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Mass vehicle induced mortalities of Giant Bullfrogs in Nylsvley Nature Reserve

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Introduction

Scientific interest in roadkill and road ecology is on the increase, given the massive role roads play in dividing the earth's terrestrial surfaces (e.g. Collinson et al. 2014; Perkins, Shilling & Collinson 2022; Sutherland, Dunning & Baker 2010). Linear structures such as road networks have the capability of substantially compromising terrestrial landscapes and their associated biota (Forman & Alexander 1998). While the threats imposed by road networks vary considerably (Trombulak & Frissell 2000), the most commonly observed impact is that of wildlife-vehicle collisions, causing what is commonly referred to as roadkill. In some regions, amphibians have been observed to be the most impacted vertebrates, by road networks (Glista, DeVault & DeWoody 2008), yet more work is needed to fully assess impacts on populations and roadkill mitigation (Beebee 2013). Certain amphibians are at risk of roadkill predominantly because of their life history strategies, where they have large congregations, often moving onto nearby roads during or after breeding seasons, while their slow movement and the lack of detectability by drivers add to their vulnerability (Puky 2005; Trombulak & Frissell 2000). When roads are built through or near wetlands and other water sources used for breeding by amphibians, the threat of amphibian roadkill is intensified.

The Nylsvley Nature Reserve (NNR) is a protected area situated in the Waterberg District of the Limpopo province, South Africa. Within the centre of the NNR is the Nyl River Floodplain, a vital inland wetland fed from the Waterberg massif. Because of its conservation importance, specifically to that of avifauna, the NNR has been declared a Ramsar site. As a result of its proximity to the major cities of Johannesburg and Pretoria, the exceptional biodiversity, and self-driving option for visitors, the reserve is a tourism hotspot, leading to heavy traffic during peak holiday periods.

Jacobsen (1977) published extensive work on the amphibian diversity of the NNR, whereby a total of 18 species of amphibians were documented within its borders. Because of taxonomic changes and increased sampling efforts, approximately 24 species are now expected to occur within NNR. *Pyxicephalus adspersus* (Giant Bullfrog) (Jacobsen 1977) and more recently *Pyxicephalus edulis* (African Bullfrog) are both species that have been recorded within the boundaries of the reserve. Members of the *Pyxicephalus* genus display mass breeding congregations, typically taking place in temporary pans or wetlands for a few weeks after heavy rainfall in their breeding season. *Pyxicephalus adspersus* and *P. edulis* are listed under the Least Concern category of the International Union for Conservation of Nature (IUCN) Red List (IUCN SSC Amphibian Specialist Group 2013, 2016). However, both species have been reported to be experiencing population declines, primarily because of urbanisation causing conversion and degradation of suitable habitats (IUCN SSC Amphibian Specialist Group 2013, 2016). Roadkill is considered a significant contributor to reductions in *Pyxicephalus* populations; however, no major work has been performed to investigate the true extent of this threat. This study reports on short-term, mass vehicle-induced mortalities of juvenile *P. adspersus* within the borders of the NNR. The study serves as an *ad hoc* observation of how even minimal vehicle traffic during spawning events can generate high levels of roadkill.

Methods

On 14 February 2021, during a relatively wet season, *ad hoc* observations of extensive dead *P. adspersus* were observed on the main road and on many other roads of the NNR. This prompted the researchers (G.K.N. and E.A.J.) to walk a transect of approximately 2.7 km on the main road (Figure 1a), starting at the campsite (24° 38′ 58″ S; 28° 40′ 24″ E) and ending at the turnoff to

