

The effect of linear infrastructure on vertebrate fauna in Hluhluwe-iMfolozi Park and northeastern KwaZulu- Natal, South Africa

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ABSTRACT

Global anthropogenic growth continues to increase, threatening not only wildlife but the health of the entire biosphere. The anthropogenic growth coincides with an increase in vehicle traffic, placing further stress on natural environments as the mortality rates of fauna from vehicle collisions rise, thereby increasing human-wildlife conflict. It is to avoid this human-wildlife conflict that protected areas are so beneficial. However, global studies have shown that public roads in protected areas cause large numbers of wildlife mortalities, threatening the stability of these wildlife refugia, and that increasing tourism rates add further ecosystem stress by increasing wildlife-vehicle collisions (WVCs). The conflict faced by wildlife is also disproportionate between taxa, with reptiles and large predators facing the highest threat levels. Therefore, effective measures must be taken to minimise the effect of anthropogenic structures on natural environments as vehicle traffic density increases globally, particularly in Africa, where little research has been conducted on the ecological impact of linear infrastructure. In light of the conservation challenges associated with linear infrastructures, this study had four aims. Firstly, to identify the species assemblage of vertebrate WVCs along the R618 road that bisects Hluhluwe-iMfolozi Park (HiP) and of the R22 road that passes through the northern border of iSimangaliso Wetland Park (ISWP). Secondly, to determine the demographics of reptiles present on paved roads in HiP, ISWP and an adjacent developing community on ISWP's border. Thirdly, to determine the amount of road avoidance by lions (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) in HiP. Finally, to determine the interaction with anthropogenic structures by cheetahs (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*) in HiP. Roadkill and herpetofauna presence data were collected along R618 and R22 in HiP, ISWP, and the ISWP-adjacent Giba Rural Community (GRC) monthly from March 2021 to March 2023. Vertebrate mortality and reptile occupancy were

determined by assessing species diversity and abundance, mapping hotspots of mortality and reptile occupancy and using a suite of ecological factors to identify drivers of vertebrate mortality and reptile occupancy. Spatial analysis programs and global positioning system (GPS) data collected from 11 lions and 21 wildebeest were used to determine the average distance to roads for the two species and to examine how this distance differed across road types. Additionally, GPS data collected from nine collared cheetahs and 17 collared African wild dogs were used to determine the average distance to anthropogenic structures and to compare these distances across structures. In addition, hotspot analyses were conducted to determine key areas of use near roads or anthropogenic structures and statistical analyses of the habitat features were performed to determine how they affected the distance kept from roads for lion and wildebeest and anthropogenic structures for cheetah and wild dogs across multiple wet and dry seasons from May 2019 to April 2022. A total of 1606 road mortalities were recorded, with small-bodied vertebrates (< 5 kg) comprising most of the identified species. Amphibians had the highest mortality rate (1066 individuals), while birds had the highest species diversity (31 identified species). High mortality levels were concentrated in areas with unsloped road surfaces and increased vehicle traffic within HiP, while the surrounding habitat had a larger influence on mortalities within ISWP. Reptile presence was significantly higher in protected areas. However, the majority (78.3%) were found as road mortalities, with a significant difference between the number of live and dead individuals observed between protected and unprotected land. Snakes comprised the majority (70.3%) of observed reptiles, with reptile observation frequencies varying according to habitat intactness and type, as well as vehicle traffic patterns and road design, with light conditions having a minor influence on reptile road occupancy. The majority of lion and wildebeest GPS fixes were within 1 km of roads in HiP, with hotspots of wildebeest activity near roads (within 400 m) occurring close

to water sources, and hotspots of lion activity near roads occurring near areas frequently utilised by tourists or park staff. Where distance to dirt and paved roads, and to tourist locations, were key driving factors, significant differences were observed between lion and wildebeest males and females, with male lions and female wildebeest maintaining the smallest distances to roads. Finally, despite cheetah and wild dog heavily utilising over 20,000 ha (21%) of the total area protected by HiP, both species only had a significant presence in less than 3% (~2,880 ha) of HiP. Cheetahs also maintained greater distances from anthropogenic structures than wild dogs, with male cheetahs and female wild dogs maintaining the smallest distances for each species. Distances from anthropogenic structures were lowest near dirt roads and unfenced structures and highest from paved roads. The results showed that intervention is needed along portions of the two public roads in both protected areas and the ISWP adjacent GRC, particularly during the wet season, with mortality rates as high as 13 carcasses per km. Mitigation measures should be implemented to reduce wildlife mortality along roads in both locations, with specific measures targeting small vertebrates. All planned mitigation measures should focus on the sections identified as high mortality zones. Further studies are needed, as roads in HiP clearly negatively affect lions and blue wildebeest, with both species avoiding them. As hotspots of lion, blue wildebeest, cheetah, and African wild dog activity were centred around areas with typically high rates of human visitation, this increases the risk of human-wildlife conflict and the influence of human activity on movement patterns in both lions and blue wildebeest. This is of concern as lions, cheetahs, and African wild dogs are species whose persistence is threatened in HiP. Therefore, this study's results provide key insights into conservation concerns and further indicate the need for more effective species management to reduce human-wildlife conflicts in the rural communities surrounding HiP and ISWP.

PREFACE

The data described in this thesis were collected in Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park, KwaZulu-Natal, Republic of South Africa, from March 2021 to March 2023. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Prof Colleen T. Downs, and co-supervised by Dr Cormac Price and Dr Jarryd P. Striecher.

This thesis, submitted for the degree of Doctor of Philosophy in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

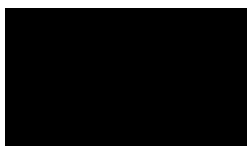


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Cameron T. Cormac

March 2025

I certify that the above statement is correct and as the candidate's supervisor, I have approved this thesis for submission.



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Prof Colleen T. Downs

Supervisor

March 2025

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DECLARATION 1 - PLAGIARISM

I, Cameron Cormac, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

PUBLICATION 1- Published – Global Ecology and Conservation

Effects of public roads on wildlife-vehicle collisions in two protected areas, Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park, in KwaZulu-Natal, South Africa

CT Cormac, C Price, W Collinson, DJ Druce, JP Streicher & CT Downs

Author contributions:

CTC conceived the paper with CP, DJD, WC and CTD. CTC collected and analysed data, and wrote the paper. CP, WC, DJD, JPS and CTD contributed valuable comments to the manuscript.

PUBLICATION 2- Published – Journal for Nature Conservation

Reptile demographics on paved roads in protected (Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park) and unprotected areas (Giba Rural Community) in north-eastern KwaZulu-Natal, South Africa

CT Cormac, C Price, JP Streicher & CT Downs

Author contributions:

CTC conceived the paper with CP, JPS and CTD. CTC collected and analysed data, and wrote the paper. CP, JPS and CTD contributed valuable comments to the manuscript.

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Road network avoidance by lion (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa

CT Cormac, C Price, DJ Druce, JP Streicher & CT Downs

Author contributions:

CTC conceived the paper with DJD and CTD. CTC collected and analysed data, and wrote the paper. CP, JPS and CTD contributed valuable comments to the manuscript.

PUBLICATION 4- not submitted

Interactions with anthropogenic infrastructure by cheetahs (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa

CT Cormac, C Price, DJ Druce, JP Streicher & CT Downs

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CHAPTER 1

Introduction

1.1 Background

As human populations increase, there is a concomitant increase in anthropogenic infrastructure and changes in land use. This anthropogenic growth coincides with increased vehicle traffic on roads, placing further stress on natural environments as the mortality rates of fauna increase because of vehicle collisions (Van der Ree et al., 2015). Additionally, in countries where large portions of the overall Gross Domestic Profit (GDP) involve income from ecotourism activities, increases in tourism also lead to higher numbers of wildlife mortalities because of collisions within protected areas (Forman and Alexander, 1998; Rendall et al., 2021). This increased growth also leads to an increase in the density of anthropogenic structures such as roads, powerlines, walls and fences. The global need for increased anthropogenic structures coincides with an increase in habitat change, habitat degradation and species loss (Ganivet, 2020). Additionally, as natural environments are altered to meet human needs, linear infrastructures such as roads and powerlines enable increased and more efficient exploitation of natural resources (Oztemel and Gursev, 2020). The loss of natural habitats also facilitates increases in the persecution of non-domesticated fauna because of inevitable increases in the conflicts between humans and wildlife (Nyhus, 2016). Therefore, effective measures must be taken to minimise the effect of anthropogenic structures on natural environments (Prodanova et al., 2023).

1.2 Effect of anthropogenic expansion

In the last century, the global human population has increased from approximately two billion in the 1900s to over eight billion at present (Ritchie et al., 2023; Worldometers, 2024). This rapid growth of the global human population has led to subsequent increases in habitat change

and species loss (Cunningham and Beazley, 2018). The United Nations Report (2019) on the global biodiversity crisis detailed that approximately one million species of plants and animals are currently at risk of extinction. There are numerous human-driven causes for this rapid rate of biodiversity loss, the largest of which are the clearance of land for agricultural purposes, particularly livestock production, and human habitation (Meyer and Turner, 1992; Thornton, 2010; Downs et al., 2021). This continual, rapid increase in anthropogenic land use change and resource demand drives an equally rapid growth in the need for structures that enhance the efficiency of resource production, transport and supply, as well as the protection of said resources. These structures include roads to access and distribute resources, power and pipelines to distribute the fuel and electricity needed to process and collect resources. Additionally, structures such as walls and fence lines are needed to protect and control the resources needed for human societal function. Structures such as roads are increasing by a rate of 5.7% (collected for years between 2009 and 2015) globally (Strano et al., 2017). However, the rate of road network growth is higher in developing countries, where population growth rates are greatest (Meijer et al., 2018). However, despite developing countries, particularly in Africa, having higher rates of development coupled with often being regions with high floral and faunal diversity, a significant deficit remains in the amount of research on the ecological effects of linear infrastructure conducted in these regions (Bennett, 2017).

As the scale of conversion of natural environments to increasingly urbanised areas continues to grow, the amount of conflict between humans and wildlife also increases (Nyhus, 2016). The exploitation of wildlife is one of the main sources of conflict between humans and wildlife (Maxwell et al., 2016). Between the years 2000 to 2015, Symes et al. (2018) found an average loss of 54% of species in the Sundaland biodiversity hotspot in Southeast Asia because of the combined effects of deforestation and wildlife trade. In Africa, the ivory and rhino horn trade has seen large reductions in rhinoceros populations, with 95% of white rhinos

(*Ceratotherium simum*) and 40% of black rhinos (*Diceros bicornis*) remaining in South Africa (Eikelboom et al., 2020) and a 4% per annum reduction in African elephant (*Loxodonta africana*) populations (Hauenstein et al., 2019). Another major source of conflict is the persecution of species that are seen as undesirable, such as snakes (Soanes et al., 2018; Parkin et al., 2020). Crawford and Andrews (2016) found that residents of Jekyll Island, Georgia, United States of America (USA), who responded to questionnaires about their views on vehicle collisions with wildlife, were least concerned about hitting snakes compared with hitting mammals or turtles. This was further supported by Beckmann and Shine (2012), who found that surveys to quantify how many drivers in the Northern Territory, Australia, intentionally targeted wildlife while driving found a discrepancy of 25% between responses stating that drivers intentionally targeted invasive cane toads (*Rhinella marina*) and how many native species were targeted. The conflict between humans and wildlife is not only caused by direct measures; conflict between humans and wildlife can arise indirectly, such as changes in available habitat, collisions with anthropogenic structures (Barrett et al., 2019), negative interactions between wildlife and farmers (Gemeda and Meles, 2018) and the spread and removal of alien species (Richardson et al., 2020).

As linear features are unnatural and the threat to the natural environment posed by roads became apparent, investigations into the threat of other linear anthropogenic structures gained attention (Geneletti, 2006; Dean et al., 2018). These structures include roads, fences, walls, powerlines, pipelines, railways and canals (Dean et al., 2018). The study of the effects of linear infrastructures on the natural environment is known as linear infrastructure ecology, a subset of which is road ecology (Dorsey et al., 2015). This is a diverse field of study, as each linear infrastructure type has unique challenges to the environments surrounding it and the researchers investigating its effects. However, all linear infrastructures have a set of common impacts that they share, such as habitat loss and fragmentation, hazards to species movements

and corridors that invasive species, particularly invasive plants and feral domestic animals, use for dispersal (Forman et al., 2003; Rahlao et al., 2010; Collinson et al., 2015; Doherty et al., 2015; Dean et al., 2018).

Roads have been a key factor, together with the development of human settlements, for as long as humans have needed to transport commodities. The oldest records of roads are adapted animal paths around springs found in Jericho, dated to 6000 BC, and the earliest paved roads date back to 4000 BC, with stone-paved roads in modern Iraq and timber-paved roads in Glastonbury, England (Lay et al., 2007). By 2000 BC, with the invention of cost-effective metal stone working tools and wheeled vehicles demanded improved roadways, paved roads became the norm. The Romans, however, were the most prolific road builders of the ancient world, with many roadway construction techniques still used in modern road development (Lay et al., 2007). Although updated with modern surfacing, several roadways in Britain from the Roman period are still in use today (Dalgaard et al., 2022).

Roads, however, pose a major threat to natural environments, particularly wildlife (Forman and Alexander, 1998). The impacts of roads are both direct and indirect, and may have both positive and negative effects on the wildlife communities in the areas where roads are placed (Vercayie and Herremans, 2015). Negative impacts of road infrastructure include barriers to species movements, habitat fragmentation, and changes in genetic diversity within communities (Soulsbury and White, 2015). Additionally, roads increase light, air, and other pollutants in areas, typically leading to habitat degradation (Soulsbury and White, 2015). As the density of road networks and the subsequent change in traffic volume (Jacobson et al., 2016), so do the impacts on the natural environments surrounding roads (Van Der Ree et al., 2015), as examples of the effect of roads on the surrounding habitat. As habitat change is the largest global cause of biodiversity decline (Elmqvist et al., 2016), measures must be taken to

reduce the impacts of roads on natural environments. For this to be achieved, the full extent of road networks' impact on surrounding environments must be understood.

The effects of road networks on the natural environment have been a known area of concern for nearly a century. The report by Stoner (1925) detailed the recording of a total of 225 identifiable vertebrate wildlife mortalities on roads in Iowa, USA, over four days between June and July 1924. However, the field of road ecology only became formalised in Europe, the United Kingdom and the USA in the 1970s (Tepper, 2011). However, as roads form a vital part of human societal development and environmental concerns continue to grow amongst the general public and scientific community, the field of road ecology is garnering increased attention. This is partly because of the seminal work conducted by Forman et al. (2003), which provided a framework that enabled city planners to directly incorporate the results of scientific research into infrastructure development.

The development of roads leads to habitat loss, increasing habitat fragmentation and reducing overall habitat permeability, which in turn reduces species movement capabilities (Bennett 2017). The genetic transfer capabilities of species are impacted, resulting in a reduction of overall biodiversity (Haddad et al., 2015; Assis et al., 2019). This reduction in species movement capabilities is greater in areas of higher road density as well as greater in species with already low movement capability because of body size and speed of movement (Assis et al., 2019). Evidence of the effect being greater on smaller or slower-moving species can be seen in Filius et al. (2020), where herpetofauna made up 72% of all road kills near Tena city, Napo, Ecuador. A significant difference was observed in the species composition of flying insects on either side of a large road near Stockholm, Sweden (Andersson et al., 2017). In a review of the causes of mortality in European wildlife from 1942 to 2017, 63% of mortality research attributed collisions with infrastructure and vehicles as the primary cause of vertebrate fauna mortality, with vehicles being the main cause in 47% of studies (Garcês et al., 2020). Of

the nine studies on amphibians reviewed, all reported collisions with vehicles as the main cause of death (Garcês et al., 2020). In addition to mortality, the barrier effect caused by roads is influenced by wildlife road avoidance behaviour, which can be divided into three separate behaviours: noise avoidance, road surface avoidance, and car avoidance (Jaeger et al., 2005; Duffett et al., 2020). Of the three, noise avoidance has the largest area of effect (Grace and Noss, 2018), with avoidance behaviours by leopards (*Panthera pardus*) in the Primorsky Krai region, Russia, displayed up to 500 m away from the nearby highway (Naidenko et al., 2021).

Herpetofauna are among the taxa whose populations are profoundly impacted by road infrastructure (Colino-Rabanal and Lizana, 2012). In addition to herpetofauna communities being affected by the barriers and pollution introduced by roads, they are also impacted by changes in drainage patterns caused by roads, particularly in the case of amphibians (Colino-Rabanal and Lizana, 2012). Morphometric and behavioural characteristics make herpetofauna susceptible to vehicle collisions (Heigl et al., 2017). Additionally, drivers may target herpetofauna, leading to disproportionate levels of mortality (de Resende Assis et al., 2022). In the case of amphibians, roads (particularly larger roads) introduce barriers that many smaller species may not be able to cross (Heigl et al., 2017). This, in turn, creates habitat fragmentation, leading to reductions in genetic diversity (Lukanov et al., 2014). Additionally, because of the susceptibility of many amphibian species, changes in the landscape, such as habitat loss and the introduction of pollutants, may lead to significant reductions in amphibian populations (Andrews et al., 2008; BeeBee, 2013; Hopkins and Brodie, 2015), leading to further reductions in genetic diversity.

In the case of reptiles, roads, in addition to causing habitat change, also introduce new artificial areas that many reptiles use to help behaviourally regulate their body temperatures, leading to increased mortality because of collisions with vehicles (Gonçalves et al., 2018). Because roads are either paved or compacted, they become effective heat sinks and can have

higher temperatures than the surrounding areas for extended periods (Malik et al., 2010). This attracts reptiles seeking heat sources to maintain their body temperatures, particularly in winter or overnight (Gonçalves et al., 2018). These factors make roads particularly detrimental to herpetofauna communities and necessitate targeted methods to mitigate their impacts on these communities.

Railways are second among anthropogenic linear structures in the level of their impact on the natural environment, where their major impacts on surrounding habitats are similar to roads in that they cause direct mortalities, habitat change and fragmentation and create a barrier effect (Van der Grift, 1999; Dorsey et al., 2015). Railways can cause mortalities in several ways because of their design and the speed at which trains travel. These include collisions between trains and wildlife, entrapment leading to dehydration and overexposure to heat and sunlight, as well as electrocution because of electrified rails and overhanging powerlines (Kornilev et al., 2006; Dorsey et al., 2015). Fence lines, while not as impactful as roads or railways, still have a variety of impacts on vertebrate fauna, impairing the movement and migration of animals between areas, which in turn reduces gene flow within a species (Hepenstrick et al., 2012). If fences are electrified, they can also cause mortalities because of electric shock (Beck, 2009; Lee et al., 2021). However, fences also provide a measure of protection to the fauna held within as they reduce the area into which humans are allowed to enter protected areas (McInturff et al., 2020), however, this is only effective for species with small ranges and where fencing aimed at guiding movements through safe crossings are effective (McInturff et al., 2020; Smith et al., 2020). They also reduce the amount of human-wildlife conflict that occurs between fauna and communities surrounding protected areas (Hayward and Kerley, 2009).

1.3 Effect of linear infrastructure on large mammals

A large portion of research into the effect linear structures have on wildlife has been framed by investigating the interactions between linear structures, humans and large mammals (mammals with body weight over 30 kg (Caughley and Krebs, 1983; Coffin, 2007). This is in large part because of the charismatic nature of most large mammals, with a significant majority of species globally being listed as threatened at various levels (Rondinini and Visconti, 2015). Additionally, many large mammals, particularly species that display herding behaviour, have large home ranges and make use of migratory pathways, which may be disrupted by linear structures (Kaphegyi et al., 2013). However, the largest likely cause of the strong focus on large mammals, and mammals in general, in linear infrastructure ecology is the risk of human death created during wildlife-vehicle collisions (WVCs) (Van der Ree et al., 2015). Between 2003 and 2017, in New England in the US state of Maine, over 57,000 WVCs with wild ungulates were recorded, 26 of which resulted in human fatalities, with the majority of the remaining cases resulting in drivers sustaining severe injuries (Clark et al., 2019). This risk of human death often drives political will, leading to calls for changes in policy and WVC mitigation (Forman and Alexander, 1998; Coffin, 2007; Van der Ree et al., 2015; Goldfarb, 2023). Beyond roads and railways and WVCs, human-wildlife conflict in the agricultural sector also drives political focus on the effect that fences have on large mammals, particularly in regions that utilize fencing in protected area management, because of the effects that large herbivores have on cropland and large predators on livestock farming (Boone and Hobbs, 2004; Zhang et al., 2023). For example, in the northern Apennines region of Italy, 165 wolf (*Canis lupus*) attributed depredation events occurred during 2011 to 2016, resulting in the loss of 528 livestock animals, while an additional 3,442 crop damage events attributed to wild boar (*Sus scrofa*) within the same time period (Davoli et al., 2022). However, roads, railways, and fences can have a significant impact on the ecology of the large mammal species that they impact.

Linear structures have a largely negative impact on large mammals, and as with environments in general, the effects of linear structures can be either direct or indirect (Van der Ree et al., 2015). Mortalities caused by WVCs, fence entanglements and electrocutions comprise the direct negative impact imposed by linear structures (Forman and Alexander, 1998; Jaeger et al., 2005; Van der Ree et al., 2015; Robb et al., 2022). While resulting indirect negatives include changes in selected habitat (Quiles and Barrientos, 2024), behaviour (Lewis et al., 2021), movement (Forman and Alexander, 1998; Ascensão et al., 2016; Wattles et al., 2018), migration patterns (Jaeger et al., 2005; Robb et al., 2022) and population size, density and health (Proctor et al., 2020). Additionally, as many large carnivores are apex predators, changes in their populations create a top-down negative trophic cascade, this cascade is not only caused by changes in the number of predators but also by changes in predator-prey interaction (Whittington et al., 2005; Proctor et al., 2020; Dennehy et al., 2021; Quintana et al., 2022; Ruiz-Capillas et al., 2022). At times, linear infrastructure can create an area where predation risk becomes negligible due to anthropogenic presence increasing avoidance behaviours in predator species (Prokopenko et al., 2016; Proctor et al., 2020; Ruiz-Capillas et al., 2021). Although anthropogenic linear infrastructures predominantly harm natural environments, they may be beneficial to certain species, particularly large herbivorous mammals. Roads may allow for easier movement through landscapes and increased hunting opportunities for species that can adapt to using them safely (Raiter et al., 2018). Fences may increase hunting success (Dupuis-Desormeaux et al., 2016). Additionally, species that employ scavenging behaviour can benefit from the mortalities that occur on roads as a result of WVCs (Hill et al., 2021) and mortalities from collisions with fences (Ruiz-Capillas et al., 2022).

1.4 Study of linear infrastructure effects in South Africa

Most research into the ecology of road and linear infrastructure is conducted in North America and Europe, with 46% and 30% of road network studies, respectively, taking place in these regions, as found in a review of road network studies conducted between 2011 and 2015 by Bennett (2017). The smallest proportion of global research on road ecology from 2011 to 2015 came from the African continent, which was responsible for only 3% of the total, with only three studies from South Africa (Bennett, 2017). As development rapidly grows within South Africa and the pressures placed on the environment and the organisations that protect it continue to increase, so does the focus on the effects of this rapid development and the call for measures to combat its negative environmental impacts. A review of the status of road ecology in Africa by Collinson et al. (2019) found that only 210 papers were published from the African continent between 1956 and 2017, with the highest number of publications (66) originating from South Africa. When using the search method employed by Collinson et al. (2019), we identified an additional 65 publications, primarily from South Africa, published between 2017 and 2025 (Supplementary Table S1.1).

As roads have the most significant direct impacts on the environment because of the continual and increasing risk of mortality because of collisions with vehicles, they have received the majority of studies in South Africa, with many examples from around the country. Bullock et al. (2011) found 184 carcasses during a study investigating bird and mammal mortalities along a road between Upington and Twee Rivieren base camp in South Africa's Northern Cape Province, with the bat-eared fox (*Otocyon megalotis*) being the most commonly found species. Another study done by Collinson et al. (2015) found 1027 carcasses along roads within the Greater Mapungungwe Transfrontier Park area in South Africa's Limpopo Province, where helmeted guinea fowl (*Numida meleagris*) and nightjars (*Caprimulgus* spp.) were the most affected. The nature of many South African herpetofauna species, particularly anurans, is

that they are endemic to small regions and under some level of threat of extinction; the impacts of roads on these species can be pronounced (Botts et al., 2013). In a short note by Coombs (2015), he described finding 36 Cape grass lizard (*Chamaesaura anguina anguina*) carcasses along a road on a farm in Grahamstown, in South Africa's Eastern Cape Province, only one day after a fynbos fire in the nearby area. However, despite the growing amount of information as to how roads and other linear structures affect South African wildlife, policy and legislative implementation that target sustainable development of transport systems is still lacking (Hlatshwayo et al., 2024) and a paucity of information on how roads and other linear structures affect wildlife remains, particularly around their effects on wildlife in protected areas.

1.5 Aims of the study

The aims of this study were fourfold. Firstly, to identify and compare the species assemblage of vertebrate wildlife-vehicle collisions (WVCs) along the ~18 km sections of the R618 road that bisects Hluhluwe-iMfolozi Park (HiP) and of the R22 road that passes through the northern border of iSimangaliso Wetland Park (ISWP). Secondly, to determine the demographics of reptiles present on paved roads in HiP, ISWP and an adjacent developing community on ISWP's border. Thirdly, to determine the rate of road avoidance by lion (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) in HiP. Finally, to determine the frequency of interaction with anthropogenic structures by cheetah (*Acinonyx jubatus*) and African wild dog (*Lycaon pictus*) in HiP.

The above four aims were achieved through the following objectives: Driven surveys were undertaken along the selected routes for all aims that concern wildlife movement and WVC-based mortalities. Species were identified using field guides, and carcasses of small to medium-sized vertebrates were collected for further studies. High-quality photographs of all specimens were taken in situ as well as from different profiles (head, side, underneath, etc.) to

show identifiable features. Data for the interaction between telemeter-collared large mammals and anthropogenic structures within HiP were collected from the relevant data holders. Maps were produced using geographical information systems (hereafter GIS) analyses and computational modelling incorporating environmental factors, as well as the number of mortalities and vertebrate activity, to determine areas of concern. Computational modelling based on the effects of environmental factors on the number of mortalities and observed reptile activity was used to determine high-risk species. These data were used to aid in mitigation measure recommendations.

Some preliminary predictions were that:

1. Herpetofauna species would make up most of the individuals killed or injured by WVCs, with small and slow-moving herpetofauna being most affected.
2. A higher number of WVCs would be observed along the ~ 18 km section of the R22 within ISWP than in HiP, as it has fewer impactful traffic control measures.
3. Higher numbers of herpetofauna WVC mortalities would be observed in protected areas.
4. Areas with smaller brush-to-road distances would contain higher numbers of WVCs because of lower visibility, reducing avoidance times for both wildlife and vehicle drivers.
5. Collared large mammals would spend most of the recorded activity away from anthropogenic structures within HiP, with areas with higher human activity being avoided more often.

1.6 Thesis structure

The main body of this thesis is organised as manuscripts prepared for publication in peer-reviewed journal articles. The first chapter (Chapter 1) is the Introduction, which provides a

review of the literature surrounding concepts covered in this study. The next four chapters (Chapters 2, 3, 4 and 5) are empirical, each covering a specific objective. Each chapter is formatted according to the journal to which it is intended to be (or has been) submitted. Because of this thesis format, a certain degree of repetition, especially in the methods section, was unavoidable. However, this is deemed to be of little concern as this format allows the reader to read each chapter separately without losing the overall context of the thesis. Chapter 2 investigated the effect of public roads on vertebrate fauna mortalities in HiP and ISWP. Chapter 3 investigated the demographics of reptile occupancy on paved roads in protected areas (HiP, ISWP) and unprotected land (Giba Rural Community) in north-eastern KwaZulu-Natal, South Africa. Chapter 4 investigated the avoidance of roads by lions (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) in HiP. Chapter 5 investigated anthropogenic infrastructure interaction by cheetahs (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*) in HiP. Chapter 6 provides a general discussion of the concepts covered in this study, as well as the overall recommendations and conclusions of the overall study.

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1.8 Supplementary information

Supplementary information Table S1.1. South African road ecology studies published from 2017 to the present.

Author (s)	Year	Publication Title	DOI Link
Joseph et al.	2017	The effect of infrastructure on the invasion of a generalist predator: Pied crows in southern Africa as a case-study	http://dx.doi.org/10.1016/j.biocon.2016.11.026
Mararakanye et al.	2017	Railway side mapping of alien plant distributions in Mpumalanga, South Africa	http://dx.doi.org/10.4102/abc.v47i1.2130
Belton et al.	2018	Anthropogenic influences on spotted hyaena diet in the Kruger National Park, South Africa	http://dx.doi.org/10.1007/s13364-018-0358-0
Dean et al.	2018	Linear structures in the Karoo, South Africa, and their impacts on biota	http://dx.doi.org/10.2989/10220119.2018.1514530
Haider et al.	2018	Mountain roads and non-native species modify elevational patterns of plant diversity	http://dx.doi.org/10.1111/geb.12727
Monti et al.	2018	The price of success: integrative long-term study reveals ecotourism impacts on a flagship species at a UNESCO site	http://dx.doi.org/10.1111/acv.12407
Patterson et al.	2018	Factors affecting presence of vervet monkey troops in a suburban matrix in KwaZulu-Natal, South Africa	http://dx.doi.org/10.1016/j.landurbplan.2017.09.016
Periquet et al.	2018	Testing the Value of Citizen Science for Roadkill Studies: A Case Study from South Africa	http://dx.doi.org/10.3389/fevo.2018.00015
Collinson et al.	2019a	Factors influencing the spatial patterns of vertebrate roadkill in South Africa: The Greater Mapungubwe Transfrontier Conservation Area as a case study	http://dx.doi.org/10.1111/aje.12628
Collinson et al.	2019b	Protecting the protected: reducing wildlife roadkill in protected areas	http://dx.doi.org/10.1111/acv.12481
Collinson et al.	2019c	Status of road ecology research in Africa: do we understand the impacts of roads, and how to successfully mitigate them?	https://doi.org/10.3389/fevo.2019.00479

Dean et al.	2019	A Review of the Impacts of Roads on Wildlife in Semi-Arid Regions	http://dx.doi.org/10.3390/d11050081
Mungate et al.	2019	Modelling the distribution of the invasive <i>Ziziphus mauritiana</i> along road corridors in Zimbabwe	http://dx.doi.org/10.1111/aje.12572
Paterson et al.	2019	Road avoidance and its energetic consequences for reptiles	http://dx.doi.org/10.1002/ece3.5515
Sibanda et al.	2019	Design of an Animal Detection System for Motor Vehicle Drivers	http://dx.doi.org/10.1016/j.procir.2019.04.175
Swart et al.	2019	The intermediate disturbance hypothesis explains arthropod beta-diversity responses to roads that cut through natural forests	http://dx.doi.org/10.1016/j.biocon.2019.03.045
Szott et al.	2019	Behavioural changes in African elephants in response to wildlife tourism	http://dx.doi.org/10.1111/jzo.12661
Williams et al.	2019	Using road patrol data to identify factors associated with carnivore roadkill counts	http://dx.doi.org/10.7717/peerj.6650
Ferreira et al.	2020	Dashcam based wildlife detection and classification using fused data sets of digital photographic and simulated imagery	http://dx.doi.org/10.23919/FUSION45008.2020.9190220
Grobler et al.	2020	Pollinator Activity and the Fecundity of a Rare and Highly Threatened Honeybush Species along a Highway in the Cape Floristic Region	http://dx.doi.org/10.1086/708385
Linden et al.	2020	Bridging the gap: How to design canopy bridges for arboreal guenons to mitigate road collisions	http://dx.doi.org/10.1016/j.biocon.2020.108560
Scholtz and van der Merwe	2020	We Can Deal with the Extra Feet, but Not the Extra Speed: the Importance of Providing a Memorable Experience in a Crowded National Park	http://dx.doi.org/10.1080/21568316.2020.1850516
Shilling et al.	2020	Designing wildlife-vehicle conflict observation systems to inform ecology and transportation studies	http://dx.doi.org/10.1016/j.biocon.2020.108797

Wiebke et al.	2020	Strength of correlation between wildlife collision data and hunting bags varies among ungulate species and with management scale	http://dx.doi.org/10.1007/s10344-020-01421-x
Young and Harrison	2020	Trends in populations of Blue Crane <i>Anthropoides paradiseus</i> in agricultural landscapes of Western Cape, South Africa, as measured by road counts	http://dx.doi.org/10.2989/00306525.2020.1781702
Henry et al.	2021	Optimising the cost of roadkill surveys based on an analysis of carcass persistence	http://dx.doi.org/10.1016/j.jenvman.2021.112664
Lala et al.	2021	Wildlife roadkill in the Tsavo Ecosystem, Kenya: identifying hotspots, potential drivers, and affected species	http://dx.doi.org/10.1016/j.heliyon.2021.e06364
Malherbe et al.	2021	Mammal road-type associations in Kruger National Park, South Africa: Common mammals do not avoid tar roads more than dirt roads	http://dx.doi.org/10.1002/ece3.8190
Muntifering et al.	2021	Black rhinoceros avoidance of tourist infrastructure and activity: planning and managing for coexistence	http://dx.doi.org/10.1017/S0030605318001606
Perumal et al.	2021	The impact of roads on sub-Saharan African ecosystems: a systematic review	http://dx.doi.org/10.1088/1748-9326/ac2ad9
Serieys et al.	2021	Road-crossings, vegetative cover, land use and poisons interact to influence corridor effectiveness	http://dx.doi.org/10.1016/j.biocon.2020.108930
Shaw et al.	2021	A large-scale experiment demonstrates that line marking reduces power line collision mortality for large terrestrial birds, but not bustards, in the Karoo, South Africa	http://dx.doi.org/10.1093/ornithapp/duaa067
Grobler et al.	2022	Road and landscape-context impacts on bird pollination in fynbos of the southeastern Cape Floristic Region	http://dx.doi.org/10.1016/j.sajb.2021.11.056
Lala et al.	2022	Influence of infrastructure, ecology, and underpass-dimensions on multi-year use of	http://dx.doi.org/10.1038/s41598-022-09555-5

		Standard Gauge Railway underpasses by mammals in Tsavo, Kenya	
Linden et al.	2022	Impact of linear infrastructure on South Africa's primate fauna: the need for mitigation	http://dx.doi.org/10.1163/14219980-20211112
Nandutu et al.	2022	Error Correction Based Deep Neural Networks for Modeling and Predicting South African Wildlife-Vehicle Collision Data	http://dx.doi.org/10.3390/math10213988
Perkins et al.	2022	Anthropause Opportunities: Experimental Perturbation of Road Traffic and the Potential Effects on Wildlife	http://dx.doi.org/10.3389/fevo.2022.833129
Alex et al.	2023	Transmission of viruses and other pathogenic microorganisms via road dust: Emissions, characterization, health risks, and mitigation measures	http://dx.doi.org/10.1016/j.apr.2022.101642
Cunneyworth et al.	2023	An effect of canopy bridges on monkey-vehicle collision hotspots: Spatial and spatiotemporal analyses	http://dx.doi.org/10.1002/ajp.23492
Davis et al.	2023	Using camera trap bycatch data to assess habitat use and the influence of human activity on African elephants (<i>Loxodonta africana</i>) in Kasungu National Park, Malawi	http://dx.doi.org/10.1007/s42991-022-00330-7
Hlatshwayo et al.	2023	An inventory of amphibian roadkill in the western Soutpansberg, Limpopo province, South Africa	http://dx.doi.org/10.1080/21564574.2022.2115154
Kissi et al.	2023	Examining green road construction components: the case of Ghana	http://dx.doi.org/10.1108/BEPAM-08-2022-0120
Krag et al.	2023	Impact of artificial waterholes on temporal partitioning in a carnivore guild: a comparison of activity patterns at artificial waterholes to roads and trails	http://dx.doi.org/10.7717/peerj.15253
Lyons et al.	2023	Forest roads: regional perspectives from around the world	http://dx.doi.org/10.1080/14942119.2022.2160916

Mambiravana and Umejesi	2023	Infrastructure development and environmental risk perceptions in the Wild Coast, South Africa	http://dx.doi.org/10.4102/jamba.v15i1.1377
Mulualem et al.	2023	Examining vertebrate road mortality on highways passing through protected areas of eastern Ethiopia	http://dx.doi.org/10.1007/s10344-023-01750-7
Munyati et al.	2023	Effects of proximity to highways on tissue properties of three tropical grazing grass species	http://dx.doi.org/10.1016/j.envdev.2023.100904
Praill et al.	2023	Road Infrastructure and Primate Conservation: Introducing the Global Primate Roadkill Database	http://dx.doi.org/10.3390/ani13101692
Reece et al.	2023	Patterns and predictors of ungulate space use across an isolated Miombo woodland reserve	http://dx.doi.org/10.1111/jzo.13059
Teichman et al.	2023	Movement Choices of Persecuted Caracals on Farmlands in South Africa	http://dx.doi.org/10.1016/j.rama.2023.02.004
Tucker et al.	2023	Behavioral responses of terrestrial mammals to COVID-19 lockdowns	http://dx.doi.org/10.1126/science.abo6499
Young et al.	2023	Where do wildlife cross the road? Experimental evaluation reveals fauna preferences for multiple types of crossing structures	http://dx.doi.org/10.1016/j.gecco.2023.e02570
Chui et al.	2024	Habitat use strategies of African elephants under different seasonal and ecological constraints	http://dx.doi.org/10.1071/WR23139
Deacon et al.	2024	Unpaved road verges are attractive habitats for dung beetles (Coleoptera: Scarabaeidae) in a plantation forestry-grassland mosaic	http://dx.doi.org/10.1007/s10841-024-00558-3
Hlatshwayo et al.	2024	Mainstreaming ecological connectivity and wildlife needs in green road transport infrastructure planning in South Africa	http://dx.doi.org/10.1016/j.jenvman.2024.123062
Horion et al.	2024	First insights into the spatio-temporal ecology of sympatric large carnivores in Niokolo-Koba National Park, Senegal	http://dx.doi.org/10.1017/S0030605323001746

Maharaj et al.	2024	Insights into the invasion of the moth catcher vine, <i>Araujia sericifera</i> (Apocynaceae), in South Africa	http://dx.doi.org/10.1016/j.sajb.2024.06.034
Selmoun-Ourdani et al.	2024	Understanding habitat suitability and road mortality for the conservation of the striped hyaena (<i>Hyaena hyaena</i>) in Batna (East Algeria)	http://dx.doi.org/10.1515/mammalia-2022-0095
Van de Wat et al.	2024	Integrating a "One Well-being" approach in elephant conservation: evaluating consequences of management interventions.	https://doi.org/10.5751/ES-15193-290315
Bhardwaj et al.	2025	Mortality on the tracks: spatiotemporal patterns to rail-kill in the Balule Nature Reserve, South Africa	http://dx.doi.org/10.1002/wlb3.01167
Cormac et al.	2025	Effects of public roads on wildlife-vehicle collisions in two protected areas, Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park, in KwaZulu-Natal, South Africa	http://dx.doi.org/10.1016/j.gecco.2024.e03368
Gomez et al.	2025	Understanding and predicting animal movements and distributions in the Anthropocene	http://dx.doi.org/10.1111/1365-2656.70040
Grilo et al.	2025	Global Roadkill Data: a dataset on terrestrial vertebrate mortality caused by collision with vehicles	http://dx.doi.org/10.1038/s41597-024-04207-x
Sempe et al.	2025	Road-associated variation in insect abundance differs between three common orders	http://dx.doi.org/10.1007/s42690-025-01467-x

CHAPTER 2

Effects of public roads on wildlife-vehicle collisions in two protected areas, Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park, in KwaZulu-Natal, South Africa

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Running header: Effects of public roads on wildlife-vehicle collisions in two protected areas

2.1 Abstract

Anthropogenic change is a global threat to wildlife and the biosphere's health. Wildlife faces growing threats from anthropogenic activities, especially infrastructure. Protected areas (PAs) are not exempt, particularly those that experience high traffic volumes on public road passages both within and on their peripheries. Globally, studies have found that public roads in PAs cause large numbers of wildlife-vehicle collisions (WVCs), threatening the stability of these wildlife refugia. As vehicle traffic density is increasing globally, particularly in Africa, where little research on the ecological impact of linear infrastructure has been done, the impact of roads on wildlife in protected areas must be determined. We determined the effect of public roads on vertebrate mortalities in the protected areas of Hluhluwe-iMfolozi Park (HiP) and iSimangaliso Wetland Park (ISWP), KwaZulu-Natal Province, South Africa, collected monthly in 2022 and 2023. Species composition of vertebrate mortalities, and heat maps of mortalities, combined with a suite of ecological factors, were used to identify drivers of wildlife-vehicle collisions. A total of 1606 road mortalities were recorded, with small-bodied vertebrates (< 5 kg) comprising most of the identified species. Amphibians experienced the highest number of mortalities, 1066 individuals, while birds had the highest species diversity in the mortality record, with 31 identified species. High mortality levels were concentrated in areas with unsloped road surfaces and increased vehicle traffic within HiP, while the surrounding habitat had a larger influence on mortalities within ISWP. Our results showed that intervention is needed along portions of the two public roads in both protected areas, particularly during the wet season, with mortality rates as high as 13 carcasses per km. Mitigation measures should be implemented to reduce the number of wildlife mortalities along the roads in both locations, with specific measures to mitigate small vertebrate road mortalities. All planned mitigation measures should focus on the sections identified as high mortality zones.

Keywords: linear infrastructure; vertebrate mortalities; road mortalities; protected areas; Africa

2.2 Introduction

Anthropogenic expansion is projected to grow exponentially over the next 30 years (Mottl et al., 2021), with most anthropogenic growth forecast for developing countries (Dulac, 2013; Zhou et al., 2019). The African road network density is projected to double by 2050 (Meijer et al., 2018) from the ~ 3 million km of road presently (Dulac, 2013; Laurance et al., 2017). Roads are a vital part of the human societal function (economics, health and recreational, etc.) by connecting communities and allowing for the transfer of goods and services (Weng et al., 2013; Freire et al., 2020). However, roads have a predominately negative impact on the natural environment (Van der Ree et al., 2015; Paterson et al., 2019) by creating obstacles to animal movement (Forman and Alexander, 1998), restricting gene flow (Strasburg, 2006), fragmenting natural habitats (Fahrig and Rytwinski, 2009), creating sources of pollution (light, noise and chemical) (Bennett, 2017), altering hydrological systems (Forman et al., 2003), and as a direct cause of mortality through wildlife-vehicle collisions (WVCs) (Grilo et al., 2009; Collinson et al., 2014). Despite the numerous negative impacts of roads on ecosystems, they may also attract species because of environmental changes (Hill et al., 2020). Attracting factors may include ease of movement for less WVC susceptible species (e.g., black bears (*Ursus americanus*) and wolves (*Canis lupus*) in Michigan, USA (Kautz et al., 2021)); food sources for scavengers (Antworth et al., 2005); heat sources aiding in thermoregulation (Sisson and Roosenburg, 2023) and more favourable habitat conditions along road edges (Hill et al., 2020).

The study of linear infrastructure ecology is a relatively new field, and road ecology, the study of the effects of roads on the natural environment (Forman et al., 2003), encompasses the largest portion of all linear infrastructure studies. It has been a field of study since the 1980s

in the Global North (van der Ree et al., 2015) and has only recently started gaining traction on the African continent (Collinson et al., 2019). Published research originates predominantly from the Global North (Collinson et al., 2015), and a review of the status of road ecology in Africa by Collinson et al. (2019) identified only 210 published road ecology studies from 1954 to 2016 across all of Africa. By contrast, Bennet (2017) found 215 papers published globally between 2011 and 2015, of which only 10 (4.7%) were from Africa, while 163 (76%) of included studies came from Europe (64; 30%) and North America (99; 46%). Despite the global understanding of the effect of road networks on natural environments, an extensive research gap persists in African-based literature (van der Ree et al., 2015; Bennett, 2017; Collinson et al., 2019).

The impacts of public roads that pass through protected areas are of particular concern, as these are designed to act as wildlife refugia (Haight and Hammill, 2020; Huang et al., 2021). Numerous studies have found that roads pose a threat to wildlife in protected areas, contravening their status as wildlife refugia (Garriga et al., 2012). The mortalities caused by roads in protected areas also place further strain on park management, which already struggles to control the wide variety of threats to fauna in reserves (Ramp et al., 2006; Garriga et al., 2012). The threat of public roads in protected areas has been an ongoing issue across the globe; an assessment of road impacts on wildlife in national parks in the United States found that over half of park management units reported that WVCs were affecting wildlife populations (Ament et al., 2008). Similarly, an assessment of WVCs on roads in Golestan National Park, Iran, found that WVC susceptible species had an average increase in mortalities of 95% between 2004 and 2013 (Kazemi et al., 2016). Studies on the African continent have also found similar trends. In Serengeti National Park, Tanzania, the main roads had the highest number of hyenas (*Crocuta crocuta*) roadkills (Naciri et al., 2023). Additionally, roads with higher traffic volumes in Serengeti National Park had higher rates of bird and mammal WVCs (Lyamuya et al., 2021).

Other major protected areas in Africa with high vehicle traffic also experience high rates of WVC on these roads within their boundaries, with 83 species of birds, mammals and reptiles identified as roadkill from 2007 to 2018 in the Tsavo Conservation Area (Lala et al., 2021). Additionally, 44 species of birds, mammals and reptiles were recorded as roadkill in four protected areas in Ethiopia (Mulualem et al., 2023), and 47 large mammal WVCs from 11 species between 2006 and 2013 were recorded (Gandiwa et al., 2020). Despite the findings detailing the effects of public roads on wildlife in protected areas in other African countries, minimal information has been published on the impact that public roads have on wildlife in protected areas in South Africa.

