Running Title: Sex bias in turtle-vehicle interactions

Revisiting the hypothesis of sex-biased turtle road mortality

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Carstairs, S., M. Dupuis-Desormeaux, and C.M. Davy. 2018. Revisiting the hypothesis of sex-biased turtle road mortality. Canadian Field-Naturalist. 132(3): xxx-xxx. https://doi.org/10.22621/cfn.v132i3.1908

Abstract

Road mortality poses a major threat to the persistence of threatened turtle populations. Several studies have suggested that additional terrestrial movements associated with nesting create greater risks to females than to males. The Ontario Turtle Conservation Centre (OTCC) is home to the Kawartha Turtle Trauma Centre, Ontario's only dedicated turtle hospital, and admits injured, wild turtles year-round. The OTCC admits up to 900+ turtles/year with road injuries being the primary cause of admission. We tested the hypothesis that road mortality in turtles is female-biased, using data from injured Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles (*Chelydra serpentina)*, Blanding's Turtles (*Emydoidea blandingii*), and Northern Map Turtles *(Graptemys geographica*) collected over an area of approximately 126 000 km^2 and admitted to OTCC's hospital from January 2013 to October 2017. There was no difference in the number of male and female admissions for painted, Blanding's, or Snapping turtles ($P > 0.05$). More female map turtles were admitted than males ($P = 0.000$). Admission of female turtles peaked in June during the nesting season, but male admissions were more evenly distributed throughout the season. Our admissions data provide a temporally un-biased and geographically broad snapshot of turtle-vehicle interactions that can directly inform conservation and management policies. Although mortality count data are not equivalent to per capita mortality rates, our results demonstrate that vehicle strikes can have substantial impacts on both female and male turtles.

Key words: *Chelydra serpentina; Chrysemys picta; Emydoidea blandingii;* Ontario; road ecology; road mortality; sex-biased dispersal; sex ratio; wildlife rehabilitation

Introduction

Roads have been called the "sleeping giant" of conservation biology (Forman and Alexander 1998) because of their pervasive impacts on biodiversity and habitat connectivity. The effects of roads include habitat fragmentation, barrier effects, genetic isolation of population fragments, and most obviously, direct mortality due to vehicle-wildlife collisions (Strasbourg 2006; van der Ree *et al.* 2011; Beebee 2013). Smaller, slower wildlife species may be more susceptible to vehicle strikes because they take more time to cross a road, increasing the probability of interaction with a vehicle, and may not use flight as a predator response, further increasing the likelihood of vehicle strikes (Fahrig and Rytwinski 2009). Turtles are particularly vulnerable to the increased mortality caused by vehicle strikes because their long-lived life-history strategy and slow population growth rates magnify the population-level impacts of small increases in adult mortality (Congdon *et al.* 1993; Gibbs and Steen 2005; Crawford *et al.* 2014; Rytwinski and Fahrig 2015).

Several studies have tested the hypothesis that female turtles are at higher risk of road mortality during the nesting season because overland movements required to find a suitable nest site may increase females' probability of contact with roads (Steen and Gibbs 2004; Aresco 2005; Gibbs and Steen 2005; Steen *et al.* 2006; Patrick and Gibbs 2010; Dorland *et al.* 2014). In addition, females that do nest on the shoulder of paved roads can spend considerable amounts of time searching the road itself, even those that have repeatedly nested at the same area, whereas males typically cross the road and do not show this nest searching behaviour (R. Brooks pers. comm. 11 May 2018). If road mortality is female-biased, then the adult sex ratios of turtle populations should gradually become male-biased (Steen and Gibbs 2004; Gibbs and Steen 2005; Steen *et al.* 2006; Patrick and Gibbs 2010; Dupuis-Desormeaux *et al.* 2017).

Road mortality studies often do not report the sex of the turtles (e.g., Ashley and Robinson 1996; Gunson *et al.* 2014; Baxter-Gilbert *et al.* 2015; Choquette and Valliant 2016; Dupuis- Desormeaux *et al.* 2017), or only report the sex of a limited sample (Haxton 2000). In addition, some road mortality surveys are carried out for a limited portion of the active season (e.g., Haxton 2000) or surveys are carried out for a limited number of days throughout the season (e.g., Cureton and Deaton 2012). A temporally unbiased dataset of road mortality occurrences in male and female turtles is required to test directly the hypothesis of sex-biased road mortality occurrences in turtles at the landscape scale.

