capturé 34 Tortues Peintes du Centre. Le rapport entre le sexe masculin:féminin étant de 21:4, représentant un des rapports sexuel les plus exagérés pour une population de tortues. Nous attribuons cet écart à un risque de mortalité routière qui diffère par sexe, et possiblement autres facteurs tels la prédation et les conditions de nidification. Les résultats de cette étude s'ajoutent à plusieurs autres révélant un biais masculin du rapport des sexes relié à la mortalité routière.**

Key Words.—mark-recapture; Painted Turtle; road mortality; sex ratio; urban; wetland

Introduction

Road mortality is one of many threats to several species of turtles (Beaudry 2008; Ives-Dewey and Lewandowski 2012) and is responsible for a wide range of negative effects on populations, such a reduction in gene flow, depletion of population size, and skewing of sex ratios towards males (Gibbs and Steen 2005; Ives-Dewey and Lewandowski 2012). High traffic roads (i.e., those having several thousand vehicles per lane per day) are relatively impassable to turtles (Gibbs and Shriver 2002). As little as 2–3% chronic additional adult mortality is more than most turtle species can absorb and still maintain healthy population dynamics (Congdon et al. 1993, 1994). Previous turtle road mortality studies in Ontario have found that Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles (*Chelydra serpentina*), and Blanding's Turtles (*Emydoidea blandingii*) living in proximity to road networks are at high risk of road mortality due to life-history traits (Ashley and Robinson 1996; Haxton 2000; Beaudry 2008). Both male and female turtles are susceptible to roadkill, as both sexes will cross roads to travel to adjacent wetlands. In one study, 100% of monitored turtles crossed at least one road during the season (Beaudry et al. 2008). Males tend to be active earlier in the season, starting in May, and later in the season through to September, while both male and female movement spike in June and July (Beaudry et al. 2010).

Female turtles are more susceptible to vehicular mortality because of their nesting habits (Aresco 2005; Steen et al. 2006; Patrick and Gibbs 2010). Evidence from various studies (e.g., Christens and Bider 1987; Conant Wood and Herlands 1997; Haxton 2000; Steen et al. 2006; Steen and Gibbs 2004) indicates that annual overland nesting migrations, usually in June and July, and the use of roadside substrate for

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nesting puts female turtles at higher risk of road mortality. Also, nesting on roadsides puts females and hatchlings at risk of collisions with vehicles that may intentionally aim for them (Ashley 2007). Over the last century, turtle populations across the United States have shown an increasingly male-biased population, coincident with the increasing road network (Gibbs and Steen 2005). In contrast, a recent study in Ontario found no significant effect of roads on Midland Painted Turtle sex ratios (Dorland et al. 2014). The Midland Painted Turtle is the most common species of turtle found in Ontario. This species inhabits various inland water bodies with a soft substrate and abundant basking sites, and is the most frequent turtle species found at urban or degraded sites (DeCatanzaro and Chow-Fraser 2010; Marchand and Litvaitis 2004). The objective of our study was to sample and document the turtle species assemblage in a wetland complex with a history of high turtle road mortality near Brampton, Ontario, Canada.

Materials and Methods

Study site.—We conducted our study in the municipality of Brampton, Ontario, Canada, at an urban wetland complex that is bisected by 2.5 km section of Heart Lake Road and is adjacent to the Heart Lake conservation area. The Heart Lake conservation area is approximately 100 ha and is characterized by a series of small surface wetlands ranging from 2–8 ha that are interconnected by small ephemeral streams and also includes a 20 ha kettle lake and one of the largest remnants of forest within the Etobicoke Creek watershed. The two-lane road has bisected this provincially significant wetland complex since 1877. The road is paved (since the mid-70s) with gravel shoulders and has a posted speed limit of 60 km/h. In many places, the road bisects a series of small wetlands with < 5 m of shoulder between road and the edge of the water.

Road mortality.—Road mortality surveys were conducted by volunteers in 2011, 2013, and 2015. In 2011, we conducted a pilot study with trained volunteers that monitored both sides of the 2.5 km stretch of the Heart Lake Road (between Sandalwood Parkway to the south and Mayfield Road to the north). Volunteers searched and recorded turtle carcasses on the road, along the road shoulder and in the ditches and embankments near the road. The volunteers identified carcasses to species, if possible; otherwise, to order or class but were not required to report sex or age of the dead animals. In 2013 and 2015, we implemented a similar protocol, but along a shorter 1.5 km segment of the same road, concentrating on the main area of high mortality as identified in the 2011 pilot study (between Sandalwood Parkway and Countryside Drive). Volunteers conducted the daily (in 2011 and 2013) or weekly (2015) road surveys on foot during daylight hours, noting the location of the carcasses and removing the carcasses afterwards. We mapped turtle mortality locations using ArcMap 10.3 (Esri, Redlands, California, USA). We measured traffic volume using automated paired axle sensors (MetroCount, Fremantle, Australia).