South Africa has 11280684 km² (9.2%) of its total land area classified as protected areas, with a total of 1527 protected areas (Department of Forestry, Fisheries and Environment, 2023). Hluhluwe-iMfolozi Park (HiP) and iSimangaliso Wetland Park (ISWP) are among the most significant because of their cultural importance as well as their contributions to conservation and ecotourism (Perissinotto et al., 2013; Cromsigt et al., 2017). Both protected areas act as large tourism magnets for the country and contribute to the overall Gross Domestic Product (GDP) of South Africa (Maniklall and Tifflin, 2023). Additionally, both these protected areas fall into the Maputaland-Pondoland-Albany biodiversity hotspot located on the eastern coast of South Africa's KwaZulu-Natal Province (Perissinotto et al., 2013; Cromsigt et al., 2017). Sections of major provincial roads also pass through both HiP and ISWP and do not mirror speed regulations of other interior protected areas roads, which have a maximum speed limit of 40 km/h as mandated by the amended Environmental Protection Act (57/2003) as part of Government Gazette 42967 (South African Government, 2020). As there is no information on how these major roads affect wildlife within these two protected areas, it is imperative that information be gathered so that mitigation may be effectively informed to curb any existing or future risk to the vital ecosystems within HiP and ISWP.

In light of the conservation challenges associated with roads, our research aimed to determine the effect of public roads on vertebrate species via mortality counts on the roads that pass through the protected areas of HiP and ISWP. Species composition of vertebrate mortalities and heat maps of mortality, combined with a suite of ecological factors, were used to identify drivers of WVCs. We predicted that small vertebrates (<5 kg) would make up the majority of mortality records because Borza et al. (2023) found that most drivers took extra measures to avoid collisions with large mammals because of the perceived increase in repair costs. In addition, we predicted that flat, straight sections of the road would have the highest mortality concentrations as these portions of roads allow for faster vehicle movement (Zolali et al., 2021). We predicted that vehicle traffic (Filius et al., 2020) and weather conditions (Mayer et al., 2021) would influence vertebrate mortality along the target roads most because of their effects on animal movement patterns.

2.3 Methods

2.3.1 Study areas

Our study took place along the two major public roads (the R618 and R22), which pass through HiP and ISWP, respectively, in the uMkhanyakude region of South Africa's KwaZulu-Natal Province (Fig. 2.1). This region is located in the northeast of the province and lies on the Mozambique coastal plain which experiences weather similar to the tropics (Cromsigt et al., 2017). The ISWP is South Africa's third-largest PA. It is classified as a Ramsar Site and was South Africa's first United Nations Educational, Scientific and Cultural Organisation (UNESCO) World Heritage Site (Perissinotto et al., 2013). The ISWP protects 1,328,901 ha of terrestrial and marine reserve area (Perissinotto et al., 2013; Isimangaliso, 2022). The HiP is one of South Africa's oldest protected areas, originally comprising two separate parks in 1895, but later joined into one entity to create the present-day park spanning 96,000 ha (Cromsigt et

al., 2017). The HiP is drier than ISWP as it falls along the edge of the geological monocline (Howison et al., 2017). The wet season is from September to April, with an average rainfall of ~950 mm, a maximum average rainfall exceeding 1500 mm, and an average maximum temperature of ~29 °C. The dry season is from May to August, with an average rainfall of ~20 mm, and an average minimum temperature of ~14 °C. Wet and dry season climatic variations are taken from a combination of historical information (Perissinotto et al., 2013; Crooms et al., 2017), as no contemporary information was available because of damaged weather monitoring equipment (pers. comm.).

The portion of the R618 road that falls within the boundaries of HiP is ~18 km in length and separates the northern (Hluhluwe) and southern (iMfolozi) portions of the park (Fig. 2.1). Present mitigation measures include cattle grids after ~400 m of fenced area at both the entrance and exit of this park's boundaries. The entrance and exit of this park also have video cameras and speed bumps, with additional speed bumps placed along the length of the public road. Structures that fauna could use to traverse the road safely include culverts. Culverts are found along all drainage lines along the road; however, they are designed chiefly for runoff dispersal, not animal movement. Due to the hilly terrain along the length of the road within this park's boundaries, V-shaped concrete runoff channels have been placed in all sloping sections of the road. The length of the R618 has also undergone resurfacing activities, with the portion of the road within park boundaries taking place from March 2021 to September 2022. The road has a speed limit of 40 km/h for ~400 m before and ~120 m after the park entrances, with the remainder having a speed limit of 60 km/h in the park and for 20 km on either side of the park. However, no structures or measures are in place that threaten drivers with financial or judicial consequences to force them to maintain these speeds. The presence of megaherbivores and other large mammals acts as additional traffic calming, with most motorists slowing or coming to a complete stop when said fauna are crossing the road (pers. comm.). Most of the road length

is surrounded by savanna-grassland with tree-dominated thickets along larger drainage lines (Howison et al., 2017). The road has an average width of 15 m and no fences that run parallel to the road.

The portion of the R22 road that falls within the boundaries of ISWP is ~15.5 km, and an additional 1.25 km on either side (Fig. 2.1) was surveyed to provide a one-to-one comparison with the HiP survey area. Present mitigation measures at the study site include cattle grids and speed bumps at the entrances of the reserve area and culverts every 300-400 m. However, there are no permanent traffic cameras, additional speed bumps past the entry sections, or a human development buffer zone past the park boundary fences. The road also has a speed limit of 60 km/h across its length, with the speed limit increasing to 80 km/h only one kilometre after this park's borders, and, as with HiP, there are no permanent measures to enforce speed limits. The road lies on predominantly flat terrain with a mixture of western Maputuland clay, western Maputuland sandy and Tembe sandy bushveld vegetation types (Kyle et al., 2021) with a dense patch of *Vachellia xanthophloea* dominated thicket along the Mkuze River. Additionally, the road runs directly through the eastern portion of Muzi Pan and several seasonal wetlands. The section of ISWP that the R22 splits is devoid of large mammals apart from the occasional presence of zebras (*Equus quagga*) and giraffes (*Giraffa camelopardalis giraffa*) in its vicinity; however, a large number of domestic cattle use the road, with their presence often slowing vehicle traffic. As with HiP, the road has an average width of 15 m and no fences that run parallel to the road.

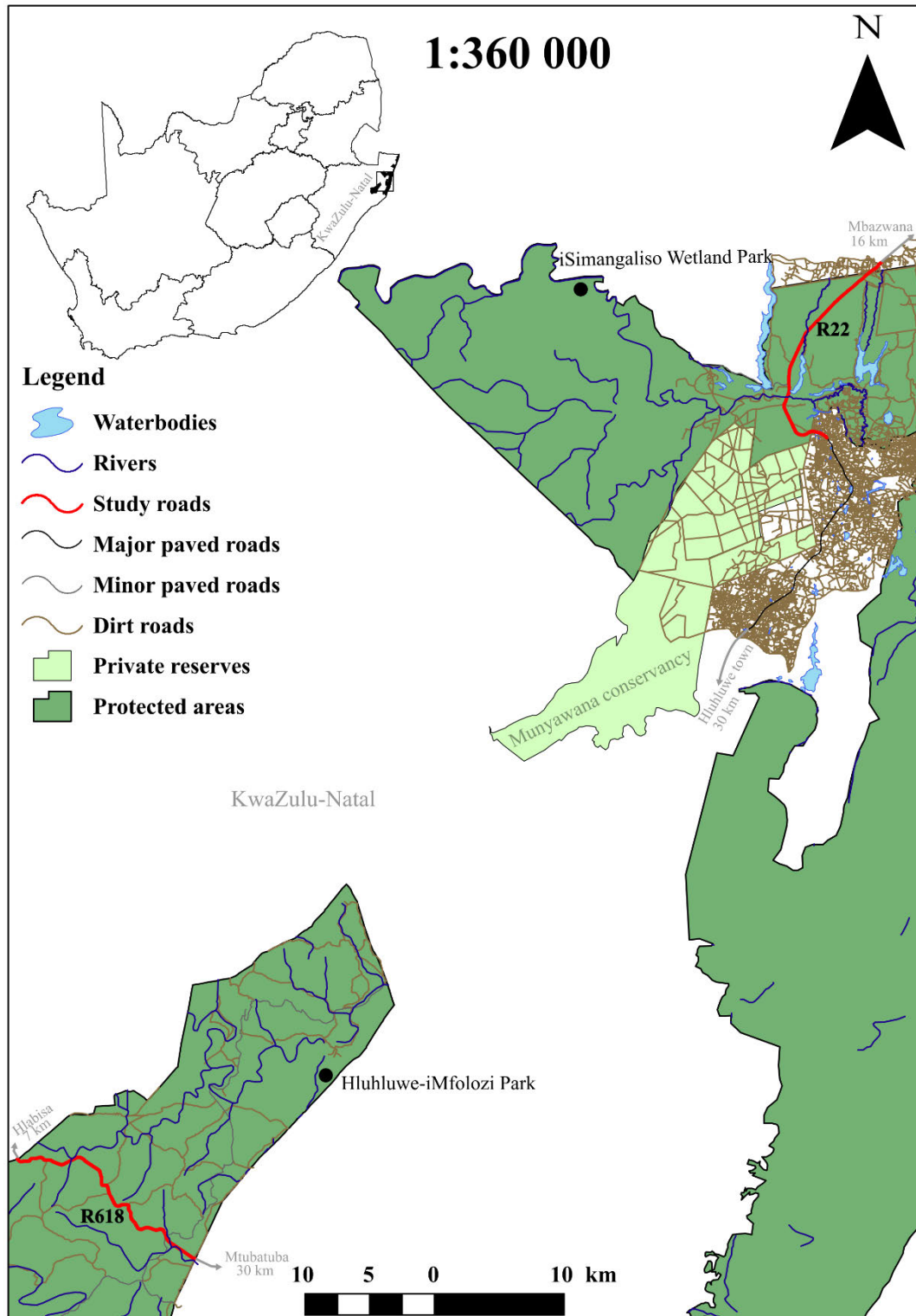


Figure 2.1: Location of study roads in Hluhluwe-iMfolozi Park (R618) and iSimangaliso Wetland Park (R22), KwaZulu-Natal Province, South Africa. (Arrows indicate the direction and distance to the nearest town labelled in grey).

2.3.2 Data collection

We collected animal-road-mortality data by conducting a week of driven surveys for two hours daily at sunrise and sunset once a month per site from March 2021 to March 2023. However, surveys could not be conducted from July 2022 till October 2022 (austral winter-early spring) because of financial constraints. Every fourth evening and the fifth morning were used as rest periods (selected based on observed reduction in detection efficiency after 3.5 consecutive days of the survey). We conducted all surveys with two observers (observers included one trained observer, with secondary observer training level varying based on personal availability; untrained observers were trained during the first 3.5 days of the consecutive survey). We conducted all surveys at an average speed of 40 km/h (minimum speed of 38 km/h and a maximum of 43 km/h) following the methods described in Collinson et al. (2014). We selected the survey times and speeds to allow for a maximum of four transects to be completed within 2 h, as well as to reduce the number of potentially missed carcasses and to include all faunal activity periods (diurnal, nocturnal, and crepuscular). We conducted surveys using a 2019 Ford Ranger XLT 2.0-L with cruise control activated to assist in speed maintenance. Additionally, we placed BrowningYo© (Birmingham, United States of America (USA)) Strike Force HD ProX trail cameras (model BTS-5HDPX) at each entrance of both protected areas to record vehicle traffic data for each survey period.

For each carcass we found, the following data were collected: identification (class-species level, depending on carcass condition); geographic location coordinates; photographs of the carcass (for verification purposes), and the surrounding environment; distance of road to nearest vegetation, as Lala et al. (2021) found that adjacent vegetation influenced WVC rates; road characteristics (surface slope presence, shape, side slope presence, shoulder vegetation presence, number of lanes) as Grilo et al. (2009) and Zolali et al. (2021) found that road design and maintenance affected both animal and driver behaviour on and around roads; habitat

characteristics (vegetation type, presence of water), as Filius et al. (2020) found that vegetation type and water presence influenced WVC rates; The number of vehicles that passed through the area on the day of discovery and the day of the week (as a proxy for vehicle traffic patterns); climatic conditions (rainfall, ambient air temperature, road surface temperature, presence of cloud cover, moon phase, moon illumination and time of day (As a proxy for solar light intensity, sunrise (AM) or sunset (PM)) as Garriga et al. (2017) and Linley et al. (2020) found that weather, temperature and light conditions influenced animal movement and WVC rates; the presence of wildlife paths and existing traffic calming measures and potential crossing structures as Hill et al. (2020) and Sisson and Roosenburg (2023) found that the presence of existing movement pathways and structures that increase traversal success rates influence road use and WVC rates.

We used a Samsung Galaxy A53 (Suwon-si, South Korea) to take photographs with geotags engaged and to collect geographical locations with a global positioning system (GPS) using the GPS logger app v.3.2.1 (BasicAirData; Paris, France). A 40 m hand-held laser distance meter with level (Mac Africa cc; Randburg, South Africa) was used to collect distance measures and slope presence. We recorded the presence/absence of wind and visual obstruction from the observed presence of wind, mist, or precipitation during sampling. We determined cloud cover categories based on the number of visible clouds: clear (no visible cloud), partly (intermittent cover), and overcast (complete cover). A non-contact forehead infrared thermometer with surface temperature mode (Medic spa; Yavne, Israel) was used for surface temperature readings. Ambient air temperatures were collected from the built-in Ford Ranger air temperature reader. Location-specific moon phases, moon illumination percentages, and sunrise and sunset times were collected using the Moon Phase Calender app v.1.54 (ProbadoSoft. Józefoslaw, Poland). On average, we spent 5 min of in-field processing time per carcass to maximise available search time. We confirmed identifications post-surveys using

morphometric traits, published field guides, and consultation with experts. This study was conducted as an Ezemvelo KZN Wildlife registered project, permit number E/5161/05.

2.3.3 Analyses

We conducted analyses using each week of surveys as a replicate, resulting in a total of 20 replicates per study area. Species Red List statuses were confirmed using International Union for Conservation of Nature (IUCN) species assessments (IUCN, 2023). To avoid excessive skewing of hotspots and statistical analyses, any cases where 20 or more mortalities of a single species were recorded on a road length of less than 50 m were removed from the data to be analysed separately. The first year of the study comprised the first 12 surveys, and the second year surveys from 13 to 20. We created heat maps of mortality using ArcGIS Pro (ESRI, 2023). These were created for each study area in the wet and dry seasons during the first and second years of data collection. We created clusters using constant density with a radius of 5 m at a scale of 1:100 000. To quantify the number of carcasses per kilometre and to conduct hotspot analyses, we divided the study roads into 1 km segments (Supplementary Information Fig. S2.1). To create road segments, we created a buffer with a width of 7.5 m (as the mean width of the paved surface was ~15 m) for the length of the study roads. The buffer was then separated into 1 km lengths using the generate points along lines tool and split using the split line at point tool. Point files were then spatially joined to produce counts per kilometre. Results of Moran's I test showed no significance from random, signifying that locations are sufficiently separated. We conducted hotspot analyses using the Getis-Ord G_i^* method (Ord and Getis, 1995) on the spatially joined point and segment files using fixed distance bands with a 1 m threshold and Euclidean distance. We did this to determine the significance of vertebrate mortality in each 1 km road segment across both locations per season. The resulting z-scores indicated clustering intensity, where positive values indicated hot spots, negative values indicated cold spots, and

p-values above 90% confidence indicated significant regions. We compared hotspot result layers using the ArcGIS Pro Hotspot analysis comparison tool (ESRI, 2023) to determine if any identified significant mortality road segments remained across seasons. Default fuzzy weights were used to compare across detected significance levels; nonsignificant features were not excluded. The number of neighbours was set to 17 to compare significances within locations. This was chosen to indicate location-specific significance changes and to avoid repetition of cross-location analysis. We conducted comparisons with the default of 499 permutations (ESRI, 2023). Monthly mortality rates (Supplementary Information Table S2.1) were calculated from average per kilometre mortality counts, with per-season mortality rate changes calculated from the changes between months surveyed within each season. This was done to account for the difficulties created by civil unrest and high fuel costs that occurred during the duration of the study.

Redundancy analyses (RDA) were conducted in RStudio (RStudio team, 2020) using the R-package “vegan” (Oksanen et al., 2013). The RDAs were conducted to determine the effects of various ecological factors, with factors grouped according to whether they had a physical presence in the environment or not (Supplementary Information Table S2.2). Physical factors included daily vehicle count, road, and habitat characteristics, while non-physical factors included light and weather characteristics. The RDAs were conducted for both between study area mortality counts and between mortalities per terrestrial vertebrate classes in each study area. We used the RStudio code to conduct analyses from the R workshop series created by the Québec Centre for Biodiversity Science (QCBS R Workshop Series, 2023). Where variables included categorical information, each category was included as a separate variable to determine the effect of each variable category on the overall system; category information is detailed in Supplementary Information Table S2.2. We selected the five most influential variables for display in the figures. Statistical analyses of the mortality demographics were also

conducted in RStudio using the “vegan” package. To determine the significance of carcass numbers between study areas, we conducted a two-sample t-test on the $\log_{10}$ transformed number of carcasses. Due to the data not conforming to the assumptions of parametric statistical tests, we ran a further Kruskal-Wallis test to determine the significance of the number of carcasses per terrestrial vertebrate class between and within study areas. We conducted pairwise Wilcox tests using a Bonferroni adjustment method for any Kruskal-Wallis tests where significance was found.

2.4 Results

2.4.1 Mortality demographics

We recorded a total of 1606 vertebrate road mortalities during our study, of which 509 and 1097 were logged for HiP and ISWP, respectively. Of the total mortalities found in ISWP, 660 individuals belonged to a single species of amphibian (*Pyxicephalus edulis*), recorded during five mass death events during a single week in March of 2023. Amphibians had the highest number of mortalities (HiP 241 (47.4%); ISWP 165 (37.7%)) followed by mammals (HiP 98 (19.3%); ISWP 105 (24.0%)) in both study areas (Fig. 2.2). Reptiles had the lowest number of road mortalities in HiP (77, 15.1%) compared with ISWP (96, 22.2%). Birds had the lowest number of road mortalities in ISWP (71, 16.2%) compared with HiP (93, 18.3%).

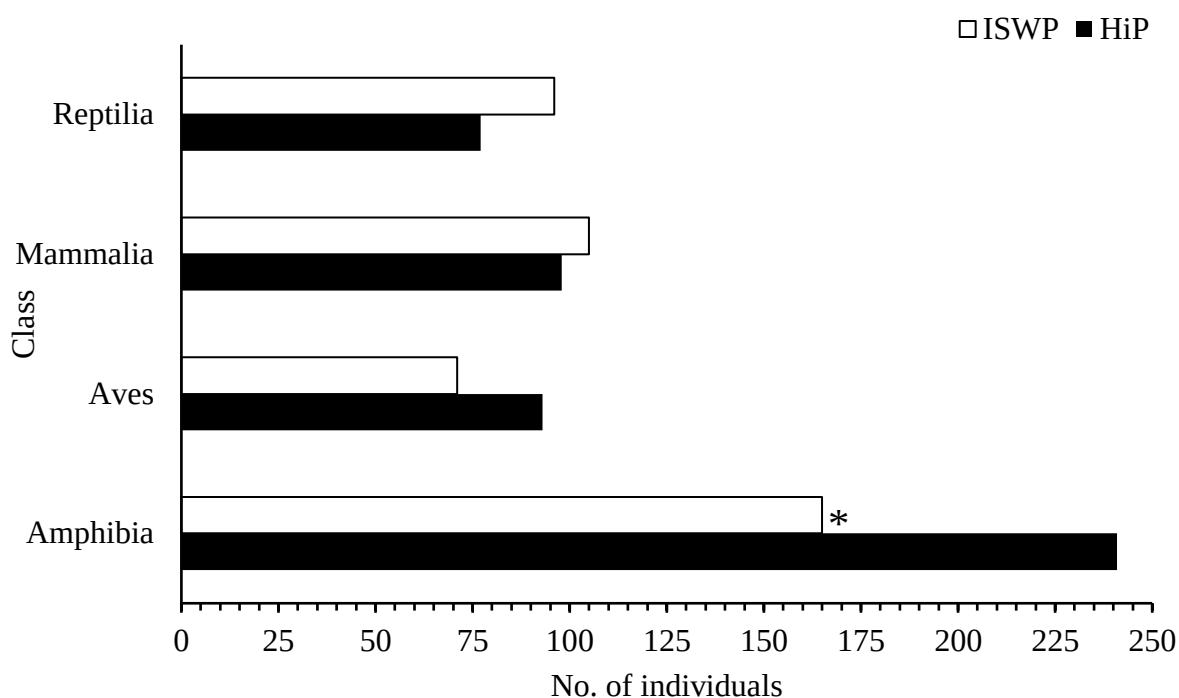


Figure 2.2: Mortalities per vertebrate taxa along survey roads in Hluhluwe-iMfolozi Park (HiP) and iSimangaliso Wetland Park (ISWP) from March 2021 to March 2023. (Note: * = 660 *Pyxicephalus edulis* mortalities that met the mass event removal criteria).

We found that of identified carcasses, HiP had higher taxonomic diversity represented in vertebrate road mortalities. Road mortalities in HiP represented a total of 76 species, 72 genera, 52 families and 21 orders. We found ISWP had a total of 73 species, 69 genera, 47 families and 18 orders represented in the vertebrate road mortality record. Of all the recorded mortalities, only seven came from species with an average body mass of over 5 kg. Species identifications, average body mass, and mortality numbers are present in Supplementary Information Table S2.3, and the demographics of unidentified mortalities are shown in Supplementary Information Table S2.4. At the species level, HiP had greater diversity in all vertebrate taxa except in reptiles. In HiP, birds comprised 29% (22) of recorded species, followed by amphibians (26%, 21), reptiles (24%, 18) and mammals (21%, 16). Reptiles comprised 32% (23) of recorded species in ISWP, followed by birds (27%, 20), amphibians

(25%, 18) and mammals (16%, 12). Above the species level, except at the order level, ISWP had greater diversity in reptile and bird road mortalities. We found no significant difference in the number of mortalities recorded between locations (T-tests, $p = 0.123$, $df = 38$, $t = 1.577$). We also found no significant difference between mortalities per vertebrate class both within ISWP (Kruskal-Wallis test, $\chi^2 = 2.703$, $df = 3$, $p = 0.440$) and between locations (Kruskal-Wallis test, $\chi^2 = -1.197$, $df = 3$, $p = 0.753$). We found an overall significant difference among mortalities per vertebrate class within HiP (Kruskal-Wallis test, $\chi^2 = 12.52$, $df = 3$, $p = 0.006$). However, we found that the significant differences were between amphibian mortalities and all other vertebrate taxa (pairwise Wilcox test, Aves: $p = 0.024$; Mammalia: $p = 0.025$; Reptilia: $p = 0.032$), but we found no significant difference between any other taxa ($p = 1.00$). Additional tables containing the listed threats to species were compiled from IUCN (2023) and South African National Biodiversity Institute (SANBI, 2023) species assessments (Supplementary Information Table S2.5).

2.4.2 Mortality hotspots

2.4.2.1 Hluhluwe-iMfolozi Park

During the first wet season (8 months – 2021-2022) in HiP (Fig. 2.3a), we recorded 234 vertebrate road mortalities with an average of 13 carcasses per km. In contrast, we recorded only 103 mortalities during the first dry season (4 months – 2021) (Fig. 2.3b), averaging 5.72 carcasses per km. During the second wet season (6 months – 2022-2023) (Fig. 2.4a), we recorded 152 vertebrate mortalities with an average of 8.44 carcasses per km compared with 20 carcasses (1.11 km^{-1}) in the second dry season (2 months – 2022) (Fig. 2.4b, Supplementary Information Table S2.1). The hotspot analysis of the first wet season identified only the second kilometre of the R618 in HiP (Supplementary Information Fig. S2.1a) as a hotspot of mortality. Analysis of the second wet season identified kilometres one ($z = 1.660$; $p = 0.097$), two ($z =$

2.432; $p = 0.015$) and four ($z = 1.917$; $p = 0.055$). During the first dry season of the first year, hotspots included kilometres seven ($z = 1.658$; $p = 0.097$), 13 ($z = 2.246$; $p = 0.025$), 15 ($z = 2.834$; $p = 0.005$) and 17 ($z = 1.658$; $p = 0.097$). While during the second dry season, mortality hotspots included kilometres two ($z = 1.739$; $p = 0.082$), 13 ($z = 2.596$; $p = 0.009$) and 18 ($z = 1.739$; $p = 0.082$). The degrees of freedom for all hotspot analyses were 35, and no significant cold spots were identified.

Hotspot comparison between wet seasons found an overall similarity of 0.93, yet only HiP kilometres one and two remained consistent across both wet seasons. Dry season comparison found an overall similarity of 0.91, with only kilometres two and 18 in HiP remaining consistent across dry seasons. The between-season hotspot comparisons found a global similarity of 0.86 between the first wet and dry seasons, with none of the hotspots remaining consistent; however, kilometre two retained a high mortality density. A global similarity of 0.88 was found between the second wet and dry seasons, with only kilometre two remaining as a consistent hotspot. A comparison of the first wet season and the second dry season found a global similarity of 0.92, with kilometre two in HiP remaining consistent. The comparison of the first dry season and second wet season had a global similarity of 0.87, with only kilometres one, two, and four remaining consistent hotspots. The first year had higher mortality rates than the second, with a 35% reduction in HiP between wet seasons and an 80% reduction between dry seasons.

2.4.2.2 *iSimangaliso Wetland Park*

During the first wet season (8 months – 2021-2022) in ISWP, we recorded a total of 229 carcasses (12.72 km^{-1}) (Fig. 2.5a) and 54 carcasses (3 km^{-1}) in the dry season (4 months – 2021) (Fig. 2.5b). During the second wet season (6 months – 2022-2023), we recorded 139 road mortalities (7.72 km^{-1}) (Fig. 2.6a) and 15 mortalities (0.83 km^{-1}) during the second dry season

(2 months – 2022) (Fig. 2.6b). The hotspot analysis of the first wet season identified the eighth kilometre of the R22 in ISWP (Supplementary Information Fig. S2.1b) with a z-score of 1.685 ($p = 0.092$). Analysis of the second wet season found only kilometre three ($z = 3.203$; $p = 0.001$) as a hotspot. During the first dry season, no hotspots were found in ISWP. An analysis of the second dry season found hotspots at kilometres eight ($z = 1.739$; $p = 0.082$) and 12 ($z = 2.596$; $p = 0.009$). The degrees of freedom for all hotspot analyses were 35, and no significant cold spots were identified.

Hotspot comparisons between wet seasons found an overall similarity of 0.93, yet no hotspots remained across wet seasons. Dry season comparisons found an overall similarity of 0.91, with no hotspots remaining across dry seasons. The between-season hotspot comparisons found a global similarity of 0.86 between the first wet and dry seasons, with none of the hotspots remaining between seasons. A global similarity of 0.88 was found between the second wet and dry seasons again, with no detected hotspots remaining across the season. Comparisons of the first wet season and second dry season found a global similarity of 0.92, with only kilometre eight remaining as a hotspot. A comparison of the first dry season and the second wet season had a global similarity of 0.87 again, with no consistent hotspots remaining. The wet season in ISWP had higher mortality rates than the dry season across both years of surveys; the first year also had higher mortality rates than the second, with a 39% reduction in mortality rate between wet seasons, and a 72% reduction between dry seasons.

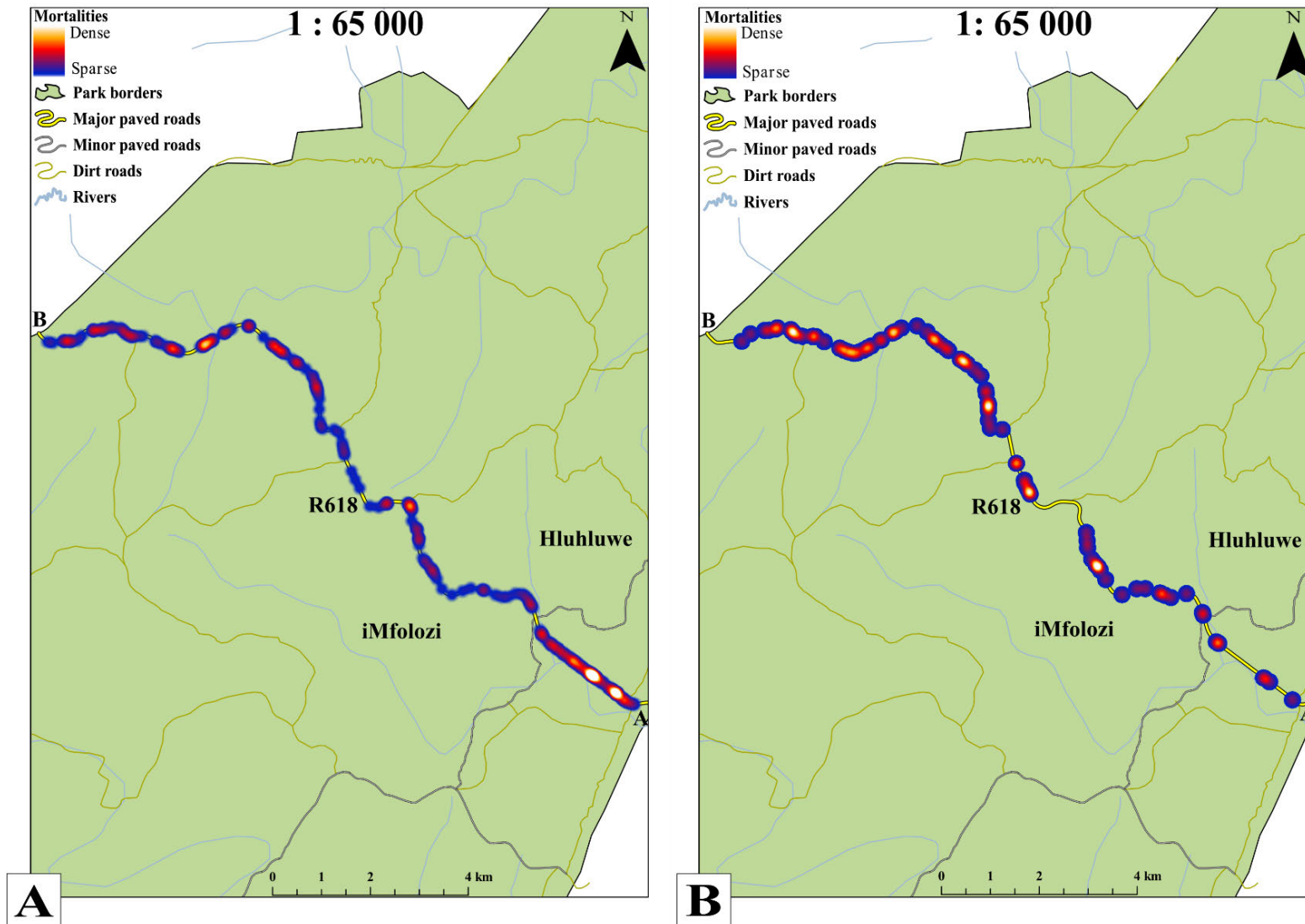


Figure 2.3: Vertebrate road mortality in Hluhluwe-iMfolozi Park from the 1st year, where a) is a heatmap of wet season mortalities, and b) a heatmap of dry season mortalities.

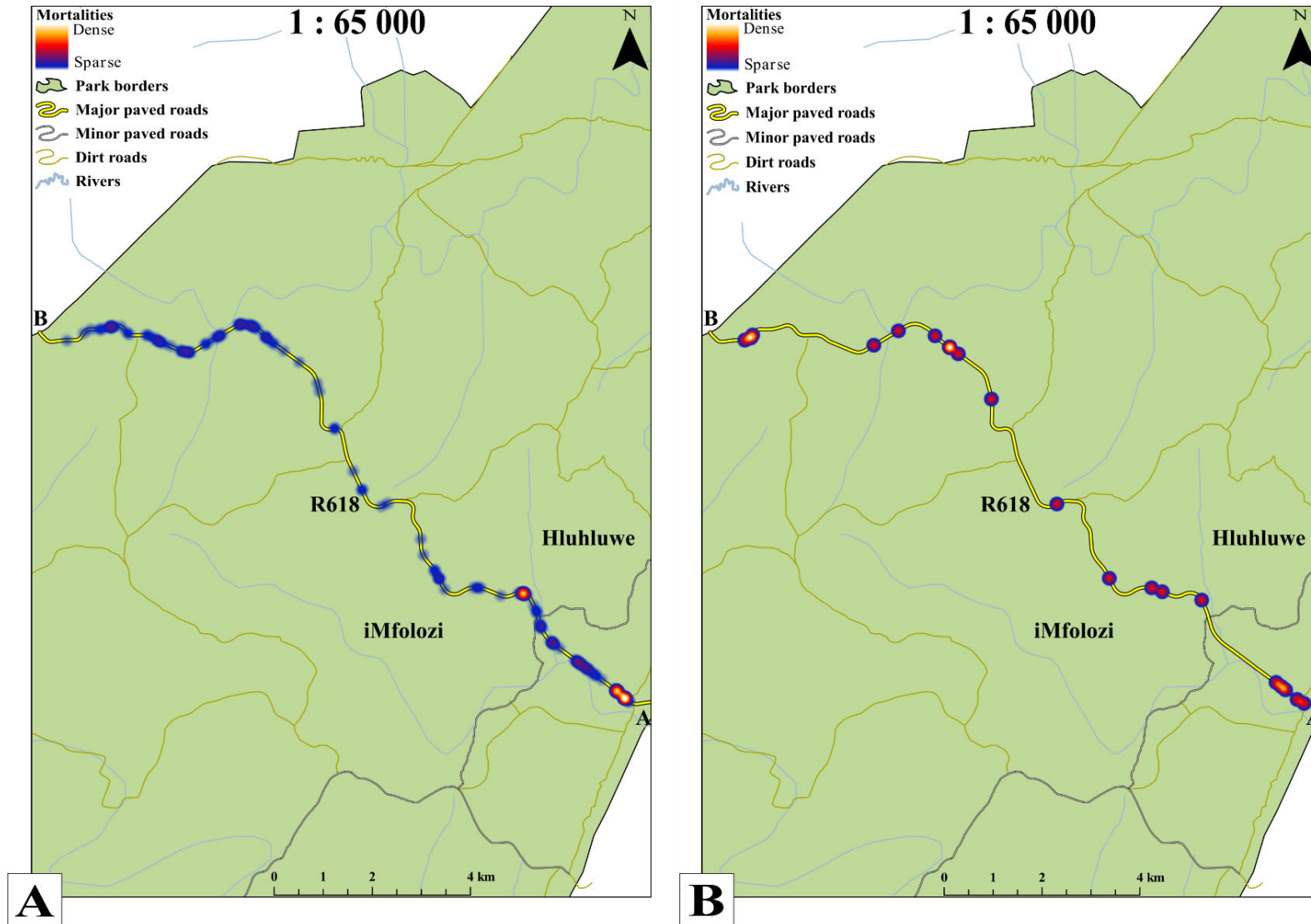


Figure 2.4: Vertebrate road mortality in Hluhluwe-iMfolozi Park from the 2nd year where a) is a heatmap of wet season mortalities, and b) a heatmap of dry season mortalities.

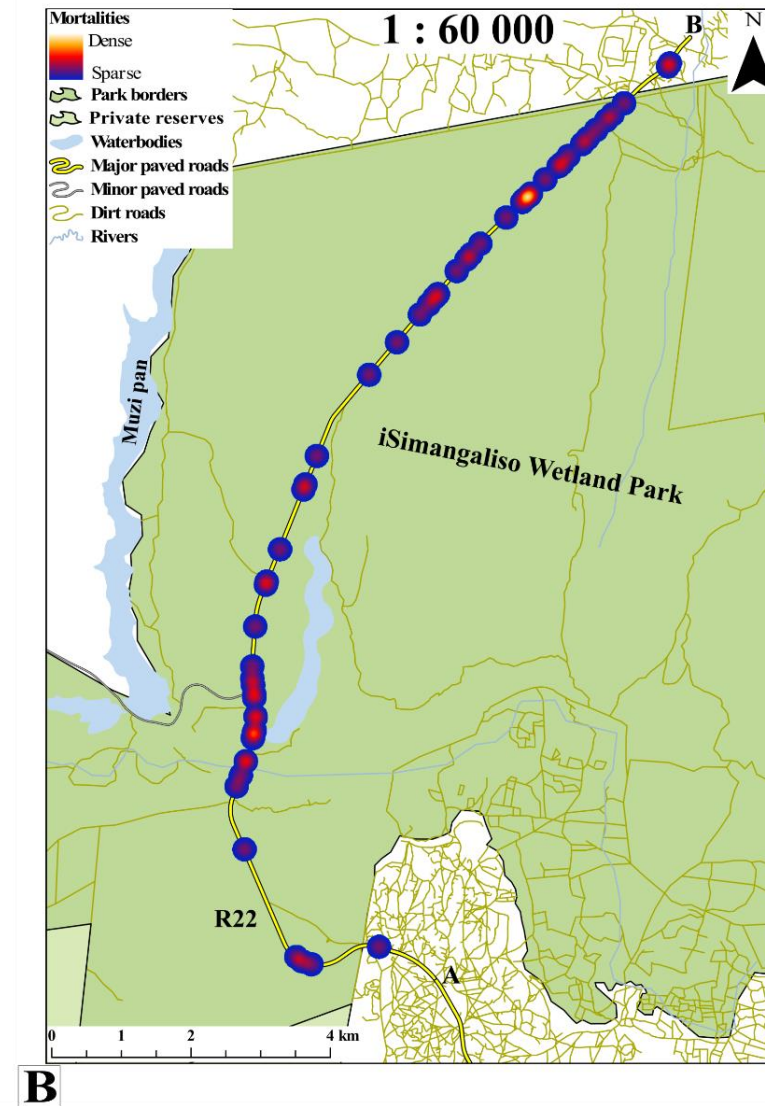
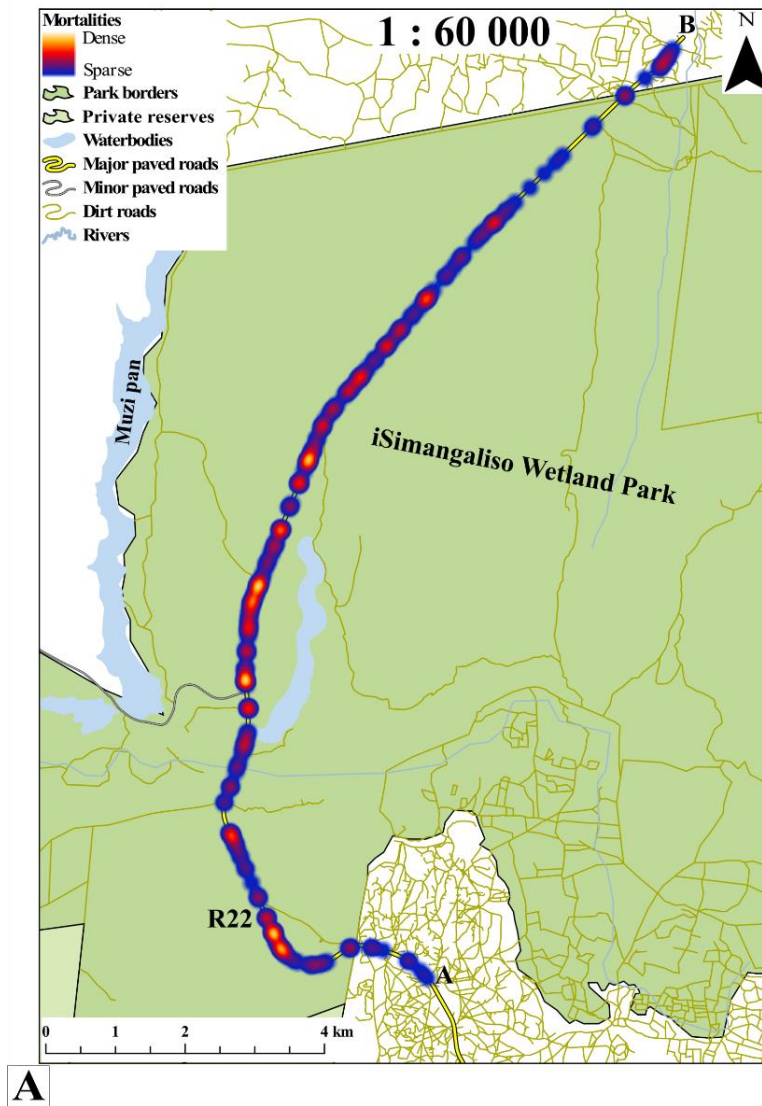


Figure 2.5: Vertebrate road mortality in iSimangaliso Wetland Park from the 1st year, where a) is a heatmap of wet season mortalities, and b) a heatmap of dry season mortalities.

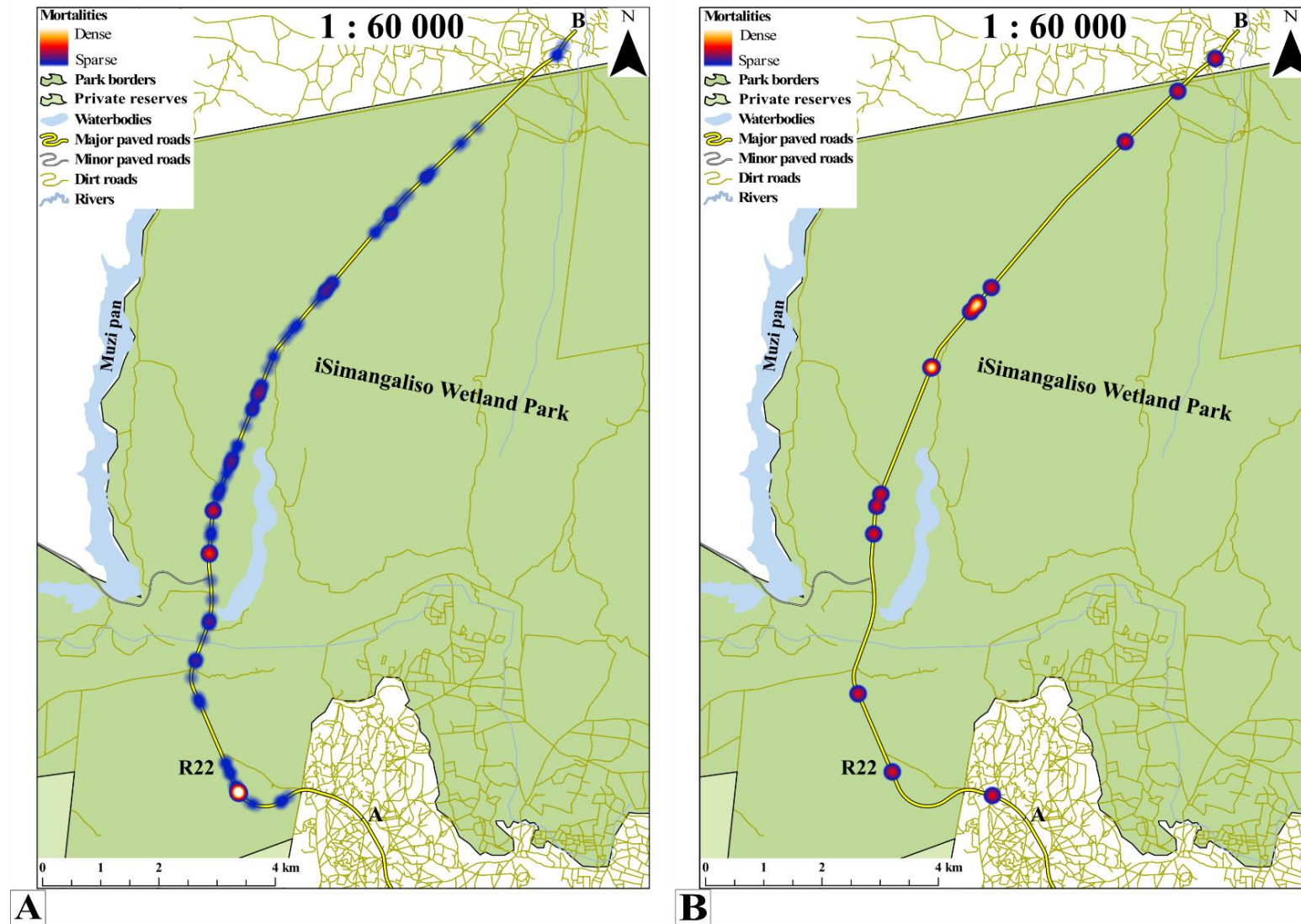


Figure 2.6: Vertebrate road mortality in iSimangaliso Wetland Park from the 2nd year where a) is a heatmap of wet season mortalities, and b) a heatmap of dry season mortalities.