The Ontario Turtle Conservation Centre (OTCC) is home to Canada's only dedicated turtle rehabilitation centre (www.ontarioturtle.ca). The OTCC admits turtles injured in a variety of ways, but the vast majority of admissions (80-95%, depending on the species) represent turtles injured on roads across southern Ontario. Southern Ontario contains 92% of the Ontario's human population, and some of the highest concentrations of roads on the planet with a road located on average every 1.5 km (Gunson 2010; Laurance *et al.* 2014). Admissions to the OTCC include all eight species of turtles native to Ontario, including the globally endangered Spotted Turtle (*Clemmys guttata*; van Dijk 2011) and Blanding's Turtle (*Emydoidea blandingii*; van Dijk and Rhodin 2011). All Ontario species except Spiny Softshell (*Apalone spinifera)* have been admitted with vehicle-related injuries.

Admissions to the OTCC have increased steadily since 2010 (Figure 1A), in part due to increased public participation following intensive public education initiatives. The OTCC admissions dataset provides an opportunity to test the hypothesis of sex-biased road effects on a large, temporally unbiased, and geographically broad sample of turtles struck by vehicles across an area of approximately 126 000 km^2. We used the OTCC data to test the hypothesis that interactions with vehicles affect more female turtles than males, predicting that if more females than males are struck by vehicles in our intake area, then counts of turtles admitted to the Centre would also be significantly female-biased.

Methods

The OTCC, home of the Kawartha Turtle Trauma Centre (KTTC), is located in Peterborough, Ontario (44.336776^{o}N, 78.348319^{o}W), and receives cases from across southern Ontario and occasionally from southern Quebec. Turtles are brought to the OTCC by members of the public and by field biologists, or transferred from other wildlife rehabilitation centres when complex veterinary care is required. Admissions include all species of turtle native to Ontario, and the majority of admissions are Midland Painted Turtles (*Chrysemys picta marginata*), Snapping Turtles (*Chelydra serpentina*), and Blanding's Turtles. During admission, OTCC staff record each turtle's species, sex, size (carapace length and width), and age class (hatchling, juvenile or adult), as well as the cause of admission and the collection location. Admissions to the hospital take place all year round, however the majority occur from mid April to late October, during the active season of turtles in southern Ontario.

Vehicle strikes typically cause life-threatening injuries in turtles (Figure 1b,c), and medical records from the Centre confirm that the successful treatment and rehabilitation of turtles that are hit by vehicles depends on rapid veterinary treatment. Therefore, admissions data for turtles struck by vehicles represent turtles that would have died in the absence of treatments, and are an appropriate proxy for vehicle-related mortalities. Successfully rehabilitated turtles are released back into the wild near their initial collection location.

We used OTCC admissions data from January 2013 to October 2017 to test whether vehicle strikes on turtles occur more frequently in one sex than the other. We removed records from turtles that were admitted due to other causes, and limited our data set to only turtles that had been hit by a vehicle. We tested for significant deviations from an unbiased sex ratio in the admissions data by performing a nonparametric binomial two-sided test based on a one-sample binomial distribution (Wilson and Hardy 2002) with the untested assumption that the populations of turtles in the study area also were not sex-biased. All statistics were performed in Microsoft Excel (Microsoft Corporation; Redmond, Washington USA 93052), and we considered results significant at $\alpha = 0.05$. Location data were mapped using ArcMap 10.1 (ESRI; Redlands, California, USA).

Results

The OTCC admitted 2355 turtles from 2013 to 2017 (Figure 2). Of these, 1722 were mature individuals that were admitted due to vehicle strikes and sexed during the admission process and used in our analyses. The majority were Midland Painted Turtles (62%), followed by Snapping Turtles (29%), and Blanding's Turtles (6.5%; Table 1). Approximately half (51%) of our admissions due to vehicle strikes were female, and admissions of female Midland Painted Turtles, Snapping Turtles and Blanding's Turtles peaked in June, concurrent with the nesting season for these species. Admissions of male turtles showed multiple clusters of admissions extending from early spring to late fall (Figure 3). Turtles were admitted for care following vehicle strikes as early as 13 March (2013), as late as 25 October (2017).

A binomial test showed that admissions of males and female turtles due to vehicle strikes when combined over all five years were not statistically different from equal for Midland

Painted Turtles (P = 0.404), Snapping Turtles (P = 0.660), or Blanding's Turtles (P = 0.110; Table 1). Admissions of Northern Map Turtles (*Graptemys geographica)* were significantly female-biased (P = 0.000), but map turtles made up only 2% of total vehicle-related admissions.