Jacana hide (24° 38′ 34″ S; 28° 41′ 37″ E). During the walked transect, the climatic conditions were hot and humid with no wind and heavy cloud cover. Each researcher walked along the main tyre tracks of the road, able to observe each half of the axial transect. The transect of the road was divided into 11 quadrants of c.a. 250 m long and dead *Pyxicephalus* spp. identified and enumerated within each section (live *Pyxicephalus* spp. were roughly estimated based on the size of groups present on the road and not counted because of the high density, of which many retreated into surrounding vegetation when approached). All roadkill consisted of individuals ~500 mm in size, clearly indicating they had recently completed metamorphosis. For identification, approximately every 20th dead on-road individual was closely inspected for the presence or absence of the following: (1) white dot on the tympanum, (2) several prominent, (3) interrupted skin ridges on the back and (4) a pale vertebral stripe, all characters that assist in distinguishing between juvenile *P. adspersus* and *P. edulis*. Because of the quantity of roadkill along the transect, and that no material was handled, the given method of identifying a subset of individuals was the most practical approach. As all tourist vehicles entering the NRR are required to sign a guest book upon entrance to the protected area, a record of traffic levels was also available. As such, the number of vehicles that entered the provincial park over the weekend was determined from the guest book. The number of times a car drove the single transect could not, however, be determined. Yet, all vehicles that enter the park will likely take the road surveyed at least once when entering or exiting the reserve.

Results

The total length of the transect produced 1005 dead juvenile *Pyxicephalus* spp. (Table 1). All ~ 50 dead bullfrogs assessed were identified as *P. adspersus*. Most roadkill observed were fresh (Figure 1b, c) and likely driven over either on 14 February, with a few expected to have been killed on the 13 February 2021. Of the 1005 dead bullfrogs, only 15, based on their dehydrated appearance, were considered to have been killed more than 48 h before the observation and were, thus, excluded from the total observations.

A total of 81 vehicles signed in and entered the gate between 13 February 2021 and 14 February 2021. The highest density of roadkill was present in the first 250 m road (1.02 dead on-road individual per meter), located in the flooded grasslands of a drainage line north of the main floodplain (Figure 1a). The density of roadkill started to decrease after 1.25 km, once the road bordered the main Nylsvley Floodplain. Estimates of live individuals also began to decline within the latter part of the transect.

On the day of the survey and the following day, many active individuals were seen in the early mornings (Figure 1d) and late afternoons. While midday had active individuals on the road, intensity of activity declined. When a vehicle approached, juveniles would scatter across to the sides of the road. Yet even with the slow speed of a tourist vehicle, many were still unable to escape death. Large congregations were often observed, these were often in muddy depressions in the

TABLE 1: Observations of *Pyxicephalus* spp. killed on a transect of the main road within the Nylsvley Nature Reserve between 24° 38′ 58″ S; 28° 40′ 24″ E and 24° 38′ 34″ S; 28° 41′ 37″ E DOR = dead on road.

Section	Number killed	Kills per meter	Start	End
1	254	1.02	24° 38′ 58″ S; 28° 40′ 24″ E	24° 39′ 02″ S; 28° 40′ 31″ E
2	135	0.54	24° 39′ 02″ S; 28° 40′ 31″ E	24° 39′ 01″ S; 28° 40′ 40″ E
3	55	0.22	24° 39′ 01″ S; 28° 40′ 40″ E	24° 38′ 59″ S; 28° 40′ 49″ E
4	129	0.52	24° 38′ 59″ S; 28° 40′ 49″ E	24° 38′ 57″ S; 28° 40′ 57″ E
5	165	0.66	24° 38′ 57″ S; 28° 40′ 57″ E	24° 38′ 55″ S; 28° 41′ 05″ E
6	73	0.29	24° 38′ 55″ S; 28° 41′ 05″ E	24° 38′ 53″ S; 28° 41′ 15″ E
7	60	0.24	24° 38′ 53″ S; 28° 41′ 15″ E	24° 38′ 55″ S; 28° 41′ 23″ E
8	85	0.34	24° 38′ 55″ S; 28° 41′ 23″ E	24° 38′ 55″ S; 28° 41′ 32″ E
9	16	0.06	24° 38′ 55″ S; 28° 41′ 32″ E	24° 38′ 47″ S; 28° 41′ 34″ E
10	14	0.06	24° 38′ 47″ S; 28° 41′ 34″ E	24° 38′ 40″ S; 28° 41′ 37″ E
11	21	0.08	24° 38′ 40″ S; 28° 41′ 37″ E	24° 38′ 34″ S; 28° 41′ 37″ E
Total	**1005**	**0.37**	-	-