Mortality rates in HiP were higher than in ISWP despite the lack of data for the missing months (July – October 2022), single-season rate differences between locations showed wet season changes below one carcass km⁻¹ (1st year = $\Delta 0.28$ carcasses km⁻¹; 2nd year = $\Delta 0.72$ carcasses km⁻¹). The dry season showed a larger variation (1st year = $\Delta 2.72$ carcasses km⁻¹; 2nd year = $\Delta 0.28$ carcasses km⁻¹). The portion of the R618 in HiP identified as a hotspot was surrounded by savanna habitat with several seasonal water bodies nearby, one of which was within 5 m of the road and 650 m away (at its furthest point) from a perennial stream (the Nyalazi River). Several animal paths intersected this portion of the road. In this kilometre, the road has been constructed in a straight line with a gentle slope in the direction of Hlabisa town, cresting shortly before the end of the portion of the road. Most of this portion of the road also has steep slopes on either side with no structures that fauna may use to traverse safely under or over the road. There are also V-shaped channels near the crest of the surface slope with two small (28 x 20 cm) speed bumps in the centre of the surface slope. These were two of only five-speed bumps that were not relocated during the resurfacing activities along the R618; however, they were altered in size previously, being 40 x 15 cm in size. This portion also lies between an unpaved road often used by park officials and tourist game-viewing vehicles, and the park's main entrance. During the dry season of the first year, resurfacing took place along this portion of the R618, and the seasonal water bodies and the Nyalazi River were completely dry of all surface water. All the remaining hotspots recorded both in the general and taxon-specific analyses were only present seasonally. Of all the recorded seasonal hotspots, only two were present across years, both of which were taxon-specific. One in the 13th kilometre of the R618 in HiP (Supplementary Information Fig. S2.1a) specific to Mammalia across dry seasons and another in the 3rd kilometre (Supplementary Information Fig. S2.1b) of the R22 in ISWP specific to Aves across wet seasons. A list of all taxon-specific mortalities can be found in Supplementary Information Table S2.6.

2.4.3 Factors influencing mortality

At the location level, the most influential physical factors leading to vertebrate road mortalities included the slope of the road, the presence of sloped edges on both sides of the road and the absence of standing water and the absence of sloped road edges (Fig. 2.7a). The list of constraining variable biplot scores is shown in Supplementary Information Table S2.7. Constraining variables explained 90.3% of the model of physical variables, while unconstrained variables explained the remaining 9.7%. The model had an adjusted R^2 score of 0.346 and a model significance of $p = 0.05$ ($F = 2.57$; $df = 5$), and Eigenvalues of 659.11 and 24.41 for Axis RDA1 and RDA2, respectively (Eigenvalues for all four axes can be found in Supplementary Information Table S2.7). Of the top influencing factors, only the absence of standing water (SW-A) showed a significant difference in effect between locations ($F = 8.33$; $df = 1$; $p = 0.001$). The most influential non-physical variables included time of day (AM and PM), the difference between road and air temperatures, the proportion of moon illumination and the absence of visual obstructions (Fig. 2.7b). The list of constraining variable biplot scores is shown in Supplementary Information Table S2.7. Constraining variables explained 40.4% of the non-physical RDA model, while unconstrained variables explained 59.6%. The model had an adjusted R^2 score of -0.259 and had no significance. With Eigenvalues of 263.57 and 88.88 for Axis RDA1 and RDA2, respectively (Eigenvalues for all four axes can be found in Supplementary Information Table S2.7). However, the absence of visual obstructions showed a mild significant difference in effect between locations ($F = 3.32$; $df = 1$; $p = 0.1$).

The RDAs of the taxa in HiP found that the most influential physical variables included the number of vehicles per day, the distance from carcass to vegetation, the presence of sloped edges on both sides of the road, the absence of sloped edges and the absence of standing water (Fig. 2.8a). The list of constraining variable biplot scores is shown in Supplementary Information Table S2.8. Constraining variables explained 90.0% of the model, while

unconstrained variables explained 10.0%. The RDA model of physical factors had an adjusted R^2 score of 0.515 and model significance ($F = 5.0$; $df = 5$; $p < 0.001$) and Eigenvalues of 182.13 and 127.77 for Axis RDA1 and RDA2, respectively (Eigenvalues for all four axes can be found in Supplementary Information Table S2.8). Of the top influencing factors, the absence of standing water (SW-A) showed a significant difference in effect between taxa ($F = 21.07$; $df = 1$; $p < 0.001$). The most influential non-physical variables included time of day (AM), the difference between road and air temperatures, day length, partial cloud cover and the absence of visual obstructions (Fig. 2.8b). The list of constraining variable biplot scores is shown in Supplementary Information Table S2.8. Constraining variables explained 57.6% of the model, while unconstrained variables explained the remaining 42.4%. The model had an adjusted R^2 score of 0.262 and significance of $p = 0.05$ ($F = 2.9259$; $df = 5$), with Eigenvalues of 119.66 and 14.97 for Axis RDA1 and RDA2, respectively (Eigenvalues for all four axes can be found in Supplementary information Table S2.8). The presence of partial cloud cover also had a significant difference in effect between taxa ($F = 8.6123$; $df = 1$, $p = 0.001$).

The RDAs of the taxa in ISWP found that the most influential physical variables included the number of vehicles per day, weekdays, straight road segments, and savanna and bushveld habitats (Fig. 2.9a). The list of constraining variable biplot scores is shown in Supplementary Information Table S2.9. Constraining variables explained 61.7% of the model, while unconstrained variables explained the remaining 38.3%. The RDA model of environmental variables had an adjusted R^2 score of 0.098 but no model significance and Eigenvalues of 146.66 and 43.05 for Axis RDA1 and RDA2, respectively (Eigenvalues for all four axes can be found in Supplementary Information Table S2.9). Of the top influencing factors, however, savanna habitat (H-Sav) showed a significant difference in effect between taxa ($F = 3.8256$; $df = 1$; $p = 0.05$). The most influential non-physical variables included time of day (AM), day length, moon illumination proportion, and no or partial cloud cover (Fig.

2.9b). The list of constraining variable biplot scores is shown in Supplementary Information Table S2.9. Constraining variables explained 25.6% of the model, while unconstrained variables explained the remaining 74.4%. The model had an adjusted R^2 score of -0.032 and had no significance, with Eigenvalues of 63.59 and 14.61 for Axis RDA1 and RDA2, respectively (Eigenvalues for all four axes can be found in Supplementary information Table S2.9). The proportion of moon illumination had a mild, significant difference in effect between taxa ($F = 3.2381$; $df = 1$, $p = 0.01$).

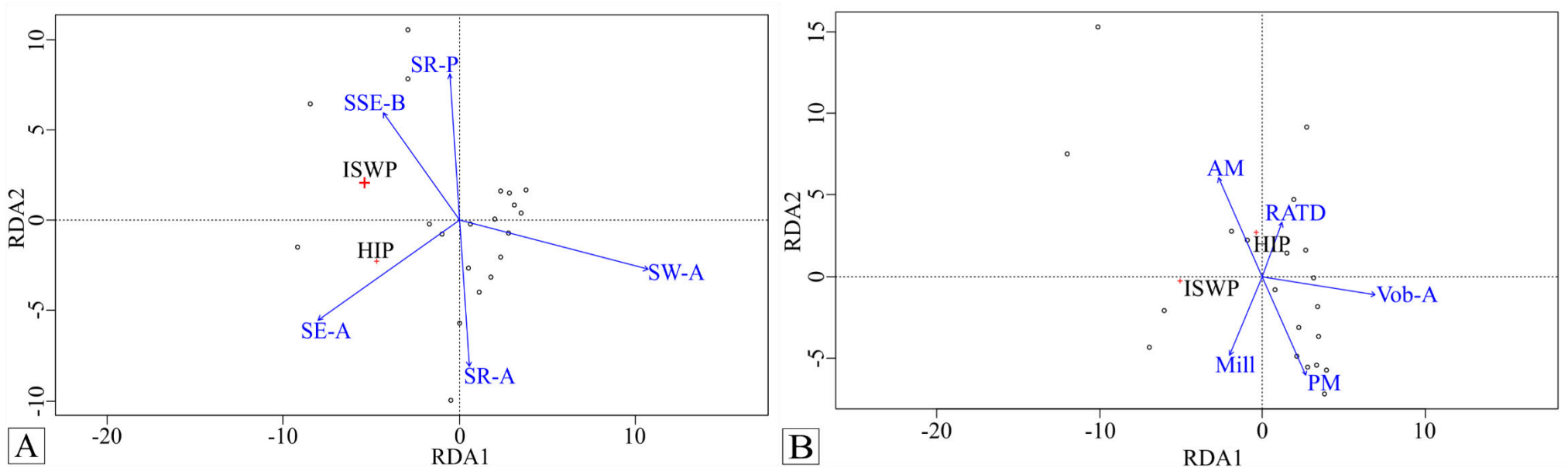


Figure 2.7: Influencing factors with the highest impact on vertebrate road mortalities in Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park, KwaZulu-Natal, South Africa, where a) is the effect of physical variables (Sloped road edge-Absent (SE-A); Sloped road surface-Absent (SR-A); Sloped road surface-Present (SR-P); Side of sloped edge-Both (SSE-B); Standing water-Absent (SW-A)) and b) the effect of non-physical variables (moon illumination (Mill); road-Air temperature differential (RATD); time of day-morning (AM); time of day-evening (PM); Visual obstruction-absent (Vob-A)).

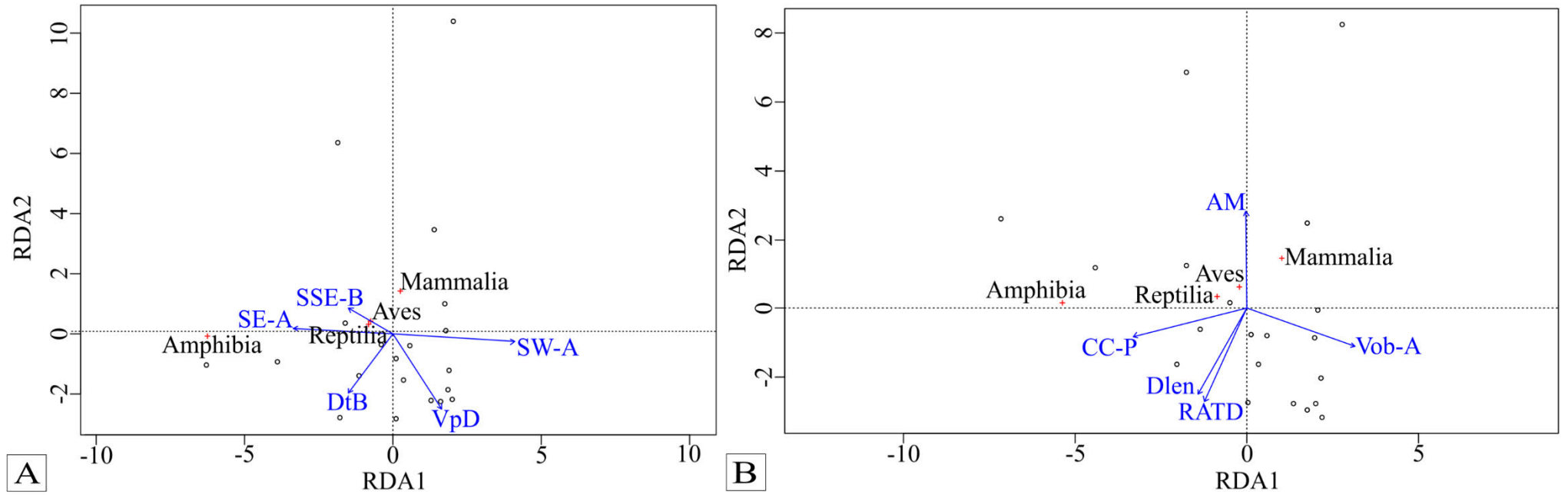


Figure 2.8: Influencing factors with the highest impact on road mortalities of vertebrate taxa within Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa, where a) is the effect of physical variables (distance to brush (DtB); sloped road edge-Absent (SE-A); side of sloped edge-both (SSE-B); standing water-absent (SW-A); vehicles per day (VpD)) and b) the effect of non-physical variables (cloud cover-partly (CC-P); day length (Dlen); road-air temperature differential (RATD); and time of day-morning (AM)).

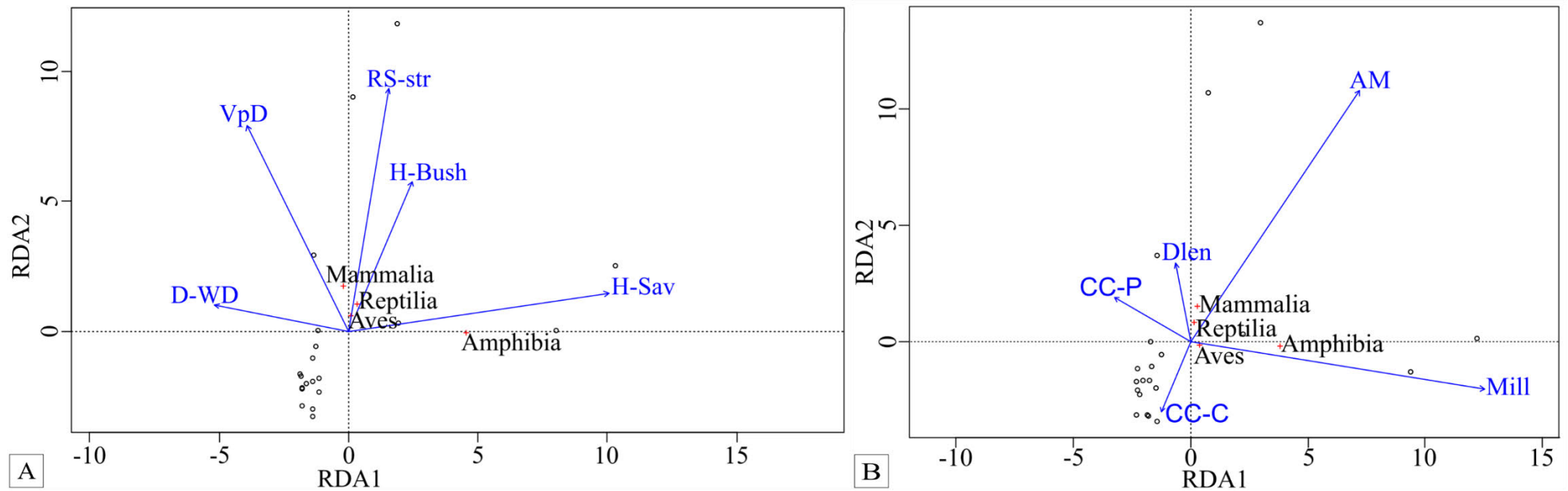


Figure 2.9: Influencing factors with the highest impact on road mortalities of vertebrate taxa within iSimangaliso Wetland Park, KwaZulu-Natal, South Africa, where a) is the effect of physical variables (day of week-weekday; habitat-bushveld (H-Bush); habitat-savanna (H-Sav); Road Shape-Straight; vehicles per day (vpd), and b) effect of non-physical variables: cloud cover-clear (CC-C); cloud cover-partly (CC-P); day length (Dlen); moon illumination (Mill); and time of day-Morning (AM)).

2.5 Discussion

Our study aimed to determine the effect of paved public roads on vertebrate road mortalities in two protected areas, HiP and ISWP. We found that the public paved roads passing through HiP and ISWP had relatively similar effects on vertebrate road mortalities. The geographic location of mortalities was near constant within each location across seasons; however, mortality rates were significantly higher during the wet season. Environmental factors showed differences in the factors most influential in mortality rates at the two sites. Mortalities were also distributed evenly across taxa and locations, with only amphibians in HiP showing a significant difference in mortalities.

2.5.1 Mortality demographics

Despite the removal of 660 *P. edulis* mortalities recorded in ISWP from the overall data set, amphibians still had the highest number of mortalities across all locations, with double the total number of mortalities than mammals, which had the second highest mortality count. When considering the taxonomic diversity of road mortalities, the trends were opposite to that of the mortality counts, with birds and reptiles having the highest taxonomic diversity in HiP and ISWP, respectively, with both reptiles and birds showing greater taxonomic diversity in ISWP. However, birds and mammals had higher numbers of individuals that could not be identified as species because the carcasses of these taxa were in poorer condition, with passerines and murids being particularly difficult to identify.

The wet season carcass km^{-1} rate of the roads surveyed in our study was relatively high for South Africa, with a rate of 13 carcass km^{-1} compared with another study that found a wet season rate of 4.3 carcass km^{-1} on paved roads (Collinson et al., 2015). Additionally, studies in Tanzania found a rate of 0.13 carcass km^{-1} (Kioko et al., 2015) and a rate of 0.11 carcass km^{-1} in the Tsavo Conservation Area between Nairobi and Mombasa, Kenya (Lala et al., 2021).

Similarly, Perumal et al. (2021) also found that roads within protected areas are no different in their level of protection to species than areas outside of protection. However, regardless of body size category or location, roads are still considered to be a significant and continually growing threat to the safety of both humans and wildlife (Forman and Alexander, 1998; van der Ree et al., 2015; Collinson et al., 2019), with anthropogenic driven habitat change in general being one of the greatest threats to biodiversity globally (Almeida-Rocha et al., 2020; Doherty et al., 2020; Prakash and Verma, 2022). However, despite this, of the 106 species identified in our study, only 26 had habitat change or road-related issues listed as threats to species at an international level and only 22 listed these issues as threats locally (Supplementary Information Table S2.5).

2.5.2 Mortality hotspots

The wet season had higher mortality rates than the dry season across both years of surveys, with mortality rates higher in HiP than in ISWP (except for the numerous mass *P. edulis* death events during the week survey in March of 2023). Despite both wet and dry seasons having above 85% similarity in the general distribution of vertebrate road mortalities across both years of our study, only one portion of the R618 in HiP remained a hotspot of mortality throughout the majority of our study, except for the dry season of the first year. The presence of this hotspot and the break in its continuous presence, caused by road resurfacing activities, is similar to results seen in studies of road avoidance in forest-dwelling caribou (*Rangifer tarandus caribou*) in the Laurentides Wildlife Reserve near Québec City, Canada, where avoidance increased as road modification increased, and movement rates increased with traffic and human activity density (Leblond et al., 2012). Additionally, both Blanding's turtles (*Emydoidea blandingii*) in Ontario, Minnesota and Maine, USA, and eastern massasaugas (*Sistrurus catenatus*) in Ontario and Michigan, USA, avoided crossing roads with higher human activity (Paterson et al., 2019).

Moreover, a review found that anthropogenic habitat alteration could have long-term effects on area avoidance by local fauna (Ewers and Didham, 2006). Alternatively, the recorded hotspots may be caused by the larger numbers of amphibian mortalities in this portion of the road, skewing the detection of hot and cold spots of mortality. Most amphibians employ an explosive breeding method, which sees large numbers of offspring produced in a small period, leading to higher counts when these juveniles begin moving away from their birth pools (Hartel et al., 2007; Ulloa et al., 2019). This was particularly apparent when looking at the mass *P. edulis* death events that took place in March of 2023 along kilometre three of the R22 in ISWP.

The avian road mortality hotspot that was present across wet seasons in ISWP is on a heavily built-up portion of the road where the road surface is higher than the natural vegetation that surrounds it; however, there are no means for animals to pass below the road, fauna have to change their path of travel therefore. Betleja et al. (2020) found that birds fly significantly lower over roads than natural vegetation. Meanwhile, Husby (2016) found that larger body-sized birds were more likely to react to oncoming traffic sooner than smaller birds. All of this makes smaller avian species more susceptible to WVCs, as was seen with the mortalities along this stretch of road. The mammalian hotspot on the R618 in HiP, present across dry seasons, is located along a section of road that lies in the middle of a grass-dominated savanna with several surrounding seasonal water sources and several animal paths. This portion of the road experienced many murid road mortalities and was also the location of an African buffalo (*Syncerus caffer*) and a zebra mortality. The presence of this hotspot during the dry season is likely because of animals having to cross over the road to find water and the presence of food sources such as spilt grain from passing vehicles, which may also be attracting murids to the road surface and the carcasses of other road mortalities. Cassimiro et al. (2023) found that the movement of mammals is related to the presence of water bodies in landscapes, while Dhiab et al. (2021) found that spilt grain and carcasses significantly attracted small mammals.

2.5.3 Factors influencing mortality

Our RDA analyses at both the between-location level and taxa within location levels showed that environmental factors significantly influenced mortalities more than climatic factors. Only the RDA of climatic factors influenced mortalities between taxa within HiP. Model significance was shown where constraining factors explained mortality distribution more than unconstrained factors, with partial cloud cover (CC-P) being the only significant factor where the number of mortalities per taxa (except for mammals) decreased as the effect of CC-P increased. Similarly to our results, in south-eastern Australia, as cloud cover increased (and therefore the amount of light decreased), the activity in mammal prey species increased, particularly in nocturnal species (Linley et al., 2020). Our RDA of environmental factors at the location level had only one significant factor in the absence of standing water (SW-A) near (within 50 m) of the road, with only five points not clustered around the gradient factor, with mortality numbers in both HiP and ISWP increasing as SW-A increased. Additionally, of the most influential environmental factors of mortality between sites, two had a negative relationship, with both being subfactors of road slope (SR). The majority of data points clustered around the absence of a sloped road (SR-A) surface, with four of the five most influential environmental factors relating to the overall design of the road.

Numerous studies have found that WVC rates are heavily influenced by the proximity of roads to water bodies (Glista et al., 2008; Langen et al., 2009; Gunson et al., 2011; Gandiwa et al., 2020). This was congruent with the mostly continuous hotspot in HiP, supporting this point further. However, other studies (Garriga et al., 2017; Gandiwa et al., 2020) also found that temporal weather and wildlife activity patterns affected WVC patterns when water sources did not come into direct contact with roads. The absence of a sloped road surface was expected

to greatly impact the number of vertebrate road mortalities, as supported by Gunson et al. (2011), who found multiple studies that showed WVC increased in areas of level terrain.

The environmental RDA for ISWP found that the presence of savanna habitat (H-Sav) had a significant difference in the number of mortalities between taxa with the greatest effect on the number of amphibian mortalities, with all other taxa being primarily found in bushveld habitat (H-bush). The factors identified either had to do with habitat type or vehicle traffic patterns, with the road straights (RS-str) being the only road design-related factor. This was expected as most of the roads in ISWP consist of straight segments. All of the topmost affecting factors increased mortality; however, the road shape and habitat factors had varying degrees of negative relationship to the vehicle traffic factors, with H-Sav and weekday (D-WD) having the most negative relationship.

Our RDA of environmental factors affecting taxa in HiP found that the SW-A had a large, significant difference in how it influenced mortalities, with mortalities in all taxa except mammals decreasing in relation to SW-A. The absence of standing water also had a near negative relationship to the absence of a sloped road edge (SE-A), with SW-A increasing as SE-A decreased. However, despite SW-A having a significant difference in its effect between taxa, the majority of records clustered closely to the number of vehicles per day (VpD), with mortalities in all taxa except amphibians increasing as the VpD increased. As with the local level, the topmost influential environmental factors included road design with VpD and SW-A, which had a mostly negative relationship to the road design factors, except for the relationship between VpD and distance to brush (DtB). The vehicle thoroughfare rate (i.e. VpD) is a well-known cause of WVC (Forman and Alexander, 1998; Seiler, 2003; Gunson et al., 2011; Shilling et al., 2021). However, the decrease in the number of amphibian mortalities as vehicle numbers increased could indicate possible road avoidance. A 58% reduction in WVCs in four states in the USA (California, Idaho, Maine, and Washington) occurred in 2020

(Shilling et al., 2021). However, another study in the USA found that despite lower traffic density during the latter half of 2020 during the COVID-19 pandemic, WVC numbers were unchanged and were even higher than the previous year at the end of 2020, indicating a WVC offset between wildlife road use and vehicle traffic density (Abraham and Mumma, 2021). Additionally, numerous studies found that when road verges had natural or favourable vegetation present, WVCs increased (Ramp et al., 2006; Grosman et al., 2009; Gunson et al., 2011; Rendall et al., 2021).

Despite the variables explaining the majority of the data's distribution, the RDA models either had low R^2 values or did not show significance from non-constrained models, indicating that the constraining variables poorly described the variability in the data. There may be several explanations for the low R^2 values or non-significance, including missing explanatory variables, such as the movement patterns of individual species, in addition to inter- and intra-species conflicts (Cassimiro et al., 2023). However, when examining the data point clustering between the RDA figures, the labelled group points (red crosses) and most unlabelled survey points (black circles) were on opposite sides of the axis centroids. This is caused by data skewing, as was the case with the explanatory variables, because of the count nature of the data. As significances were found despite the skewing present, this indicated that another potential cause of the low R^2 scores was the structure of the data used. Several included physical and non-physical factors involved counts based on qualitative rather than quantitative data because of the unavailability of information concerning road construction and climatic data. Several studies have shown that quantitative data are far superior to qualitative in their ability to describe the variation seen in natural data, allowing for more rigorous statistical analysis (Brannen, 2017), with the standard method of investigating factors influencing mortality using generalised linear models. Further study is required to determine the effect of the identified significant factors on road mortality using quantitative data, which were not

readily available at the time of our study, with data being collected manually in cases of any data sets not being available from management entities.

2.5.4 Implications for Africa

In addition, our results showed that both the R618 in HiP and R22 in ISWP have the highest roadkill rates recorded to date in South Africa. The previous highest was found by Collinson et al. (2015) in the Greater Mapungubwe Transfrontier Conservation Area. Our study also had relatively higher results than studies on paved roads in other African countries, showing a ten-fold increase to that of a national road in Tanzania that passed through a mixed land use (protected and unprotected land) area dominated by savanna habitat (Kioko et al., 2015) and a national road that passed through a savanna dominated portion of the Tsavo Conservation Area a tourist hotspot in Kenya (Lala et al., 2021). This drastic increase in recorded mortalities is likely because of two factors: the taxa included and the survey length. As is the case with most road ecology studies (Gardner et al., 2007; Tingley et al., 2016; Doherty et al., 2020; Tipton et al., 2023), studies from the African continent did not include all terrestrial vertebrate taxa, either excluding amphibians (Drews, 1995; Lala et al., 2021; Mulualem et al., 2023), only focusing on birds and mammals (Lyamuya et al., 2021), large mammals (Gandiwa et al., 2020) or single species (Naciri et al., 2023). Additionally, our study had 240 surveys conducted over 120 days across two consecutive years, with 12 surveys per month. Other studies were either conducted infrequently, once or twice a month over multiple years (Gandiwa et al., 2020; Lala et al., 2021), for less than a year (Drews, 1995; Lyamuya et al., 2021; Mulualem et al., 2023) or opportunistically (Naciri et al., 2023). These practices may lead to an underrepresentation of the scale of the impact of roads on protected areas and reduce the ability of researchers to create accurate models of the impact of roads on surrounding ecosystems (Goosem, 2009; Balčiauskas et al., 2020). Future studies should employ methods that consider all terrestrial

vertebrate taxa and as much relevant data that could affect the movement of each taxon when designing studies.

2.5.5. Recommendations, limitations and future work

Our study represented the first of its kind for the areas included. While park authorities may have conducted other internal studies, no published information regarding vertebrate road mortalities within HiP and ISWP existed. Our study also represents the first study of vertebrate road mortalities on major provincial roads that pass through protected areas in South Africa, which had one of the country's highest recorded counts of vertebrate road mortalities. However, this study was not without its limitations. Foremost of these was the gap in data collection (July 2022 – October 2022, austral winter-spring) because of increased fuel prices in South Africa and financial constraints. The presence of large, dangerous mammals, severe weather events and the level of training of observers also limited the capability to conduct surveys and collect data. Inexperienced observers detect significantly fewer carcasses (Collinson et al., 2014). Additionally, the collection of data may also be influenced by the time taken to process carcasses, the length of survey time and scavenging rates (Antworth et al., 2005). However, it is impossible to get an exact number of faunal mortalities, as it is presently impossible to constantly monitor large portions of roads, leading to a need for estimations of total impact (Schwartz et al., 2020), nor can all off-road carcasses (animals that are flung or that die off the road post impact) be accounted for. In the federal district of Brasilia, Brazil, it was estimated that recorded mortality values were 2-10 fold lower than the actual mortality value (Santos et al., 2016). Another limitation of our study was that we experienced a lack of highly accurate (below 10 m) GIS data on both environmental characteristics and climatic trends.

South Africa is recognised as the third most biologically diverse country in the world (Bartels and Kotze, 2006; Hoveka et al., 2020) and is the 25th largest country in the world

(CIA, 2021). As of 2021, road networks in South Africa are the tenth longest in the world (Department of Transport, 2021). South Africa has the most advanced knowledge of the effect of roads on its biodiversity within Africa, with 66 of the 210 (31.4%) publications recorded (Collinson et al., 2019). Comparatively, Tanzania, the country with the second-highest number of publications, only had 21 (10%) publications between 1954 and 2016 (Collinson et al., 2019). However, there are relatively few publications detailing the effect of public roads that traverse protected areas in Africa as a whole (Collinson et al., 2014; Lala et al., 2021). Present road planning in South Africa follows the guidelines set in the White Paper on National Transport Policy as part of the amendments made to the South African Roads Policy (Department of Transport, 2018), which seeks to optimise capacity, maintain current roads, and develop further road networks. However, because road ecology is still a relatively new field of study in Africa, most road development plans do not include the results of road ecology studies, leading to the development of roads that are sub-optimally designed for the conservation of surrounding environments (Roberts and Sjölund, 2015).

Given that South Africa holds a disproportionate amount of biodiversity, the lack of information concerning the environmental impacts of roads, especially those that pass through protected areas, is of concern. Our results show that additional studies should be conducted within HiP and ISWP to investigate the rate of scavenging on these roads, studies on behavioural responses to roads and vehicle traffic by resident vertebrate species and the frequency and demographics of use of any existing structures present. Only once these studies have been conducted can one accurately generate models of how to mitigate the threats posed by these roads to humans and wildlife in their respective areas. Despite this, some preliminary, inexpensive mitigation measures could be employed to address the issues. These include placing or relocating speed bumps and other speed-reducing structures to slow driver speeds. Studies show that slower vehicle speeds increase the time between vehicles, allowing for more

successful road traversal in local species (Seiler, 2005; Jaarsma et al., 2006). Further signage could also help reduce mortality rates. For example, signs changed the driving behaviour of 61% of drivers, and 98% of drivers who changed their driving habits avoided collisions with placed dummy snakes (Collinson et al., 2019). Additionally, slotted culverts were effective and could provide smaller vertebrates with a means of escape (Grilo et al., 2010), particularly in areas where water bodies are nearby, such as in kilometre 2 in HiP. However, expensive mitigation measures, such as the combination of fencing and crossing structures, were significantly more effective than inexpensive measures, such as animal detection systems or wildlife reflectors (Rytwinski et al., 2016).

2.6 Conclusions

As predicted, small vertebrates and herpetofauna made up the majority of road mortalities within HiP and ISWP, with most of the mortalities located in flat areas. Although vehicle density affected mortality rates, it was not the largest influencing factor. Our results showed that there is a need for intervention, such as extra or better-placed speed bumps, animal movement tunnels, cameras, etc, along certain portions of the R618 in HiP and the R22 in ISWP, as these areas had some of the highest recorded vertebrate road mortality rates in South Africa to date. However, future studies need to be conducted within protected areas to show the accurate scale of the impact of roads in protected areas in South Africa. Mitigation measures to reduce the impacts of the roads in both locations should include measures that are specifically designed to reduce vertebrate road mortalities, with measures to reduce small vertebrate road mortalities of particular importance. All planned mitigation measures should focus on the areas with the highest road mortalities, with implemented measures designed to maximise successful traversal of the fauna based on the species composition of each road mortality hotspot. It is suggested that areas of the roads near waterbodies should have measures

present to reduce wildlife-vehicle collisions, and all water channelling structures should be kept clear at all times to prevent debris and water collection. The threat of wildlife road mortality should also be included in all road planning and design processes across South Africa and in all species assessments.

CRedit authorship contribution statement

Cameron Cormac: Conceptualisation, Methodology, Data collection, Formal analysis, Data curation, Writing – original draft. Cormac Price, Wendy Collinson, Dave J. Druce, Jarryd P. Streicher: Conceptualisation, Methodology, Formal analysis, Writing – review and editing, Supervision. Colleen T. Downs: Conceptualisation, Methodology, Formal analysis, Writing – review and editing, Funding, Project administration, Supervision.

Declaration of competing interest

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

Data availability

Data belong to the University of KwaZulu-Natal and will be made available on reasonable request.

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Ethics statement

Not applicable: This manuscript does not include human or live animal research. We collected roadkills.

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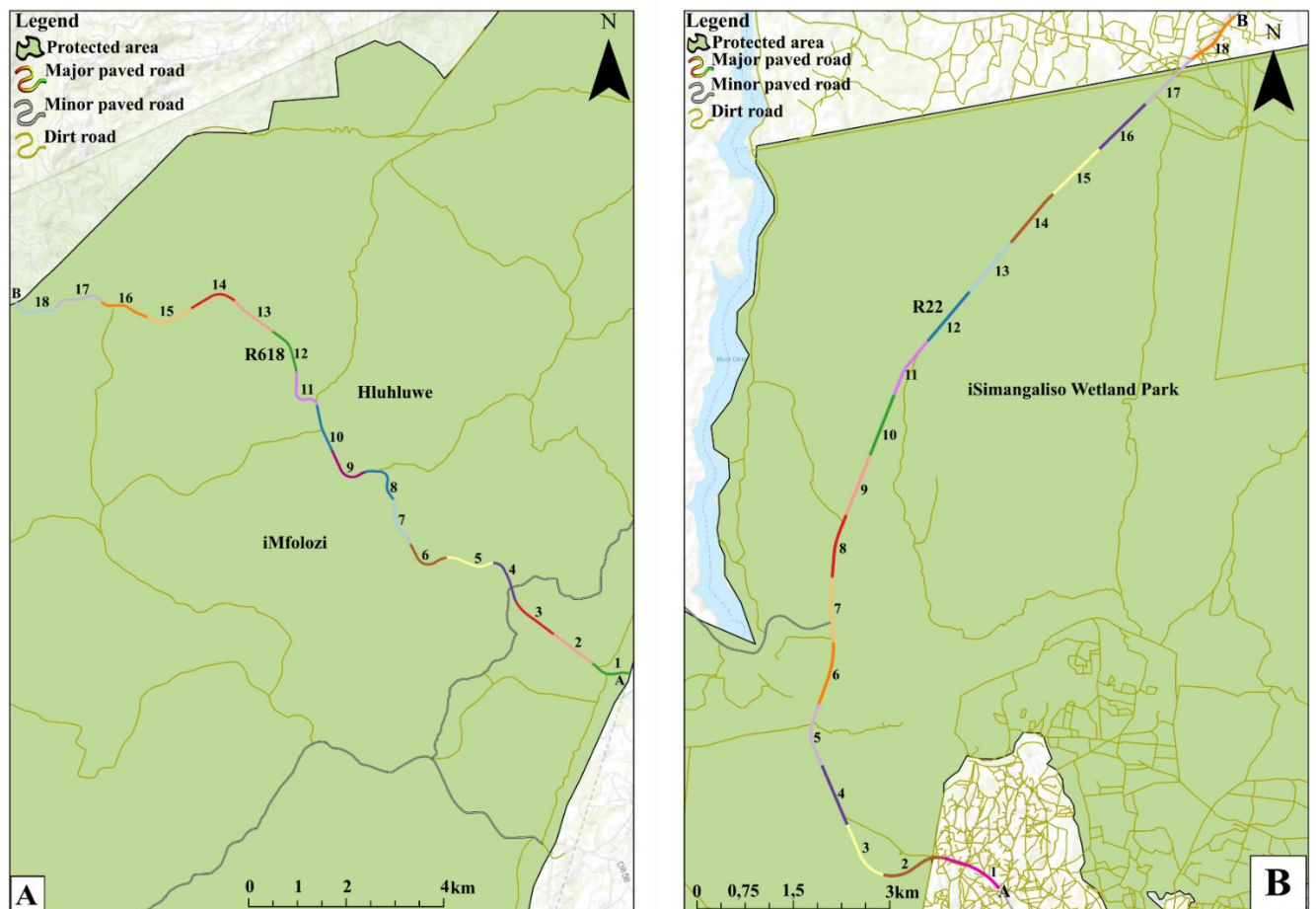
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2.9 Supplementary Information



Supplementary Information Fig. S2.1: Segments of vertebrate road mortality study locations in northern KwaZulu-Natal, South Africa. A) R618 in Hluhluwe-iMfolozi Park. B) R22 in iSimangaliso Wetland Park. (Note: Survey start and end points are represented by map lettering, A and B, respectively).

Supplementary Information Table S2.1: Vertebrate mortalities per kilometre in Hluhluwe-iMfolozi Park (HiP) and iSimangaliso Wetland Park (ISWP), KwaZulu-Natal, South Africa.

Survey	Carcasses km ⁻¹	
	HiP	ISWP
1	4.0	3.9
2	2.8	4.5
3	1.3	1.0
4	0.5	0.5
5	1.6	0.4
6	0.8	0.3
7	1.6	0.8
8	1.1	0.3
9	0.4	0.2
10	1.2	2.9
11	1.3	0.3
12	2.1	0.6
13	1.8	1.4
14	0.9	0.6
15	0.2	0.2
16	2.8	0.3
17	0.6	0.7
18	0.5	0.3
19	0.9	3.2
20	1.9	1.7

Supplementary Information Table S2.2: Physical and non-physical factors with associated abbreviations used in the ordination analyses of vertebrate road mortalities recorded in Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park.

Variable name	Subcategories	Abbreviation
Physical factors		
Vehicle traffic variables		
Vehicles per day	none	VpD
Day of week	Workday	D-WD
	Weekend	D-WE
Road construction variables		
Road shape	Straight	RS-str
	Curved	RS-cur
No. of lanes	Two	L2
	Three	L3
Additional structure	Absent	AS-A
	Present	AS-P
Sloped road	Absent	SR-A
	Present	SR-P
Sloped edge	Absent	SE-A
	Present	SE-P
Side of sloped edge	Both	SSE-B
	One	SSE-1
Habitat variables		
Habitat	Savanna	H-Sav
	Bushveld	H-Bush
	Altered	H-Alt
Animal path	Absent	Ap-A
	Present	Ap-P
Side of road	Larger protected area	Sor-L
	Smaller protected area	Sor-S
Non-physical factors		
Light variables		
Length of Day	none	Dlen
Moon illumination	none	Mill
Time of day	Morning	AM
	Evening	PM
Weather variables		
Obstruction of driver visibility	Absent	Vob-A
	Present	Vob-P
Cloud cover	Clear	CC-C
	Partly cloudy	CC-P
	Overcast	CC-O
Wind	Absent	W-A
	Present	W-P

Supplementary Information Table S2.3: Vertebrate species identified from vertebrate road mortalities in Hluhluwe-iMfolozi Park (HiP) and iSimangaliso Wetland Park (ISWP) between 2021 and 2023.

Species name	Common name	# per species HiP	# per species ISWP
Anura (frogs)			
<i>Afrixalus delicatus</i>	Delicate leaf folder	1	0
<i>Amietia delalandii</i>	Common river frog	10	0
<i>Arthroleptis stenodactylus</i>	Shovel-footed squeaker	1	0
<i>Chiromantis xerampelina</i>	Grey foam-nest treefrog	23	5
<i>Hemius marmoratus</i>	Mottled snout-burrower	1	0
<i>Hyperolius marmoratus</i>	Painted reed frog	0	14
<i>Hyperolius pusillus</i>	Water lily reed frog	0	1
<i>Hyperolius semidiscus</i>	Yellow striped reed frog	0	4
<i>Hyperolius tuberilinguis</i>	Tinker reed frog	1	1
<i>Kassina maculata</i>	Red-legged kassina	0	1
<i>Kassina senegalensis</i>	Bubbling kassina	3	8
<i>Leptopelis mossambicus</i>	Brown backed tree frog	8	0
<i>Phrynomantis bifasciatus</i>	Banded rubber frog	34	4
<i>Ptychadena anchietae</i>	Plain grass frog	15	3
<i>Ptychadena mascareniensis</i>	Mascarene grass frog	1	3
<i>Ptychadena mossambica</i>	Broad-banded grass frog	7	1
<i>Ptychadena porosissima</i>	Striped grass frog	2	0
<i>Pyxicephalus edulis</i>	Lesser bullfrog	33	667
<i>Schismaderma carens</i>	Red toad	52	0
<i>Sclerophrys capensis</i>	Raucous toad	0	4
<i>Sclerophrys garmani</i>	Eastern olive toad	4	74
<i>Sclerophrys gutturalis</i>	Guttural toad	2	1
<i>Tomopterna cryptotis</i>	Tremolo sand frog	4	4
<i>Xenopus laevis</i>	Common platanna	15	3
<i>Xenopus muelleri</i>	Müller's platanna	0	1
Unknown individuals: 21 HiP; 21 ISWP			
Aves (birds)			
<i>Amblyospiza albifrons</i>	Thick-billed weaver	0	1

<i>Andropadus importunus</i>	Sombre greenbul	2	0
<i>Bubo africanus</i>	Spotted eagle owl	0	2
<i>Burhinus capensis</i>	Spotted thick-knee	1	5
<i>Caprimulgus europaeus</i>	European nightjar	0	1
<i>Caprimulgus pectoralis</i>	Fiery-necked nightjar	1	7
<i>Centropus superciliosus</i>	Burchell's coucal	6	1
<i>Charadrius tricollaris</i>	African three-banded plover	1	0
<i>Cisticola ayresii</i>	Wing snapping cisticola	2	0
<i>Cisticola chiniana</i>	Rattling cisticola	2	0
<i>Cisticola juncidis</i>	Zitting cisticola	1	0
<i>Cisticola natalensis</i>	Croaking cisticola	2	0
<i>Colius striatus</i>	Speckled mousebird	1	1
<i>Coracias garrulus</i>	European roller	1	0
<i>Corythornis cristatus</i>	Malachite kingfisher	1	3
<i>Dicrurus adsimilis</i>	Fork-tailed drongo	1	0
<i>Emberiza flaviventris</i>	Golden breasted bunting	1	0
<i>Guttera edouardi</i>	Southern Crested guineafowl	0	1
<i>Halcyon albiventris</i>	Brown-hooded kingfisher	1	8
<i>Lamprotornis nitens</i>	Cape glossy starling	1	0
<i>Lanius collaris</i>	Southern fiscal shrike	0	1
<i>Lophoceros alboterminatus</i>	Crowned hornbill	1	1
<i>Macronyx croceus</i>	Yellow-throated longclaw	0	1
<i>Melaniparus niger</i>	Southern black tit	0	1
<i>Ploceus cucullatus</i>	Village weaver	2	2
<i>Pycnonotus barbatus</i>	Dark-capped bulbul	5	4
<i>Ortygornis sephaena</i>	Crested francolin	1	2
<i>Trachyphonus vaillantii</i>	Crested barbet	2	0
<i>Trochocercus cyanomelas</i>	Blue-mantled crested flycatcher	0	1
<i>Turnix sylvaticus</i>	Common buttonquail	1	0
<i>Zosterops virens</i>	Cape white-eye	1	1
Unknown individuals: 56 HiP; 26 ISWP.			
Mammalia (mammals)			

<i>Aepyceros melampus</i>	Impala	1	0
<i>Aethomys ineptus</i>	Tete veld rat	18	4
<i>Chlorocebus pygerythrus</i>	Vervet monkey	1	1
<i>Grammomys dolichurus</i>	Woodland thicket rat	0	4
<i>Equus quagga</i>	Plains zebra	1	0
<i>Herpestes ichneumon</i>	Large grey mongoose	0	5
<i>Herpestes sanguineus</i>	Slender mongoose	3	2
<i>Ictonyx striatus</i>	Striped polecat	0	2
<i>Hystrix africaeaustralis</i>	Cape porcupine	1	0
<i>Lemniscomys rosalia</i>	Single striped mouse	4	1
<i>Lepus saxatilis</i>	Scrub hare	4	2
<i>Mastomys natalensis</i>	Natal multimammate mouse	15	2
<i>Mus minutoides</i>	Pygmy mouse	1	0
<i>Mungos mungo</i>	Banded mongoose	1	0
<i>Myosorex varius</i>	Forest shrew	1	0
<i>Nycteris thebaica</i>	Cape slit-faced bat	1	0
<i>Petrodromus tetradactylus</i>	Four-toed sengi	0	3
<i>Scotophilus dinganii</i>	Yellow-bellied house bat	1	0
<i>Steatomys pratensis</i>	Fat mouse	3	3
<i>Syncerus caffer</i>	African buffalo	3	0
<i>Thallomys paedulcus</i>	Acacia thicket rat	0	15
Unknown individuals: 39 HiP; 61 ISWP.			
Reptilia (reptiles)			
<i>Acontias plumbeus</i>	Giant legless skink	4	1
<i>Afrotyphlops bibronii</i>	Bibron's blind snake	0	1
<i>Afrotyphlops schlegelii</i>	Schlegel's blind snake	0	4
<i>Atractaspis bibronii</i>	Bibron's stiletto snake	1	0
<i>Bitis arietans arietans</i>	Puff adder	11	27
<i>Boaedon capensis</i>	Brown house snake	8	7
<i>Causus rhombeatus</i>	Rhombic night adder	0	1
<i>Chamaeleo dilepis dilepis</i>	Common flap-neck chameleon	3	2
<i>Chamaesaura macrolepis</i>	Large-scaled grass lizard	1	0

<i>Crotaphopeltis hotamboeia</i>	Red-lipped snake	1	4
<i>Dasypeltis scabra</i>	Rhombic egg eater	5	2
<i>Dispholidus typus</i>	Boomslang	5	2
<i>Dipsadoboa aulica</i>	Marbled tree snake	0	1
<i>Gerrhosaurus flavigularis</i>	Yellow-throated plated lizard	1	1
<i>Homopholis wahlbergii</i>	Wahlberg's velvet gecko	0	1
<i>Limaformosa capensis</i>	Common file snake	1	1
<i>Lycophidion capense</i>	Common wolf snake	1	1
<i>Naja mossambica</i>	Mozambique spitting cobra	4	1
<i>Nucras ornata</i>	Ornate sandveld lizard	1	0
<i>Pelomedusa galeata</i>	Marsh terrapin	4	1
<i>Pelusios sinuatus</i>	Serrated hinge terrapin	1	1
<i>Psammophis mossambicus</i>	Olive grass snake	3	1
<i>Pseudaspis cana</i>	Mole snake	0	1
<i>Python natalensis</i>	Southern African python	2	5
<i>Stigmochelys pardalis</i>	Leopard tortoise	5	7
<i>Telescopus semiannulatus</i>	Eastern tiger snake	3	2
<i>Thelotornis capensis</i>	Southern twig snake	5	9
<i>Varanus albigularis albigularis</i>	White-throated monitor lizard	0	3
<i>Xenocalamus transvaalensis</i>	Speckled quill-snouted snake	0	1
Unknown individuals: 6			
HiP; 9 ISWP			