Mark-recapture study.—We conducted a mark-recapture population study within one of the many bisected wetlands inside the wetland complex to investigate the demographics of the painted turtle population. The chosen wetland for the population study was located nearest one of the previously identified hotspots of turtle road mortality. This wetland measured approximately 6.5 ha and was bisected by approximately 300 m of the Heart Lake Road. We captured turtles using hoop traps (76 cm × 64 cm × 119 cm collapsible turtle trap (Wildlife Control Supplies, East Granby, Connecticut, USA) and basking traps (made in-house). We distributed the hoop traps along the shallow margins of the pond and secured them with stakes. A portion of the hoop traps remained above the water to permit captive turtles to breathe. The basking traps were set in deeper areas of the wetland, near basking logs. We baited the hoop traps with a variety of baits including frozen trout, sardines, chicken, and cat food and normally checked traps daily. When staff was not able to attend the site, hoop traps were left open to allow animals to swim through and freely exit. For basking traps, one end of the ramp portion was set inside the trap to allow turtles to climb out when traps remained *in situ*. In 2014, we placed eight traps (five hoop traps and three basking traps) for 5 d (5–9 August) resulting in a 40 trap-days effort. In 2015, we placed six traps (three hoop traps and three basking traps) in the wetlands during various times for a cumulative 30 d (12 June to 16 September) resulting in 180 trap-days.

For each captured turtle, we recorded the species, mass, measured the foreclaw and the precloacal tail length with vernier calipers, and assigned a sex, if possible. We used the length of the foreclaw and the precloacal tail length as determinants of sex (Ernst and Lovich 2009). To permit unique identification upon re-capture, we notched carapaces following a modified Cagle method (Cagle 1939). We also injected a 9 mm passive integrated transponder (PIT) tag (Biomark, Boise, Idaho, USA) in turtles with a straight carapace length of 80 mm or longer. To determine if the sex ratio of painted turtles was unbiased, we performed a binomial test (i.e., a nonparametric two-sided test based on a one-sample binomial distribution) on the number of males and females captured (Wilson and Hardy 2002).