Discussion

Our temporally unbiased, five-year admissions dataset from the OTCC does not support the hypothesis of sex-biased road mortality in Midland Painted, Snapping, or Blanding's Turtles, but suggests that roads may have a greater impact on female Northern Map Turtles than on males. The OTCC admits turtles year-round, enabling continuous collection of road mortality data over five years, and accurate sexing of each turtle admitted. Our road mortality data are count data, like those of most other road ecology studies, and cannot be converted to mortality rates because robust demographic data are available for only a few well-studied turtle populations. However, the even distribution of road mortality occurrences among males and females of the most commonly-hit species in our dataset suggests that the impacts of roads on turtles are more evenly shared between the two sexes than previous studies have suggested.

Painted, Snapping, and Blanding's turtles frequently move overland to find mates, to access resources such as foraging or over-wintering sites, or to find a suitable nest site (Pettit *et al*. 1995; Tuberville *et al.* 1996; Ernst and Lovich 2009). These activities bring both males and females of these three species into contact with roads, as reflected in our data. Nesting season is clearly associated with increased risk of road injury for female turtles in Ontario. However, our results also provide empirical support to a recent modelling approach (Beaudry *et al.* 2010) which suggest that male mortality is similar to female mortality when the entire active season of mid-April to the end of October is considered. Beaudry *et al.* (2010) found that male and female

Blanding's Turtles had similarly long movement patterns, and were both similarly exposed to road mortality. However, males moved more than females, before and after nesting season. Although our sample of Northern Map Turtles was small (34 individuals), the sample was heavily biased towards females (94%). The behaviour of Northern Map Turtles is consistent with this observation: male Northern Map Turtles are almost exclusively aquatic, whereas females emerge rarely, and usually only to find a suitable nest site (Ernst and Lovich 2009).

Survivorship in female turtles has been a focus of turtle ecologists and conservation biologists for good reason; it has an extremely high impact on the growth rates of turtle populations (Congdon *et al.* 1993; Heppell *et al.* 1996; Heppell 1998; Mitrus 2005; Enneson and Litzgus 2008). Therefore, maximizing female survivorship is considered a key component of effective turtle conservation. Nevertheless, reproductive males and females are required to sustain a viable turtle population, and conservation programs should ensure adequate survival rates in both sexes. Most methods used to mitigate road impacts on turtles (ecopassages, drift fencing, etc.) probably provide equal protection to both sexes. Road closures during the nesting season may adequately protect nesting female turtles but fail to protect males and females moving overland during the rest of the active season.

One tool used to recover threatened turtle populations is "headstarting", in which eggs are hatched in artificial conditions that maximize hatching success and eliminate nest depredation. Hatchlings are often also reared to a larger size before release, which may reduce the risk of early juvenile mortality (e.g., Iverson 1991; Haskell *et al*. 1996; Bennett *et al.* 2017). In species with temperature-dependent sex determination, incubation conditions can be controlled to produce a predetermined sex ratio in hatchlings. Setting aside the many factors that can prevent recruitment of headstarted or wild-hatched juveniles to a turtle population, our data add evidence to suggest that headstarting projects or other attempts to augment populations should consider both sexes, and not focus solely on females. The fact that females spend more time on roads, and yet males are struck in equal numbers, additionally adds support for the high impact of roads on the male population.

Converting mortality count data such as those presented here to mortality rates for male and female turtles in a population, and thus inferring and projecting population level impacts requires knowledge of the sex ratio of the underlying population—a major limitation of our study and of many others. Accurately estimating population sex ratios requires substantial survey effort, and some survey methods do not have equal detection rates for both sexes. For example, hoop traps may capture male-biased samples of painted turtle populations (Ream and Ream 1996), while surveys of turtle nesting sites are necessarily female-biased. Furthermore, counts of road mortality for long-lived animals such as turtles do not accurately represent demographic trends (Rytwinski and Fahrig 2015), and unequal male and female road mortality rates could cause yearly fluctuations in the population's sex ratio as the population nears extinction.

The underlying reasons for the discrepancy between the equal sex ratio in road injuries we found and the increasing male-biased population sex ratios correlated with higher road densities found by numerous other studies (e.g., Marchand and Livaitis 2004; Steen and Gibbs 2004; Aresco 2005; Gibbs and Steen 2005; Steen *et al.* 2006; Patrick and Gibbs 2010) remain uncertain. We urge road ecologists to remain critical of the underlying assumptions in the interpretation of mortality counts, and we urge turtle researchers to be cautious of assumptions that could inadvertently prioritize protection of one sex over the other.