Supplementary Information Table S2.4: Taxonomic classification of unidentified vertebrate road mortalities recorded in Hluhluwe-iMfolozi Park (HiP) and iSimangaliso Wetland Park (ISWP) from March 2021 to March 2023

Classification name	Taxonomic level	Location
Amphibia		
Anura	Order	ISWP (8); HiP (10)
Bufo	Family	ISWP (2); HiP (1)
Afrixalus	Genus	ISWP (2)
Breviceps	Genus	HiP (3)
Hyperolius	Genus	ISWP (6)
Ptychadena	Genus	HiP (7)
Xenopus	Genus	ISWP (3)
Aves		
Aves	Class	ISWP (13); HiP (20)
Passeriformes	Order	ISWP (1); HiP (9)
Accipitridae	Family	HiP (1)
Alaudidae	Family	ISWP (1)
Cisticola	Genus	ISWP (4); HiP (13)
Caprimulgus	Genus	ISWP (5); HiP (4)
Ploceus	Genus	HiP (1)
Tchagra	Genus	ISWP (4); HiP (16)
Mammalia		
Mammalia	Class	ISWP (2)
Yungipetra	Suborder	ISWP (1); HiP (1)
Yungipetra	Suborder	ISWP (1); HiP (3)
Muridae	Family	ISWP (56); HiP (28)
Rhinolophidae	Family	HiP (1)
Soricidae	Family	HiP (1)
Crocodyria	Genus	HiP (4)
Dendromys	Genus	HiP (1)
Steatomys	Genus	ISWP (1)
Reptilia		
Serpentes	Suborder	ISWP (3); HiP (2)
Colubridae	Family	ISWP (1)
Pelomedusidae	Family	ISWP (2)
Testudinidae	Family	HiP (2)
Chamaesauridae	Genus	HiP (1)
Naja	Genus	HiP (1) ISWP (2)
Philothamnus	Genus	ISWP (1)

Supplementary Information Table S2.5: IUCN and SANBI Assessments and threats to species identified from road mortalities recorded in Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park. (Note: LC = Least concern; NT = Near Threatened; *=Different SANBI name)

Species name	IUCN listing	Year	IUCN listed threats	SANBI listing	Year	SANBI listed threats
Aves						
<i>Amblyospiza albifrons</i>	LC-Stable	2018	None	LC-unknown	2015	None
<i>Andropadus importunus</i>	LC-Stable	2018	None	LC-unknown	2015	None
<i>Bubo africanus</i>	LC-Stable	2022	Exploitation; human disturbance; roads and railroads	LC-unknown	2015	None
<i>Burhinus capensis</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Caprimulgus europaeus</i>	LC-decreasing	2016	Habitat change	LC-unknown	2015	None
<i>Caprimulgus pectoralis</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Centropus superciliosus*</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Charadrius tricollaris</i>	LC-unknown	2016	Disease	LC-unknown	2015	None
<i>Cisticola ayresii</i>	LC-decreasing	2016	None	LC-unknown	2015	None
<i>Cisticola chiniana</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Cisticola juncidis</i>	LC-increasing	2016	Habitat change	LC-unknown	2015	None
<i>Cisticola natalensis</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Colius striatus</i>	LC-increasing	2016	None	LC-unknown	2015	None
<i>Coracias garrulus</i>	LC-decreasing	2019	Exploitation; Habitat change	NT-decreasing	2015	Habitat change
<i>Corythornis cristatus*</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Dicrurus adsimilis</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Emberiza flaviventris</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Guttera edouardi*</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Halcyon albiventris</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Lamprotornis nitens</i>	LC-Stable	2018	None	LC-unknown	2015	None
<i>Lanius collaris</i>	LC-increasing	2016	None	LC-unknown	2015	None
<i>Lophoceros alboterminatus*</i>	LC-decreasing	2016	None	LC-unknown	2015	None
<i>Macronyx croceus</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Melaniparus niger*</i>	LC-Stable	2016	None	LC-unknown	2015	None
<i>Ploceus cucullatus</i>	LC-Stable	2018	None	LC-unknown	2015	None

<i>Pycnonotus barbatus</i> *	LC-increasing	2018	None	LC-unknown	2015	None
<i>Ortygornis sephaena</i> *	LC-Stable	2016	None	LC-unknown	2015	None
<i>Trachyphonus vaillantii</i>	LC-decreasing	2016	None	LC-unknown	2015	None
<i>Trochocercus cyanomelas</i>	LC-decreasing	2016	None	LC-unknown	2015	None
<i>Turnix sylvaticus</i>	LC-decreasing	2016	Habitat change	LC-unknown	2015	None
<i>Zosterops virens</i> *	LC-unknown	2016	None	LC-unknown	2015	None
Anura						
<i>Afraxalus delicatus</i>	LC-unknown	2013	Disease; habitat change; invasive species	LC-stable	2013	Habitat change
<i>Amietia delalandii</i>	LC-Stable	2017	None	LC-stable	2017	None
<i>Arthroleptis stenodactylus</i>	LC-Stable	2015	None	LC-stable	2015	None
<i>Chiromantis xerampelina</i>	LC-unknown	2013	Exploitation	LC-stable	2013	Exploitation
<i>Hemisus marmoratus</i>	LC-unknown	2013	Exploitation	LC-stable	2013	Exploitation
<i>Hyperolius marmoratus</i>	LC-increasing	2013	None	LC-increasing	2013	None
<i>Hyperolius pusillus</i>	LC-unknown	2013	None	LC-stable	2013	None
<i>Hyperolius semidiscus</i>	LC-unknown	2013	Habitat change	LC-stable	2013	None
<i>Hyperolius tuberilinguis</i>	LC-unknown	2013	Habitat change	LC-stable	2013	None
<i>Kassina maculata</i>	LC-unknown	2013	Disease; habitat change; invasive species	LC-stable	2013	Exploitation; habitat change
<i>Kassina senegalensis</i>	LC-unknown	2013	Habitat change	LC-stable	2013	Habitat change
<i>Leptopelis mossambicus</i>	LC-unknown	2013	Habitat change	LC-stable	2013	Habitat change
<i>Phrynomantis bifasciatus</i>	LC-unknown	2013	None	LC-stable	2013	None
<i>Ptychadena anchietae</i>	LC-unknown	2015	None	LC-stable	2015	None
<i>Ptychadena mascareniensis</i>	LC-unknown	2015	None	LC-unknown	2015	None
<i>Ptychadena mossambica</i>	LC-unknown	2013	None	LC-stable	2013	None
<i>Ptychadena porosissima</i>	LC-unknown	2013	None	LC-stable	2013	None
<i>Pyxicephalus edulis</i>	LC-decreasing	2015	Exploitation; habitat change	LC-decreasing	2015	Exploitation; habitat change
<i>Schismaderma carens</i>	LC-unknown	2013	None	LC-stable	2013	None
<i>Sclerophrys capensis</i>	LC-decreasing	2013	Habitat change	LC-decreasing	2013	Habitat change
<i>Sclerophrys garmani</i>	LC-unknown	2013	Habitat change	LC-stable	2013	Habitat change

<i>Sclerophrys gutturalis</i>	LC-increasing	2013	None	LC-increasing	2013	None
<i>Tomopterna cryptotis</i>	LC-Stable	2016	None	LC-stable	2015	None
<i>Xenopus laevis</i>	LC-increasing	2016	None	LC-increasing	2008	None
<i>Xenopus muelleri</i>	LC-Stable	2016	Exploitation	LC-stable	2013	None
Mammalia						
<i>Aepyceros melampus</i>	LC-Stable	2016	Exploitation; habitat change	LC-increasing	2016	None
<i>Aethomys ineptus</i>	LC-unknown	2016	None	LC-stable	2016	None
<i>Chlorocebus pygerythrus</i>	LC-decreasing	2022	Exploitation; habitat change; persecution	LC-stable	2016	Exploitation; habitat change; persecution; Wildlife-vehicle collisions
<i>Equus quagga</i>	NT-decreasing	2016	Climate change; exploitation; fencing; habitat change; human conflict	LC-increasing	2016	Exploitation; population isolation; fencing
<i>Grammomys dolichurus</i>	LC-unknown	2016	None	LC-stable	2016	Domestic cats
<i>Herpestes ichneumon</i>	LC-Stable	2016	Persecution	Not listed	NA	NA
<i>Herpestes sanguineus</i>	LC-Stable	2015	Exploitation	LC-stable	2016	None
<i>Hystrix africaeaustralis</i>	LC-Stable	2016	None	LC-stable	2016	Exploitation; persecution
<i>Ictonyx striatus</i>	LC-Stable	2015	Persecution, Invasive species	LC-stable	2016	Domestic dogs; persecution; wildlife-vehicle collisions
<i>Lepus saxatilis*</i>	LC-decreasing	2018	Exploitation; habitat change	LC-stable	2016	Exploitation; habitat change
<i>Lemniscomys rosalia</i>	LC-Stable	2016	None	LC-stable	2016	None
<i>Mastomys natalensis</i>	LC-Stable	2016	None	LC-stable	2016	None
<i>Mus minutoides</i>	LC-Stable	2016	None	LC-stable	2016	None
<i>Mungos mungo</i>	LC-Stable	2015	Exploitation	LC-stable	2016	None
<i>Myosorex varius</i>	LC-Stable	2016	None	LC-stable	2016	None
<i>Nycteris thebaica</i>	LC-unknown	2016	Habitat change, roost disturbance	LC-stable	2014	Habitat change; human disturbance

<i>Petrodromus tetradactylus</i>	LC-unknown	2013	None	NT-unknown	2013	Exploitation; habitat change
<i>Scotophilus dinganii</i>	LC-unknown	2016	None	LC-stable	2014	Habitat degradation
<i>Steatomys pratensis</i>	LC-Stable	2016	None	LC-stable	2016	None
<i>Syncerus caffer</i>	NT-decreasing	2018	Climate change; disease; exploitation; habitat change; human conflict	LC-increasing	2016	Climate change; disease; exploitation; habitat degradation
<i>Thallomys paedulcus</i>	LC-unknown	2016	None	LC-unknown	2016	Habitat change
Reptilia						
<i>Acontias plumbeus</i>	LC-stable	2019	None	LC-stable	2017	None
<i>Afrotyphlops bibronii</i>	LC-stable	2021	None	LC-stable	2017	None
<i>Afrotyphlops schlegelii</i>	LC-stable	2019	None	LC-stable	2017	None
<i>Atractaspis bibronii</i>	LC-unknown	2014	None	LC-stable	2017	None
<i>Bitis arietans</i>	LC-stable	2021	Exploitation; persecution	LC-stable	2017	Persecution
<i>Boaedon capensis</i>	LC-stable	2014	None	LC-stable	2017	None
<i>Causus rhombeatus</i>	LC-stable	2019	None	LC-stable	2017	None
<i>Chamaeleo dilepis</i>	LC-stable	2013	Exploitation	LC-stable	2017	None
<i>Chamaesaura macrolepis</i>	LC-stable	2021	Habitat change	LC-stable	2017	Habitat change
<i>Crotaphopeltis hotamboeia</i>	LC-stable	2012	None	LC-stable	2017	None
<i>Dasypeltis scabra</i>	LC-unknown	2014	Habitat change; exploitation; persecution	LC-stable	2017	None
<i>Dispholidus typus</i>	LC-stable	2012	None	LC-stable	2017	None
<i>Dipsadoboa aulica</i>	LC-stable	2019	None	LC-stable	2017	None
<i>Gerrhosaurus flavigularis</i>	LC-unknown	2014	None	LC-stable	2017	None
<i>Homopholis wahlbergii</i>	LC-stable	2021	None	LC-stable	2018	None
<i>Limaformosa capensis</i>	LC-unknown	2014	Wildlife-vehicle collisions	LC-stable	2017	None
<i>Lycophidion capense</i>	LC-unknown	2012	None	LC-stable	2017	None
<i>Naja mossambica</i>	LC-unknown	2019	None	LC-stable	2017	None
<i>Nucras ornata</i>	LC-Stable	2019	None	LC-stable	2017	None
<i>Pelomedusa galeata</i>	LC-unknown	2018	Habitat degradation	LC-unknown	2017	Habitat degradation
<i>Pelusios sinuatus</i>	Not listed	NA	NA	LC-unknown	2017	Habitat degradation

<i>Psammophis mossambicus</i>	LC-Stable	2019	None	LC-stable	2017	None
<i>Pseudaspis cana</i>	LC-unknown	2014	Persecution	LC-stable	2017	None
<i>Python natalensis</i>	LC-decreasing	2019	Exploitation; Habitat change; habitat degradation; persecution	LC-increasing	2017	Exploitation; fencing; habitat change; persecution
<i>Stigmochelys pardalis</i>	LC-unknown	2014	Exploitation; habitat change; habitat degradation	LC-stable	2017	Exploitation; habitat degradation; persecution
<i>Telescopus semiannulatus</i>	LC-Stable	2019	None	LC-stable	2017	None
<i>Thelotornis capensis</i>	LC-Stable	2019	None	LC-stable	2017	None
<i>Varanus albigularis</i>	LC-Stable	2019	None	LC-stable	2017	None
<i>Xenocalamus transvaalensis</i>	LC-Stable	2021	None	LC-stable	2017	None

Supplementary Information Table S2.6: Location of taxon-specific mortality hotspots across two wet and dry seasons in Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park. Percentages represent hotspot confidence interval.

Segment	Taxa	HiP hotspot presence				ISWP hotspot presence			
		Season				Season			
		Wet Y1	Dry Y1	Wet Y2	Dry Y2	Wet Y1	Dry Y1	Wet Y2	Dry Y2
1	Amphibia	Absent	Absent	Present (95%)	Absent	Absent	Absent	Absent	Present (99%)
	Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Reptilia	Absent	Absent	Absent	Present (99%)	Absent	Absent	Absent	Absent
2	Amphibia	Present (99%)	Absent	Present (99%)	Absent	Absent	Absent	Absent	Absent
	Aves	Present (99%)	Present (90%)	Absent	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Reptilia	Present (95%)	Absent	Present (95%)	Present (99%)	Absent	Absent	Absent	Absent
3	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Present (99%)	Absent
	Aves	Absent	Absent	Absent	Absent	Present (99%)	Absent	Present (95%)	Absent
	Mammalia	Absent	Absent	Absent	Absent	Present (95%)	Absent	Absent	Absent
	Reptilia	Absent	Absent	Absent	Absent	Present (90%)	Absent	Absent	Absent
4	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Aves	Absent	Absent	Present (95%)	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

5	Reptilia	Absent	Absent	Present (95%)	Absent	Present (90%)	Absent	Absent	Absent
	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
			Present	Present					
	Aves	Absent	(90%)	(95%)	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
			Present						
	Mammalia	Absent	(99%)	Absent	Absent	Absent	Absent	Absent	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Present (95%)	Absent	Absent
8	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present (99%)
	Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present (95%)
	Mammalia	Absent	Absent	Absent	Absent	Present (99%)	Absent	Absent	Absent
	Reptilia	Absent	Absent	Absent	Absent	Present (90%)	Absent	Absent	Absent
	Amphibia	Absent	Present (99%)	Absent	Absent	Absent	Absent	Absent	Absent
9	Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Present (99%)	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
10	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Present (99%)	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

	Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
11	Aves	Absent	Absent Present	Absent	Absent	Absent	Absent	Absent Present	Absent (95%)
	Mammalia	Absent	(95%)	Absent	Absent	Absent	Absent	(95%)	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent Present
	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	(99%) Present
12	Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent Present	(95%)
	Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	(95%)	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Amphibia	Absent	Absent Present	Absent	Absent	Absent	Absent	Absent	Absent
13	Aves	Absent	(90%) Present	Absent	Absent Present	Absent	Absent	Absent	Absent
	Mammalia	Absent	(99%)	Absent	(99%)	Absent	Absent	Absent	Absent
	Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
14	Aves	Absent	Absent (95%)	Absent	Absent	Absent	Absent	Absent	Absent
	Mammalia	Absent	Absent Present	Absent	Absent	Absent	Absent	Absent	Absent
	Reptilia	Absent	Absent (95%)	Absent	Absent	Absent	Absent	Absent	Absent
15	Amphibia	Absent	Absent Present	Absent	Absent	Absent	Absent	Absent	Absent
	Aves	Absent	(90%)	Absent	Absent	Absent	Absent	Absent	Absent

		Mammalia	Present (90%)	Absent Present (95%)	Absent	Absent	Absent	Absent	Absent	Absent
		Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
							Present (90%)			
16		Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
				Present (95%)						
		Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
17		Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
				Present (90%)						
		Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Amphibia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Aves	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
18		Mammalia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Reptilia	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Supplementary Information Table S2.7: Redundancy analysis biplot scores of all constraining variables of per location vertebrate road mortality

Variable	RDA1	RDA 2	R2 adjusted
Physical factors			
VpD	0.30289	-0.008383	0.017673
DtB	-0.33769	-0.099658	0.036990
Sor-S	-0.15653	-0.057681	-0.03549
RS-str	-0.14318	0.219587	-0.03174
L2	0.12501	0.157591	-0.03924
AS-A	-0.19400	0.111496	-0.02360
AS-P	0.19427	-0.109081	-0.02360
SR-A	0.05624	0.423752	-0.02525
SR-P	-0.05639	-0.423530	-0.02527
SE-A	-0.45908	0.426642	0.14080
SSE-B	-0.27336	-0.246510	0.01348
H-Sav	-0.14200	-0.051464	-0.03905
H-Bush	-0.26166	0.104149	0.00076
Ap-A	0.01574	0.259722	-0.04492
Sw-A	0.64053	-0.029119	0.27201
D-WD	0.28204	-0.005924	0.00794
Axis Eigenvalues			
RDA1	RDA2	PC1	PC2
659.1119	127.7712	77.7952	7.08747
Non-Physical factors			
Dlen	-0.02064	0.02622	-0.05535
Mill	-0.15447	-0.52317	-0.01849
RATD	0.09175	0.35683	-0.03917
Vob-A	0.71512	0.10550	0.10884
CC-C	0.14292	-0.09926	-0.04798
CC-P	0.10488	0.30422	-0.04209
W-A	-0.16698	0.05930	-0.04628
W-P	0.16863	-0.06457	-0.04603
AM	-0.33403	0.49708	0.00664
PM	0.33278	-0.49790	0.00646
Axis Eigenvalues			
RDA1	RDA2	PC1	PC2
263.5663	88.8752	468.3119	51.01236

Supplementary Information Table S2.8: Redundancy analysis biplot scores of all constraining variables of per vertebrate class mortality in Hluhluwe-iMfolozi Park

Variable	RDA1	RDA 2	R2 adjusted				
Physical factors							
VpD	0.34233	0.36096	0.05262				
DtB	-0.30980	0.22999	0.03205				
Sor-S	-0.30233	-0.20587	0.02277				
Sor-L	0.30271	0.20595	0.02296				
RS-str	-0.02544	-0.01819	-0.05479				
L2	0.10203	0.03114	-0.04357				
AS-A	0.04792	-0.19205	-0.03542				
SR-A	-0.40148	0.17152	0.08034				
SE-A	-0.68653	-0.02546	0.32109				
SSE-B	-0.30444	-0.11388	0.02054				
SSE-O	0.30390	0.11443	0.02031				
H-Sav	-0.04064	0.07364	-0.05139				
Ap-A	-0.06132	-0.22072	-0.04489				
Sw-A	0.84393	0.01787	0.51480				
D-WD	-0.12470	0.18805	-0.03188				
Non-physical factors							
Dlen	-0.32257	-0.5781	0.02127				
Mill	0.17257	-0.4745	-0.02451				
RATD	-0.27799	-0.6230	0.01108				
Vob-A	0.73997	-0.1198	0.23388				
CC-C	0.44333	0.2797	0.05279				
CC-P	-0.77089	-0.2948	0.26198				
W-A	0.05740	0.2387	-0.04992				
AM	-0.01777	0.5178	-0.02957				
Physical factor Eigenvalues							
RDA1	RDA2	RDA3	RDA4	PC1	PC2	PC3	PC4
182.1324	24.4134	8.6044	1.6506	19.6125	3.4762	0.9256	7.677e ⁻⁰⁴
Non-physical factor Eigenvalues							
119.6554	14.9733	3.7905	0.3793	81.2844	14.8214	4.6354	1.2760

Supplementary Information Table S2.9: Redundancy analysis biplot scores of all constraining variables of per vertebrate class mortality in iSimangaliso Wetland Park

Variable	RDA1	RDA 2	R2 adjusted				
Physical factors							
VpD	-0.177916	0.46560	-0.00837				
DtB	0.136198	0.10233	-0.04017				
Sor-S	0.056923	0.02958	-0.05169				
RS-str	0.130844	0.40918	-0.01836				
AS-A	0.142213	0.01468	-0.04079				
SR-A	0.072033	0.17153	-0.04625				
SE-A	0.005102	-0.20231	-0.04735				
SSE-B	0.242694	-0.04980	-0.02440				
H-Sav	0.562176	-0.12679	0.10140				
H-Bush	0.165130	0.23696	-0.03381				
Ap-A	0.135871	-0.12408	-0.04255				
Sw-A	-0.220321	0.11233	-0.03004				
D-WD	-0.282824	0.13581	-0.01210				
Non-physical factors							
Dlen	-0.04540	0.246545	-0.04834				
Mill	0.93635	-0.136657	0.13046				
RATD	-0.07772	-0.001850	-0.05230				
Vob-A	-0.29445	0.008645	-0.03420				
CC-C	-0.09391	-0.216194	-0.04993				
CC-P	-0.24184	0.128074	-0.04133				
W-A	0.07101	-0.146750	-0.05155				
AM	0.53710	0.785540	0.03539				
Physical factor Eigenvalues							
RDA1	RDA2	RDA3	RDA4	PC1	PC2	PC3	PC4
146.6629	43.0518	5.1959	1.2529	100.4980	18.7528	1.9154	0.6522
Non-physical factor Eigenvalues							
63.5900	14.6147	2.2116	1.1238	184.0440	48.1833	2.7403	1.4751

CHAPTER 3

Reptile demographics on paved roads in protected (Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park) and unprotected areas (Giba Rural Community) in northeastern KwaZulu-Natal, South Africa

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Running header: Effects of roads on reptiles in northeastern South Africa

3.1 Abstract

Anthropogenic change is a global threat to reptiles, and species face numerous threats, including habitat change, exploitation, and persecution. Much anthropogenic change is dependent on road networks, and reptiles are particularly susceptible to mortality related to the direct and indirect effects of roads on surrounding habitats. Our study aimed to determine the demographics and mortalities of reptiles on paved roads in protected and unprotected areas in Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park and the adjacent rural community of Giba in KwaZulu-Natal Province, South Africa. We achieved this by observing live and dead individuals to determine reptilian species diversity, and abundance, mapping occupancy hotspot distributions and determining the effect of a suite of ecological factors on observed reptile presence. Our study included sections of paved roads along the R618 in Hluhluwe-iMfolozi Park and the R22, which passes through iSimangaliso Wetland Park and the rural community of Giba. Presence data were collected during driven surveys at sunrise and sunset from March 2021 to March 2023. Total reptile numbers were not significantly different between protected and unprotected areas. However, the majority (78.3%) were found as road mortalities, with a significant difference between the number of live and dead individuals observed between protected and unprotected areas. Snakes comprised the majority (70.3%) of observed individuals. Reptile observation frequencies decreased with habitat degradation and the absence of water, while they increased with increases in vehicle density. Our study highlights the impact of roads in protected and unprotected areas on reptile species, and that mitigation is necessary for more effective conservation.

Keywords: linear infrastructure; reptiles; road mortalities; protected areas; Africa; roadkill; hotspots

3.2 Introduction

The Anthropocene is marked by rapid, pervasive human expansion into natural habitats (Nixon, 2018), resulting in challenges for wildlife (Davies et al., 2006; Lal and Nadim, 2021). Globally, many reptile species face declines and possible extinction because of threats caused by human encroachment (Gibbons et al., 2000; Paterson et al., 2021). These include habitat loss/fragmentation (Böhm et al., 2013; Jones et al., 2022) and climate change (Reading et al., 2010; Farooq et al., 2021, 2023), which results in behavioural (Tipton et al., 2023), developmental (Janzen and Paukstis, 1991; Carter and Jansen, 2021) and range distribution shifts (Sillero, 2021; Mi et al., 2023). In addition, reptiles face increased pressures from persecution (Crawford and Andrews, 2016) and exploitation (chiefly for consumption or illegal pet trade) (Fitzgerald et al., 2004; Warwick, 2014). Road infrastructure also significantly contributes to global reptile decline (Forman and Alexander, 1998; Gibbons et al., 2000; Lal and Nadim, 2021; Paterson et al., 2021), as reptiles are often drawn to roads for use as basking sites (Brattstrom, 1965; McCardle and Fontenot, 2016) and food sources for predatory (Berna and Gibbons, 1991; Tipton et al., 2023) and scavenging (Antworth et al., 2005; Karunarathna et al., 2017) species. Additionally, reptiles may attempt to traverse roads during breeding seasons and dispersal (Steen et al., 2006; Jones et al., 2022). Consequently, roads constantly threaten any reptiles that use them because of potential reptile-vehicle collisions (Bernardino and Dalrymple, 1992; Doherty et al., 2020; Paterson et al., 2021; Jones et al., 2022; Grilo et al., 2025). Direct mortality through vehicle collisions is a significant concern, particularly for slow-moving reptiles like Testudines and large-bodied snakes, which have higher collision rates (Shine et al., 2004; Brehme et al., 2018; Paterson et al., 2021).

Roads also impose indirect negative effects, such as shifts in behavioural patterns in response to road presence, which can act as barriers to reptile movement, potentially disrupting access to crucial resources and limiting genetic diversity (Shine et al., 2004; Aresco, 2005;

Herrmann et al., 2017; Paterson et al., 2019; Jones et al., 2022). The barrier effects created by roads increase as road network density increases (Patrick and Gibbs, 2010; Heigl et al., 2017), with the number of paved roads in a network correlating with road avoidance and mortality rates (Brehme et al., 2013; Paterson et al., 2019). Prevention of movement can also increase inbreeding rates and vulnerability to disease, leading to further declines in population density and persistence (Frankham, 2005; Herrmann et al., 2017). Roads also alter the local environment by introducing noise, light (Dean et al., 2019) and water pollution (Szklairek et al., 2022) and by facilitating the spread of invasive species (Forman et al., 2003; Quintero-Ángel et al., 2012), particularly alien vegetation, altering vegetation structure in habitats that surround roads (Lemke et al., 2021).

Despite existing knowledge demonstrating the effects of roads on reptiles, globally, there are few studies on the effects of roads on reptile ecology when compared with other vertebrate taxa (Gardner et al., 2007; Tingley et al., 2016; Doherty et al., 2020; Tipton et al., 2023; Arzamendia et al., 2024) and this neglect is also present in South Africa (Tolley et al., 2016, 2024). Of reptile species found in the Greater Mapungubwe Transfrontier Conservation Area in South Africa, Collinson et al. (2015) found that 28% of reptile species had high mortality rates on roads, with temperature and rainfall influencing road mortalities. Furthermore, snakes made up >70% of roadkill along a road in the Limpopo Province, South Africa, with puff adders (*Bitis arietans arietans*) comprising the majority of mortalities (Schmidt, 2018). In Grahamstown, Eastern Cape Province, South Africa, Coombs (2015) collected 32 dead Cape grass lizards (*Chamaesaura anguina anguina*), a rarely encountered species, killed by vehicles along a 1.5 km patch of gravel road after a single brush fire. In Beaufort West, Western Cape Province, South Africa, 48 greater padloper (*Homopus femoralis*) were killed by vehicles across four days along a low-traffic road (~62 vehicles per day) (Loehr, 2012). What remains unclear from these studies is the impact of surrounding

habitat conditions on the presence of reptiles on roads. The investigation of reptile road use and the effects of surrounding road characteristics could allow more effective conservation planning in protected and unprotected areas.

We aimed to determine the reptile occurrence and mortalities along public paved roads in protected and unprotected areas in Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park and Giba Rural Community in South Africa's KwaZulu-Natal Province. Details for both live and dead reptiles were recorded to determine total species composition, heat maps of occurrence and a suite of ecological factors were used to identify the driving elements of reptile-road presence. Snakes were predicted to have the highest detection (including both live and dead individuals) rates across all areas based on evidence found by previous studies (Collinson et al., 2015; Crawford and Andrews, 2016; Schmidt, 2018; Paterson et al., 2021; Jones et al., 2022). Additionally, it was predicted that regions outside of protected areas would see the lowest reptile species diversity because of lower levels of biodiversity in altered landscapes (Filgueiras et al., 2021), with habitat type predicted to be a key driving factor in the number of reptiles detected (MacKinnon et al., 2005; Anđelković and Bogdanović, 2022). However, detection rates may be equal in protected areas, as reptile species in more urban landscapes may have higher abundance because of increased resource availability (Reynolds et al., 2021). Vehicle traffic density, time of day, temperature and rainfall were predicted to be the driving factors of reptile road-use patterns, with reptile detection highest during the warm, wet season (austral spring-summer) because of the effects on behavioural and movement patterns (Colino-Rabanal and Lizana, 2012).

3.3 Methods

3.3.1 Study areas

The uMkhanyakude district, a region with predominantly isiZulu-speaking inhabitants in KwaZulu-Natal Province, South Africa, has several protected areas, including the greater iSimangaliso Wetland Park (ISWP), which stretches from the northern boundary bordering Mozambique to the southern boundary south of St. Lucia (Perissinotto et al., 2013). The region also has one of South Africa's oldest protected areas, Hluhluwe-iMfolozi Park (HiP), originally proclaimed as two separate reserves in 1895 and joined with the so-called "Corridor Reserve" containing the R618 in 1983 (Cromsigt et al., 2017). Despite being a haven for wildlife because of the number of protected areas, uMkhanyakude remains one of the most underdeveloped and neglected regions while having a large human population (Mavundza et al., 2011). A lack of service delivery and developmental oversight within the uMkhanyakude region places protected areas and reptile populations under increased stress (Farooq and Geldmann, 2024). Additional stress is placed on reptiles, particularly snakes, because of cultural stigmas that persist within the region, with beliefs applied to groups of reptiles rather than specific species and include beliefs such as reptiles being either the agents of witch doctors (Simelane and Kerley, 1997) or that encourage the killing for reptiles or that perpetuate general negative views for reptiles (Phaka et al., 2023).

Our study included three 18 km stretches of paved roads along two major arterial routes in the uMkhanyakude region. The two roads included in our study were the R618, which runs through HiP, and the R22, which passes through the KwaJozana region (parts of lower Mkuzi and Sodwana (Perissinotto et al., 2013) section of ISWP and Giba Rural Community (GRC) (Fig. 3.1). The area that falls within the boundaries of ISWP (R22a, Fig. 3.1) is ~15.5 km long; an additional 1.25 km of road on either side of the park boundaries was surveyed to have equivalent lengths across all three survey areas. The sections of the study road in the GRC area

and ISWP were separated by a kilometre of unstudied road; this was done in an attempt to avoid autocorrelation between the ending point of R22a and start point of R22b (Fig. 3.1). This method also maximised the study area that contained settlements that have been constructed along areas with seasonally present water bodies and non-perennial streams in the GRC region which is encircled by protected areas. A gradient of human density existed across all three locations. The HiP section constituted an area free of human dwellings, as no homes/permanent habitations were present within 500 m of the R618. Despite the ISWP being listed as a protected area, its portion of road still contained a small number of permanent human dwellings that did not belong to park staff, thereby representing an area with sparse human occupation (≤ 7 dwellings per kilometre). This sparse occupation was also the case within the 1.25 km of survey road on either side of the protected land at the outset of the survey. The GRC survey area was characterised by a range of human dwelling densities, with only areas that had permanent water or collected water having no human dwellings present at the outset of the survey, with roughly four to ten new dwellings constructed per month, rapidly transforming land that was usable for construction. Further location description details are presented in Cormac et al. (2025).

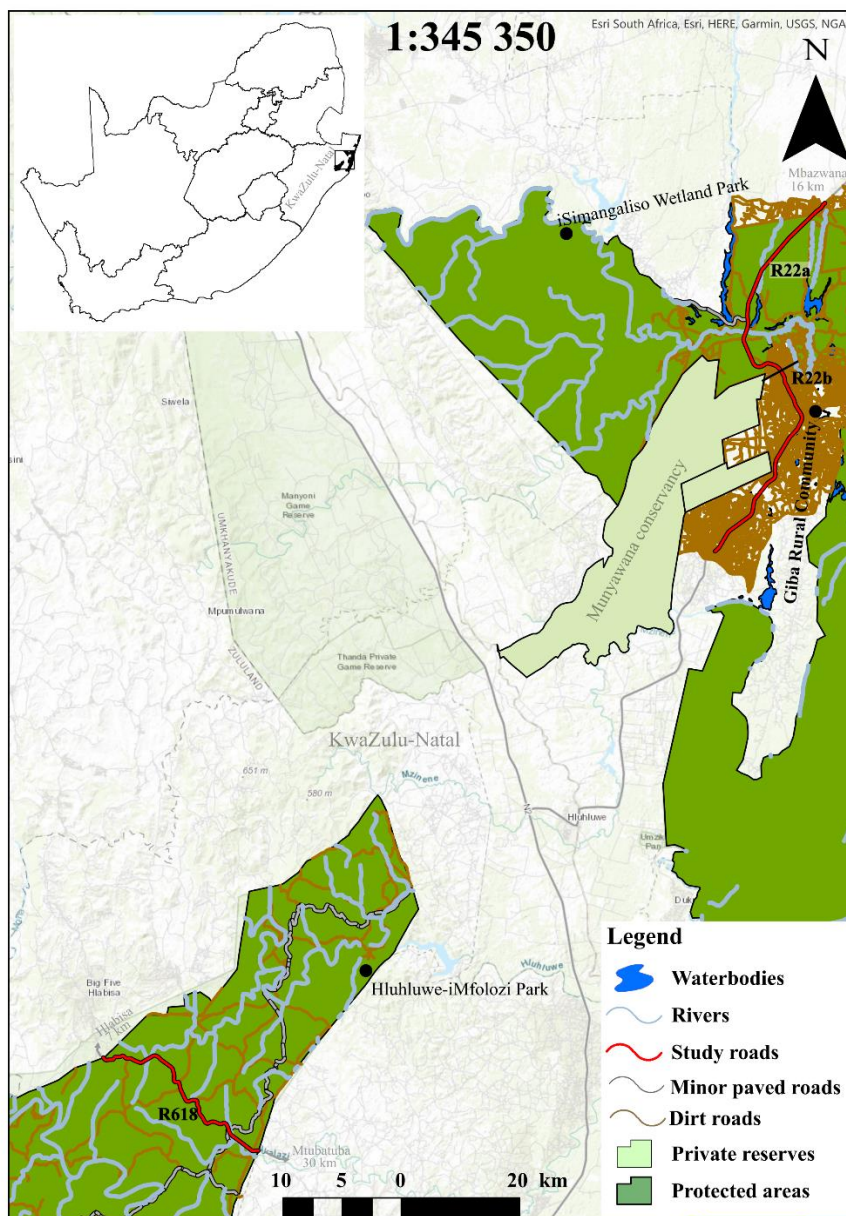


Figure 3.1: Location of study roads in protected and unprotected land in northeastern KwaZulu-Natal, South Africa. (Note: The black line along R22 indicates a separation between iSimangaliso and Giba study sections).

3.3.2 Data collection

Our study was an Ezemvelo KZN Wildlife registered project, permit number E/5161/05. We collected reptile occurrence data from observed live-on-road (LOR) and dead-on-road (DOR) specimens by conducting driven surveys for an hour daily at sunrise and sunset for a week,

once a month per site, from March 2021 to March 2023. Surveys in ISWP and GRC were conducted concurrently. Every fourth evening and the fifth morning were used as rest periods (selected based on observed reduction in detection efficiency after 3.5 consecutive days of survey). All surveys had two observers. All surveys were conducted at an average speed of $40 \text{ km h}^{-1} \pm 5 \text{ km h}^{-1}$ following the methods in Cormac et al. (2025). We placed BrowningYo© (Birmingham, USA) Strike Force HD ProX trail cameras (model BTS-5HDPX) at each entrance of both protected areas to record vehicle traffic data for each survey period; no trail cameras were placed in the community portion because of theft risk. Data for each reptile specimen found followed the protocol of Cormac et al. (2025). This included identification, geographic location coordinates, photographs (for verification purposes), distance of road to nearest vegetation (m), road characteristics (surface slope presence, shape, side slope presence, shoulder vegetation presence, number of lanes), and habitat characteristics (vegetation type, presence of water). The number of vehicles that passed through the area on the day of discovery and the day of the week (as a proxy for vehicle traffic patterns, the number of vehicles passing through both portions of the R22 was assumed equal because of equipment theft). In addition, climatic conditions (rainfall (ml), ambient air temperature ($^{\circ}\text{C}$), road surface temperature ($^{\circ}\text{C}$), presence of cloud cover); moon phase, moon illumination (%), length of day (h) and time of day (as a proxy for solar light intensity), sunrise (AM) or sunset (PM)). We also recorded the presence of wildlife paths, existing traffic calming measures and potential crossing structures using a global positioning system (GPS) using the GPS logger app v.3.2.1 (BasicAirData; Paris, France).

3.3.3 Analyses

To determine the significance of the number of DOR and LOR records between protected and unprotected areas, we used the number of records per month (across 20 months) to conduct a

paired sample t-test, with data conforming to all test assumptions. We ran further Kruskal-Wallis tests to determine the significance of each reptile suborder within study areas because the data did not conform to the assumptions of parametric statistical tests (T-test or ANOVA). We conducted pairwise Wilcoxon tests using a Bonferroni adjustment method for any Kruskal-Wallis tests where significance was found. We conducted analyses using each week of a survey as a replicate, resulting in a total of 20 replicates per study area (Cormac et al., 2025). Statistical analyses of reptile occurrence demographics were conducted in RStudio using the “vegan” package. The listed threats to species found were compiled from IUCN (2023) and South African National Biodiversity Institute (SANBI, 2023) species assessments to show the number of identified reptile species found for which roads are indicated as threats. Species Red List statuses were confirmed using International Union for Conservation of Nature (IUCN) species assessments (IUCN, 2023).

We created heat maps of reptile occurrence (collected from LOR and DOR records) using ArcGIS Pro (ESRI, 2023). During the first and second years of data collection, we created these for wet and dry seasons in each study area. We created clusters using constant density with a radius of 5 m at a scale of 1:100 000. To quantify the number of reptile occurrences per kilometre and to conduct hotspot analyses (Ord and Getis, 1995), we divided the study roads into 1 km segments (Supplementary Information Fig. S3.1). We compared hotspot result layers using the ArcGIS Pro Hotspot analysis comparison tool (ESRI, 2023) to determine if any identified significant road segments remained across seasons. A Moran’s I test was conducted on the 1 km segments to test for autocorrelation using Euclidean fixed distance bands with row standardisation, with results showing no difference from a random pattern (Z-score: -0.156, $p = 0.876$).

We conducted redundancy analyses (RDA) in RStudio (RStudio Team, 2020) using the R-package “vegan” (Oksanen et al., 2013). The RDAs were conducted to determine the effects

of various ecological factors, with factors grouped according to whether they were environmental (vehicle traffic, road construction and habitat) or climatic (light and weather) in nature (Supplementary Information Table S3.1). Numerical variables were centred and standardised, and all variables were tested for collinearity; any variables that showed above 70% correlations were removed (only included variables are contained in Table S3.1). Variables were separated because environmental variables masked climatic variables. The RDAs were conducted between protected and unprotected areas and between counts per reptile suborder in each study area. We used the RStudio code to conduct analyses from the R workshop series created by the Québec Centre for Biodiversity Science (QCBS, 2023), where variables included categorical information, and each category was included as a separate variable to determine the effect of each variable category on the overall system (category information is detailed in Supplementary Information Table S3.1). Variables with significant or near significant ($p \leq 0.1$) influential effects were included in RDA figures; if no significance was detected, the five most influential factors were displayed, as these variables may be important for mitigation purposes.

3.4 Results

3.4.1 Occurrence demographics

A total of 300 individual reptiles were recorded across the two years of surveys, with the largest number of reptiles recorded in ISWP ($n = 118$, 39.3%) (Table 3.1) where HiP had 106 (35.3%) individuals and GRC had the remaining 76 (25.4%) records. Of the 300 recorded individuals, 267 were identified to species level, with the remainder identified to varying taxonomic levels (Supplementary Information Table S3.2). Of the identified species, puff adders (*Bitis arietans*) had the highest number of recorded individuals in all three study areas, with a total of 61 individuals. Of the 34 identified species, only nine had more than 50% of recorded individuals

found alive and only the rhombic egg eater had over ten live individuals found (Supplementary Information Table S3.2). The puff adder, a slow-moving snake species, the most recorded species had the highest number of mortalities with 47 DORs recorded. Comparatively, for other slow-moving species like the south-eastern hinged-back tortoise (*Kinixys zombensis*), despite having less than ten individuals found, 86% (n = 6) of individuals were found alive. No live individuals were recorded for any of the five observed (identified) semi-arboreal snake species. However, only seven of the 34 confirmed reptilian species (Supplementary Information Table S3.2) recorded during the survey listed habitat change as being a threat to the species globally or domestically, however only *Kinixys natalensis* had an evaluation other than least concern (Supplementary Information Table S3.3). Although *Limaformosa capensis*, a least concern species mentioned roads directly as a threat to the species (Supplementary Information Table S3.3).

Table 3.1: Numbers of recorded reptiles according to suborder in Hluhluwe-iMfolozi Park (HiP), iSimangaliso Wetland Park (ISWP) and Giba Rural Community (GRC) in KwaZulu-Natal, South Africa, from March 2021 to March 2023.

Suborder	Location numbers			Total
	HiP	ISWP	GRC	
Cryptodira	10	8	12	30
Lacertilia	15	12	4	31
Pleurodira	5	7	16	28
Serpentes	76	91	44	211
Total	106	118	76	300

Table 3.2: Numbers of reptiles found alive and dead on roads in and outside of protected areas across Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park and Giba Rural Community in KwaZulu-Natal, South Africa.

Suborder	Alive		Dead	
	Protected	Unprotected	Protected	Unprotected
Cryptodira	4	4	14	8
Lacertilia	8	1	16	4
Pleurodira	3	6	10	11
Serpentes	36	3	124	48
Total	51	14	164	71

We did not find a significant difference in the number of reptiles recorded on the roads in each location (Kruskal-Wallis test, $\chi^2 = 4.028$, $df = 2$, $p = 0.134$); however, we did find significant differences in the number of individuals belonging to different reptile suborders (Kruskal-Wallis test, $\chi^2 = 59.982$, $df = 3$, $p < 0.001$), with significantly more snakes (Serpentes) recorded than any of the other reptile suborders (pairwise Wilcox test, Serpentes vs: Cryptodira: $p < 0.001$; Lacertilia: $p < 0.001$; Pleurodira: $p < 0.001$), we did not find any significant differences between any of the other suborders. As with the location-specific counts, snakes comprised the majority of records in protected and unprotected areas, with counts of 160 (53.3%) and 51 (17.0%), respectively (Table 3.2). Significantly more reptiles were recorded as DORs ($t = -3.407$, $df = 38$, $p = 0.002$), with 235 (78.3%) found dead (Table 3.2) and no significance between the number of dead reptiles found between protected and unprotected land, with DORs representing 76.3% (164) and 83.5% (71) of records in protected and unprotected areas respectively. In HiP, representing a wholly protected area, 72.6% (77) of reptiles were found DOR. In GRC, a wholly unprotected area, 82.9% (63) of reptiles were

found DOR and 80.5% (95) of reptiles in ISWP, an area with low permanent human presence, were DOR.

3.4.2 Hot spots of occurrence

In HiP, averages of 2.4 reptiles km⁻¹ and 2.3 reptiles km⁻¹ during first and second wet seasons respectively, whereas respective first and second wet season reptile occurrence rates of 3.9 reptiles km⁻¹ and 1.7 reptiles km⁻¹ were recorded in ISWP and respective wet season occurrence rates of 2.6 reptiles km⁻¹ and 1.4 reptiles km⁻¹ in GRC. During respective dry seasons in HiP average reptile occurrence rates of 0.6 reptiles km⁻¹ and 0.5 reptiles km⁻¹ were recorded. ISWP had lower dry season rates of reptile occurrence with 0.5 reptiles km⁻¹ and 0.2 reptiles km⁻¹ respectively. Dry season occurrence rates were lowest in GRC which saw average rates of 0.1 reptiles km⁻¹ and 0.2 reptiles km⁻¹ (see seasonal per kilometre reptile occurrence rates for each location in Supplementary Information Table S3.4).