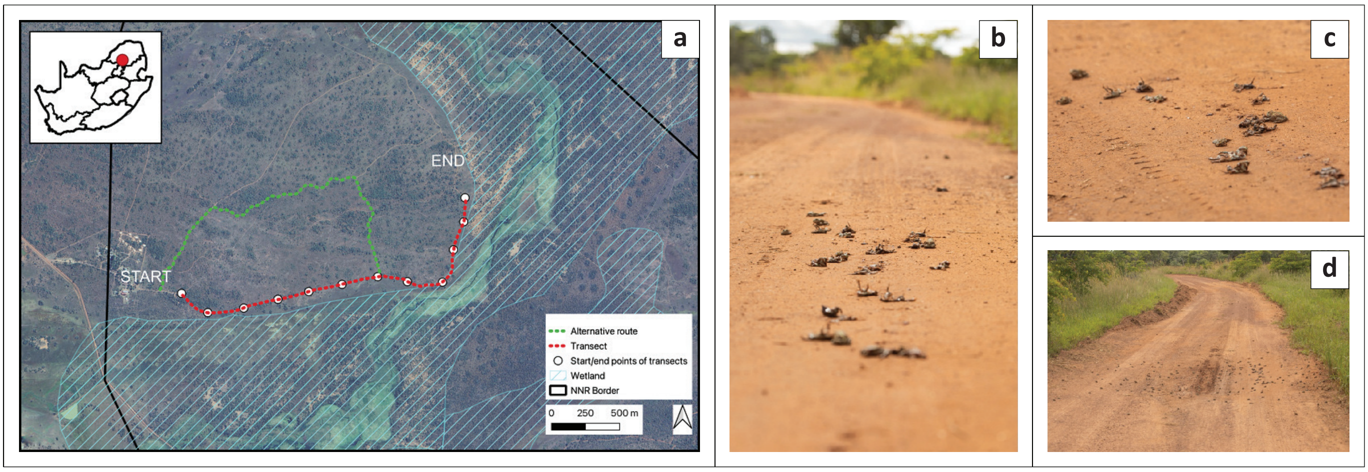

FIGURE 1: (a) Study site: Red Line – Transect walked to identify *Pyxicephalus* spp. roadkill on the main road within the Nylsvley Nature Reserve, Limpopo, South Africa. White dots – Start and end of each section of the transect. Green Line – Proposed alternative route to mitigate excessive roadkill during breeding season. Blue – Nylsvley Floodplain. Insert – Location of Nylsvley Nature Reserve. (b) Dead on road juvenile *Pyxicephalus* spp. on the main tyre tracks in Nylsvley Nature Reserve, Limpopo, South Africa. (c) A juvenile *Pyxicephalus* spp. scavenging on other juvenile *Pyxicephalus* spp. killed on the road. (d) A depression in the road formed high congregations of juvenile *Pyxicephalus* spp.

road (Figure 1d) or in sections were marshy grassland bordered the transect.

Discussion

Despite minimal, slow-moving traffic within a protected area, excessive *Pyxicephalus* roadkill was observed along a single road in the protected area NNR. While all inspected *Pyxicephalus* dead on-road were identified as *P. adspersus*, the presence of *P. edulis* among all the mortalities is plausible given that they have been recorded in the NNR. These findings further highlight how road networks that skirt wetlands are likely to increase vehicle-collision risk for certain taxa. These water bodies, embedded within a terrestrial matrix, serve as foraging and breeding patches for various invertebrate and vertebrate species (Cuthbert et al. 2022). Amphibians, as semi-aquatic organisms that typically require waterbodies to complete their life-cycles, are vulnerable to road mortalities in these areas. Those species with explosive breeding strategies, such as *Pyxicephalus* spp., are likely particularly vulnerable given their proclivity for mass aggregation by juveniles upon metamorphosis.

While this brief *ad hoc* study aimed to highlight the levels of bullfrog roadkill in a protected area over a short period, the quantified results only represented a fraction of mortalities that took place over the 2 days within the reserve's boundaries and the surrounding roads. Other roads within the park showed mortalities of similar quantities. In contrast, road networks outside the park appeared to have lower mortality numbers but would have likely had a greater traffic volume. It is possible that this is either because of more suitable breeding habitats within the reserve, these roads are less accessible for the young *Pyxicephalus* or that populations along the roads had already decreased because of roadkill.