Comment [DAWL1]: retain

Comment [DAWL2]: delete as AU requested

The morbidity and mortality of reptiles admitted to wildlife care facilities in North America has been described previously (Hartup 1996; Brown and Sleeman 2002; Rivas *et al*. 2014), but these studies focus on the veterinary medicine aspects of rehabilitation. Our study demonstrates how admission data from a wildlife rehabilitation centre can be used to address broader questions in conservation and draw inferences about threats to wild populations. Perhaps a future approach to these and new data would be to examine the sex ratio variation with locations and compare traffic, road density, or population composition. There are numerous possibilities, but our large sample at a landscape level is unique and could be explored further. Turtles are long-lived species which are slow to mature and have low survival of eggs and hatchlings. Turtle populations cannot recover quickly from increased adult mortality (Brooks *et al*. 1991). Conversely, offsetting increased mortality can have a relatively large impact on demographic rates, such that rehabilitation and release of injured turtles may have a population-level effect.

Comment [DAWL3]: retain as suggested ; AU agreed to retain

Acknowledgements

We thank Lindsay Maxim, Donnell Gasbarrini, and all the staff and volunteers at the Ontario Turtle Conservation Centre who admitted, cared for, and released the turtles described in this study. Thanks also to Lucy Brown for preparing Figure 2. The Ontario Turtle Conservation Centre is accredited as a wildlife rehabilitation centre by the Government of Ontario. We thank the many generous individuals and organizations who support the Centre, including the Ontario Ministry of Natural Resources and Forestry. R. Brooks (Professor Emeritus, Department of

Integrative Biology, College of Biological Science, University of Guelph) provided information from his many years observing wild turtles.

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Comment [DAWL4]: Not deleted, retain reference.

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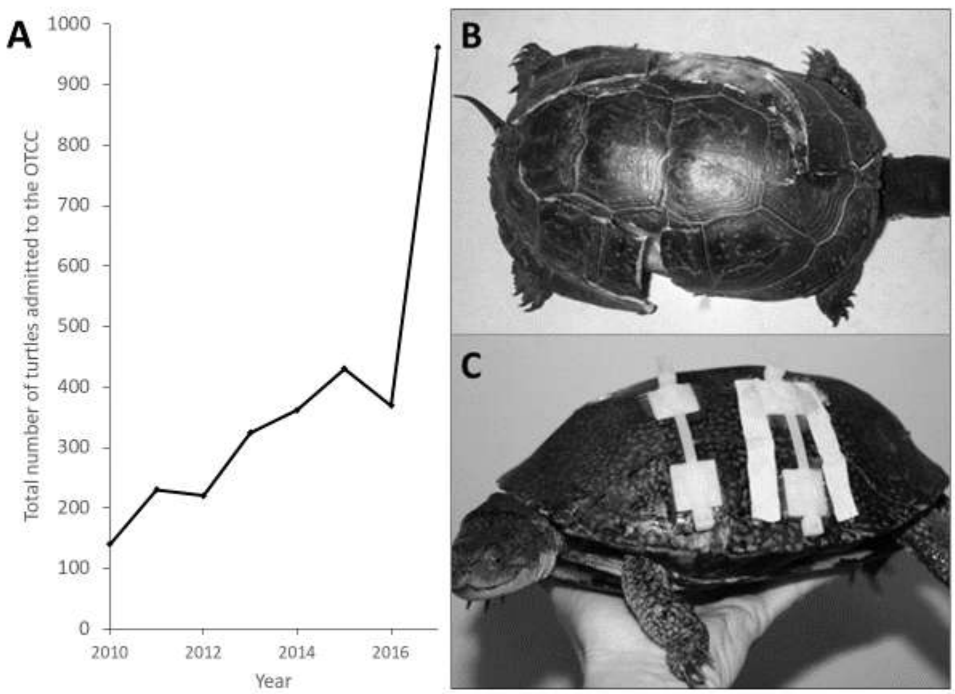
Received 10 February 2017
Accepted 6 July 2018

Figure 1.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Total number of turtles admitted to the OTCC
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Figure 2.