The hotspot analysis of the first wet season identified six significant reptile occupancy hotspots across all three sites, one HiP (Fig. 3.2) and the other five in ISWP (Fig. 3.3), and no hotspots were identified in GRC (Fig. 3.4). Additionally, four significant cold spots were identified for reptiles, with three in HiP and one in GRC. The hotspot analysis of the second wet season identified only one significant hotspot for reptiles in each of the three study sites. No additional cold spots were identified for reptiles. The hotspot analysis of the first dry season identified only three significant reptile occupancy hotspots across all three sites, two in HiP (Fig. 3.5) and one in ISWP (Fig. 3.6), no statistically significant hotspots for reptiles were identified in GRC (Fig. 3.7) The hotspot analysis of the second dry season identified four significant hotspots for reptiles across all three study sites. Three hotspots were located in HiP at the edges of the park, and one in GRC; no reptile hotspots were identified in ISWP. No

reptile cold spots were identified during dry season periods. The degrees of freedom were 53 across both wet and dry season hotspot analyses.

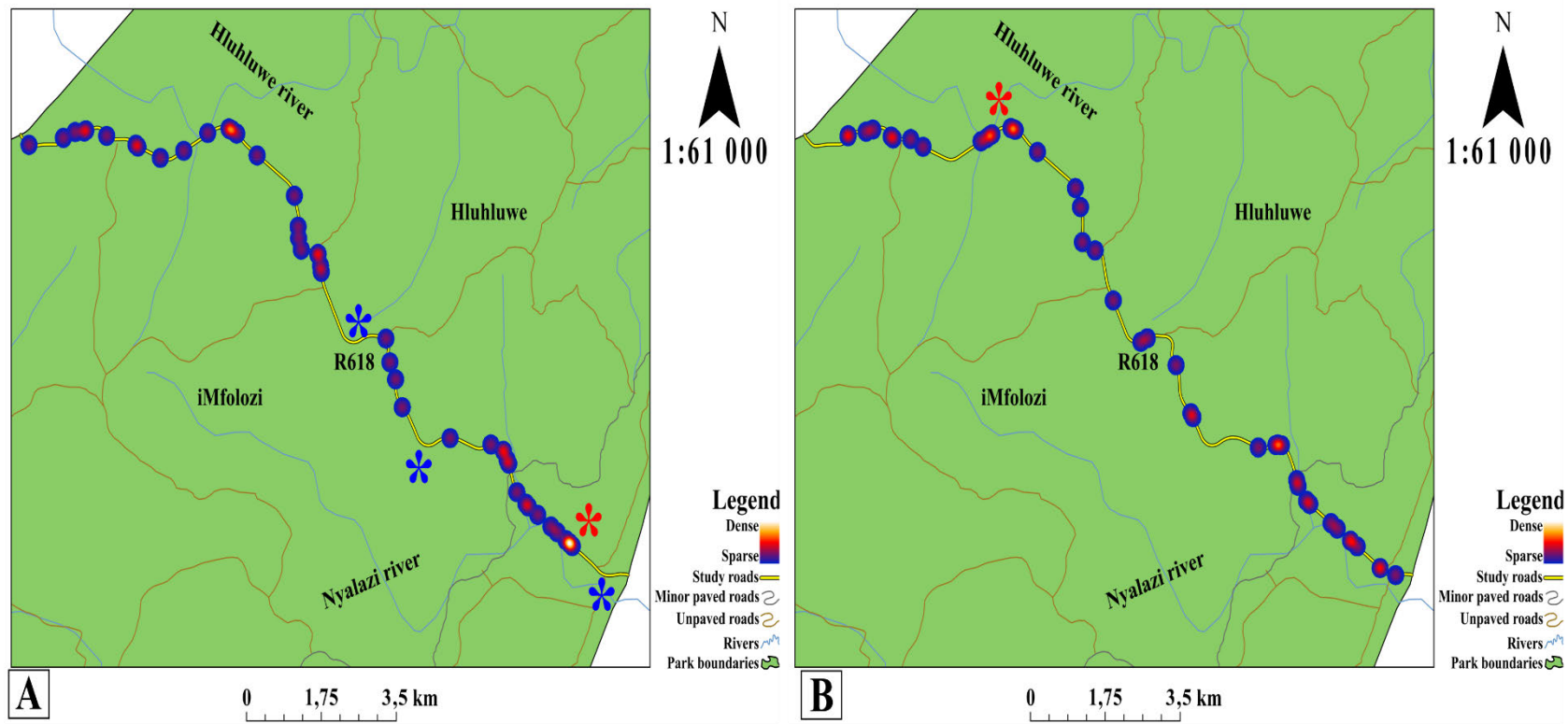


Figure 3.2: Wet season reptile occurrence along a major public paved road in Hluhluwe-iMfolozi Park, where a) is a heatmap of the first year and b) is for the second year of surveys. (Note: Red asterisks represent significant hotspots, and blue asterisks represent significant cold spots).

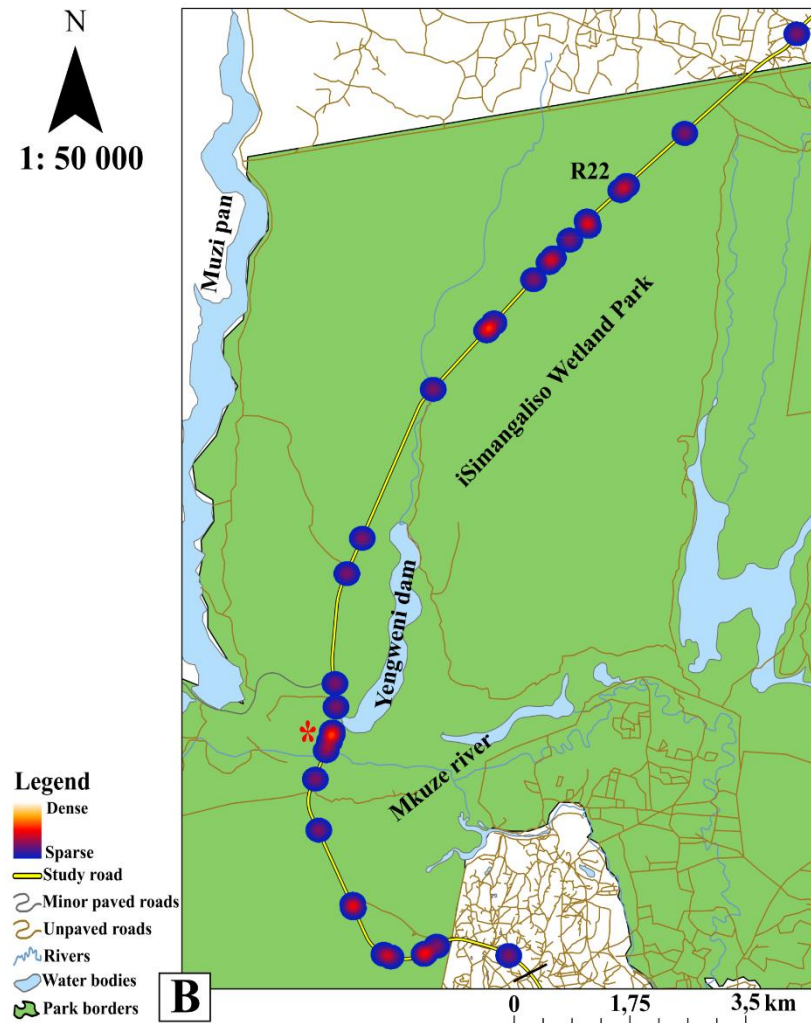
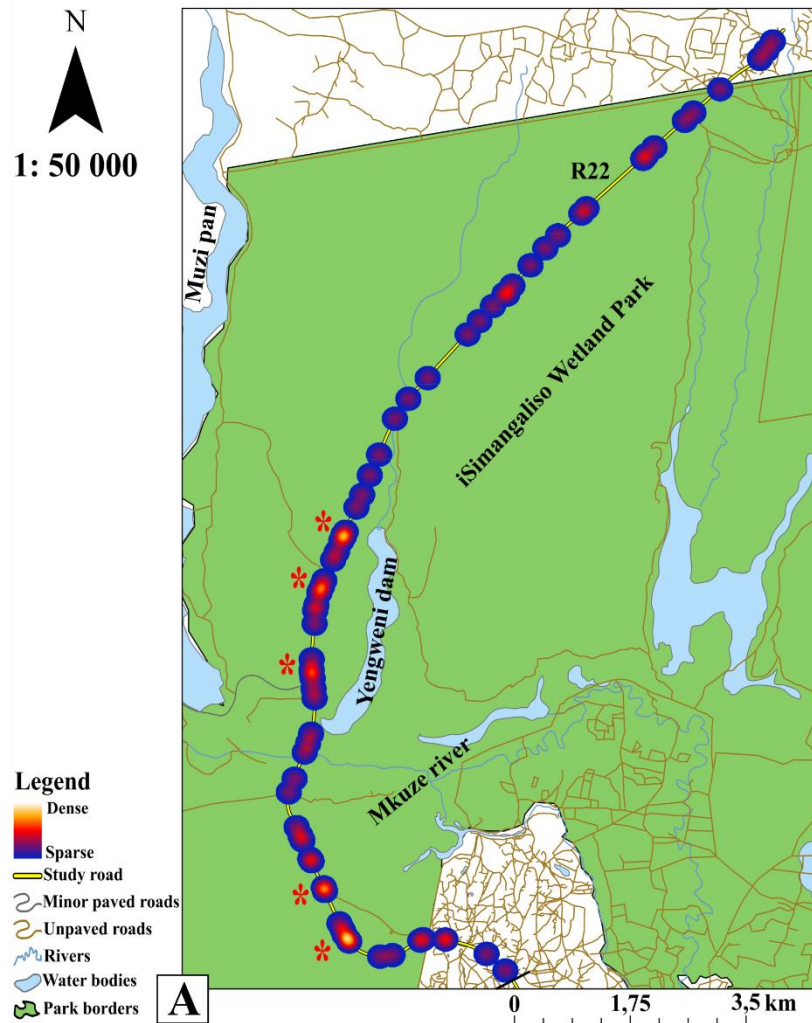


Figure 3.3: Heatmap of wet season reptile occurrence along a major public paved road in iSimangaliso Wetland Park, where a) represents the first year and b) represents the second year of surveys. (Note: The black line represents the separation between the iSimangaliso and Giba community survey areas. Red asterisks represent significant hotspots).

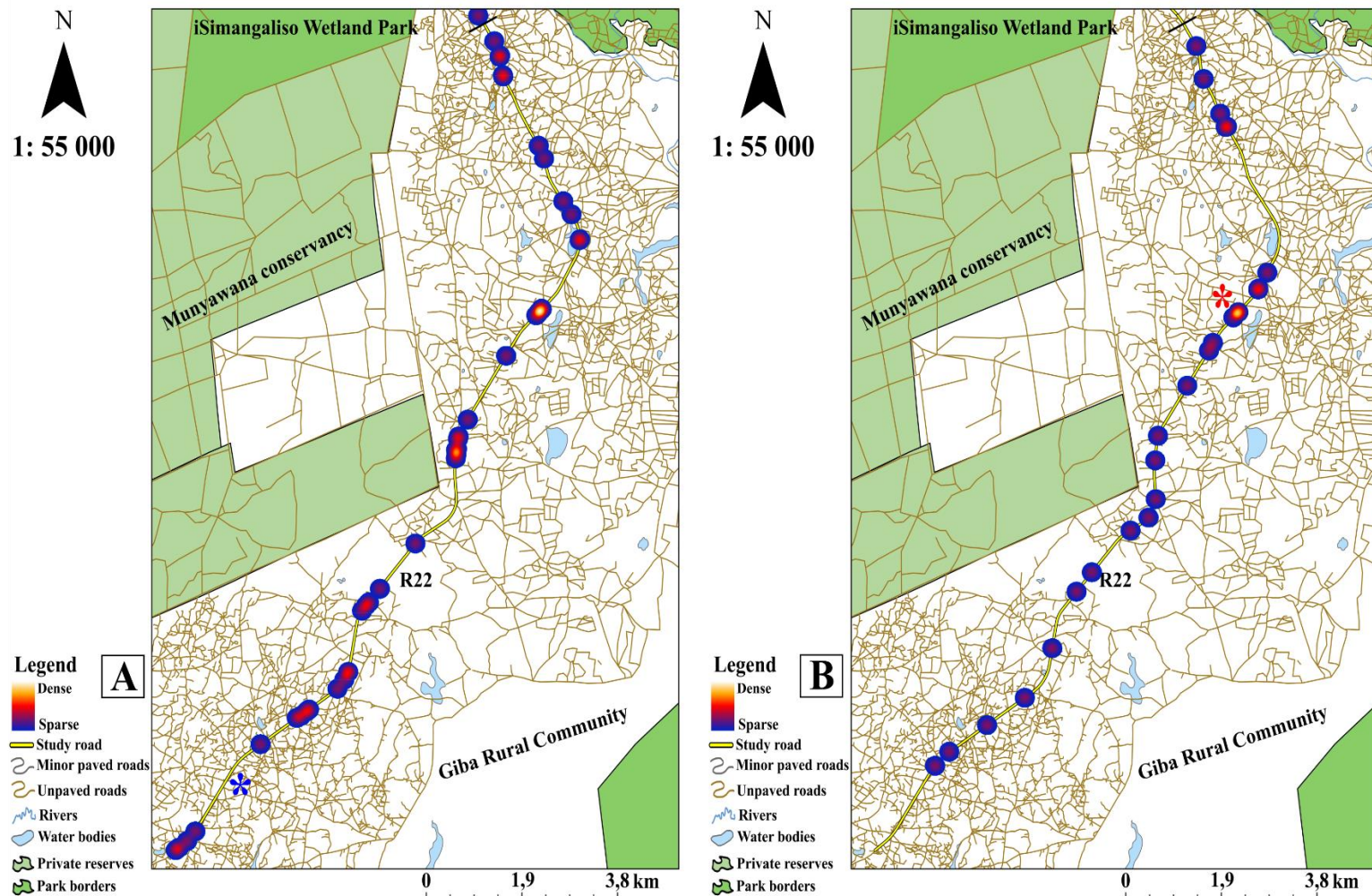


Figure 3.4: Heatmap of wet season reptile occurrence along a major public paved road in iSimangaliso Wetland Park, where a) represents the first year and b) represents the second year of surveys. (Note: The black line represents the separation between iSimangaliso and Giba community survey areas. Red asterisks represent significant hotspots, and blue asterisks represent significant cold spots).

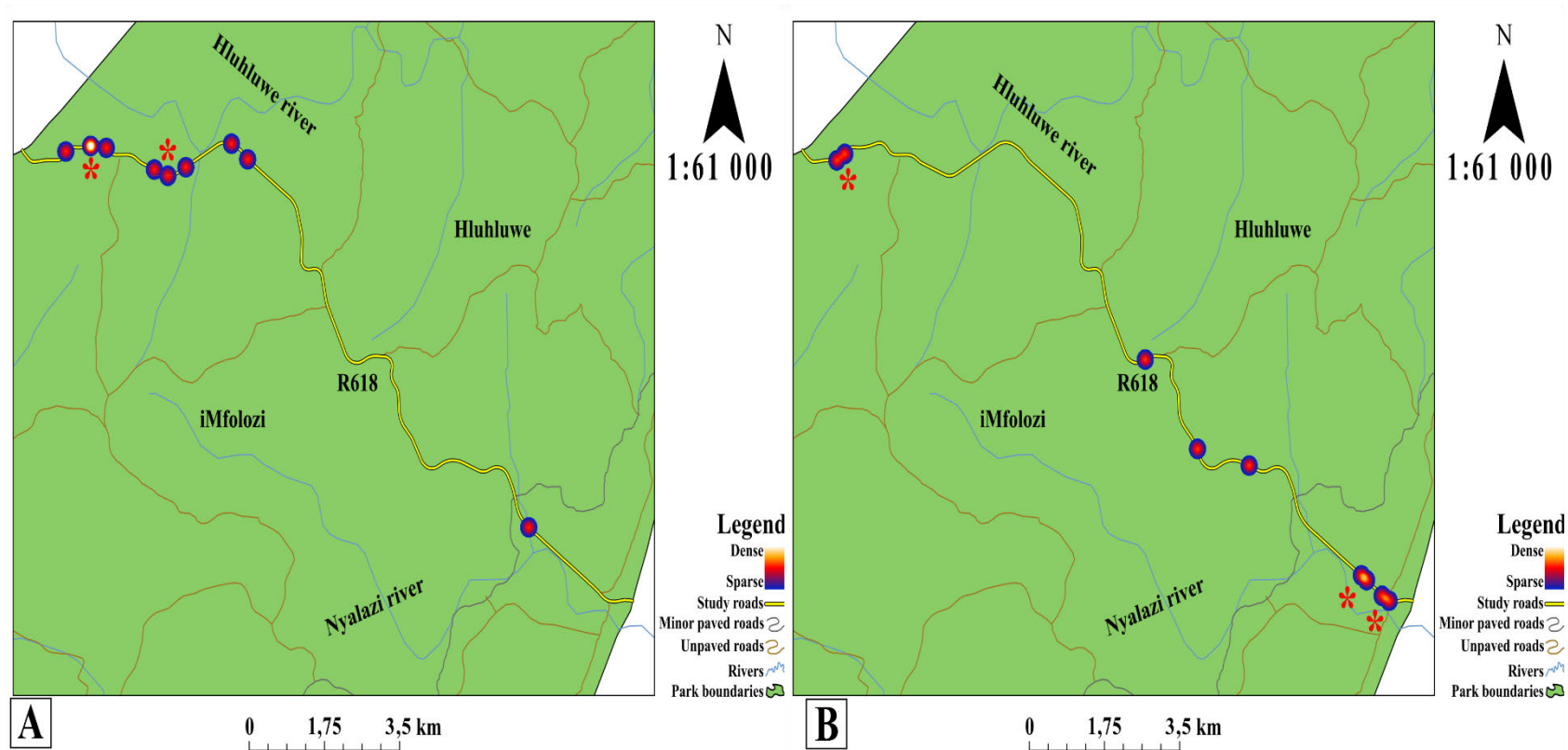


Figure 3.5: Heatmap of dry season reptile occurrence along a major public paved road in Hluhluwe-iMfolozi Park, where a) represents the first year and b) represents the second year of surveys. (Note: Red asterisks represent significant hotspots.).

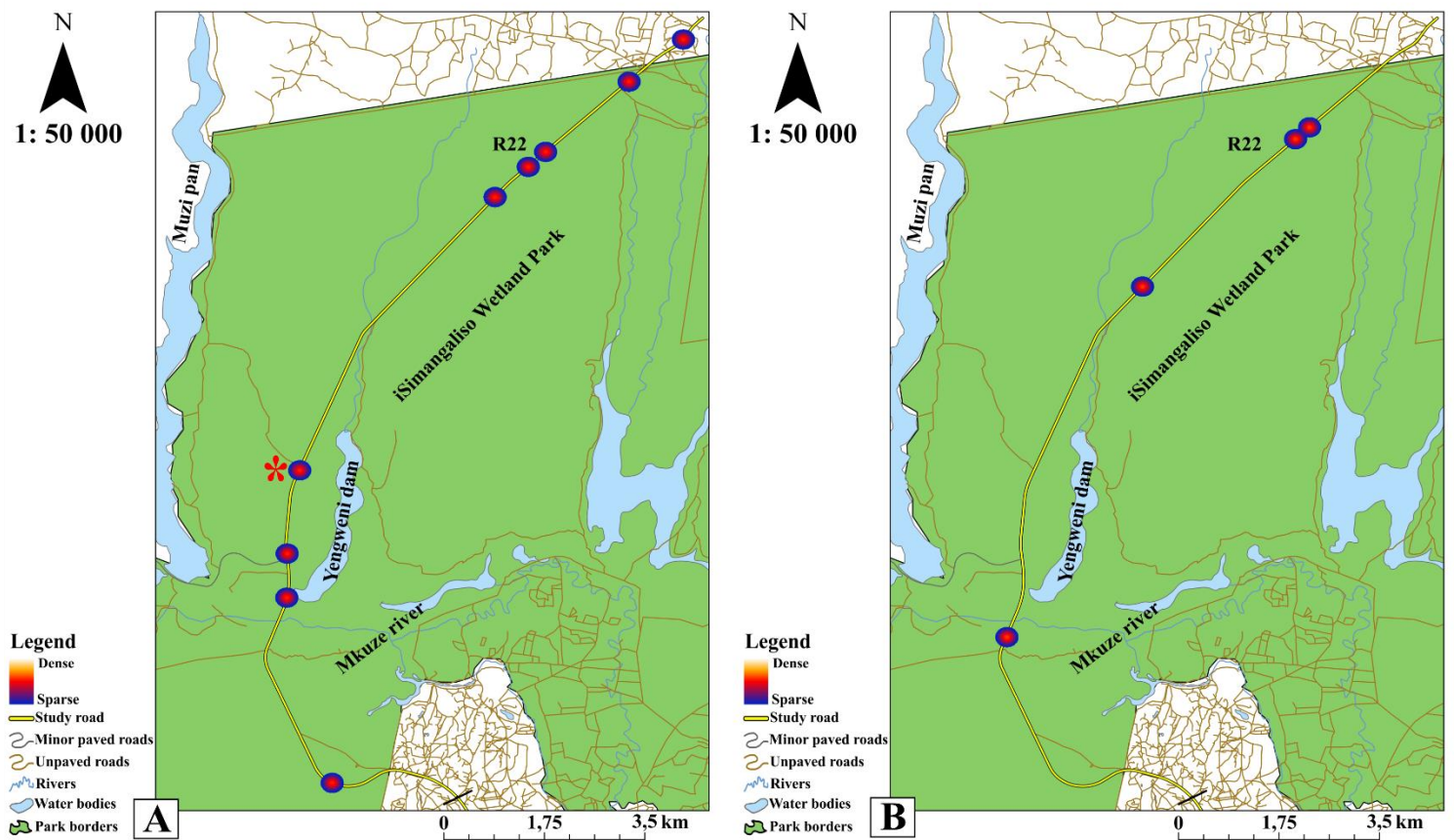


Figure 3.6: Heatmap of dry season reptile occurrence along a major public paved road in iSimangaliso Wetland Park, where a) represents the first year and b) represents the second year of surveys. (Note: The black line represents the separation between iSimangaliso and Giba community survey areas. The red asterisk represents a significant hotspot).

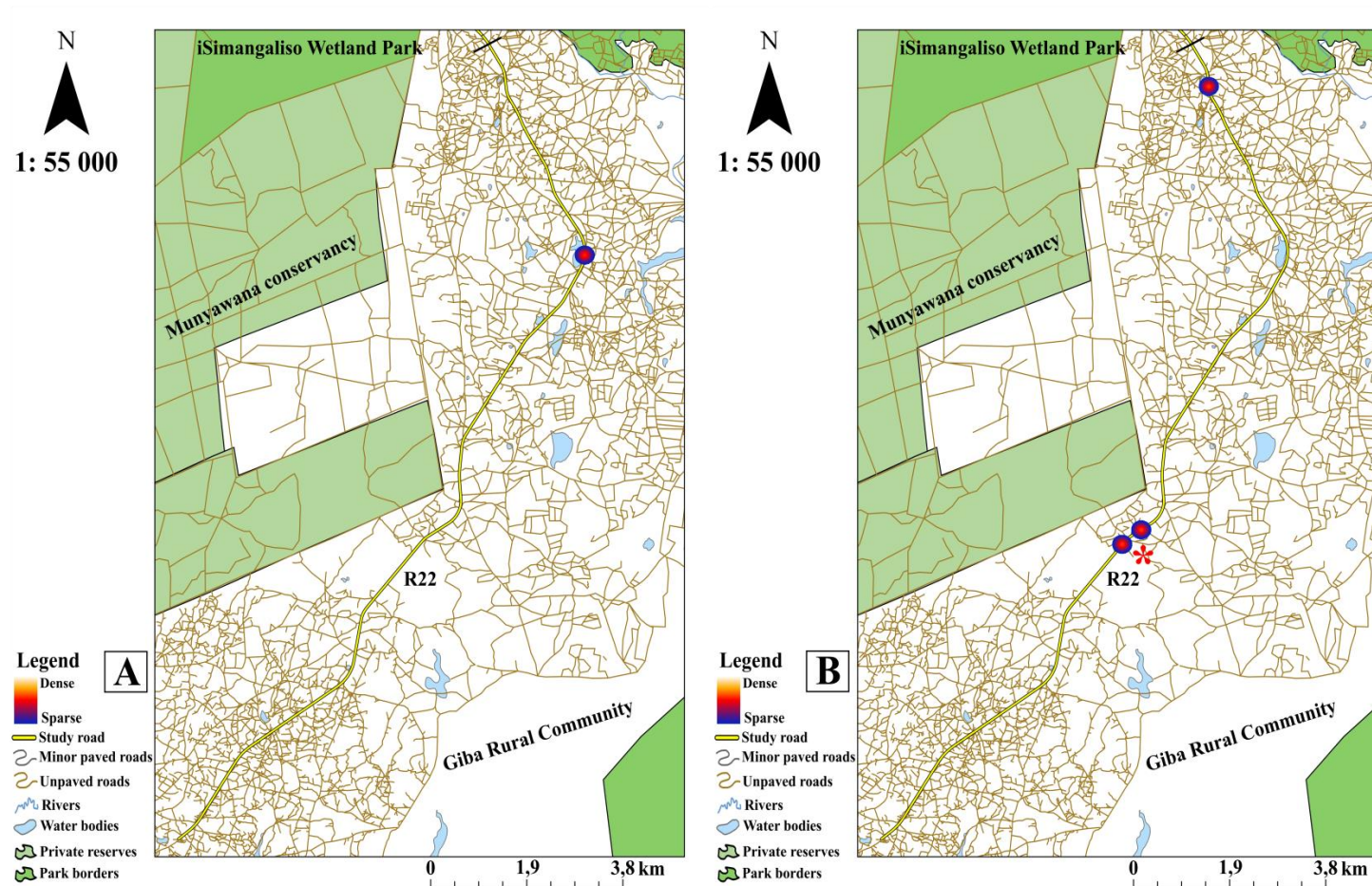


Figure 3.7: Heatmap of dry season reptile occurrence along a major public paved road in iSimangaliso Wetland Park, where a) represents the first year and b) represents the second year of surveys. (Note: The black line represents a separation between the iSimangaliso and Giba community survey areas. The red asterisk represents a significant hotspot).

The comparison of wet and dry season hotspots found an overall similarity of 0.79 in the first year and an overall similarity of 0.93 in the second year. Hotspot comparison between wet seasons found an overall similarity of 0.85. Of all the significant locations identified within single seasons, no cross-season locations were found for reptiles in any of the three study areas. Hotspot comparisons between dry seasons found an overall similarity of 0.93, with only kilometre two in HiP identified as a cross-season hotspot. Comparisons of the first-year wet season and second-year dry season found an overall similarity of 0.80, and the dry season of the first year and wet season of the second year had an overall similarity of 0.95 with kilometres two and four remaining hotspots in HiP with no hotspots remaining in ISWP nor GRC.

3.4.3 Occurrence influencing factors

The absence of standing water significantly increased the number of reptiles recorded along roads in protected and unprotected areas, with a larger effect on occurrence rates in unprotected areas (Table 3.3). The presence of road-adjacent altered habitat significantly increased the number of reptiles recorded in unprotected areas despite the altered habitat being present in both ISWP and GRC (Table 3.3, Fig. 3.8a). The number of vehicles per day, while not having a significant effect, had the largest negative influence of reptile detection along roads in both protected and unprotected land, reptile detection decreasing as the number of vehicles increased in protected areas and increasing in unprotected areas. No climatic variables were found to have a significant effect on reptile detection rates (Table 3.3, Fig. 3.8b) however, the most influential climatic variables included length of day (h) (Dlen), presence of partial cloud cover (CC_P), the difference between road and air temperatures (°C) (RATD), the proportion of moon illumination (%) (Mill) and the absence of visual obstructions (Vobs_A) (Fig. 3.8b). Where reptile detection decreased in both protected and unprotected land as days got longer and partial cloud cover was present, and reptile detection decreased as moonlight increased.

Reptile detection increased with the absence of visual obstructions in both protected and unprotected areas, and increased in protected areas as the road-air temperature difference increased.

Table 3.3: Comparative analytics of significant ecological factors affecting reptile road occupancy in protected and unprotected areas (PA), and reptile suborders in Hluhluwe-iMfolozi Park (HiP), iSimangaliso Wetland Park (ISWP) and Giba Rural Community (GRC).

Figures	Constraining variables (%)	df	F	R ₂	p	Eigen values	Significant effect
10a (PA)	94.4	3	9.45	0.571	0.001	65.87; 16.22	Absence of standing water (0.001); Presence of altered habitat (0.025)
10b (PA)	30.8	1	5.33	-0.195	-	21.07; 5.75	None
11a (HiP)	98.3	3	6.15	0.49	0.001	6.36; 1.18	Presence of savanna habitat (0.001); Weekend (0.005)
11b (HiP)	42.4	1	8.38	-0.15	-	2.97; 0.33	None
12a (ISWP)	69.2	3	1.037	0.025	-	18.39; 0.44	None
12b (ISWP)	16.1	1	0.264	-0.045	-	3.85; 0.36	None
13a (GRC)	80.0	2	4.77	0.32	0.009	13.91; 1.52	Vehicles per day (0.02); Distance to brush (0.03)
13b (GRC)	68.1	2	6.96	0.361	-	12.05; 1.06	Partial cloud cover (0.05)

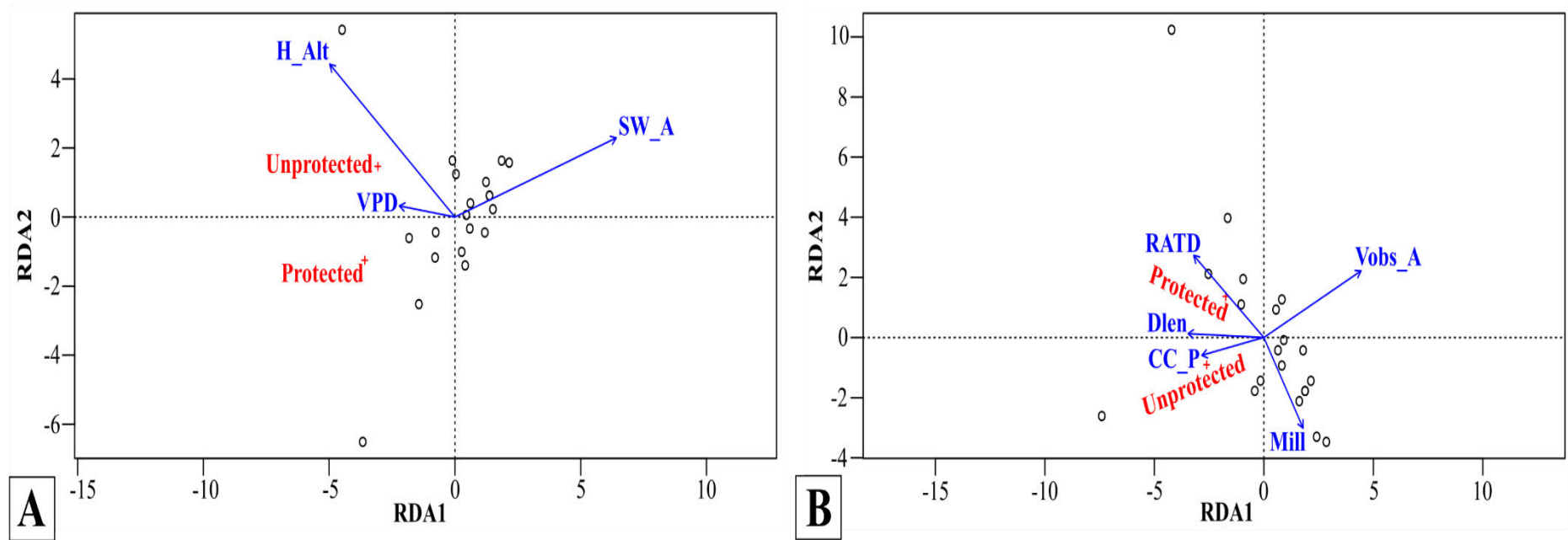


Figure 3.8: Effect of ecological factors on reptile road occupancy in protected and unprotected areas, where A) are environmental variables and B) are climatic variables. (Note: Abbreviations represent: Partial cloud cover (CC_P); Length of day (hrs) (Dlen); altered habitat (H_alt); Moon illumination (%) (Mill); Road-air temperature difference (°C) (RATD); Absence of standing water (SW_A); Absence of visual obstructions (Vobs_A) and the number of vehicles per day (VPD). Hollow circles represent survey months).

The RDA of reptile suborder road occupancy in HiP found that the most influential environmental variables included weekend days (Weekend), the presence of savanna habitat (H_Sav) (Fig. 3.9a) (list of constraining variable biplot scores in Supplementary Information Table S3.6). The presence of road adjacent savanna habitat significantly decreased the detection of all reptile suborders, decreasing the detection of lizards (Lacertilia) the most. Reptile detection significantly increased for all reptile suborders except lizards on weekend days. No climatic variables were found to have a significant effect on detection rates across reptilian suborders in HiP (Table 3.3, Fig. 3.9b); however, the most influential climatic variables included length of day, presence of partial cloud cover, the difference between road and air temperatures, the absence of wind (Wind_A) and of visual obstructions (Fig. 3.9b). Where detection rates of all suborders, except tortoises (Cryptodira) increased according to decreases of wind presence, day length, road-air temperature and lack of visual obstructions and increases in partial cloud cover.

The RDA of environmental factors on reptile suborder road occupancy in ISWP found no significant effects; however, the most influential variables included the number of vehicles per day, Sloped road edges on both sides (SSE_B), and the absence of sloped road surfaces (SR_A), Sloped edges (SE_A) and animal paths (AP_A) (Fig. 3.10a) (list of constraining variable biplot scores in Supplementary Information Table S3.7). Snake (Serpentes) detection decreased along road portions with flat edges or sloped edges on both sides, with no animal paths. Detection for all reptile suborders decreased in areas with flat road surfaces. Squamate (Lacertilia and Serpentes) detection decreased during sunrise (AM) as road-air temperature differences and day lengths decreased on days that had clear skies (CC_C) with no visual obstructions, whereas Testudines (Cryptodira and Pleurodira) detection increased according to these factors. The RDA of reptile suborder road occupancy in GRC found that the number of vehicles per day and the distance to brush (DTB) had a significant effect (Fig. 3.11a) (list of

constraining variable biplot scores in Supplementary Information Table S3.8). Detection of all reptile suborders decreased as vehicle numbers and distance to brush increased. The only climatic factor that showed a significant effect was the presence of partial cloud cover (Fig. 3.11b), with the detection of all suborders increasing except Lacertilia.

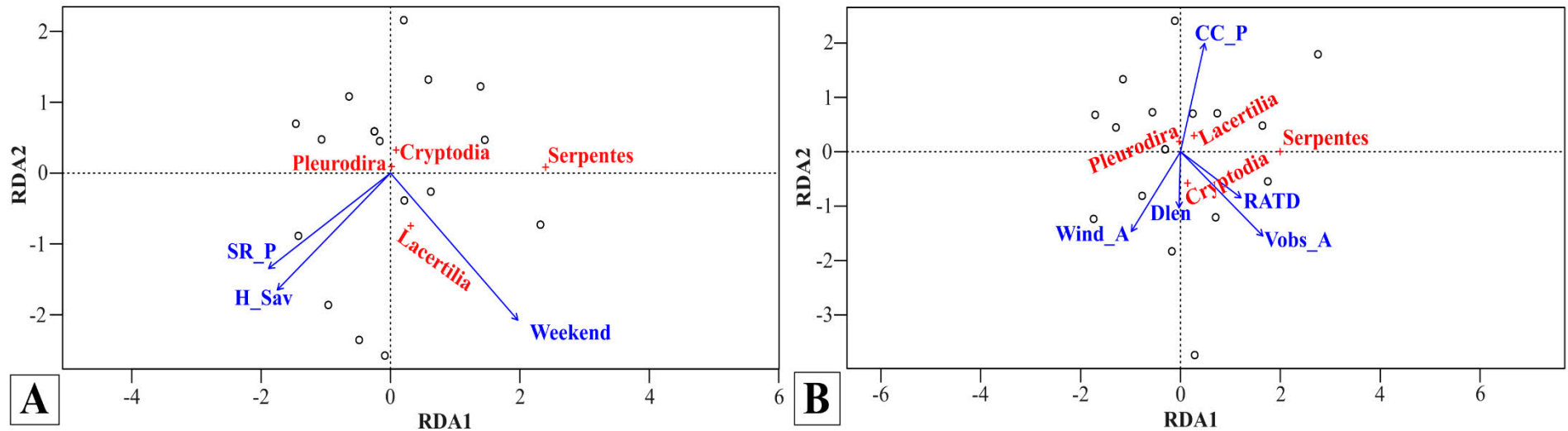


Figure 3.9: Effect of ecological factors on road occupancy of reptilian suborders in Hluhluwe-iMfolozi Park, where A) are environmental variables and B) climatic variables. (Note: Abbreviations represent: Partial cloud cover (CC_P); Length of day (hrs) (Dlen); Savanna habitat (H_Sav); Road-air temperature difference (°C) (RATD); Presence of a sloped road surface (SR_P); Absence of visual obstructions (Vobs_A); Absence of wind (Wind_A) and weekend days (Weekend). Hollow circles represent survey months).

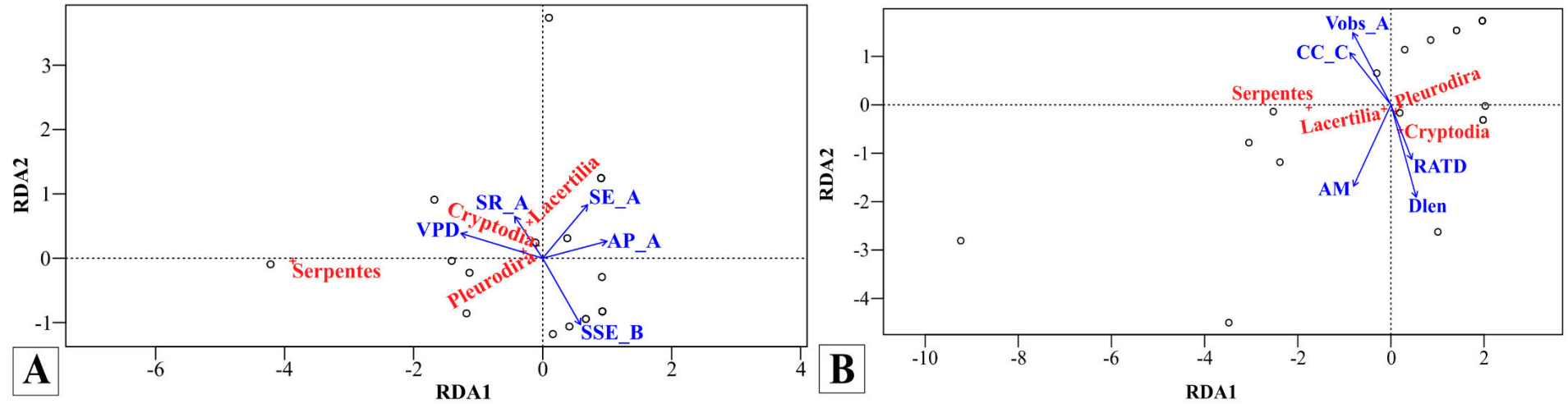


Figure 3.10: Effect of ecological factors on road occupancy of reptilian suborders in iSimangaliso Wetland Park, where A) are environmental variables and B) climatic variables. (Note: Abbreviations represent: Mornings (AM); Absence of animal paths (AP_A); No cloud cover (CC_C); Length of day (hrs) (Dlen); Road-air temperature difference (°C) (RATD); Absence of sloped road edge (SE_A); Absence of a sloped road surface (SR_A); Sloped edges of both sides of the road (SSE_B); No visual obstructions (Vobs_A), the number of vehicles per day (VPD). Hollow circles represent survey months).

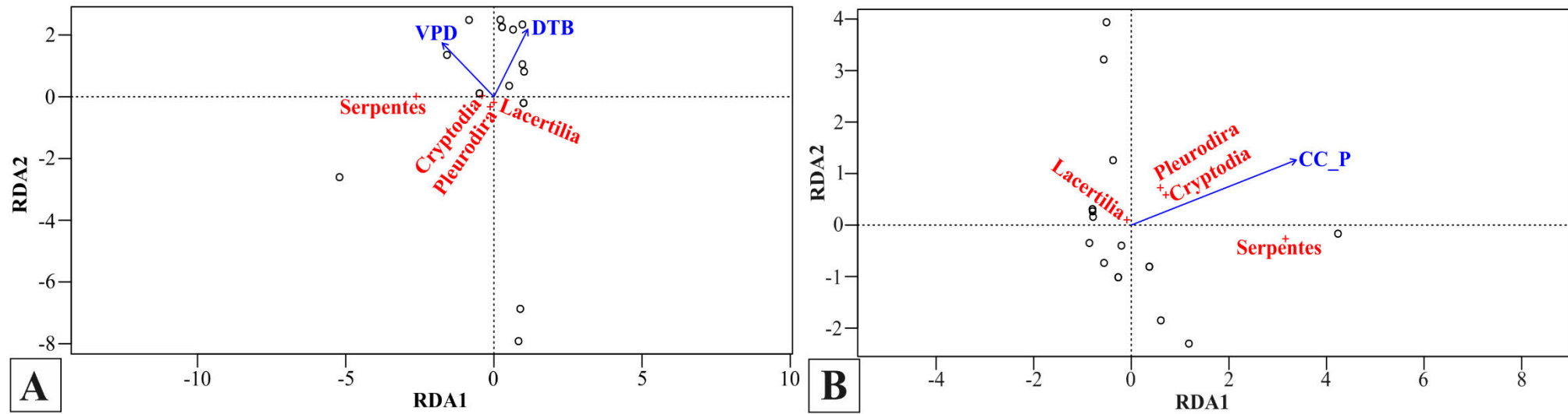


Figure 3.11: Effect of ecological factors on road occupancy of reptilian suborders in the Giba Rural Community, where A) are environmental variables and B) climatic variables. (Note: Abbreviations represent: Partial cloud cover (CC_P); Distance to brush (m) (DTB), and the number of vehicles per day (VPD)).

3.5 Discussion

Our study aimed to determine the effect of land protection on reptile use of public paved roads. We found no significant difference in the total numbers of reptiles on roads in protected and unprotected areas. However, most were dead individuals, with snakes comprising most reptiles recorded. Seasonal differences in reptile counts were observed, with wet seasons having higher detections than dry seasons, and higher detection rates were found within protected areas, as predicted. However, the general distribution of reptile recordings did not change between years. However, HiP had the only hotspot present for most of the study. With said hotspot being characterised by savanna habitat with steep slopes on either side of the road, no structures that fauna could use to traverse safely and no traffic calming measures present during the three seasons that this portion became a hotspot. Additionally, no animal paths surrounded this portion of the road, but the road ran perpendicularly through drainage lines leading to the nearby Hluhluwe River. The hotspots recorded at the park entrances in HiP during the dry season were unexpected as these areas should have the lowest vehicle speeds because of the presence of large speed bumps; however, these areas were characterised by dense vegetation and steep road edges on either side of the road, which could be contributing to slower movement and reaction speeds in the reptiles attempting to cross these portions of the road. The portions of the R22 that fell outside of protected areas had lower reptile presence. They had no sites of consistent reptile presence, with near-zero presence recorded during dry seasons, particularly in the GRC portion of the R22. Generally, environmental factors had a larger effect on reptile road use in protected and unprotected areas than climatic factors. The absence of standing water was the most significant factor in overall reptile detection in both protected and unprotected land; however, the effects on different reptile suborders varied between study locations.

Across all three study areas, snakes comprised most of the total reptiles recorded, making up 211 of the overall 300 records, matching our predictions. However, most snakes recorded, as with the overall number of reptiles recorded, were found dead, with only 39 individuals found alive. Previous studies have found that snakes have been disproportionately affected by roads (Forman and Alexander, 1998; Andrews et al., 2015; Lal and Nadim, 2021; Paterson et al., 2021) being particularly susceptible to vehicle collisions because of physiological and behavioural traits including basking behaviour and slow movement speeds (Shine et al., 2004; McCardle and Fontenot, 2016; Paterson et al., 2021; Jones et al., 2022), as well as anthropogenic persecution (Crawford and Andrews, 2016; Farooq et al., 2023). Of the reptile species recorded, puff adders had the highest number of individuals (61 across all three sites). This species, as with most species recorded, is slow-moving, terrestrial and displays typical “pauser” behaviour to vehicle traffic, similar to many reptiles' behavioural responses to vehicle traffic (Jacobson et al., 2016). However, for southern African pythons (*Python natalensis*), which have large body sizes as adults, only juveniles were found dead, and several larger-bodied individuals that were observed attempting to traverse the R618 in HiP were seen quickly retreating to nearby brush while within the first metre over the road shoulder. This could indicate potentially learned behaviour; however, no studies have been done on learning capability in this python species. A recent review of learning behaviour in non-avian reptiles found several snake species with recorded evidence of the ability for conditional learning, learned avoidance behaviour, and spatial reference memory (Szabo et al., 2021). Although, the low number of large-bodied pythons may be because drivers avoid them to reduce damages caused by collisions (Crawford and Andrews, 2016). However, this species may also be targeted due to their prevalent use in traditional healing practices and bush meat collection, particularly within the areas included in this study (Phaka et al., 2023) No live individuals of the observed semi-arboreal snake species were recorded, being particularly apparent with

southern twig snakes (*Thelotornis capensis*), which were the fourth most common snake species recorded. Semi-arboreal snake species also fall into the “pauser” category despite their typical use of high-speed movements because of their use of camouflage for protection from predators (Jacobsen et al., 2016). In addition to pausing behaviour, stress from being in unnatural terrain makes semi-arboreal snakes more vulnerable to traffic-related mortality (Hartmann et al., 2011; Maschio et al., 2016).