It is possible that *Pyxicephalus* roadkill numbers were an underestimation, given that an abundance of predators were observed scavenging the dead on road and alive juvenile *Pyxicephalus* spp. These were not only predominantly avifauna but also included other opportunists such as a Southern Tree Agama (Jackson & Nicolau 2022) and other *Pyxicephalus* individuals (Figure 1c). Indeed, Santos, Carvalho and Mira (2011) found that amphibian carcases typically do not last long on the road relative to other taxonomic groups, with scavenging contributing to this observation. In several cases during this study, scavenging events by juvenile *Pyxicephalus* on dead on-road *Pyxicephalus* resulted in further deaths, after failing to move for oncoming vehicles.

It has been estimated that roadkill can significantly influence certain amphibian populations. Hels and Buchwald (2001) reported that up to 10% of certain populations of *Rana temporaria* (Common Frog) and *Rana arvalis* (Moor Frog) were killed annually through vehicle-induced mortalities. Smith and Dodd (2003), over a period of 1 year, found that 91% of all mortalities documented on a single road were herpetofauna, of which the majority were amphibians. Similar results were found by Glista et al. (2008), where 95% of all roadkill were reptiles and amphibians. The findings of the present study suggest that *Pyxicephalus* spp. may be particularly vulnerable to roadkill at certain times of the year. Similarly, a study assessing amphibian roadkill in the north of the Limpopo province found that *P. edulis* roadkill densities were among the highest of all species observed (Hlatshwayo et al. 2022). Areas of concern are likely those where breeding and tadpole metamorphosis takes place. This was presumably the case within the first section of the transect at NNR, where both roadkill and live estimates were elevated. There are many variables that could influence congregations of metamorphs, with the most likely being that this area was the most suitable breeding ground during the season.

It is plausible that simple mitigation measures for the species are possible, particularly in protected areas. Practical roadkill mitigation measures for amphibians have been through volunteer groups, along with temporary fencing and pitfall traps, allowing amphibians to be captured and moved across the road (Beebee 2013). Mitigation efforts such as small road tunnels and fences have also proven to be effective in reducing amphibian deaths (Beebee 2013), with one study showing a decrease in roadkill between 85% and 100% (Helldin & Petrovan 2019). Similarly, signage has been proven to be successful in limiting roadkill (Beebee 2013), through the alerting of drivers to the potential presence of fauna. In the NRR specifically, there is no dangerous megafauna within the reserve, which often hinders the implementation of temporary fencing and pitfall traps (Jordaan 2022). A simple culvert and drift fences over the first 250 m of the transect could potentially have mitigated much of the mortalities that occurred over 2 days. Similarly, as the public road network within NNR is a looped route, tourists could be restricted to entering and exiting the high-mortality sections during key periods. A short alternative road leading to the jacana hide is present within the reserve (Figure 1a). Despite still going past the floodplain, this route mitigates driving through the section with the highest mortalities from this study. Should the decision of alternative routes be taken by conservation officials, it is essential to determine whether the spawning events take place in the same locations each season. Alternative routes would likely be more successful than signs, given that the observed numbers of *Pyxicephalus* were so high that even cautious drivers would not be able to avoid all individuals on the road.

Conclusion

This study further highlights that even in formally protected areas, roadkill can be significant and warrants continuous management consideration. The extent a short road network with minimal traffic had on amphibian mortalities was considerable. This protected area could represent an ideal pilot site to investigate the efficacy of local simple and cost-effective mitigation measures on road networks in the proximity of wetland ecosystems, within the context of amphibian roadkill. As a part of this process, further data and monitoring of *Pyxicephalus* spp. roadkill and population levels within the Ramsar-protected area are required.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

G.K.N. and E.A.J. carried out the data collection. G.K.N. wrote the article with input from all authors E.A.J. and R.J.W. All authors G.K.N., E.A.J. and R.J.W. conceptualised the discussed project, discussed the results of the findings and contributed to the final manuscript. G.K.N., E.A.J. and R.J.W. contributed funds to the project.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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Data availability

Data sharing is not applicable to this article, as no new data were created or analysed in this study.

Disclaimer

The views expressed in the article are that of the authors and not an official position of the institution or funder.

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