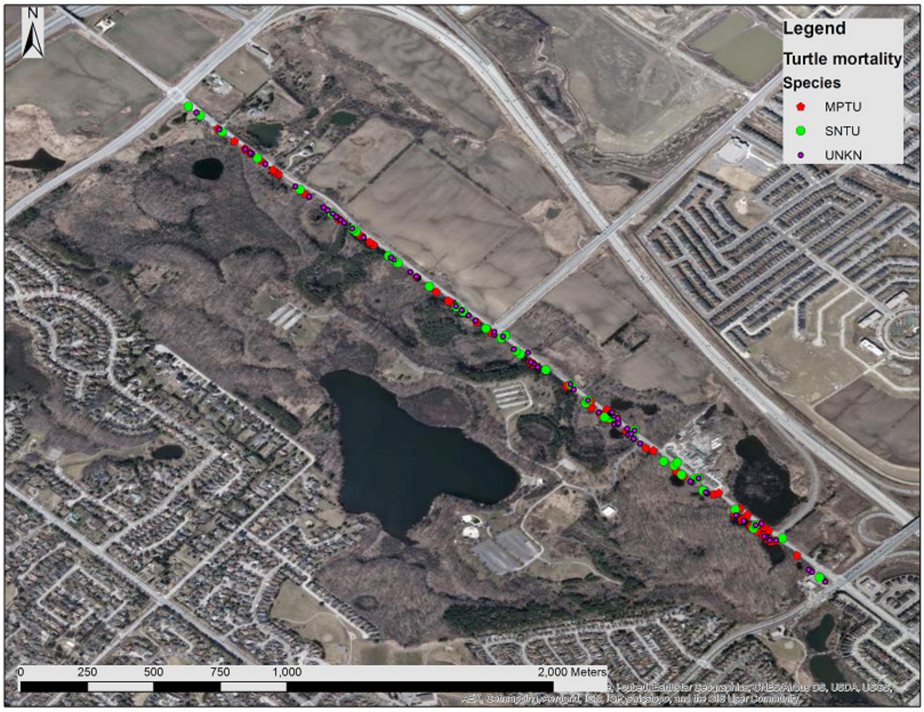

Figure 1. Turtle mortality along Heart Lake Road, Brampton Ontario, Canada in 2011, 2013, and 2015. Each turtle carcass is represented by a point on the map (there may be overlap). The acronyms in the legend are: MPTU (Midland Painted Turtle), SNTU (Eastern Snapping Turtle), and UNKN (unknown) where turtle carcasses were not identified on the data sheets.

We performed the statistical analysis using Microsoft Excel v.14.6.9 (Microsoft Corporation, Redmond, California, USA).

Results

In June 2013, we conducted a weeklong traffic survey and recorded 41,613 vehicles. Average traffic was 5,435 vehicles/d for weekdays and 7,073 vehicles/d for the weekend. Average speed was 78.1 km/h. We detected carcasses of 217 turtles over 335 survey days of road mortality surveys in 2011, 2013, and 2015. We documented turtle carcasses along the entire surveyed length of Heart Lake Road (Fig. 1) and a high concentration of mortality near the sampled wetland (Fig. 2). Of the 217 turtle carcasses discovered, we positively identified 118 Midland Painted Turtle and 40 Eastern Snapping Turtle (Table 1).

Table 1. Wildlife-vehicle collisions along Heart Lake Road, Brampton, Ontario, Canada in 2011 (9 May to 31 October; 176 d), 2013 (7 April to 30 September; 134 d), and 2015 (10 June to 6 October; 25 d) with monitoring effort in days and length of road surveyed.

Wildlife-Vehicle Collisions	2011 2.5 km	2013 1.5 km	2015 1.5 km
Midland Painted	27	76	15
Snapping Turtles	19	15	6
Unidentified turtles	48	10	1
Total	94	101	22

Over the two years of mark-recapture study (220 trap-days), we captured 52 turtles, including 34 individual Midland Painted Turtles (Appendix I) and seven Eastern Snapping Turtles. We averaged 0.24 turtles per trap day, with 41 new captures and 11 recaptures. Of these, 46 were captured in hoop traps and six in basking traps. The male to female ratio of Midland Painted Turtles was 21:4 or 84.0% in favor of males, the binomial test indicated that the proportion of males of 0.84 was higher than the expected ratio of 0.5 ($P < 0.001$). Our sample size of Eastern Snapping Turtles makes the male to female ratio of 2:3 insufficiently small to report a test statistic.

Discussion

The male to female sex ratio in our Midland Painted Turtle population is one of the most skewed sex ratios recorded for any individual wetland within a turtle population study in North America (Gibbs and Steen 2005; Marchand and Litvaitis 2004). Healthy turtle populations typically possess a stable sex ratio of 1:1 among adults (Gibbons 1968); however, forest coverage, proximity to roads and varying levels of predation can significantly alter this ratio (Marchand and Livaitis 2004). We posit four potential sources for the

Figure 2. Turtle mortality locations along the sampled wetland section of the Heart Lake Road, Brampton, Ontario, Canada in 2011, 2013, and 2015. Each turtle carcass is represented by a point on the map (there may be overlap). The acronyms in the legend are: MPTU (Midland Painted Turtle), SNTU (Eastern Snapping Turtle), and UNKN (unknown) where turtle carcasses were not identified on the data sheets.

male-biased sex ratio we observed in Midland Painted Turtles. First, high road mortality at our site might be a factor in the skewed sex ratio as the series of small interconnected wetlands are located very close to the road (< 10 m). In contrast, the study by Dorland (2014) found that wetland proximity to roads had no effect on the sex ratio of Midland Painted Turtles. However, that study focused on isolated ponds within 65 m of roads that had no other source of water within 250 m. Both the wetland proximity to the road and the interconnected nature of our studied wetland complex might explain why our results differ from the study by Dorland (2014). Turtles at our study site have greater opportunity for exploratory movement between wetlands and a greater risk of encountering vehicles due to the proximity to the road.