The trends seen in reptiles in all three study areas, as well as between protected and unprotected areas, were influenced by at least one vehicle traffic pattern variable, with either the number of vehicles per day (as predicted) or by weekends (days with higher vehicle traffic because of tourist vehicles) causing an increase in the number of reptile DORs. Similar trends were observed surrounding a Greek protected area where over 80% of reptile road kills occurred in areas with higher traffic volumes (Zevgolis et al., 2023). Andrews et al. (2015) found that vehicular traffic patterns influenced the number of reptile road deaths and reptiles using paved roads. Additionally, at the location level, aspects of road design such as the presence of a sloped road surface in HiP and ISWP and the distance to the natural vegetation in GRC had considerable influences on the detection of reptiles on the paved roads (Sahlean et al., 2024), with the majority again being found DOR. Previous studies have also found similar trends. Roadside banks caused a significant increase in the presence of wildlife mortalities in the Greater Mapungubwe Transfrontier conservation area, South Africa (Collinson et al., 2019). A horizontal and a vertical curve in the shape of a road significantly increased wildlife mortalities in San Jerónimo, Colombia (Lozano and Patiño-Siro, 2020).

Habitat alteration also played a role in reptile road use, with reptile numbers decreasing with altered habitats, as predicted. Gonçalves et al. (2018) and Collinson et al. (2019) found that wildlife mortalities were higher in natural habitats. The relationship between the savanna habitat and the sloped road surfaces also played a role in reptile road use demographics in HiP

where almost all reptile suborders were found less frequently in areas where a sloped road surface passed through the savanna. This trend is similar to the results found by the review by Gunson et al. (2011), which found that multiple studies had recorded the presence of road-associated slopes as a key predictor in road mortalities, with most finding that mortalities decreased where roads bisected areas with steep slopes.

Most reptiles were detected during the wet season as predicted, with dry season average detection rates reaching a maximum of 0.6 reptiles km⁻¹ in HiP. Multiple studies have observed similar temporal patterns with wet season peaks of reptile roadkill. In Rio Grande do Sul State, Brazil, reptile roadkill was concentrated in the summer (Gonçalves et al., 2018). In the nature reserve, “Obedska bara”, Serbia, reptile roadkill double-peaked with a peak at the beginning of spring and a second at the end of summer (Anđelković and Bogdanović, 2022). The double-peaked pattern of reptile roadkill occurred for all reptile taxa on roads in Catalonia and northeastern Spain (Garringa et al., 2017). Additionally, the higher rates of roadkill during the wet season could indicate a reduction in the fleeing capability of reptiles attempting to cross over the road, as crossing structures or refugia that could otherwise be used may be flooded during the wet season (Tassi et al., 2023). However, most climatic factors had no significant influence on reptile detection. Only partial cloud cover in GRC significantly affected the number of reptiles on the paved road. However, factors such as day length and a lack of visual obstructions, i.e. precipitation or mist, were influential in all three locations and in protected, as well as unprotected areas. The change in temperature between ambient air temperature and the temperature of the tarred road surface also showed some influence on reptile detection in protected land. Numerous studies have shown that reptiles alter their behaviour with thermoregulatory needs, with movement patterns (time and type of movements), habitat preferences (basking site location, orientation, etc.), and activity being heavily affected by the mode of thermoregulation employed (Brattstrom, 1965; Clement et al., 2023). Effects of

changes in light and faunal activity rhythms vary according to diurnal rhythms, i.e., the daytime light period (Kleitman, 1949). Consequently, the time of day that a reptile species is most active is directly proportional to the threat of traffic collision (Andrews et al., 2006).

The results of our study can be used to provide key insights to aid in the determination of effective mitigation measures that could be placed to reduce reptile mortality along the R22 and R618 to improve conservation efforts in HiP, ISWP and GRC, with mitigation measures necessary because the area is home to a number of threatened species and high species diversity (Tolley et al., 2024). These measures could include slotted culverts, which have been found to be effective measures to reduce WVCs with small-bodied vertebrates (Grilo et al., 2010) particularly near water sources, these would provide a rapid escape route allowing small bodied species to escape through the road rather than having to reach the road edge. Additionally, seasonally altering speed limits could be used to protect reptiles and other species during periods of high movement activity, such as migration and nest site location. Seasonal changes to speed limits and vehicle access to roads during key activity periods has shown some success at reducing WVCs, particularly when paired with measures that force drivers to obey speed limits (Garrah et al. 2015). Effective short term measures that reduce the negative impacts of the paved roads passing through HiP, ISWP and GRC, would include increasing vehicle speed dampening measures, speed bumps and speed traps, and signage focusing of areas near activity hotspots. These measures have had past success at reducing WVCs in protected areas. (Jaarsma et al., 2006; Collinson et al. 2019). The effective placement of crossing and barrier structures could be used as a long-term protection option; however, further research is needed to determine such structures' environmental impact. Across multiple studies, the placement of crossing structures and temporary mitigation measures were effective forms of wildlife road mortality mitigation; however, these measures depended on the accuracy of structure

placement and seasonal animal movement behaviour (Grilo et al., 2010). Education campaigns to inform local communities surround HiP and ISWP may also prove effective.

However, our study was limited by the need to conduct driven surveys along these roads, as a small number of live specimens were not included because we could not reach them in time for identification. The use of driven surveys to conduct linear infrastructure ecology studies has been well established to provide under representative observations (Teixeira et al., 2013; Santos et al., 2015; Sahlean et al., 2024), however, because of the dangers posed by local wildlife and the need for an armed park ranger to be present foot surveys may prove impossible to conduct as rigorously as driven surveys and would also be restricted to diurnal surveys. Additionally, having secondary observers who were untrained in handling snakes also hindered specimen recovery and data collection speed. Furthermore, scavengers and poachers could remove specimens before being recorded and the lack of available quantitative information of both road design and weather variables may have negatively affected the strength of statistical tests of variables that may be affecting reptile occurrence rates. Additional studies should be conducted to determine the mortality and road use by reptiles on the road networks surrounding the R618 and R22 in the areas surveyed in this study, as well as to determine the full effect that the road networks and anthropogenic changes have on reptiles within all protected areas within South Africa. Scavenging rates of reptilian carcasses along the R618 and R22 should also be conducted to allow for more accurate predictions of the total number of reptiles that fall victim to roadkill. This could be achieved by placing camera traps on a time-lapse at a recorded carcass (carcasses transferred to the road edge to minimise the likelihood of new roadkill), particularly in areas recorded as mortality hotspots. These studies should also be conducted in tandem with studies of the perceptions of reptiles by locals and tourists and drivers' responses to different reptiles along these tarred roads to determine better the full breadth of the risk posed to reptiles by the public paved roads.

The threats reptiles face along the portions of major paved roads that pass through the HiP and ISWP protected areas and adjacent unprotected areas of GRC must be addressed so that reptiles within these regions can be more effectively protected. Both HiP and ISWP are critical conservation areas due to their presence within a biodiversity hotspot region, the subsequent high level of species diversity that they support, and the extreme cultural importance that both parks possess. The GRC region is also of key importance as this region may pose considerable threat to wildlife because of high rates of habitat alteration, and with the area being completely encircled by protected areas it forms an area of concentrated human presence in an otherwise largely unaltered wilderness. It is also apparent that threats to species and protected areas need to be more thoroughly assessed so that threat levels to both can be more accurately relayed, and management and conservation plans can be made more effective, with mitigation measures being made at a site-specific scale. Understanding the full depth of the effect that roads have on the natural environments, particularly on the data-poor African continent, is becoming of ever-increasing importance because of the rate at which anthropogenic changes are occurring and the level of developmental change that the continent is predicted to experience (Dulac, 2013).

CRedit authorship contribution statement

Cameron Cormac: Conceptualisation, Investigation, Formal analysis, Writing – original draft. **Cormac Price:** Conceptualisation, Writing – review & editing. **Jarryd Streicher:** Conceptualisation, Writing – review & editing. **Colleen T. Downs:** Conceptualisation, Sourcing funding, Writing – review & editing.

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Declaration of competing interests

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

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Data availability

Data belong to the University of KwaZulu-Natal and will be made available on reasonable request.

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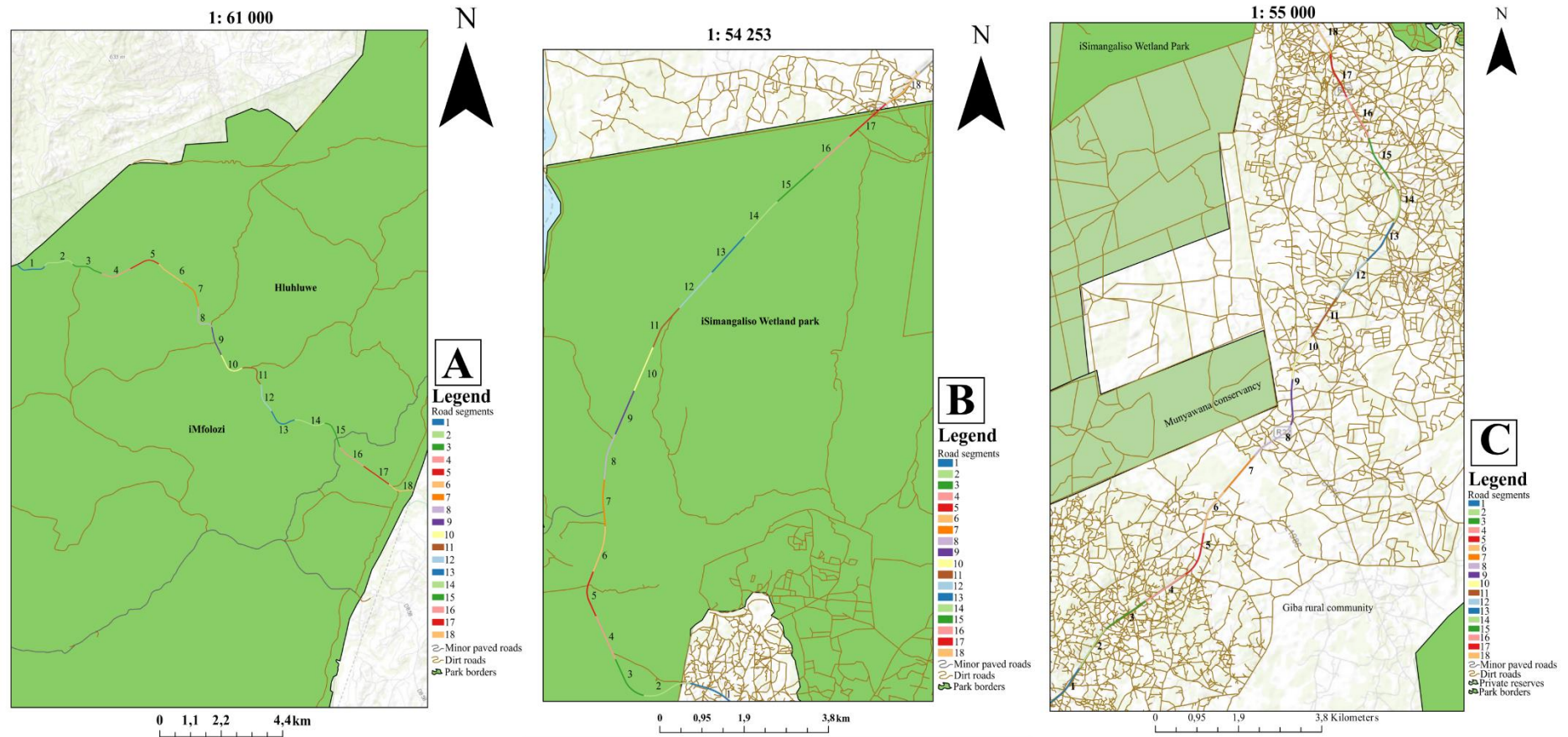
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3.8 Supplementary information



Supplementary Information Figure S3.1: Segments of reptile road occupancy study locations in northern KwaZulu-Natal, South Africa where A) is R618 in Hluhluwe-iMfolozi Park, B) R22 in iSimangaliso Wetland Park, and C) R22 in Giba Rural Community.

Supplementary Information Table S3.1: Factors with associated abbreviations used in the ordination analyses of reptile road occupancy in Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park and Giba Rural Community.

Variable name	Subcategories	Abbreviation
Vehicle traffic variables		
Vehicles per day	none	VpD
Day of week	Weekday	Weekday
	Weekend	Weekend
Road construction variables		
Road shape	Straight	RS_str
	Curved	RS_cur
No. of lanes	Two	Bi-lane
	Three	Tri-lane
Additional structure	Absent	RST_A
	Substructure	RST_Sub
	Superstructure	RST_Sup
Sloped road	Absent	SR_A
	Present	SR_P
Sloped edge	Absent	SE_A
	Present	SE_P
Side of sloped edge	Both	SSE_B
	One	SSE_1
Side Channel	Absent	SC_A
	Present	SC_P
Shoulder Brush	Absent	SB_A
	Present	SB_P
Habitat variables		
Habitat	Savanna	H_Sav
	Bushveld	H_Bush
	Altered	H_Alt
Animal path	Absent	AP_A
	Present	AP_P
Side of road	Larger protected area	Sor_L
	Smaller protected area	Sor_S
Standing water	Absent	SW_A
	Present	SW_P
Light variables		
Length of Day	none	Dlen
Moon illumination	none	Mill
Time of day	Morning	AM
	Evening	PM
Weather variables		
Obstruction of driver visibility (Rain, mist, fog)	Absent	Vobs_A
	Present	Vobs_P
Cloud cover	Clear	CC_C
	Partly cloudy	CC_P
	Overcast	CC_O
Wind	Absent	Wind_A
Road-air temperature difference	none	RATD

Supplementary Information Table S3.2: Reptiles recorded along major paved roads over two years within Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park and Giba Rural Community in northeastern KwaZulu-Natal, South Africa.

Identified individuals											
Scientific name	Common name	Live				Dead				%Live	%Dead
		HIP	ISWP	GRC	Total	HIP	ISWP	GRC	Total		
Cryptodira – Tortoises											
<i>Kinixys natalensis</i>	Natal hinge tortoise	1	0	1	2	0	0	1	1	0.7	0.3
<i>Kinixys zombensis</i>	East African hinge tortoise	2	1	3	6	0	0	1	1	0.9	0.1
<i>Stigmochelys pardalis</i>	Leopard tortoise	0	0	0	0	5	7	5	17	0.0	1.0
Lacertilia – Lizards											
<i>Acontias plumbeus</i>	Giant legless skink	3	1	0	4	4	1	1	6	0.4	0.6
<i>Chamaeleo dilepis</i>	Common flap-neck chameleon	1	0	1	2	4	2	1	7	0.2	0.8
<i>Gerrhosaurus flavigularis</i>	Yellow-throated plated lizard	0	0	0	0	1	1	0	2	0.0	1.0
<i>Homopholis wahlbergii</i>	Walhberg's velvet gecko	0	3	0	3	0	1	0	1	0.8	0.3
<i>Nucras ornata</i>	Ornate sandveld lizard	0	0	0	0	1	0	0	1	0.0	1.0
<i>Varanus albigularis</i>	White-throated monitor	0	0	0	0	0	3	1	4	0.0	1.0
Pleurodira – Terrapins											
<i>Pelomedusa galeata</i>	South African helmeted terrapin	0	1	1	2	4	1	1	6	0.3	0.8
<i>Pelusios castanoides</i>	Yellow-bellied hinged terrapin	0	1	3	4	0	0	1	1	0.8	0.2
<i>Pelusios sinuatus</i>	Serrated hinge terrapin	0	2	1	3	0	1	0	1	0.8	0.3
Serpentes – Snakes											
<i>Afrotyphlops bibronii</i>	Bibron's blind snake	0	0	0	0	0	1	1	2	0.0	1.0
<i>Afrotyphlops schlegelii</i>	Schlegal's blind snake	0	0	0	0	0	4	2	6	0.0	1.0
<i>Atractaspis bibronii</i>	Bibron's stiletto snake	0	0	0	0	1	0	2	3	0.0	1.0
<i>Boaedon capensis</i>	Brown house snake	1	2	0	3	8	7	4	19	0.1	0.9
<i>Bitis arietans</i>	Puff adder	6	6	1	13	11	27	9	47	0.2	0.8
<i>Causus rhombeatus</i>	Rhombic night adder	0	0	0	0	0	1	0	1	0.0	1.0
<i>Crotaphopeltis hotamboeia</i>	Herald snake	0	2	1	3	1	3	4	8	0.3	0.7
<i>Dasypeltis scabra</i>	Rhombic egg eater	10	2	0	12	5	2	2	9	0.6	0.4
<i>Dispholidus typus</i>	Boomslang	0	0	0	0	5	2	2	9	0.0	1.0
<i>Dipsadoboa aulica</i>	Marbled tree snake	0	0	0	0	0	1	0	1	0.0	1.0

<i>Limaformosa capensis</i>	Cape file snake	1	0	1	2	1	1	4	6	0.3	0.8
<i>Lycodonomorphus obscuriventris</i>	Floodplain water snake	0	1	0	1	0	0	0	0	1.0	0.0
<i>Lycodonomorphus rufulus</i>	Common brown water snake	1	0	0	1	0	0	0	0	1.0	0.0
<i>Lycophidion capense</i>	Common wolf snake	1	0	0	1	1	1	1	3	0.3	0.8
<i>Naja mossambica</i>	Mozambique spitting cobra	0	0	0	0	4	1	0	5	0.0	1.0
<i>Philothamnus semivariatus</i>	Spotted bush snake	0	0	0	0	0	1	0	1	0.0	1.0
<i>Psammophis mossambicus</i>	Olive grass snake	0	0	0	0	3	1	1	5	0.0	1.0
<i>Pseudaspis cana</i>	Mole snake	0	0	0	0	0	1	0	1	0.0	1.0
<i>Python natalensis</i>	Southern African python	2	0	0	2	3	5	0	8	0.2	0.8
<i>Telescopus semiannulatus</i>	Eastern tiger snake	0	0	0	0	3	2	1	6	0.0	1.0
<i>Thelotornis capensis</i>	Southern twig snake	0	0	0	0	5	9	4	18	0.0	1.0
<i>Xenocalamus transvaalensis</i>	Speckled quill-snouted snake	0	1	0	1	0	1	0	1	0.5	0.5
Unknown individuals											
Chamaesaura	unknown grass lizard	0	0	0	.	2	0	0	2	.	1.0
Naja	unknown cobra	0	0	0	.	1	2	0	3	.	1.0
Philothamnus	unknown green snake	0	0	0	.	0	1	2	3	.	1.0
Colubridae	unknown colubrid	0	0	0	.	0	1	0	1	.	1.0
Pelomedusidae	unknown terrapin	0	0	0	.	1	2	8	11	.	1.0
Testudinidae	unknown tortoise	0	0	0	.	2	0	1	3	.	1.0
Serpentes	unknown snake	0	0	0	.	2	3	1	6	.	1.0

Supplementary Information Table S3.3: IUCN and SANBI red list assessment of species recorded along R618 and R22 arterial roads over two years in Hluhluwe-iMfolozi Park, iSimangaliso Wetland Park and Giba Rural Community in KwaZulu-Natal, South Africa. DD- Data Deficient, LC-Least concern, VU- Vulnerable.

Species name	IUCN listing	Year	SANBI listing	Year	Listed threats
Cryptodira					
<i>Kinixys natalensis</i>	VU-decreasing	2017	VU-decreasing	2017	Habitat change; exploitation
<i>Kinixys zombensis</i>	Not listed	NA	LC-unknown	2017	Habitat change; exploitation
<i>Stigmochelys pardalis</i>	LC-unknown	2014	LC-stable	2017	Habitat change; exploitation
Lacertilia					
<i>Acontias plumbeus</i>	LC-stable	2019	LC-stable	2017	None
<i>Chamaeleo dilepis</i>	LC-stable	2013	LC-stable	2017	Exploitation
<i>Gerrhosaurus flavigularis</i>	LC-unknown	2014	LC-stable	2017	None
<i>Homopholis wahlbergii</i>	LC-stable	2021	LC-stable	2018	None
<i>Nucras ornata</i>	LC-Stable	2019	LC-stable	2017	None
<i>Varanus albigularis</i>	LC-Stable	2019	LC-stable	2017	Exploitation
Pleurodira					
<i>Pelomedusa galeata</i>	LC-unknown	2017	LC-stable	2017	Habitat degradation; exploitation
<i>Pelusios castanoides</i>	LC-unknown	1996	VU-unknown	2017	unknown
<i>Pelusios sinuatus</i>	Not listed	NA	LC-stable	2017	NA
Serpentes					
<i>Afrotyphlops bibronii</i>	LC-stable	2021	LC-stable	2017	None
<i>Afrotyphlops schlegelii</i>	LC-stable	2019	LC-stable	2017	None
<i>Atractaspis bibronii</i>	LC-unknown	2014	LC-stable	2017	None
<i>Boaedon capensis</i>	LC-stable	2021	LC-stable	2017	None
<i>Bitis arietans</i>	LC-stable	2014	LC-stable	2017	Exploitation; persecution
<i>Causus rhombeatus</i>	LC-stable	2019	LC-stable	2017	None
<i>Crotaphopeltis hotamboeia</i>	LC-stable	2012	LC-stable	2017	None
<i>Dasypeltis scabra</i>	LC-unknown	2014	LC-stable	2017	Habitat change
<i>Dispholidus typus</i>	LC-stable	2012	LC-stable	2017	None
<i>Dipsadoboa aulica</i>	LC-stable	2019	LC-stable	2017	None

<i>Limaformosa capensis</i>	LC-unknown	2014	LC-stable	2017	Exploitation; roads
<i>Lycodonomorphus obscuriventris</i>	DD-unknown	2019	LC-stable	2017	unknown
<i>Lycodonomorphus rufulus</i>	LC-stable	2019	LC-stable	2017	None
<i>Lycophidion capense</i>	LC-unknown	2012	LC-stable	2017	None
<i>Naja mossambica</i>	LC-unknown	2019	LC-stable	2017	None
<i>Philothamnus semivariegatus</i>	LC-unknown	2014	LC-stable	2017	Exploitation
<i>Psammophis mossambicus</i>	LC-Stable	2019	LC-stable	2017	None
<i>Pseudaspis cana</i>	LC-unknown	2014	LC-stable	2017	Exploitation; persecution
<i>Python natalensis</i>	LC-decreasing	2019	LC-increasing	2017	Habitat change; exploitation; persecution
<i>Telescopus semiannulatus</i>	LC-Stable	2019	LC-stable	2017	Exploitation
<i>Thelotornis capensis</i>	LC-Stable	2019	LC-stable	2017	None
<i>Xenocalamus transvaalensis</i>	LC-Stable	2021	LC-stable	2017	Habitat change

Supplementary Information Table S3.4: Seasonal reptile count per kilometre road segment across two years in Hluhluwe-iMfolozi Park (HiP), Isimangaliso Wetland Park (ISWP) and Giba Rural Community (GRC), in KwaZulu-Natal, South Africa.

Road segment	Wet 1			Wet 2			Dry 1			Dry 2			Average	Total
	HiP	ISWP	GRC	HiP	ISWP	GRC	HiP	ISWP	GRC	HiP	ISWP	GRC		
1	1	4	4	0	1	0	0	0	0	2	0	0	1.0	12
2	4	4	0	4	3	1	5	0	0	1	0	0	1.8	22
3	3	6	1	4	3	1	0	1	0	0	0	0	1.6	19
4	2	6	4	0	0	2	3	0	0	0	0	0	1.4	17
5	4	4	4	8	2	1	1	0	0	0	1	0	2.1	25
6	1	3	3	1	5	1	1	1	0	0	2	0	1.5	18
7	1	6	1	2	1	1	0	1	0	0	0	0	1.1	13
8	4	7	1	2	1	2	0	3	0	0	0	2	1.8	22
9	2	7	2	1	1	2	0	0	0	0	0	0	1.3	15
10	0	3	5	2	0	1	0	0	0	1	0	0	1.0	12
11	2	2	1	1	1	1	0	0	0	0	0	0	0.7	8
12	2	2	5	2	3	6	0	0	0	0	1	0	1.8	21
13	0	4	2	0	1	3	0	0	0	0	0	0	0.8	10
14	2	3	3	1	4	0	0	1	1	1	0	0	1.3	16
15	4	2	1	4	2	0	0	2	0	0	1	0	1.3	16
16	5	2	2	3	1	2	1	0	0	0	1	0	1.4	17
17	7	3	4	4	0	1	0	1	0	2	0	1	1.9	23
18	0	3	3	3	1	1	0	1	0	2	0	0	1.2	14
Average	2.4	3.9	2.6	2.3	1.7	1.4	0.6	0.6	0.1	0.5	0.3	0.2	1.4	16.7
Total	44	71	46	42	30	26	11	11	1	9	6	3	25.0	300

Supplementary Information Table S3.5: Redundancy analysis biplot scores of all constraining variables of reptile road occupancy per land protection status

Variable	RDA1	RDA 2	R2 adjusted
Environmental factors			
VPD	-0.26347	0.08817	0.00147
DTB	-0.29947	-0.31287	0.03541
Weekday	0.251467	0.05115	-0.00449
Bi-lane	-0.09851	0.02737	-0.04765
RS_Str	-0.03395	-0.02199	-0.05454
SR_A	-0.2276	0.09036	-0.01254
SE_A	0.100642	-0.27226	-0.03286
SSE_B	0.32611	0.04965	0.02995
SC_A	0.089114	0.35646	-0.02419
RST_A	-0.06718	-0.13946	-0.04812
RST_Sub	0.14334	0.13027	-0.03579
SB_A	0.353801	0.2761	0.05952
H_Sav	0.231568	-0.27932	0.00267
H_Bush	0.131933	-0.07663	-0.04048
SW_A	0.781504	0.06239	0.43346
AP_A	-0.00834	0.02124	-0.05541
Axis Eigenvalues			
RDA1	RDA2	PC1	PC2
65.8660	16.2218	4.02348	0.857125
Climatic factors			
Dlen	-0.5808	0.02128	0.03073
Mill	0.3011	-0.50574	-0.01450
RATD	-0.5377	0.45857	0.03308
AM	-0.2352	-0.05184	-0.04122
Vobs_A	0.7448	0.37203	0.09598
Wind_A	-0.1406	-0.08482	-0.05000
CC_C	0.5434	0.12138	0.02099
CC_P	-0.4747	-0.09963	0.00277
Axis Eigenvalues			
RDA1	RDA2	PC1	PC2
21.0705	5.75402	51.3568	8.787

Supplementary Information Table S3.6: Redundancy analysis biplot scores of all constraining variables of per reptile suborder road occupancy in Hluhluwe-iMfolozi Park

Variable	RDA1	RDA 2	R2 adjusted				
Environmental factors							
VPD	0.5299	0.2183	0.1499				
DTB	-0.3491	0.1750	0.0311				
Weekday	0.2520	-0.0923	-0.0144				
RS_Str	0.0487	0.2824	-0.0581				
SR_A	-0.1048	-0.4232	-0.0437				
SE_A	-0.1499	0.1340	-0.0459				
SSE_B	0.1070	0.5964	-0.0261				
STR_A	-0.4043	0.3843	0.0710				
STR_Sub	0.3725	-0.3100	0.0516				
H_Bush	0.0231	0.0515	-0.0636				
SW A	-0.2938	0.7336	0.0424				
Climatic factors							
Dlen	0.1720	0.5109	-0.0320				
Mill	-0.1624	0.8194	-0.0108				
RATD	-0.0455	0.1712	-0.0631				
AM	-0.0304	0.6137	-0.0396				
Vobs_A	-0.5343	-0.2163	0.1269				
Wind_A	0.1443	-0.0177	-0.0513				
CC_C	-0.4454	0.0841	0.0640				
CC_P	0.8821	0.3298	0.4469				
Environmental factor Eigenvalues							
RDA1	RDA2	RDA3	RDA4	PC1	PC2	PC3	PC4
6.3638	1.1801	0.6156	0.1766	0.1419	0.0000	0.0000	0.0000
Climatic factor Eigenvalues							
2.9748	0.3324	0.2128	0.0767	3.5527	0.9033	0.3716	0.0537

Supplementary Information Table S3.7: Redundancy analysis biplot scores of all constraining variables of per reptile suborder road occupancy in iSimangaliso Wetland Park

Variable	RDA1	RDA 2	R2 adjusted				
Environmental factors							
VPD	-0.4750	0.1450	0.1015				
DTB	-0.2242	-0.0862	-0.0191				
Weekday	0.1857	-0.1215	-0.0312				
RS_Str	-0.1291	-0.0807	-0.0432				
SR_A	-0.1620	0.2432	-0.0363				
SE_A	0.2612	0.3118	-0.0064				
SSE_B	0.2201	-0.3866	-0.0155				
STR_A	0.1466	0.0775	-0.0401				
STR_Sub	-0.1022	-0.1883	-0.0474				
H_Sav	-0.0568	-0.2109	-0.0522				
H_Bush	0.2352	-0.1226	-0.0170				
AP_A	0.3740	0.0994	0.0528				
SW_A	0.0179	-0.2173	-0.0538				
Climatic factors							
Dlen	0.2099	-0.7379	-0.0394				
Mill	0.1654	-0.1594	-0.0500				
RATD	0.1735	-0.4361	-0.0466				
AM	-0.3109	-0.6501	-0.0345				
Vobs_A	-0.3161	0.5765	-0.0365				
Wind_A	0.1961	-0.1335	-0.0494				
CC_C	-0.3416	0.4160	-0.0361				
CC_P	0.1158	-0.0613	-0.0515				
Environmental factor Eigenvalues							
RDA1	RDA2	RDA3	RDA4	PC1	PC2	PC3	PC4
18.3941	0.44117	0.4264	0.1549	8.3316	0.2130	0.0746	0.022
Climatic factor Eigenvalues							
3.8538	0.35561	0.2683	0.04502	22.8080	0.3655	0.2797	0.0819

Supplementary Information Table S3.8: Redundancy analysis biplot scores of all constraining variables of per reptile suborder road occupancy in Giba Rural Community.

Variable	RDA1	RDA 2	R2 adjusted				
Environmental factors							
VpD	-0.177916	0.46560	-0.00837				
DtB	0.136198	0.10233	-0.04017				
Sor_S	0.056923	0.02958	-0.05169				
RS_str	0.130844	0.40918	-0.01836				
RST_A	0.142213	0.01468	-0.04079				
SR_A	0.072033	0.17153	-0.04625				
SE_A	0.005102	-0.20231	-0.04735				
SSE_B	0.242694	-0.04980	-0.02440				
H_Sav	0.562176	-0.12679	0.10140				
H_Bush	0.165130	0.23696	-0.03381				
Ap_A	0.135871	-0.12408	-0.04255				
SW_A	-0.220321	0.11233	-0.03004				
D_WD	-0.282824	0.13581	-0.01210				
Climatic factors							
Dlen	-0.04540	0.246545	-0.04834				
Mill	0.93635	-0.136657	0.13046				
RATD	-0.07772	-0.001850	-0.05230				
Vobs_A	-0.29445	0.008645	-0.03420				
CC_C	-0.09391	-0.216194	-0.04993				
CC_P	-0.24184	0.128074	-0.04133				
Wind_A	0.07101	-0.146750	-0.05155				
AM	0.53710	0.785540	0.03539				
Environmental factor Eigenvalues							
RDA1	RDA2	RDA3	RDA4	PC1	PC2	PC3	PC4
13.9116	1.5151	0.2524	0.1134	2.9469	0.7679	0.1745	0.0462
Climatic factor Eigenvalues							
12.0514	1.0556	0.2417	0.0772	5.3981	0.7014	0.1703	0.0322

CHAPTER 4

Road network avoidance by lion (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa

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Running header: Lion and wildebeest road avoidance in Hluhluwe-iMfolozi Park

4.1 Abstract

Protected areas globally are key for wildlife conservation and biodiversity protection. These areas often undergo minimal landscape transformation; however, they are heavily dependent on tourism for their continued functioning. Tourism activities, while necessary, present opportunities for human-wildlife conflict that roads facilitate. Understanding the factors that affect wildlife road avoidance in protected areas is crucial for ongoing conservation efforts. Our study aimed to determine factors affecting road avoidance by lions (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. The distance to roads, hotspots of activity near roads, and the effect of associated spatial information were assessed to determine how they influenced road avoidance and how roads impacted interactions between lions and blue wildebeest. We used data collected from 11 lions and 21 wildebeest, along with global information systems (GIS), to determine near-road hotspots and the average distance to roads maintained by the two species across different road types. Statistical analyses were used to determine habitat factors influencing the distance kept from roads from May 2019 to April 2022. We found that a significant difference in the distance kept from roads exists between species and between road types, with dirt roads having the lowest distance kept across all separations. Year and road type were the only factors that significantly influenced the average distance to roads across both species. Near-road wildebeest hotspots were centred near water sources, while hotspots of near-road lion activity were centred around areas with typically high rates of human visitation, increasing the risk of human-wildlife conflict and the influence of human activity on lion movement patterns. As roads in HiP may be restrictive for lion and blue wildebeest populations, with both species avoiding roads, a need exists for policy reform for all roads within HiP's boundaries.

Keywords: Human-wildlife conflict; protected areas; roads; linear infrastructure; predator; prey

4.2 Introduction

Protected areas globally hold immense significance for wildlife conservation, serving as vital refugia for threatened species (Oldekop et al., 2016) and being key to biodiversity conservation (Cantú-Salazar and Gaston, 2010). They are often viewed as areas with minimal landscape transformation (Maxwell et al., 2020). However, the majority of protected areas are dependent on tourism activities (Holmes and Brockington, 2012). Tourism dependence is especially prominent in developing nations (Garriga et al., 2012). In 2024, South Africa ranked third in global tourism revenue, with tourism accounting for 8.8% of the country's Gross Domestic Product (GDP) and 10.2% of employment (SA Government News, 2025). Tourism activities, while necessary for the maintenance of protected areas, also introduce opportunities for human-wildlife conflict to occur (Ramp et al., 2006). This conflict can manifest in both direct and indirect forms (Mekonen, 2020). Direct forms of conflict include wildlife-vehicle collisions (WVCs) (Trombulak and Frissell, 2000; Cormac et al., 2025) and theft of crops (Sillero and Switzer, 2001; Kiffner et al., 2021). Indirect issues include avoidance of areas of high human activity, which previously could have been suitable or valuable habitat for a particular species (Lewis et al., 2021), reduced patterns of wildlife movement (Forman and Alexander, 1998; Robb et al., 2022), and habitat degradation (Miranda et al., 2017). As tourism increases globally, the associated human-wildlife conflict also rises, leading to increased stressors on wildlife in protected areas (Garriga et al., 2012). Road networks are set to become increasingly significant areas of human-wildlife conflict in protected areas as tourism levels rise.

Globally, roads significantly impact the behaviour and movement of wildlife (Barbosa et al., 2020; Hlatshwayo et al., 2024; Van der Ree et al., 2015). Roads have been found to act as both ecological barriers (Ascensão et al., 2016) and movement pathways (Bernes et al., 2017). Heavily influencing wildlife distribution, migration patterns, and human-wildlife interactions (Quiles and Barrientos, 2024). The effect of roads on ecosystems can extend to shaping the interspecific behaviours of wildlife (Quiles and Barrientos, 2024). Predator-prey relationships can be influenced by species' preference or avoidance of roads (Ruiz-Capillas et al., 2021). In Alberta and British Columbia, Canada, roads and the presence of motorised vehicles affected grizzly bears (*Ursus arctos*) at both the population and individual levels, altering spatial ecology, so impacting the conservation status of the species (Proctor et al., 2020). In addition, elk (*Cervus elaphus*) in Alberta, Canada, responded to roads as they would respond to the presence of predators (Prokopenko et al., 2016). In Galicia, Spain, the frequency with which grey wolves (*Canis lupus*) crossed paved roads was determined by the size of the road, traffic volume and speed limit, crossing more frequently in low-traffic, low-speed portions of the road, with the majority of crossings taking place at night (Dennehy et al., 2020). Similarly, moose (*Alces americanus*) in Massachusetts, USA, increased their movement speeds when crossing roads, with intensities of movement speed increases corresponding to road density and ultimately affecting the habitat areas used and how they moved between habitat patches (Wattles et al., 2018). Moose have also been recorded shifting their birthing sites towards paved roads in Yellowstone National Park, USA, as brown bears (*Ursus arctos*), a major neonate predator, in the area display traffic aversion behaviour; this therefore provides moose with safer birthing areas despite the presence of roads and human traffic (Berger, 2007). Lions (*Panthera leo*) in Addo Elephant Park in South Africa displayed significantly higher rates of disturbance behaviours when in areas with tourists present

(Hayward and Hayward, 2009), and Burak et al. (2023) found that despite a lack of physical barriers, lions in Laikipia, Kenya, selected for habitat within wildlife areas during times of increased human activity. As human understanding of wildlife movement and behaviour in protected areas is vital for biodiversity conservation and ecological maintenance, particularly the intricate dynamics between predators and prey, it is important to determine the effects of road networks on these relationships in any given protected area.

Predation risk and fear are concepts that have been integral to animal behaviour ecology since the 1980s, when Sih (1980) described how prey species altered their habitat usage based on predation risk, with the concept of “landscape of fear” introduced by Laundré et al. (2001). The study on how the reintroduction of grey wolves to Yellowstone National Park affected foraging behaviours in elk and bison (*Bison bison*), where Laundré et al. (2001) first described the concept of the landscape of fear, found that the prey species became more vigilant as wolves expanded their range within the park. However, many interpretations of this concept have since emerged, though when interpreted as a species perception of the spatial variation of predation risk modelling shows this fear of predation across a landscape with peaks in areas of high risk and valleys in areas low risk, with risk being affected by habitat type, and terrain characteristics (Gaynor et al., 2019; Laundré et al., 2010). Apex predators have a significant impact on the landscape of fear, with prey species selecting habitats to maximise survival and reproduction activities while minimising predation risk (Palmer et al., 2022). Reindeer (*Rangifer tarandus tarandus*) experience predation risk from brown bears (*Ursus arctos*), which serves as the largest factor influencing reindeer habitat selection (Sivertsen et al., 2016). In Africa, ungulates the size of wildebeest (*Connochaetes*) and smaller are susceptible to range restrictions caused by the interaction between resource availability and risk of predation (Owen-Smith, 2019). Human presence has also been shown to

heavily influence the landscapes of fear, altering both the habitat selection of prey species (Moleón and Sánchez-Zapata, 2023) and the habitats selected by predatory species (Everatt et al., 2022), as well as predator-prey interactions (Palmer et al., 2023). However, the effects generated by humans are not limited to their physical presence, but also include the structures they use. Anthropogenic structures, such as roads and buildings, consistently influence habitat selection because of perceived risk of human encounters (Palmer et al., 2023).

Hluhluwe-iMfolozi Park (HiP) and the surrounding areas have a long history of shifts in large herbivore and carnivore populations (Le Roux et al., 2017). The majority of these shifts were human-driven, with periods of intense hunting taking place during the late nineteenth century, with once prominent populations of elephant (*Loxodonta africana*) (Vincent, 1970) and eland (*Taurotragus oryx*) (Vincent, 1970) hunted to local extinction in the current boundaries of HiP (Le Roux et al., 2017). From 1929 to 1950, before the HiP was consolidated into a single reserve, 1155 blue wildebeest (*Connochaetes taurinus*) were culled as part of campaigns to remove ungulates from the Zululand region because of disease outbreaks affecting domestic cattle, with the wildebeest population eradicated in the iMfolozi area by 1950 (Bourquin and Hitchens, 1979; Le Roux et al., 2017; Mentis, 1970). Post-1950 and the consolidation of both park sections in 1989, during population recovery campaigns, wildebeest began to repopulate, moving south from the Hluhluwe portion of HiP (Deane, 1966; Le Roux et al., 2017; Vincent, 1970). However, because of overgrazing concerns, wildebeest populations were culled in large numbers from 1960 to 1980 (Brooks and Macdonald, 1983; Le Roux et al., 2017). Despite management changes, the present wildebeest population has not regained the numbers seen during the 1960s because of steep declines in 2008 and the reintroduction of predators (Le Roux et al., 2017). Carnivore populations exhibited similar population trends to those of the large ungulates, with most large carnivores in

HiP going locally extinct at some point, and reintroduction programs formed a significant focus of park management activities for four decades (Somers et al., 2017). Before reintroduction in the 1960s, lions in HiP were hunted to local extinction in the early 1940s (Player, 1997; Somers et al., 2017). Reintroduced populations reached a high of 140 individuals during the 1980s; however, populations saw a steady decline thereafter, despite multiple translocations, because of poor genetic quality resulting from inbreeding (Somers et al., 2017). Inbreeding depression has a well-known cost to heterosis, leading to increased disease susceptibility, with the high inbreeding coefficient found in the lion founder population in HiP being one of the driving factors attributed to the major population decline that was observed (Trinkel et al., 2011).

The reintroduction of predators also coincided with declines in prey species, such as zebra (*Equus quagga*), nyala (*Tragelaphus angasii*), impala (*Aepyceros melampus*) and blue wildebeest in HiP, as they are all the preferred prey species comprising four out of five of the most common species found in lions' diets in HiP (Le Roux et al., 2017; Somers et al., 2017). Lions are also the sole source of wildebeest predation in HiP, with lion activity the only evidence of predation in 692 wildebeest carcasses recorded between 1983 and 2010 (Somers et al., 2017). Lions were also responsible for evidence of predation of 933 African buffalo (*Syncerus caffer*) carcasses between 1983 and 2010 (Somers et al., 2017), leading to a decline in the prevalence of bovine tuberculosis (*Mycobacterium bovis*) in HiP. As lions are an apex predator in African savannas, lion avoidance trends are similar to those of other apex predators, such as grey wolves and bears (Laundré et al., 2001; Laundré et al., 2010; Sivertsen et al., 2016), where species prone to lion predation alter foraging sites according to lion encounter risk and forage quality (Davies et al., 2021). These interspecific dynamics further enforce the need to understand how roads in HiP affect the movements of both lions and wildebeest.

Understanding the use of roads by wildlife in protected areas is crucial for continuing conservation efforts, enabling effective management to address these evolving challenges. This study aimed to determine the predator-prey dynamics of lions and blue wildebeest in relation to road infrastructure in HiP. Distance to roads, hotspots of activity near roads, and the effect of associated spatial information were assessed as proxies to determine the impact of roads on movement and species interactions between the two species. The average distance each species kept from the roads and how it varied between road types was determined using spatial analysis programs. In addition to creating hotspot maps of near-road lion and blue wildebeest activity in HiP and the habitat features present at these hotspots, the effect of the habitat features on road interaction per species was examined using data collected from collared individuals of each species across three dry and wet seasons. We predicted that lions would spend the majority of their time avoiding areas with roads, particularly paved roads, and that wildebeest would prefer to remain near road networks because of reduced predator presence. This prediction aligns with the findings reported by Berger (2007). Additionally, we predicted that road design and the distance between roads and standing water would have the largest effect on road interaction rates. Where road design (verge size, wildlife passage concentration) affects predation risk, predation increases in areas with smaller verges and single passages, thereby increasing the fear of these locations (Quiles and Barrientos, 2024). Roads that are closer to standing waterbodies would have higher detections for both species because of their water dependency (Sirot et al., 2016), and predation risk is less impactful during droughts (Riginos, 2015).

4.3 Methods

4.3.1 Study areas

Our study took place in the uMkhanyakude region, the northernmost district of South Africa's KwaZulu-Natal Province, on the east coast of the country, and included the entirety of the road network contained in HiP (Fig. 4.1), which contains several gravel roads, both private, staff use only, and public access. A park-governed paved road connects the main entrances of the park, Memorial and Nyalazi gates, as well as the main park accommodations, Hilltop and Mpila camps. Additionally, a major paved public road governed by the South African National Roads Agency Limited (SANRAL), the R618, splits the park in two, with the Hluhluwe portion in the north and the iMfolozi portion in the south. These roads experience varying levels of traffic, with control roads seeing the lowest amount compared to the major public road, which is constantly busy 24 h a day, all year round. The R618 also has the current highest recorded wildlife mortality rate in South Africa, peaking at 13 caracasses^{-km} during wet seasons (Cormac et al., 2025).

The Hluhluwe-iMfolozi Park area is one of South Africa's oldest protected areas; it originally comprised two separate parks in 1895 but was later merged into one entity to create the present-day park (Cromsigt et al., 2017). The park is presently only separated by the R618, which was originally a dirt track present before the original proclamation of the separated parks and connected the towns of Mtubatuba and Hlabisa; the road was subsequently paved in 2004 (Cromsigt et al., 2017). The HiP spans a total of 96,000 ha and aims to protect a wide array of vegetation types, with many of South Africa's most iconic fauna found in its borders, with all the “big five” (lion, African savanna elephant, black rhinoceros (*Diceros bicornis*), African buffalo, leopard (*Panthera pardus*)) present as well as white rhinoceros (*Ceratotherium simum*); African wild dog (*Lycaon pictus*), cheetah (*Acinonyx jubatus*) and ground pangolin (*Smutsia temminckii*)

(Cromsigt et al., 2017). All of these species are listed with conservation statuses ranging from Near Threatened to Critically Endangered, with decreasing populations, except for the black rhinoceros, which is increasing in number globally (IUCN, 2023). This region lies on the Mozambique coastal plain, which experiences weather similar to that of the tropics (Cromsigt et al., 2017). The HiP lies along the edge of a geological monocline (Howison et al., 2017). The wet season occurs from September to April, with an average rainfall of ~950 mm, a maximum average rainfall exceeding 1500 mm, and an average maximum temperature of ~29°C. The dry season occurs from May to August, with an average rainfall of ~20 mm and an average minimum temperature of ~14°C. Wet and dry season climatic variations are derived from a combination of historical information (Perissinotto et al., 2013; Cromsigt et al., 2017), as no contemporary information was available because of damaged weather monitoring equipment (South African National Weather Service, pers. comm.).