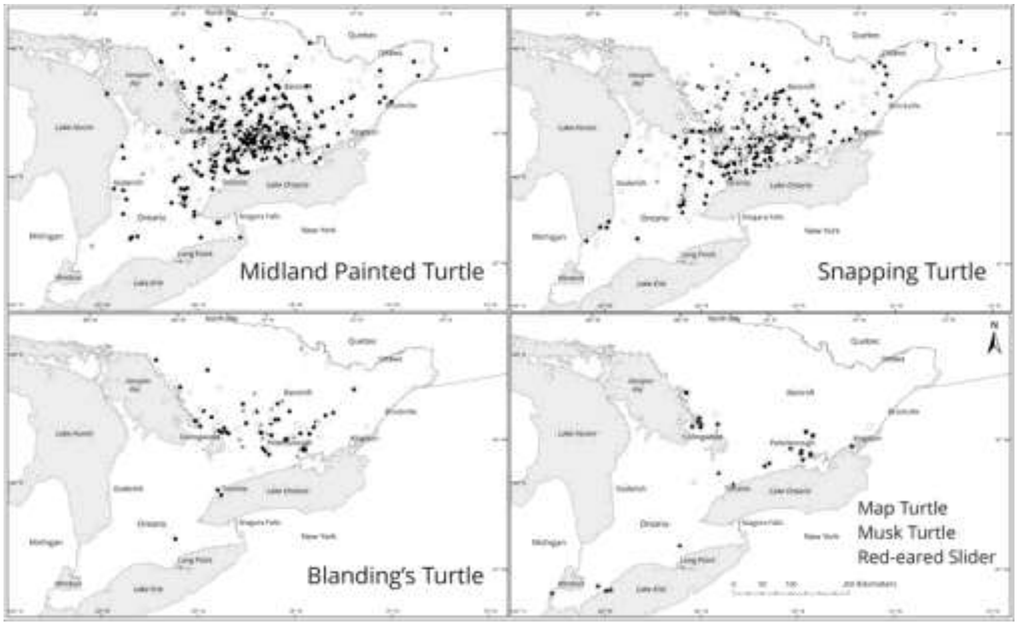
Midland Painted Turtle
Snapping Turtle
Blanding's Turtle
Map Turtle
Musk Turtle
Red-eared Slider

Figure 3. **[USE SEPARATE FIGURE FILE: colour and/or black-white]**

Figure Captions

Figure 1. a.) Admissions to the turtle hospital at the Ontario Turtle Conservation Centre (OTCC) have generally increased since 2010, providing a large sample of turtles that have been hit by vehicles. b.) Injuries from vehicle strikes are typically life-threatening, as in this Blanding`s Turtle (*Emydoidea blandingii*), but rapid surgical attention often allows turtles to recover (c). This individual was successfully rehabilitated and released back into the wild. Photos: S. Carstairs.

Figure 2. Locations of vehicle strikes on turtles admitted to the Ontario Turtle Conservation Centre, 2013-2017. Open symbols = males; black-filled symbols = females; grey-shaded symbols = juveniles. In the lower right panel, stars indicate Northern Map Turtles (*Graptemys geographica*); squares indicate Eastern Musk Turtles (*Sternotherus odoratus*); and triangles indicate Red-eared Sliders (*Pseudemys scripta*).

Figure 3. Admissions of male and female turtles struck by vehicles were comparable for Midland Painted (*Chrysemys picta*), Snapping (*Chelydra serpentina*) and Blanding's Turtles (*Emydoidea blandingii*). Admissions of female turtles (black lines) peaked during the nesting season, while admissions of males (grey lines) were more evenly spread through the season. Northern Map Turtle (*Graptemys geographica*) females were more likely to be admitted than males. November – February are omitted from the y-axis, as no admissions due to vehicle strikes occurred during these months.

TABLE 1. Species and sex distribution for 1722 turtles admitted to the Ontario Turtle Conservation Centre due to vehicular collisions from 2013 to 2017. Raw numbers of admissions for each sex, proportion of males in the sample (males/(males + females)), and the *P*-value from the binomial test, indicating the cumulative probability value that the observed sex ratio reflects an unbiased binomial distribution centered around 0.5. * indicates that the sample is significantly biased towards one sex or the other (α = 0.05).

Species	**Females**	**Males**	**Proportion of males**	***P*-value**
Painted Turtle (*Chrysemys picta*)	541	532	0.496	0.404
Snapping Turtle (*Chelydra serpentina*)	249	254	0.505	0.606
Blanding's Turtle (*Emydoidea blandingii*)	63	49	0.438	0.120
Northern Map Turtle (*Graptemys geographica*)	32	2	0.059	0.000*
Total	885	837	0.486	0.129