Secondly, nest temperature in the wild is a determinant of sex in painted turtles (Janzen 1994; Riley et al. 2014) and cooler nests tend to produce more males. The forested stands at the study site could provide shade to cool the temperature of nests located under tree cover. Consequently, the forest cover at our site might also be contributing to the male biased sex ratio that we observed.

Thirdly, Coyote (*Canis latrans*), Northern Raccoon (*Procyon lotor*), Mink (*Neovison vison*), and Virginia Opossum (*Didelphis virginiana*) are present at our study site. All of these mammal species are well known turtle predators. Females turtles often show more extensive injuries (i.e., missing limbs, cracked or indented shells, stubbed tails, or facial injuries) than males (Marchand and Litvaitis 2004). These injuries were attributed to encounters with vehicles and/or predators and the authors concluded that females might be exposed to more predators than males while searching for nest sites and during oviposition. This factor could also be partially responsible for the observed skew in the sex ratio at our study site.

Lastly, we captured significantly more turtles using hoop traps than with basking traps (46 versus six). It is therefore possible that our trapping technique biased our results toward capturing more males than females (Ream and Ream 1966). This factor could also have contributed to the biased male sex ratio. In conclusion, we observed a highly male-skewed population of Midland Painted Turtles in an urban forested wetland area bisected by a high traffic road with documented road mortality. Although all four factors may have contributed to the skewed sex ratio, our road mortality data suggest that the loss of adult turtles to vehicle encounters is the most probable cause.

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Marc Dupuis-Désormeaux is Wildlife Biologist and reformed Banker. Marc's interests lie in human-wildlife conflicts, specifically livestock depredation by carnivores and crop raiding by elephants. He has field sites in Kenya, where he studies carnivore behavior, landscape prey-traps, and wildlife fencing as well as issues arising in communities living on the borders of wildlife conservancies. He is currently a Post-Doctoral Fellow at York University, Ontario, Canada, where he works closely with the Toronto and Region Conservation Authority (TRCA) and focuses on studying turtle demographics in urban and created wetlands. He has also designed and implemented herp-specific wildlife passages and fencing that mitigate the risk of road mortality. Whenever possible, Marc also likes to conduct research with members of the North American Freshwater Turtle Research Group. (Photographed by Dr. Brian Hauge).

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Appendix I. Mark-recapture survey data for Midland Painted Turtles at Heart Lake Road, Brampton, Ontario, Canada in 2014–2015. Abbreviations are PCL = precloacal length, PL = plastron length, and CL = carapace length.

Turtle #	SEX	PCL (mm)	Foreclaw (mm)	PL (mm)	CL (mm)	Width (mm)	Body Depth (mm)	Mass (g)
1	J	5.7	--	85	92	76	37	123.5
2	J	3.5	--	76	82	72	34	85.0
3	J	2.7	--	69	78	70	31	83.0
4	J	6.1	--	92	101	83	37	152.0
5	M	14.3	--	127	136	105	44	329.0
6	M	17.4	14	94	105	81	36	156.5
7	M	14.0	10	96	102	84	40	169.5
8	M	15.9	15	104	113	91	40	204.5
9	M	12.9	--	105	112	91	40	211.5
10	M	15.0	11	111	120	98	40	236.0
11	M	19.0	13	114	129	99	44	281.0
12	F	8.0	8	150	162	119	57	551.0
13	J	5.0	--	71	78	69	30	75.0
14	M	13.0	13	109	118	99	42	245.0
15	M	17.0	14	135	139	102	47	350.0
16	J	5.0	4	79	87	76	33	100.0
17	M	10.0	11	97	109	89	39	194.0
18	M	19.0	13	126	138	103	45	344.0
19	M	13.7	14	127	138	109	44	331.0
20	F	8.1	7	151	155	122	58	571.0
21	M	14.2	13	119	129	103	43	286.0
22	M	16.1	15	127	143	107	46	350.0
23	M	14.1	15	120	131	103	43	300.0
24	M	14.6	14	114	120	92	37	222.0
25	F	7	6	104	107	83	30	192.0
26	J	5.6	--	75	78	71	33	83.0
27	J	5.5	--	71	78	70	32	84.0
28	F	7.2	9	167	175	127	59	713.0
29	M	15.1	15	123	189	109	46	356.0
30	M	14.3	14	116	125	100	42	260.0
31	M	14.1	11	97	104	82	39	170.0
32	M	18.6	16	126	139	109	46	350.0
33	M	13.9	13	126	137	107	45	331.0
34	J	3.7	--	51	55	54	20	30.0