Roads cover 318.7 ha (0.33%) of the 96,000 ha area within HiP's boundaries, with a total of 89.3 ha of paved road and 229.4 ha of unpaved road within the park, with 60% of the park's road network found in the iMfolozi portion of the park, however because the Hluhluwe portion is smaller than the iMfolozi portion it has a higher concentration of roads. The portion of the R618 road that falls within the boundaries of HiP is ~18 km in length and separates the northern (Hluhluwe) and southern (iMfolozi) portions of the park (Fig. 4.1). Present mitigation measures include cattle grids after ~400 m of fenced area at both the entrance and exit of this park's boundaries. The entrance and exit of this park also have video cameras and speed bumps, with additional speed bumps placed along the length of the public road. Structures that fauna could use to traverse the road safely may include culverts. Culverts are found along all drainage lines along the road; however, they are designed chiefly for runoff dispersal, not animal movement (Cormac

et al., 2025). Because of the hilly terrain along the length of the road in this park's boundaries, V-shaped concrete runoff channels have been placed in all sloping sections of the road (Cormac et al., 2025). The length of the R618 has also undergone resurfacing activities, with the portion of the road in park boundaries taking place from March 2021 to September 2022. The road has a speed limit of 40 km/h for ~400 m before and ~120 m after the park entrances, with the remainder of the R618 having a speed limit of 60 km/h in the park and for 20 km on either side of the park. However, no structures or measures are in place that threaten drivers with financial or judicial consequences to force them to maintain these speeds. The presence of megaherbivores and other large mammals acts as additional traffic calming, with most motorists slowing or coming to a complete stop when said fauna are crossing the road. Most of the road length is surrounded by savanna grassland with tree-dominated thickets along larger drainage lines (Howison et al., 2017). The road has an average width of 15 m and no fences that run parallel to the road. All internal park roads had a speed limit of 40 km/h, and the private-paved road within HiP had the highest traffic volume of all internal park roads. However, the area of effect that roads have on surrounding environments is not restricted to the road's immediate area, Naidenko et al. (2021) found that roads can affect wildlife behaviour up to 400 m away. When applied to the road network, this increase in the sphere of influence increases roads' area of effect to 32,163.9 ha, potentially impacting 33.5% of HiP.

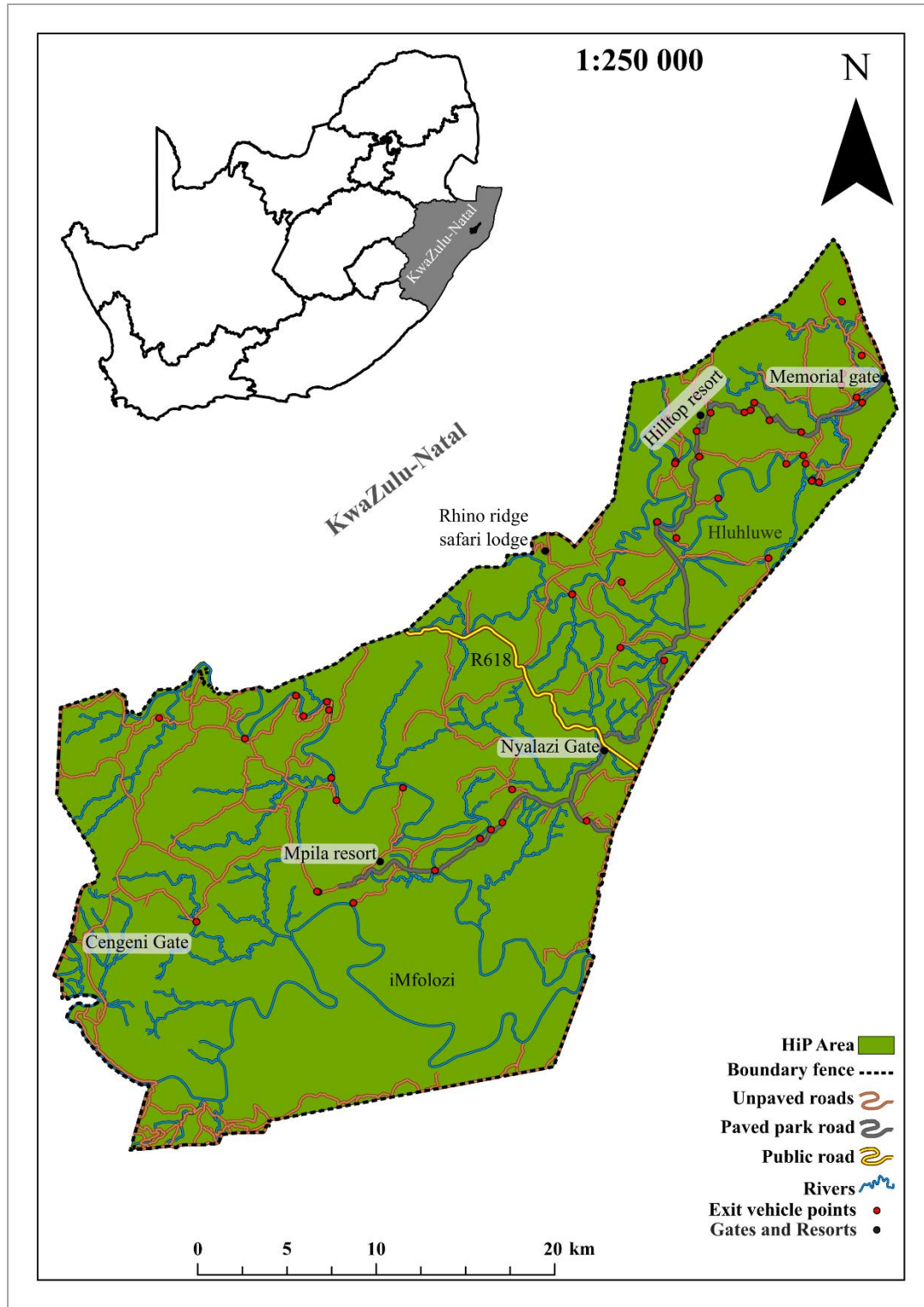


Figure 4.1: Road network of Hluhluwe-iMfolozi Park in KwaZulu-Natal Province, South Africa.

4.3.2 Spatial analyses

Our study included global positioning system (GPS) data collected for 11 lions (2 males, 9 females) and 21 blue wildebeest (1 male, 20 females) from the 1st of May 2019 to the 30th of April 2022. The methods for collaring are described in Dejeante et al. (2024). The GPS collars recorded a GPS fix ~ every 6 h; however, as collars were not all placed on individuals at the same time or did not function for the full three-year duration, data were standardised by selecting only GPS fixes from the hour before and after each of the four daily call-in (GPS fixing) periods during the collection period. A total of 9131 GPS points were collected for lions (average of 803 (Stdev = 478) fixes per individual) and 79506 for wildebeest (average of 3312 (Stdev = 3312) fixes per individual). Collar data were provided by Dr Simon Chamaillé-Jammes of the Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, and the analyses presented here follow the data use agreements made with Dr Chamaillé-Jammes. We conducted spatial analyses using ArcGIS Pro 3.5 (ESRI, 2025).

Line shapefiles of roads in HiP were created using the Earthstar Graphics satellite imagery basemap in ArcGIS Pro (ESRI, 2025), and a point shapefile for areas where tourists are typically permitted to exit their vehicles along roads was created using data provided by Ezemvelo KZN Wildlife. The average distance maintained from roads by lions and wildebeest in HiP was calculated using the Near tool with a Geodesic method, and this was repeated for each road type (dirt, private, and public), tourist points, and the distance to water. Additional information was collected on vegetation, biome, and elevation. Elevation information (SRTM 30, South African Department of Science and Technology, 2018) was collected using the Add Surface Information tool, employing a bilinear method with a 5 m search distance. Vegetation and biome information (Vegmap, South African National Biodiversity Institute, 2018) was added using the spatial join

tool with a 5 m search radius. Temperature, year, and season information were obtained from recorded collar data.

A 1 x 1 km grid was created for the park area and spatially joined to both lion and wildebeest datasets; Moran's I was then used to test for spatial autocorrelation, using species counts per kilometre. Significant clustering was detected for both species (lion – $I = 0.72$, $z = 29.45$, $p < 0.001$; wildebeest – $I = 0.75$, $z = 29.53$, $p < 0.001$). We then calculated the number of neighbours using the polygon neighbours tool; this was done to account for the observed clustering (with both datasets displaying three neighbours). Line-centred buffers were created to incorporate the different widths of each road type using the buffer tool with flat ends. Buffer widths were set to 3 m for dirt roads, 5 m for the park's private paved road and 7.5 m for the R618. We then joined the buffers for each road type using the merge tool to create a map of the HiP road network. An additional 400 m (based on the findings of Naidenko et al. (2021)) was added to the road network map using the pairwise buffer tool to create a map of the road area of influence. The resulting feature layer was clipped to exclude any areas that fell outside of the HiP boundary fence. Seasonal hotspots of lion and wildebeest activity across HiP's area were calculated using the find hotspot tool with bin size set to 400 m and neighbourhood size set to 1,000 m. These were chosen to produce hotspots on a 400 m² square grid and to incorporate the clustering observed at the 1 km² scale.

4.3.3 Statistical analyses

We conducted statistical analyses using R (version 4.5.1; Team, 2020) plug-in in Google Collab (Sukhdeve and Sukhdeve, 2023). The statistical analysis workflow described was used for both the lion and wildebeest collected data. Scale fixed effects (Supplementary information Table S4.1)

were Z-transformed to normalise the data following normality and assumption testing using the car package (Fox and Weisberg, 2019). A generalised linear mixed-effects model (GLMM) using the lme4 package (Bates et al., 2015) was used to determine the effect of collected fixed and random variables (Supplementary information Table S4.1) on the maintained distance in each respective species and between both species. We ran correlation tests to determine if any of the included variables displayed strong correlations (correlations greater than 70%), and no strongly correlated variables were found. We analysed variance (ANOVA) tests to determine differences in the distance kept from different road types between the two species using base R packaging (Team, 2020). We conducted the post-hoc analyses using the emmeans package (Lenth, 2017). Graphical representations were visualised using the ggplot2 package (Wickham, 2016).

4.4 Results

4.4.1 *Near road hotspots*

We found that lions had a higher detection rate across wet and dry seasons within the area of anthropogenic influence, within 400 m of roads in HiP. However, despite having a higher density within this area, only two significant hotspots of GPS fixes were recorded during the wet season (Fig. 4.2a), one of which was found in the northwest area of the Hluhluwe portion of HiP near the Hilltop Resort and the surrounding staff accommodations. The number of significant hotspots of GPS fixes for lions within 400 m of roads in HiP during the dry season (Fig. 4.2b) rose to six, with four areas in the iMfolozi portion of HiP, three of which were near areas with higher human activity. The remaining two in the northern region were between the Maphumulo picnic site, a large, unfenced area where tourists may exit vehicles to eat and prepare food (including meat preparation), Memorial Gate and Hilltop Resort, all of which see high levels of human presence.

However, only the GPS-fix hotspot surrounding the Hilltop Resort area was consistent across both seasons.

In contrast, wildebeest, despite having a lower overall detection rate within 400 m from roads, five GPS-fix hotspots were recorded along dirt roads during wet seasons (Fig. 4.3a), three in the southwest portion where dirt roads were near water courses, one along the dirt road used to maintain the border fence along a tributary of the Hluhluwe River in the northwest and one in the Seme region of the Hluhluwe portion of HiP in an area with several seasonal watering holes. During the dry season, the number of wildebeest GPS-fix hotspots near roads decreased to four again, with hotspots in areas where dirt roads were near water sources (Fig. 4.3b). Three hotspots in the iMfolozi portion of HiP, near a meandering section of the Black Umfolozi River, and the final hotspot was again in the Seme region. No hotspots were consistent across species for either season.

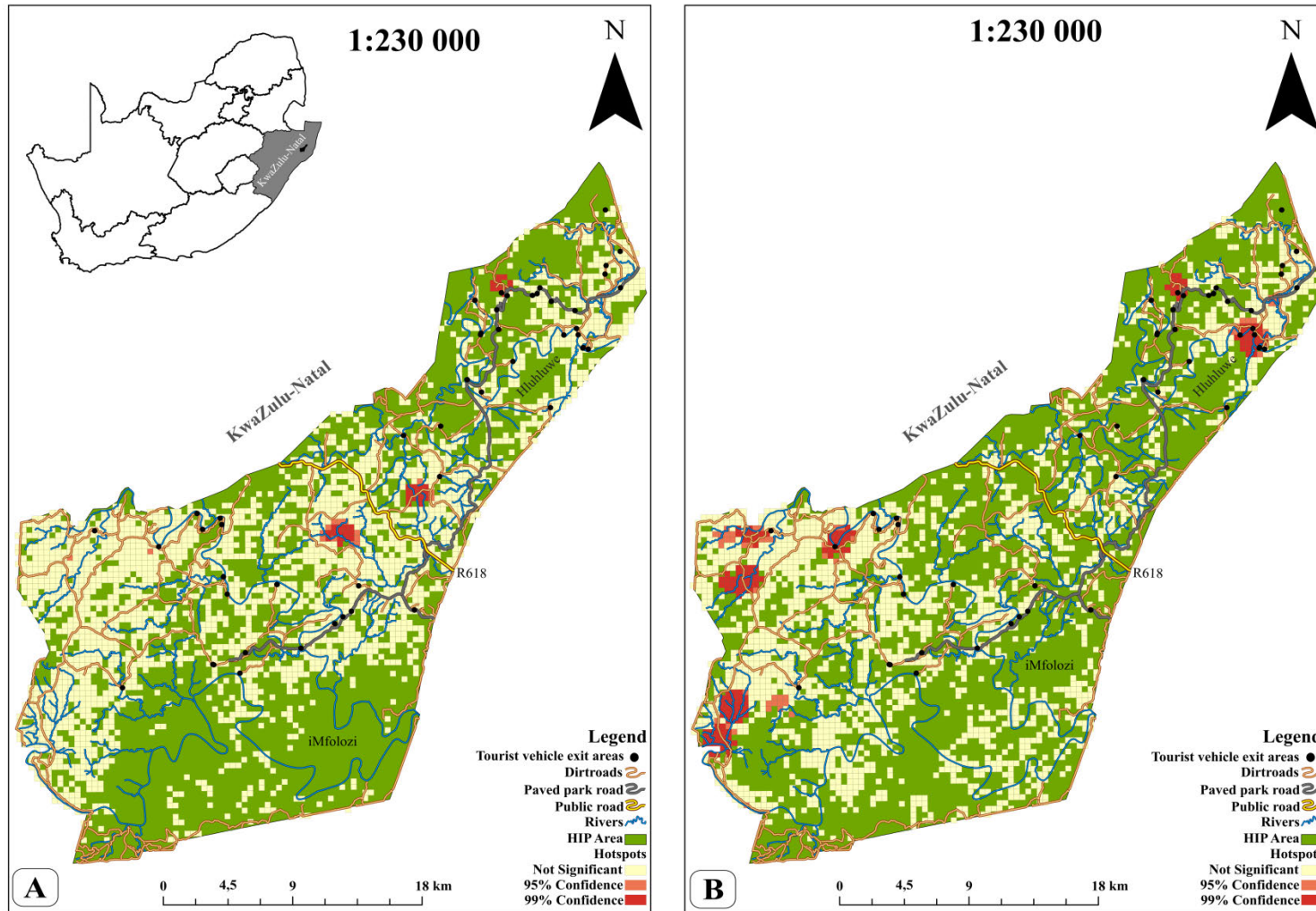


Figure 4.2: Hotspots of lion (*Panthera leo*) activity near roads from May 2019 to April 2022 in Hluhluwe-iMfolozi Park, South Africa. A) Wet season near road activity. B) Dry season near road activity.

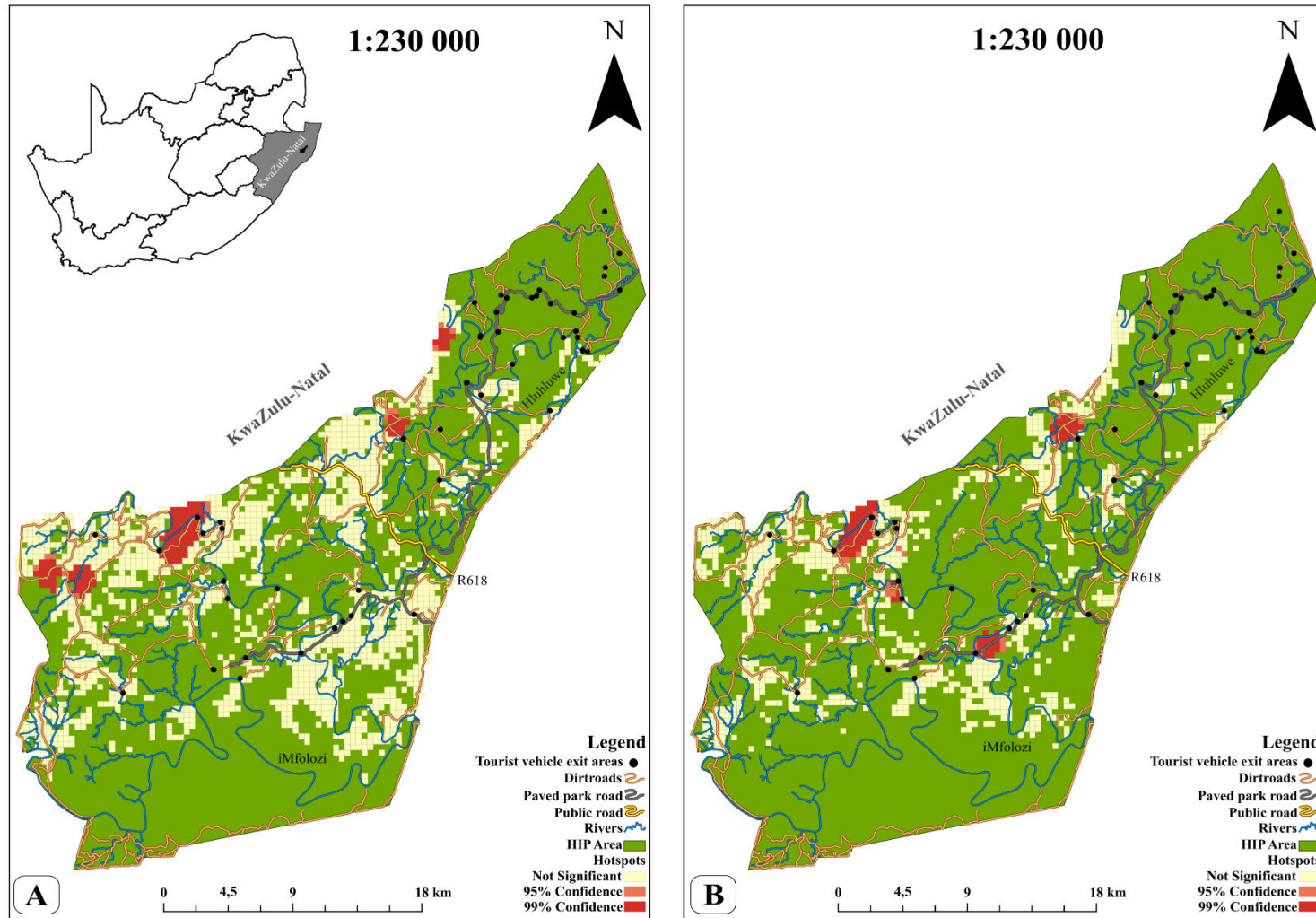


Figure 4.3: Hotspots of blue wildebeest (*Connochaetes taurinus*) activity near roads from May 2019 to April 2022 in Hluhluwe-iMfolozi Park, South Africa. A) Wet season near road activity. B) Dry season near road activity.

4.4.2 Factors affecting distance from roads

The distance maintained from roads in HiP showed a significant difference between the species. We initially found significant differences in distance, mediated by road type, in the distance maintained from roads by both species (Table 4.1). Further analysis revealed that the distance maintained by lions from roads decreased in response to the type of road, distance to tourist areas, distance to water, and presence of wildebeest (Table 4.2). Interestingly, we found that the distance maintained from roads by wildebeest decreased according to the road type, distance to tourist areas and presence of lions (Table 4.3). When comparing species, the distance maintained from roads by both lions and wildebeest was influenced by the distance to tourist areas, elevation, and the presence of other species (Table 4.4). Wildebeest maintained a closer distance to dirt and public roads than private roads when compared with lions (Fig. 4.4). The GLMMs showed that the highest variance in the distance kept from roads in all datasets occurred across years. Whereas the distance kept from roads showed the lowest amount of variance between night and day in lions and season in wildebeest, and the combined species dataset.

Table 4.1: Analysis of variance results of distance kept by lion (*Panthera leo*) and wildebeest (*Connochaetes taurinus*) from types of roads in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

	Df	Sum Squares	Mean Squares	F value	p-value
Species	1	2.395e+09	2.395e+09	152.3	<2e-16 ***
Road type	2	4.665e+12	2.332e+12	148303.9	<2e-16 ***
Species X Road type	2	1.027e+11	5.136e+10	3266.0	<2e-16 ***
Residuals	265905	4.182e+12	1.573e+07		

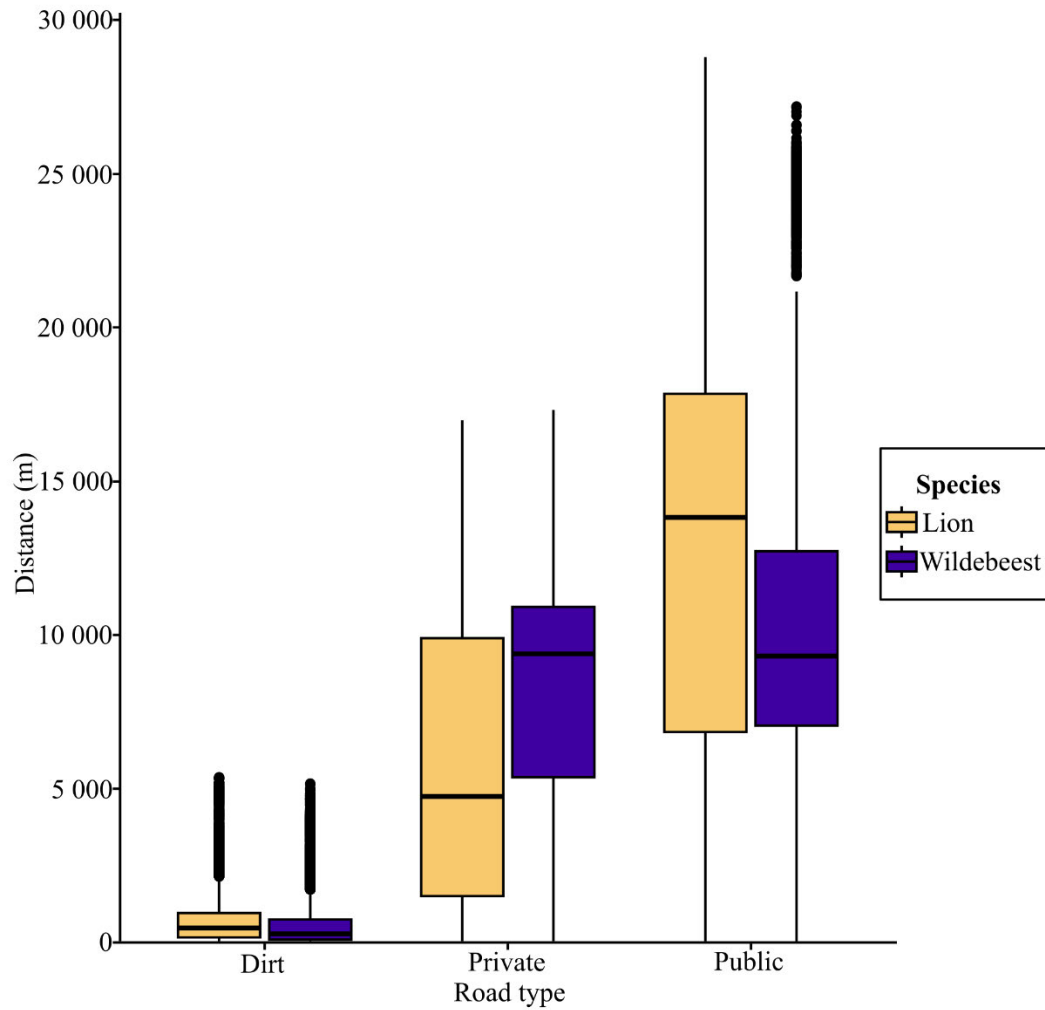


Figure 4.4: Distance kept by lion (*Panthera leo*) and wildebeest (*Connochaetes taurinus*), from types of roads in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Table 4.2: Generalised linear mixed-effects model of factors driving maintained distance from roads by lions (*Panthera leo*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Random effects	Variance	Standard Deviation	
Month	1.080e-03	0.032861	
Year	1.787e-02	0.133670	
Time of Day	4.987e-05	0.007062	
Season	9.442e-04	0.030728	
Residual	5.240e-02	0.228920	
Fixed Effects	Estimates	Standard Error	t-value
Intercept	0.081960	0.071410	1.148
Dirt road	0.967337	0.002724	355.122
Private road	0.073670	0.004131	17.835
Public road	0.024169	0.002941	8.218
Elevation	0.008901	0.003225	2.760
Water	-0.007193	0.003142	-2.289
Tourists	-0.049444	0.003560	-13.889
Wildebeest	-0.025040	0.003037	-8.246

Table 4.3: Generalised linear mixed-effects model of factors driving maintained distance from roads by wildebeest (*Connochaetes taurinus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Random effects	Variance	Standard Deviation	
Month	1.039e-03	0.032226	
Year	2.468e-03	0.049681	
Time of Day	6.919e-05	0.008318	
Season	<0.001	<0.001	
Residual	5.180e-02	0.227589	
Fixed Effects	Estimates	Standard Error	t-value
Intercept	0.0005747	0.0307545	0.019
Dirt road	0.9866323	0.0010171	970.085
Private road	0.0295798	0.0010967	26.972
Public road	0.0681218	0.0012816	53.135
Elevation	0.0051224	0.0011235	4.559
Water	0.0241638	0.0009000	26.850
Lions	-0.250841	0.0011312	-22.174
Tourists	-0.0449705	0.0011065	-40.642

Table 4.4: Generalised linear mixed-effects model of factors driving maintained distance from roads by an apex predator (lion (*Panthera leo*)) and key prey species (wildebeest (*Connochaetes taurinus*)) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Random effects	Variance	Standard Deviation	
Month	9,182e-04	3.030e-02	
Year	1.410e-03	3.755e-02	
Time of Day	6.754e-05	8.218e-03	
Season	2.193e-09	4.683e-05	
Residual	5.326e-02	2.308e-01	
Fixed Effects	Estimates	Standard Error	t-value
Intercept	0.0183780	0.0219200	0.838
Dirt road	0.9910291	0.0009088	1090.452
Private road	0.0381832	0.0010240	37.288
Public road	0.0516945	0.0009591	53.899
Elevation	-0.0014385	0.0009546	-1.507
Water	0.0211950	0.0008774	24.157
Tourists	-0.0381832	0.0010341	-37.117
Species	-0.0128463	0.0027719	-4.635

4.5 Discussion

Our study aimed to determine the dynamics of lion and blue wildebeest presence near and avoidance of roads in HiP. We found that most recorded GPS data fixes for both species were within one kilometre of roads; however, there were outlier cases where the distance from roads was over five kilometres, which in the case of lions is double the species' daily movement capabilities (Yiu et al., 2015). However, the mean average distance was the smallest from the dirt roads and largest from the public paved road. The near-road hotspots of lion presence were recorded along roads near areas with staff accommodation or areas where park visitors are permitted to exit their vehicles. Meanwhile, the near-road hotspots of blue wildebeest presence were centred near water sources. The factors that influenced the distance kept from roads included road type, with unpaved roads favoured over paved roads with significantly lower average

distances from dirt roads both within species and between species; the distance from tourist areas, with distance from roads increasing for both species (less so in wildebeest) as the distance to tourist areas decreased. The distance kept from roads also increased as the density of lion presence (i.e., perceived predation threat) increased. High levels of yearly variation in the average distance kept from roads were observed in wildebeest; however, the average distance maintained from roads did not vary greatly according to season, remaining fairly stable between subsequent seasons (i.e., between wet seasons or between dry seasons).

The pattern of general avoidance of roads by lions was similar to patterns observed in other reintroduced populations (Yiu et al., 2015), where lions avoided areas of human activity, but the distance kept from human structures decreased over time as habituation levels increased. However, in the areas that lions do utilise roads, particularly near tourist sites, this may be linked to food supplementation, as lions do engage in scavenging behaviour, particularly when resources are scarce (Pereira et al., 2014). The patterns of general avoidance by blue wildebeest also followed similar trends found elsewhere in Africa. For example, in the Athl-Kaputlel Plains, Kenya, blue wildebeest consistently avoided anthropogenic features, particularly primary roads, irrespective of the season, because of increased human activity and predation (Stabach et al., 2016). However, the pattern that we observed may be linked to how roads can provide prey species with a refuge from predators (Berger, 2007).

The avoidance patterns are likely linked to the level of human activity surrounding the various road types, which was supported by the results as road type may be used as a proxy for vehicle speed and traffic volume (Robson and Blouin-Demers, 2013), with vehicle speed and traffic volume being the greatest along the public paved roads and lowest along the dirt (unpaved) roads (Laurance and Balmford, 2013). Similarly, Hayward and Hayward (2009) found that lions

were significantly more likely to display disturbance behaviours when tourists were present, causing increased stress levels and decreased overall fitness in individuals as tourist presence increased. The patterns detected in the average distance to roads, coupled with changes in the number of records near roads according to year but not season, further supported the argument that movement patterns around roads in HiP are heavily influenced by human presence. Furthermore, it coincided with the global COVID-19 pandemic, with the first year of study taking place before 2020 lockdown procedures and the closure of the park to the public, and the final year of study taking place post-lockdown restrictions, which saw May 2020 to April 2021 having drastically reduced human activity along the private paved road and dirt roads in HiP. These trends also correspond to changes in wildlife avoidance of anthropogenic structures globally in non-protected areas (Tucker et al., 2023). The hotspots of lion activity around the areas with higher levels of human activity may also indicate dispersal into areas with temporarily reduced human activity, as a significant increase and subsequent decrease in the number of records near the private paved road and dirt roads was detected, again coinciding with COVID-19 restrictions on human movements. The non-detection of any significant change around the R618 further supports this, as this road only saw a minor reduction in activity because of its nature as a major thoroughfare. These changes in human avoidance in and around protected areas also coincide with changes in human avoidance patterns seen in other regions globally (Jones et al., 2022; Seiler et al., 2020). For example, terrestrial mammal movement rates in Golden Ears Provincial Park and adjacent land in Vancouver, Canada, had subsequent reductions post-reopening (Procko et al., 2022). These changes in movement patterns are also indicative of human-wildlife conflict occurring in the boundaries of the protected area, as the avoidance of these road structures because of their permeating nature reduces the ability of species to move in the park boundaries, further

compounding the effects of the small size of the park on migration and movement patterns (Higginbottom, 2004).

Our study was limited by the resolution of the available data, as the period between recordings did not allow for finer-scale non-assumptive spatial analyses to determine the accurate scale of road network usage. Additionally, as accelerometer data were unavailable, no analyses could be conducted to assess activity patterns on or near roads. In addition to the lack of fine-scale GPS-linked movement data, the lack of fine-scale climatic data restricted the secondary information that could be used to investigate the factors driving lion and blue wildebeest near-road activity. Future work should include collecting GPS records taken every hour and include accelerometer data so that questions regarding time spent near roads, direction of movement, speed of movement and activity patterns near roads can be addressed. Future studies should also be done to investigate the non-consumptive effects of predators on prey species (Courbin et al., 2016; Say-Sallaz et al., 2019; Valeix et al., 2009) to further disentangle natural and anthropogenic stressors on their presence, movement patterns and habitat selection. Additionally, studies should be expanded to include other anthropogenic structures in HiP, and studies should be expanded to additional protected areas, particularly protected areas with historically present lineages and to protected areas with a mixture of historically present and translocated lineages of these species. This should be done to determine if translocated individuals (particularly those kept in boma structures) are less likely to avoid human structures. These results do, however, provide vital information for protected areas management, providing insights into key areas of concern, i.e. areas that may need further safety measures put in place to reduce potentially deadly human-wildlife conflict events and possible avenues for improved road design to minimise stress caused by human presence. As roads and tourist presence in HiP are vital for the parks function as with

most protected areas globally, measures aimed at combating the negative effects of roads and tourism on animal behaviours need to be made to meet the specific needs of each protected area. Need to be accounted for, in the case of HiP mitigations that could reduce the impact of roads and tourism on species within its bounds might include measures such as placing fences around any areas that tourists might exit their vehicles. Additionally, placing restrictions on crowding by safari vehicles may also yield positive results.

4.6 Conclusions

Roads in HiP had an apparent negative effect on lions and blue wildebeest, with both species exhibiting high levels of avoidance; two-thirds of recorded GPS positions for both species fell outside the area of road effect. The avoidance of roads was also heavily influenced by the type of road, with paved roads being avoided more frequently. This calls for a need to reform policies surrounding the regulations of park roads, as well as a significant reform to the rules of the public paved road that separates the two portions of the park, along with the implementation of mitigation strategies to increase movement across the HiP road network and reduce present stressors to the already struggling populations of lion and blue wildebeest in HiP.

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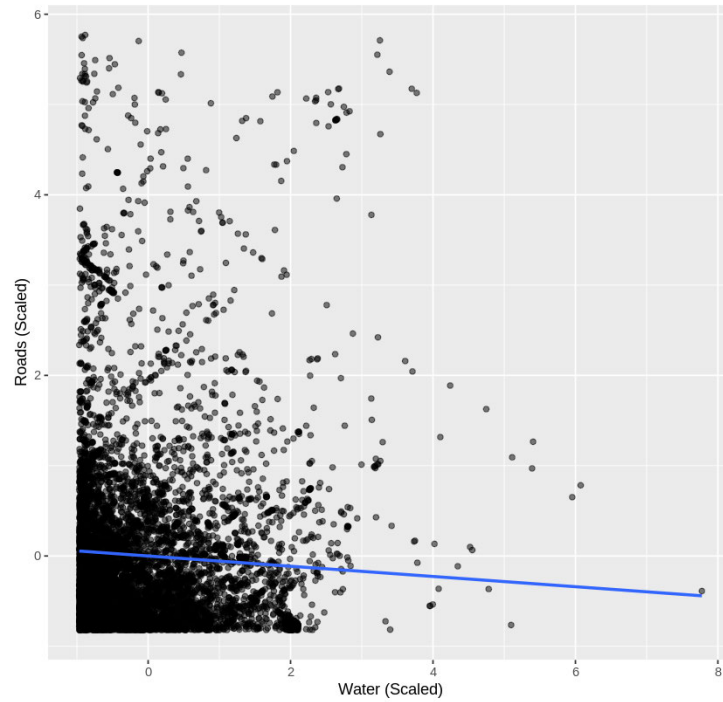
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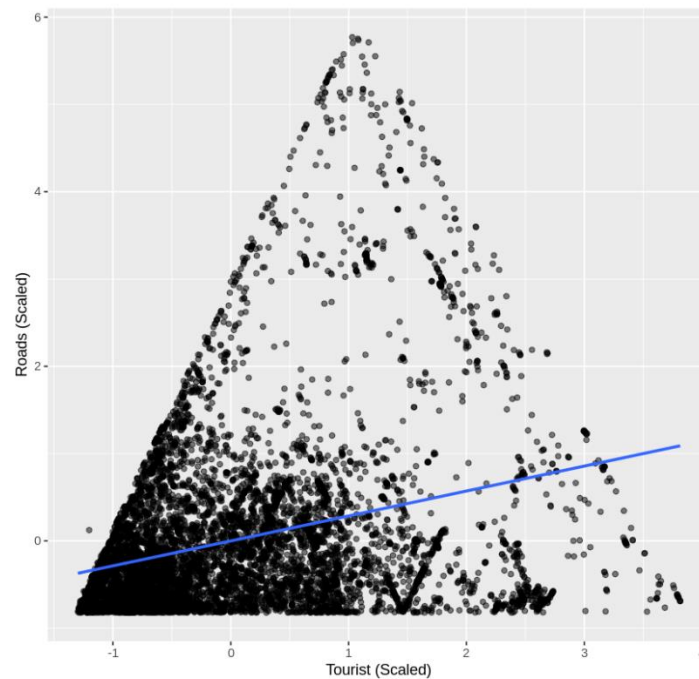
4.9 Supplementary information

Supplementary information Table S4.1: Variables used in generalised linear mixed-effects models

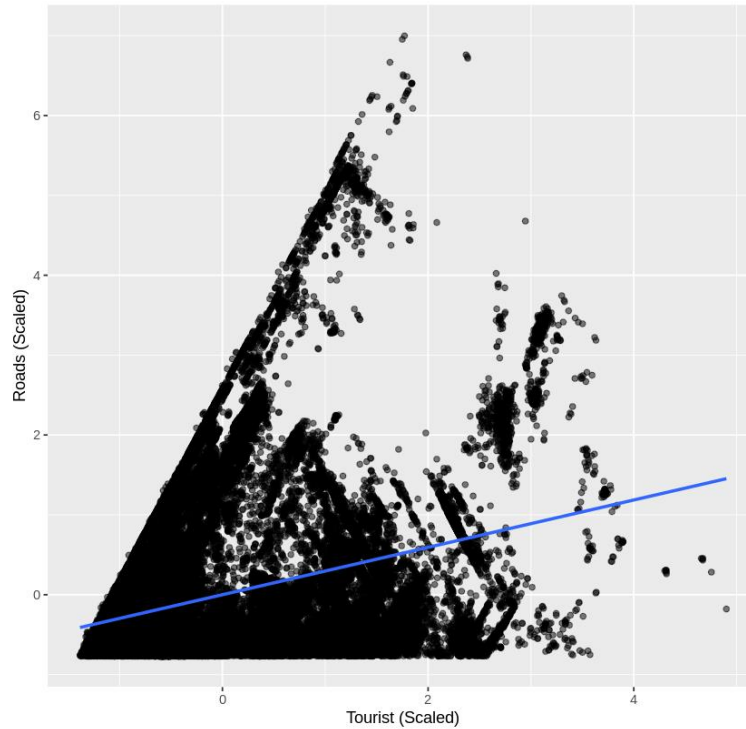
Variable	Effect type	Data type
Distance to roads (Roads) (m)	Dependant	Scale
Distance to public paved road (Public) (m)	Fixed	Scale
Distance to private paved road (Private) (m)	Fixed	Scale
Distance to unpaved roads (Dirt) (m)	Fixed	Scale
Distance to permitted vehicle exit point (Tourist) (m)	Fixed	Scale
Distance to water source (Water) (m)	Fixed	Scale
Elevation (m a.s.l)	Fixed	Scale
Vegetation	Fixed	Nominal
Biome	Fixed	Nominal
Predator/ Prey density	Fixed	Scale
Species	Fixed	Binomial
Year	Random	Nominal
Season	Random	Binomial
Month	Random	Nominal
Time of Day	Random	Binomial



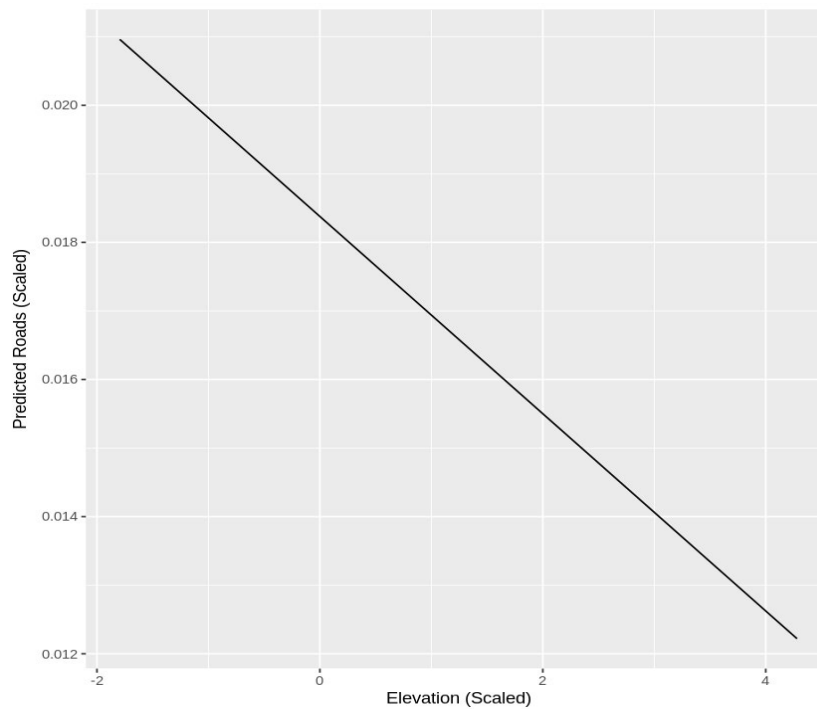
Supplementary information Figure S4.1: Distance maintained by lions (*Panthera leo*) from roads in relation to distance from water in Hluhluwe-iMfolozi Park.



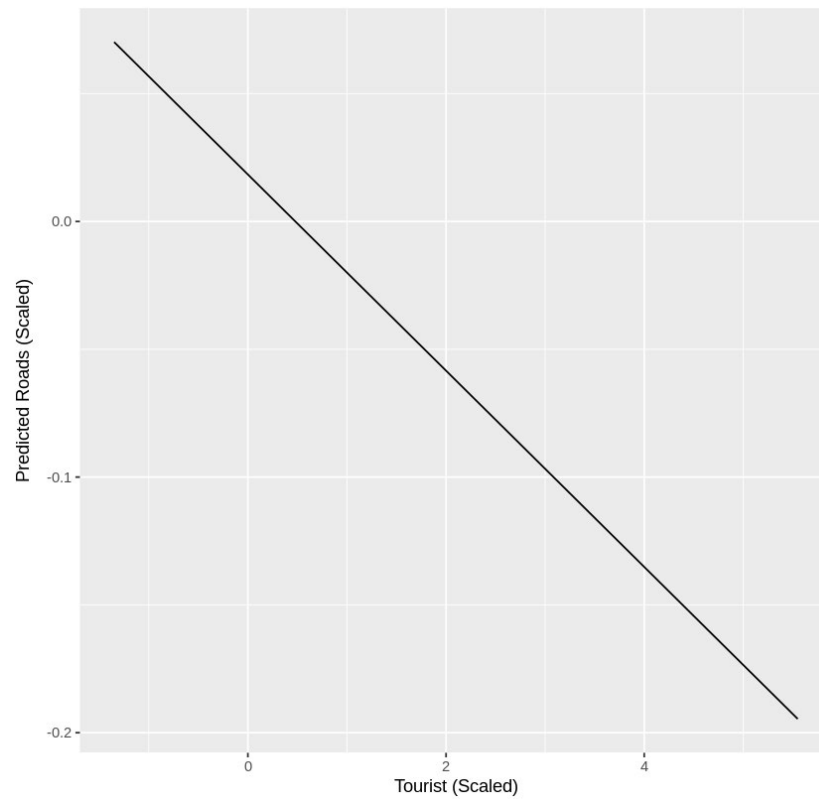
Supplementary information Figure S4.2: Distance maintained by lions (*Panthera leo*) from roads in relation to the distance from tourist areas in Hluhluwe-iMfolozi Park.



Supplementary information Figure S4.3: Distance maintained by blue wildebeest (*Connochaetes taurinus*) from roads in relation to the distance from tourist areas in Hluhluwe-iMfolozi Park.



Supplementary information Figure S4.4: Distance maintained by lions (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) from roads in relation to elevation in Hluhluwe-iMfolozi Park.



Supplementary information Figure S4.5: Distance maintained by lions (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) from roads in relation to tourist areas in Hluhluwe-iMfolozi Park.

CHAPTER 5

Interactions with anthropogenic infrastructure by cheetahs (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa

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Running header: Effects of anthropogenic structures on cheetahs and wild dogs' movement in Hluhluwe-iMfolozi Park

5.1 Abstract

Predator conservation is a crucial aspect of global biodiversity conservation, as predators play a vital role in maintaining ecosystem health. The effects of anthropogenic land use change have been shown to have numerous and wide-reaching negative impacts on the natural environment. Anthropogenic linear infrastructures have a near-ubiquitous negative impact on natural environments, yet South African protected areas extensively utilise fences in their conservation models. Little information is available on how these anthropogenic structures within protected areas affect large predators. Our study investigated the effects of anthropogenic structures on the human avoidance patterns of African wild dogs (*Lycaon pictus*) and cheetahs (*Acinonyx jubatus*) in Hluhluwe-iMfolozi Park (HiP), South Africa. This was achieved by determining the average distance kept from roads, fences and unfenced structures in HiP, in addition to creating hotspot maps of activity near the anthropogenic structures through the use of spatial analyses and the use of statistical analyses to determine driving habitat characteristics using data collected from nine collared cheetahs and 17 collared wild dogs, across three dry and wet seasons from May 2019 to April 2022. We found that, despite both species having a recorded presence across most of the park, they only make significant use of 1.7% and 2.1% of the park's area, respectively. The cheetah selected areas away from humans, while the wild dog selected areas near anthropogenic structures. This is of concern as cheetahs and wild dogs are species whose persistence is threatened in HiP, particularly wild dogs, as this species often actively leaves the park boundaries. Therefore, this study's results provide key insights into areas of conservation concern for both species and further indicate the need for more effective species management to reduce human-wildlife conflicts in the rural communities surrounding HiP.

Keywords: Predator conservation; Habituation, linear infrastructure; roads; fences; protected area.

5.3 Introduction

Globally, conservation of predators is a critical facet of biodiversity preservation and ecosystem health, as predators contribute to the regulation of prey population health and density (Quintana et al., 2022). Additionally, with the rising amount of global evidence of the impact that anthropogenic linear infrastructure has on wildlife, a significant focus has been placed on the effect these structures have on large carnivores (Whittington et al., 2005; Proctor et al., 2020; Dennehy et al., 2021). The effects of roads and fences have been a particular focus, as evidence shows that these structures have numerous and wide-ranging negative impacts on the natural environment (van der Ree et al., 2015). Roads are direct causes of wildlife mortality (Collinson et al., 2014), causing changes in movement patterns (Wattles et al., 2018). Evidence also shows that roads have effects on intra- and inter-species dynamics, such as disruptions to gene flow (Epps et al., 2005; Strasburg, 2006; Holderegger and Di Giulio, 2010), changes in population structures (Proctor et al., 2020) and trophic level disruptions (Ruiz-Capillas et al., 2022). Fences cause disruptions to species movement and migratory patterns (Jaeger et al., 2005; Robb et al., 2022) and are direct causes of wildlife mortality (Jaeger et al., 2005; Mbaiwa and Mbaiwa, 2006; Robb et al., 2022). However, many countries, particularly on the African continent, use fences to enclose protected wildlife areas (Hayward et al., 2009; Jakes et al., 2018). Although anthropogenic linear infrastructures have a near-ubiquitous negative effect on natural environments, they provide positives for some species. Roads facilitate easier movement through landscapes and increase hunting opportunities for species that can adapt to utilise them (Raiter et al., 2018). Fences reduce human-wildlife conflict and protect threatened species (Woodroffe et al., 2014; Jakes et al., 2021). Fences can also aid

predators by increasing their hunting success rate (Dupuis-Desormeaux et al., 2016). Species that use scavenging to provide a portion of their diet can benefit from the mortalities that occur on roads as a result of wildlife-vehicle collisions (Hill et al., 2021) and mortalities from collisions with fences (Ho et al., 2023).

Hluhluwe-iMfolozi Park (HiP) is one of South Africa's oldest protected areas, located in KwaZulu-Natal and holds significant importance in carnivore conservation, serving as a crucial habitat for cheetahs (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*) (Cromsigt et al., 2017). Both of these species, along with other predators such as lion (*Panthera leo*) and brown hyena (*Hyaena brunnea*), were extinct within HiP's borders at one point (Somers et al., 2017). African wild dogs are presently listed as endangered, with a declining population, and only 1409 mature individuals reported globally (IUCN, 2024). In KwaZulu-Natal, wild dogs are primarily found in HiP and other reserves located in the uMkanyekude district in the northeast of the province (Somers et al., 2017). Since reintroduction and translocation activities in HiP began in 1980, wild dogs have left the park's boundaries, with departures taking two forms: long-distance dispersal and range expansion (Somers et al., 2017). Of the observed wild dog dispersal events, long-distance dispersals were influenced by land cover, elevation, road and human density (Whittington-Jones et al., 2014; Somers et al., 2017); while range expansion events were influenced by repeated river flooding, pack space requirements, interspecies conflict with lions, a proclivity to use fences in hunting and poor fence maintenance (Creel and Creel, 2002; van Dyke and Slotow, 2003; Somers et al., 2017). In contrast, cheetahs are presently listed as vulnerable with a decreasing population, and only 6,517 mature individuals are reported globally (IUCN, 2024). The southernmost cheetah population is in HiP, and much less is known about the distribution and population of cheetahs in HiP (Somers et al., 2017). Cheetahs disappeared from records in HiP

and surrounding areas in the 1920s, and despite the introduction of 64 individuals in the 1960s and several subsequent translocations, a photographic census in 2015 indicated that fewer than 15 individuals were present in HiP's borders (Rowe-Rowe, 1992; Hunter, 1998; Somers et al., 2017).

In HiP, cheetahs and wild dogs both favour nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*) as prey sources, with these two prey species making up the majority of their diets (Somers et al., 2017). Wild dogs favour nyala over impala because of a higher capture rate in HiP, unlike in other portions of their global range (Kruger et al., 1999; Hayward and Kerley, 2005; Somers et al., 2017). In contrast, cheetahs favour impala despite prey availability similar to other portions of its range (Hayward et al., 2006; Somers et al., 2017). However, both nyala and impala are heavily contested prey species, with apex predators, such as lions and leopards (*Panthera pardus*), also hunting them. Inter-species conflict over resources is highest between lions and wild dogs, with lions often forcing wild dogs off carcasses, overpowering or killing individual wild dogs (Somers et al., 2017). This strong interspecific conflict has led to lion-avoidance behaviours in wild dogs in HiP (Creel and Creel, 1998), with the majority of activity occurring during diurnal hours, with avoidance peaking during the denning season (Saleni et al., 2007; Darnell et al., 2014; Somers et al., 2017). The inter-species conflict between cheetahs and lions is much less apparent (Swanson et al., 2014; Somers et al., 2017); however, as with wild dogs, lion-avoidance behaviour has also been observed from cheetah individuals in HiP.

Our study aimed to determine the effect of anthropogenic structures on the human avoidance patterns of wild dogs and cheetahs in HiP. These species were selected because they both fill a similar niche within their environment and are both affected by larger predators within HiP. Additionally, both species are reintroduced populations, allowing for inferences to be made regarding the effect of anthropogenic structures on the behaviour of the reintroduced populations.

Our study focused on the perspective of the distance kept from human structures, as other studies in HiP have focused on various other factors that may influence habitat selection. In HiP, the movements and habitat selections of wild dogs were heavily influenced by the presence of lions, with dogs constantly moving to find areas with low lion probability in the park (Graf, 2008; Davies et al., 2021). Cheetah movement and habitat selection patterns were also influenced by lion presence; however, they were not as heavily influenced as wild dogs (Somers et al., 2017). The aim was achieved by determining the average distance kept from roads, fences and buildings in HiP, in addition to creating hotspot maps of activity near the linear structures and buildings for cheetahs and wild dogs within HiP and investigating the driving habitat characteristics using data collected from collared individuals of each species, across three dry and wet seasons. We predicted that cheetahs would spend the majority of their time avoiding anthropogenic structures, and that wild dogs would prefer to remain near them, as previous studies have found the use of fence structures in hunting activities (van Dyk and Slotow, 2003; Davies-Mostert et al., 2013). Additionally, we predicted that lion presence and rainfall would affect interaction rates, as habitats that allowed for lower risk of lion encounters may be selected (Swanson et al., 2014), and that areas with higher human activity may provide areas of perceived safety (Berger, 2007) and rainfall has been recorded affecting wild dog movement patterns (Somers et al., 2017).

5.4 Methods

5.4.1 Study areas

Our study took place in the uMkhanyakude region, the northernmost district of South Africa's KwaZulu-Natal Province, on the east coast of the country and included the entirety of the road network contained within HiP (Fig. 5.1). A park-governed paved road that connects the main

entrances of the park, Memorial and Nyalazi gates, as well as the main park accommodations, Hilltop and Mpila camps. Additionally, a major paved public road, present since 1895 and tarred in 2003, governed by the South African National Roads Agency Limited (SANRAL), the R618, splits the park in two, with the Hluhluwe portion in the north and the iMfolozi portion in the south. These roads see varying traffic levels, with control roads seeing the lowest amount of traffic compared with the major public road, which sees traffic 24 h a day, all year round. The R618 also has the highest wildlife mortality rate recorded to date in South Africa, with a wet season high of 13 caracasses^{-km} (Cormac et al., 2025).

This region is located northeast of the province and lies on the Mozambique coastal plain, which experiences weather similar to the tropics (Cromsigt et al., 2017). The HiP area is one of South Africa's oldest protected areas and initially comprised two separate parks in 1895, but they were later joined into one entity to create the present-day park (Cromsigt et al., 2017). The HiP spans a total of 96,000 ha and aims to protect a wide array of vegetation types, with many of South Africa's most iconic fauna found within its borders, with all the "big five" (lion, African savanna elephant (*Loxodonta africana*), black rhinoceros (*Diceros bicornis*), African buffalo (*Syncerus caffer*), and leopard) present as well as African wild dog, cheetahs and white rhinoceros (*Ceratotherium simum*) (Cromsigt et al., 2017). All of these are listed with conservation statuses ranging from Near Threatened to Critically Endangered, with decreasing populations, with only the black rhinoceros increasing in numbers globally (IUCN, 2025). The HiP falls along the edge of the Mozambique geological monocline (Howison et al., 2017). Wet seasons are longer than dry seasons, with wet seasons typically occurring between September and April and dry seasons between May and August (Perissinotto et al., 2013; Cromsigt et al., 2017).

Human structures cover 338.3 ha (0.35%) of HiP's 96000 ha protected area, with a total of 89.3 ha of paved road and 229.4 ha of unpaved road within the park, 19.6 ha of fences (border and interior) and 110 unfenced structures used by either tourists or park staff. The iMfolozi portion has the majority (> 60%) of the area covered by these anthropogenic structures, however, because the Hluhluwe portion is smaller in total size there is a higher concentration of anthropogenic structures, with the R618 separating the northern Hluhluwe portion from the southern iMfolozi portion, representing an area of 26.2 ha along its length that has a lower level of protection than the remainder of the HiP. Present vehicle traffic control measures along the R618 include cattle grids, speed bumps and video cameras at both entrances, with additional speed bumps placed along the length of the public road at areas where high-use animal paths intersect the road. Fauna may use structures to traverse the road safely, such as culverts, which are found along all drainage lines along the road; however, they are designed for runoff dispersal, and V-shaped concrete runoff channels have been placed in all sloping sections of the road. The length of the R618 has also undergone resurfacing activities, with the portion of the road within park boundaries taking place from March 2021 to September 2022. The road has a speed limit of 40 km/h for ~400 m before and ~120 m after the park entrances, with the remainder having a speed limit of 60 km/h in the park and for 20 km on either side of the park. However, no permanent measures are in place that threaten drivers with financial or judicial consequences to force them to maintain these speeds. The presence of megaherbivores and other large mammals acts as additional traffic calming, with most motorists slowing or coming to a complete stop when said fauna are crossing the road. Most of the road length is surrounded by savanna grassland with tree-dominated thickets along larger drainage lines (Howison et al., 2017). The road has an average width of 15 m and no fences that run parallel to the road. One paved road exists within HiP running from the Memorial Gate

entrance to Hilltop and Mpila resorts. This road acts as the main thoroughfare through the park, with all other roads being unpaved, and areas with a permanent human presence (resorts, education centres, tourist and staff camps) have fences around them.

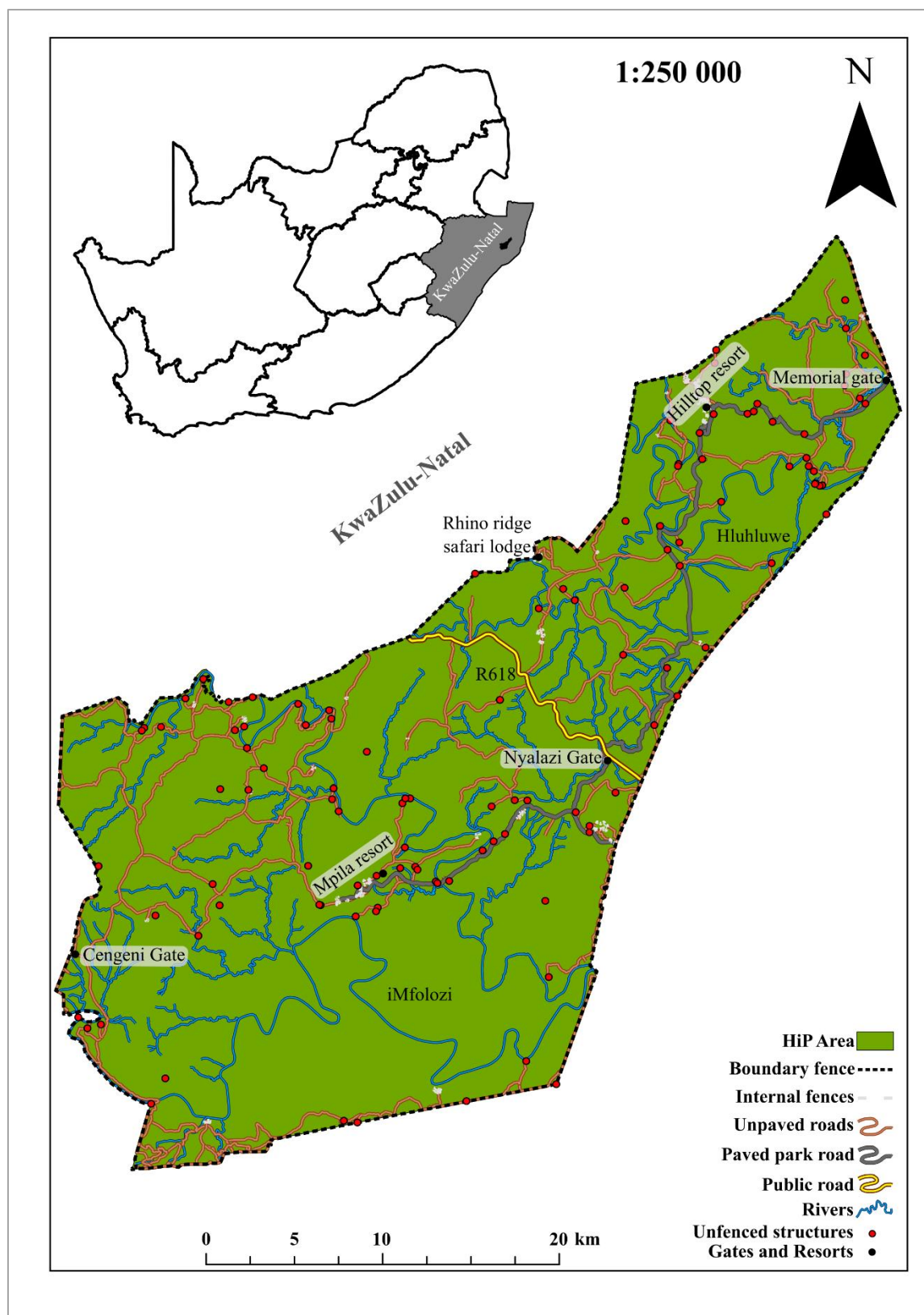


Figure 5.1: Anthropogenic structures (roads, fences and buildings) in Hluhluwe-iMfolozi Park.

5.4.2 Spatial analyses

Our study included global positioning system (GPS) data collected from May 1st, 2019, to April 30th, 2022. The GPS collars recorded a geographical location ~ every 6 h. As collars were not all placed on individuals simultaneously or did not function for the full three-year duration, data were standardised to include GPS fixes recorded within the hour before and after the standard GPS fix collection period during the data collection period, in the case of wild dog, points were only collected for one male and one female per pack. A total of 15969 GPS fixes were collected from nine (5 males, 4 females) cheetah (average of 803 (Stdev = 478) fixes per individual) and 12969 GPS fixes (average of 803 (Stdev = 478) fixes per individual) from 17 wild dogs (9 males, 8 females) belonging to 11 packs. Collar data were obtained and provided by the staff of WildlifeACT stationed in HiP; collars were placed using the methods described in Stone et al. (2022). Spatial analyses were conducted using ArcGIS Pro 3.5 (ESRI, 2025).

We created line shapefiles of linear structures in HiP using the Earthstar Graphics satellite imagery basemap in ArcGIS Pro (ESRI, 2025), and a point shapefile for unfenced structures was created using data provided by Ezemvelo KZN Wildlife. We calculated the average distance maintained from structures by cheetah and wild dog in HiP using the Near tool using a Geodesic method, and this was repeated for each dirt, private, and public roads, border and interior fences and unfenced structures and the distance to water. Additional information was collected on vegetation, biome, and elevation. Elevation information (SRTM 30, South African Department of Science and Technology, 2018) was collected by using the add surface information tool, using a bilinear method with a 5 m search distance. Vegetation and biome information (Vegmap, South African National Biodiversity Institute, 2018) was added using the spatial join tool with a 5 m search radius. The number of wild dog and lion GPS fixes (provided by Simon Chamaille-Jammes

of Centre d'Ecologie Fonctionnelle et Evolutive, CNRS) within 1,000 m of each cheetah GPS fix was also added using the spatial join tool, using the within distance (geodesic) match method. This was done to provide a proxy for the spatial density of wild dogs and lions. The same process was repeated for cheetah and lion GPS fixes, with 1,000 m of each wild dog GPS fix. Temperature, year, and season information were obtained from recorded collar data.

Cheetah and wild dog home ranges were determined using the standard deviational ellipses tool, set to one standard deviation. Deviational ellipses were produced for each individual in the cheetah dataset, while ellipses for wild dogs were created for each pack. This was done to account for the autocorrelation between male and female individuals within a single wild dog pack. A 1 x 1 km grid was created for the park area and spatially joined to both cheetah and wild dog datasets; Moran's I was then used to test for spatial autocorrelation, using species counts per kilometre, low levels of clustering were found for both species (Cheetah – $I = 0.32$, $z = 22.95$, $p < 0.001$; Wild dog – $I = 0.16$, $z = 10.58$, $p < 0.001$). Analysis of the number of nearest neighbours (polygon neighbours tool) produced results with a value below one (# of neighbours = 0.42), indicating a single neighbour. Line-centred buffers were created to incorporate the different widths of each linear structure type using the buffer tool with flat ends. We set buffer widths to 3 m for dirt roads, 5 m for the park's private paved road and 7.5 m for the R618, 2.5 m for fences (based on area cleared on either side of the fence). A 5 m buffer was created around each unfenced structure point to create polygon layers for all anthropogenic features. The buffers for each structure type were then joined using the merge tool to create a map of the HiP anthropogenic structures. An additional 400 m (based on the findings of Naidenko et al. (2021)) was added to the structures map using the pairwise buffer tool to create a map of the anthropogenic area of influence. Hotspots of cheetah and wild dog activity across HiP's area, in addition to the area of anthropogenic influence, were

calculated using the find hotspot tool with bin size set to 400 m and neighbourhood size set to 1,000 m. These were chosen to produce hotspots on a 400 m² square grid and to incorporate the clustering observed at the 1 km² scale.

5.4.3 Statistical analyses

We conducted statistical analyses using R (version 4.5.1; Team, 2020) plug-in in Google Collab (Sukhdeve and Sukhdeve, 2023). The statistical analysis workflow described was used for data collected for the cheetah and wild dog. Scale-fixed effects (Supplementary information Table S5.1) were Z-transformed to normalise the data, following normality and assumption testing using the car package (Fox and Weisberg, 2019). Residual plots of influencing effects are presented in the Supplementary information Fig. S5.1-S5.5. A generalised linear mixed-effects model (GLMM) using the lme4 package (Bates et al., 2015) was used to determine the effect of collected fixed and random variables on the maintained distance between sexes of each respective species and between both species. Correlation tests were run to determine if any of the included variables displayed strong correlations (correlations greater than 70%); no strongly correlated variables were found. Analysis of variance (ANOVA) tests were conducted to determine differences in the distance maintained from different structure types, according to the sex of each respective species and between both species. Base R-packing was used (Team, 2020). The post-hoc analyses were done using the emmeans package (Lenth, 2025). Graphical representations were visualised using the ggplot2 package (Wickham, 2016).

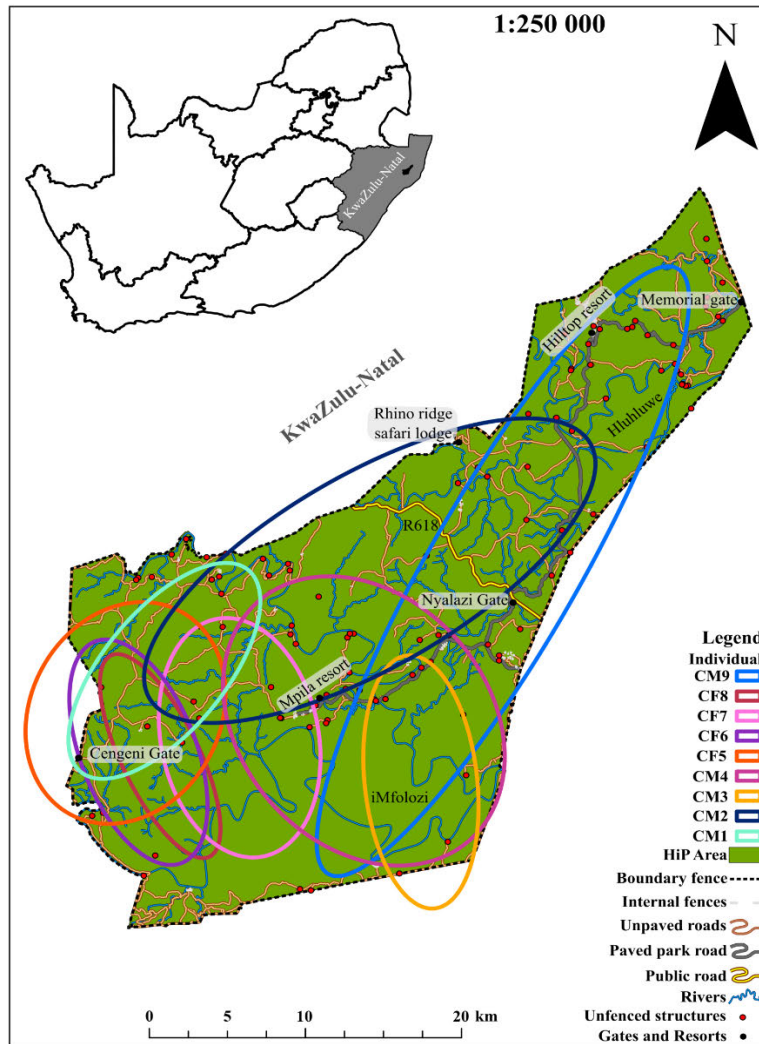
5.5 Results

5.5.1 Spatial analyses

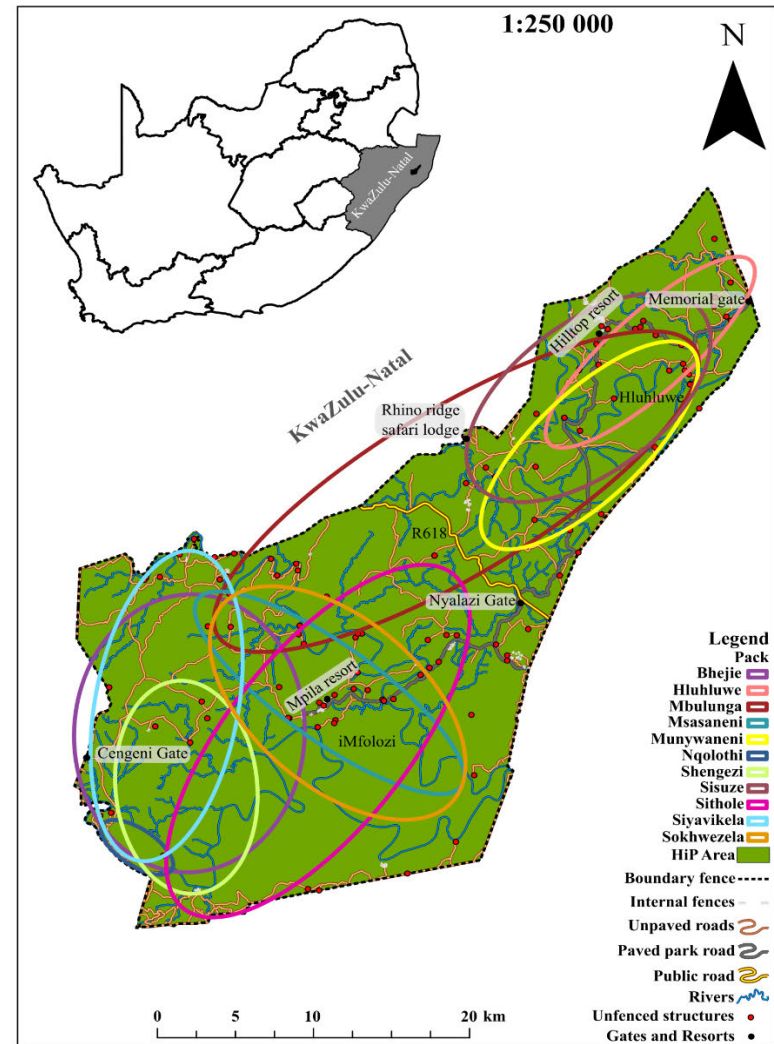
The home range ellipses created for the nine cheetahs included, found that all nine had portions of their home range areas within the iMfolozi portion of the HiP (Fig. 5.2a), however, only the home ranges of two males crossed over the R618 into the Hluhluwe portion with the home range of CM9 reaching from the north of the park to the wilderness region in HiP's southeastern region. Conversely, the home range ellipses created for the 11 wild dog packs included (Fig. 5.2b), found that only one (Mbulunga pack) of the 11 had a home range that crossed the R618, also forming the largest home range within HiP. However, the home ranges for all nine cheetah and the four wild dog packs present in the Hluhluwe portion of HiP were completely enclosed in the park (as all GPS-fixes fell within park boundaries prior to clipping). In contrast, the home ranges displayed by iMfolozi wild dog packs did not include all the GPS-fixes recorded during the collection period, as a large quantity of data points outside of the bounds of HiP were clipped before analysis. Although the combined home range for each species provided near total coverage of the area within park bounds.

The results of hotspot analysis of cheetah GPS-fixes found clustering over 21,328 ha during the wet season and 21 568 ha during the dry season; however, all areas of significant clustering were in the southern regions of the iMfolozi portions of HiP, with six areas of significant clustering (1,664 ha) during the wet season, the largest of which covered an area of 608 ha (Fig. 5.3a). When restricted to the area of anthropogenic influence, only two hotspot areas remained unchanged, the largest of which corresponded to the largest non-restricted area, covering exactly half the area (Fig. 5.3b). During the dry season, only two significant clustering areas (1,568 ha) were identified (Fig. 5.4a). Only 5 squares (80 ha) remained hotspots when only considering near-structure GPS fixes

(Fig. 5.4b). All areas with significant clustering of recorded cheetah presence were located near water sources. In contrast wild dog showed clustering across 34,144 ha of HiP during the wet season and 23,200 ha during the dry season, 11 areas of significant clusters (1,968 ha) of recorded wild dog presence were identified during the wet season, with the two largest covering 320 ha, both centred around animal housing facilities (Fig. 5.5a), with only one significant cluster not forming around an anthropogenic structure. Although when restricted to near structure records, only one area in the northeast of the Hluhluwe portion in HiP remained close to its original size, reducing by only 48 ha (Fig. 5.5b). During the dry season, wild dogs were significantly clustered (1,328 ha) in the centre of the Hluhluwe portion of HiP in an area with a high concentration of dirt roads and rivers (Fig. 5.6a). The only area that remained significant was identified in the region where dirt roads and private paved roads intersected, as well as the animal housing facility in the iMfolozi portion of HiP (Fig. 5.6b). None of the areas with significant clusters of cheetahs and wild dogs were present across species for either the wet or dry seasons.



A



B

Figure 5.2: Home range areas of A) cheetahs (*Acinonyx jubatus*) and B) wild dog (*Lycaon pictus*) packs in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

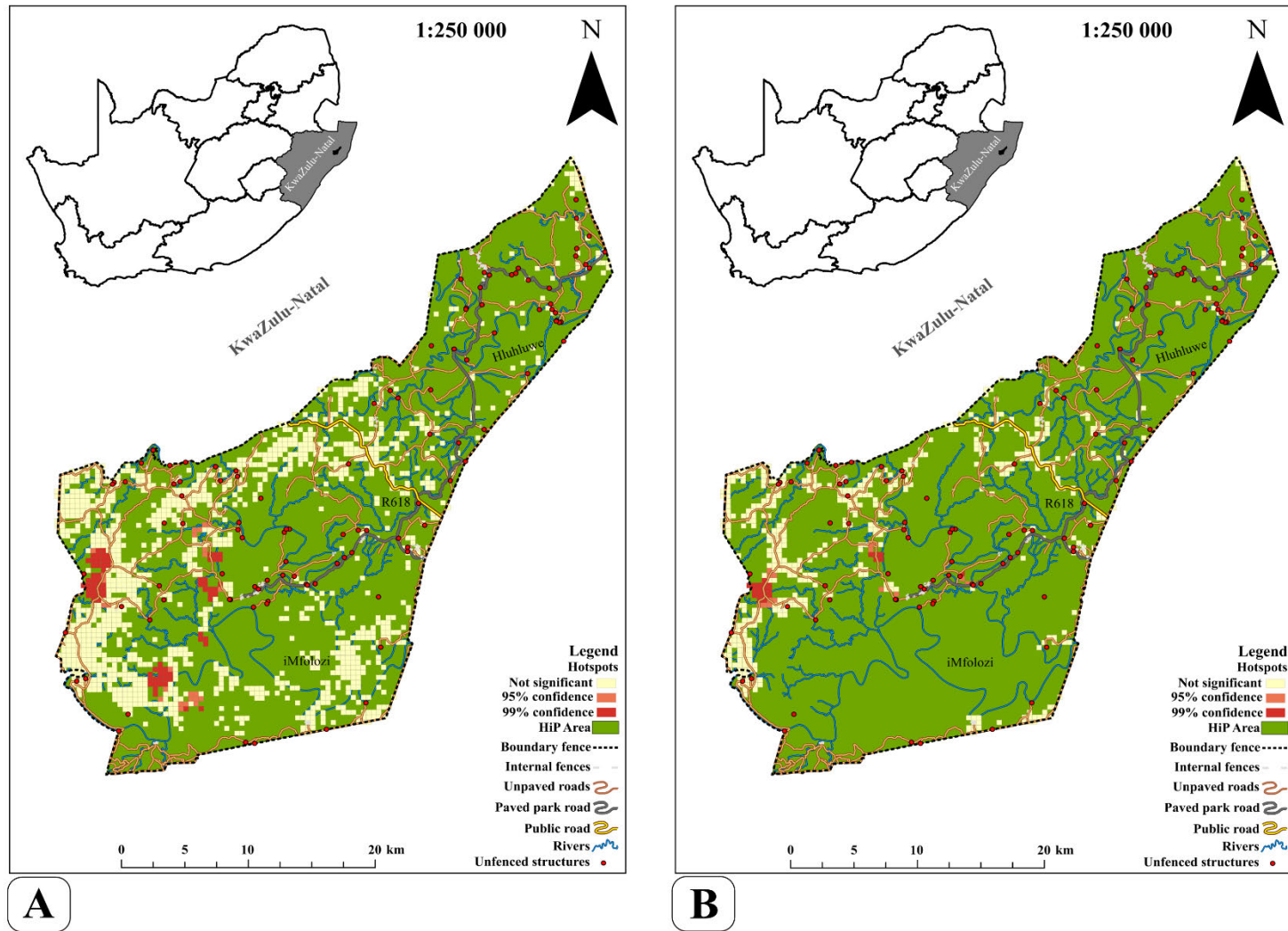


Figure 5.3: Hotspots of cheetah (*Acinonyx jubatus*) wet season activity in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa; A) total park area and B) near anthropogenic structures.

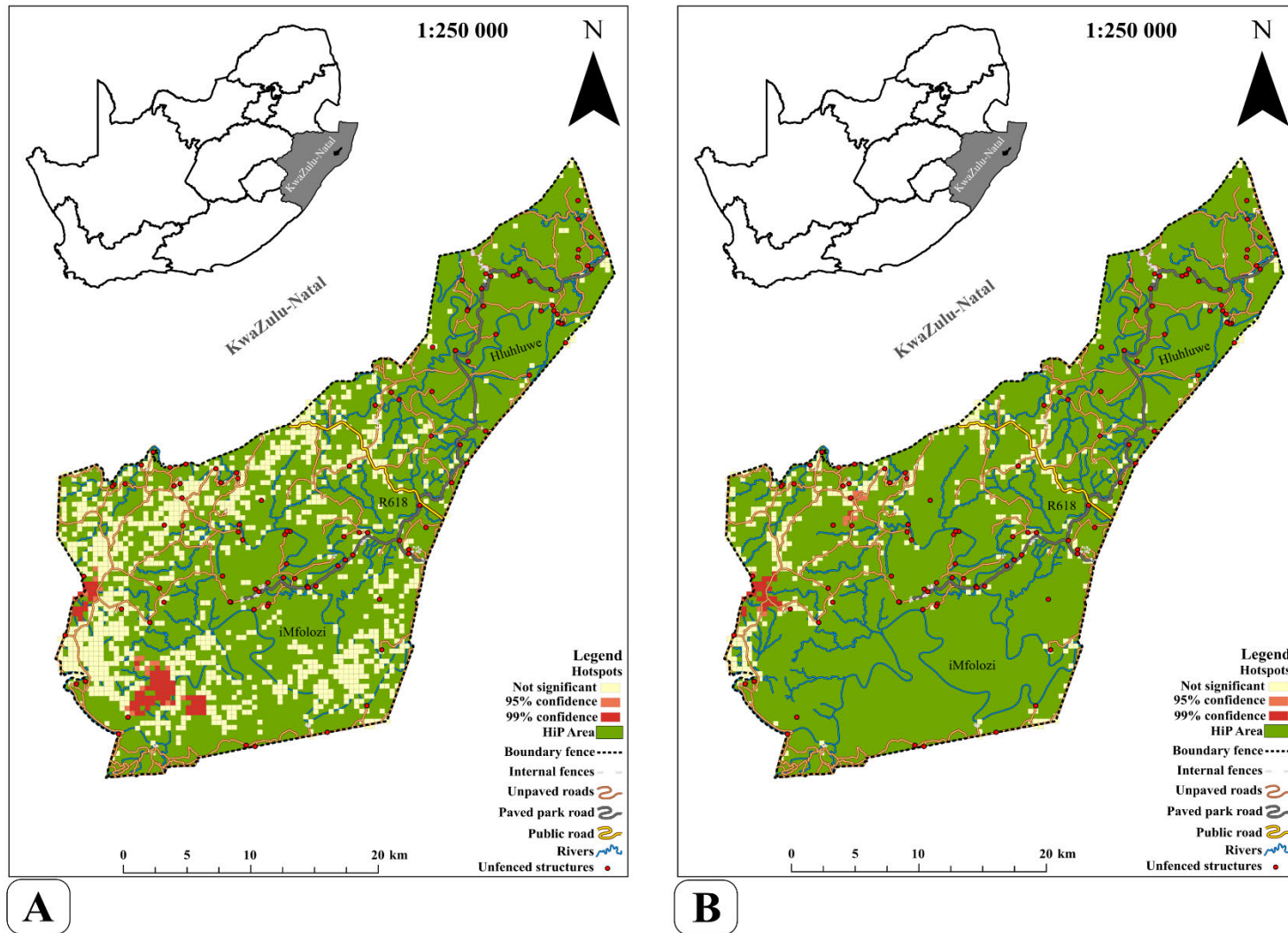


Figure 5.4: Hotspots of cheetah (*Acinonyx jubatus*) dry season activity in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. A) total park area and B) near anthropogenic structures.

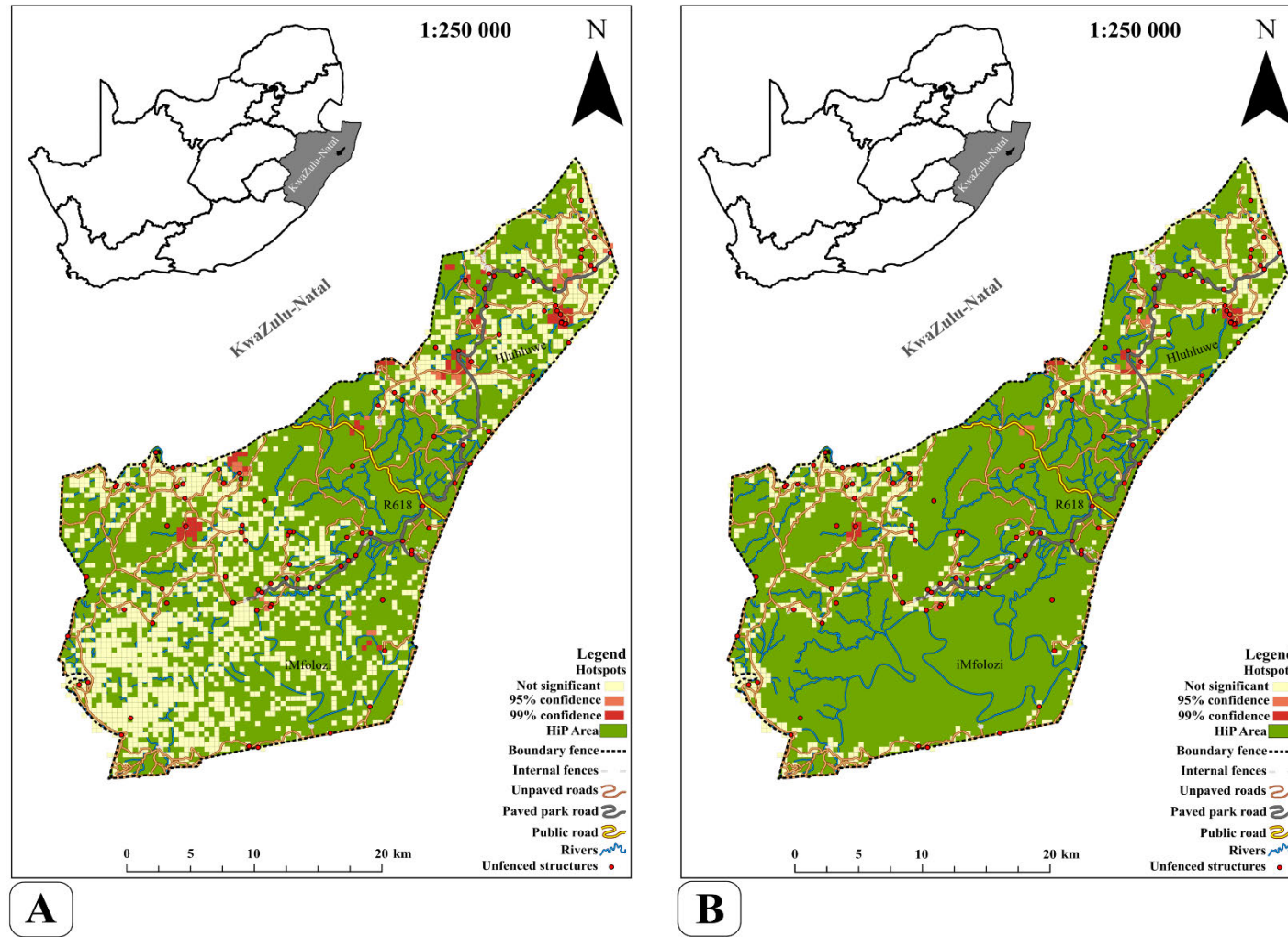


Figure 5.5: Hotspots of wild dog (*Lycaon pictus*) wet season activity in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. A) total park area, and B) near anthropogenic structures.

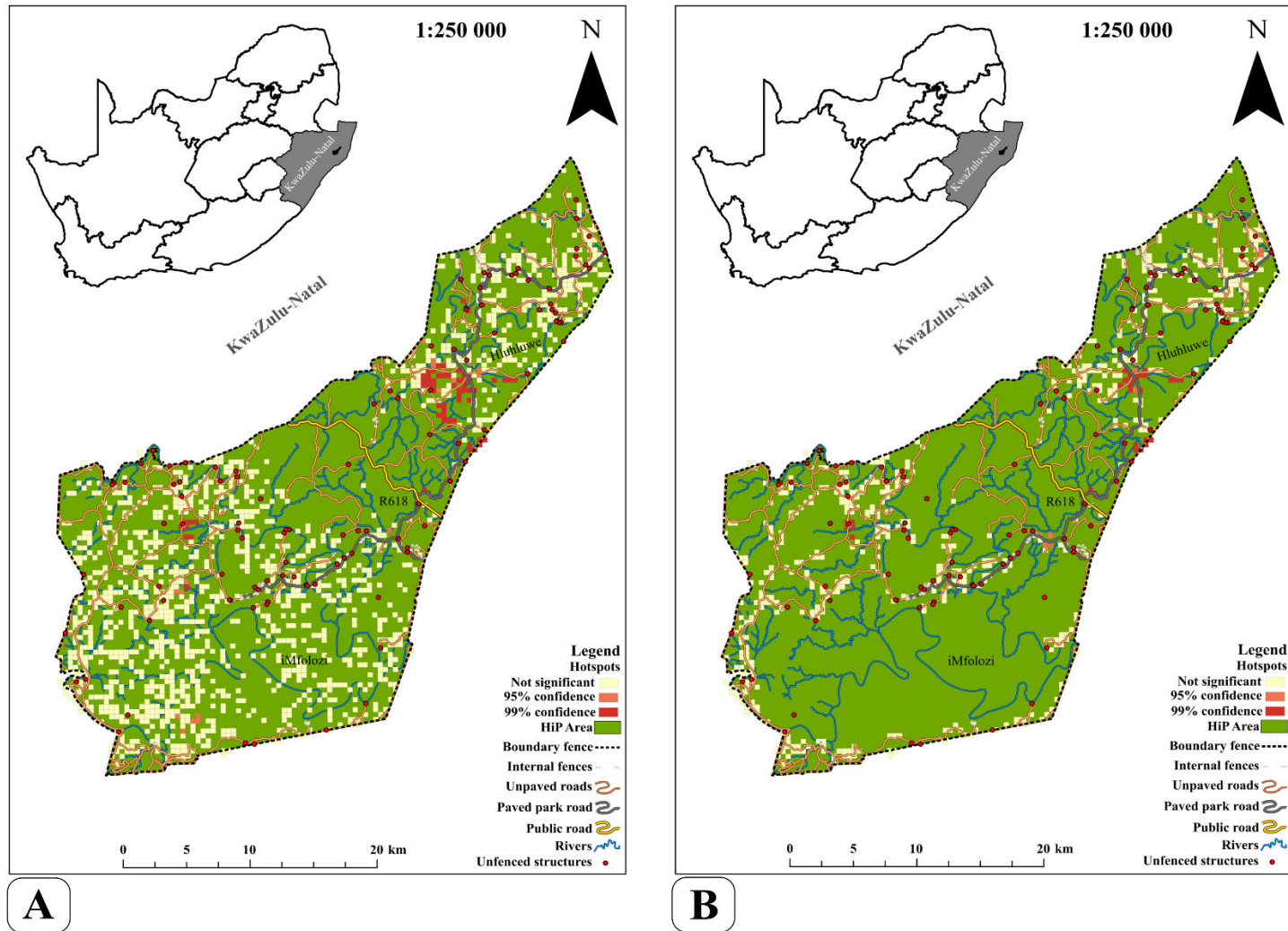


Figure 5.6: Hotspots of wild dog (*Lycaon pictus*) dry season activity in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. A) total park area, and B) near anthropogenic structures.

5.5.2 Statistical analysis of maintained distance from anthropogenic structures

The distance maintained from anthropogenic structures in HiP showed a significant difference between cheetah, wild dog, and both species. We initially found significant differences in distance mediated by sex and road type in the distance maintained from roads in both species (Table 5.1). Female cheetahs maintained a significant difference in the distance from all structure types (Table 5.1), with the highest distance from the public road and the lowest distance from dirt roads (Fig. 5.7a). Further analysis revealed that increasing elevation, the presence of wild dogs, and sex mediated the decreased distance maintained by cheetahs to anthropogenic structures (Table 5.3). However, the distance between wild dogs mediated by sex was influenced by the type of anthropogenic structure. Male wild dogs maintained the highest distance from the public road and the lowest distance from dirt roads; however, female wild dogs used areas closer to the border fence, unfenced structures, and private park roads (Fig. 5.7b). Further analysis of wild dogs revealed that increasing elevation, distance to water, and sex mediated the decreased distance maintained by these wild dogs (Table 5.4). As with the individual species, when combined, a significant difference was observed between species and the different structure types (Table 5.2). The highest distance was kept from the public road, and the lowest distances were kept from dirt roads, with cheetah maintaining higher distances from all anthropogenic structure types (Fig. 5.8). Further analysis in both species showed that increasing elevation, distance to water and presence of the other species mediated the decreased distance maintained by both species (Table 5.5).

Table 5.1: Analysis of variance results of distance kept by male and female cheetah (*Acinonyx jubatus*) and wild dog (*Lycaon pictus*) from types of anthropogenic structure in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Cheetah					
	Df	Sum Squares	Mean Squares	F value	p-value
Sex	1	3.034e+12	6.068e+11	64369	<2e-16 ***
Structure type	5	8.157e+10	8.157e+10	8653	<2e-16 ***
Sex X structure type	5	1.285e+11	2.571e+10	2727	<2e-16 ***
Residuals	95802	9.031e+11	9.427e+06		
Wild dog					
Sex	1	1.848e+12	3.696e+11	28452.2	<2e-16 ***
Structure type	5	2.817e+09	2.817e+09	216.9	<2e-16 ***
Sex X structure type	5	3.707e+10	7.451e+09	570.8	<2e-16 ***
Residuals	77802	1.011e+12	1.299e+07		

Table 5.2: Analysis of variance results of distance kept by cheetah (*Acinonyx jubatus*) and wild dog (*Lycaon pictus*) from types of anthropogenic structure in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

	Df	Sum Squares	Mean Squares	F value	p-value
Species	1	8.272e+10	9.646e+11	77393.8	<2e-16 ***
Structure type	5	4.823e+12	8.272e+10	6637.3	<2e-16 ***
Species X structure type	5	5.924e+10	1.185e+10	950.7	<2e-16 ***
Residuals	173616	2.164e+12	1.246e+07		

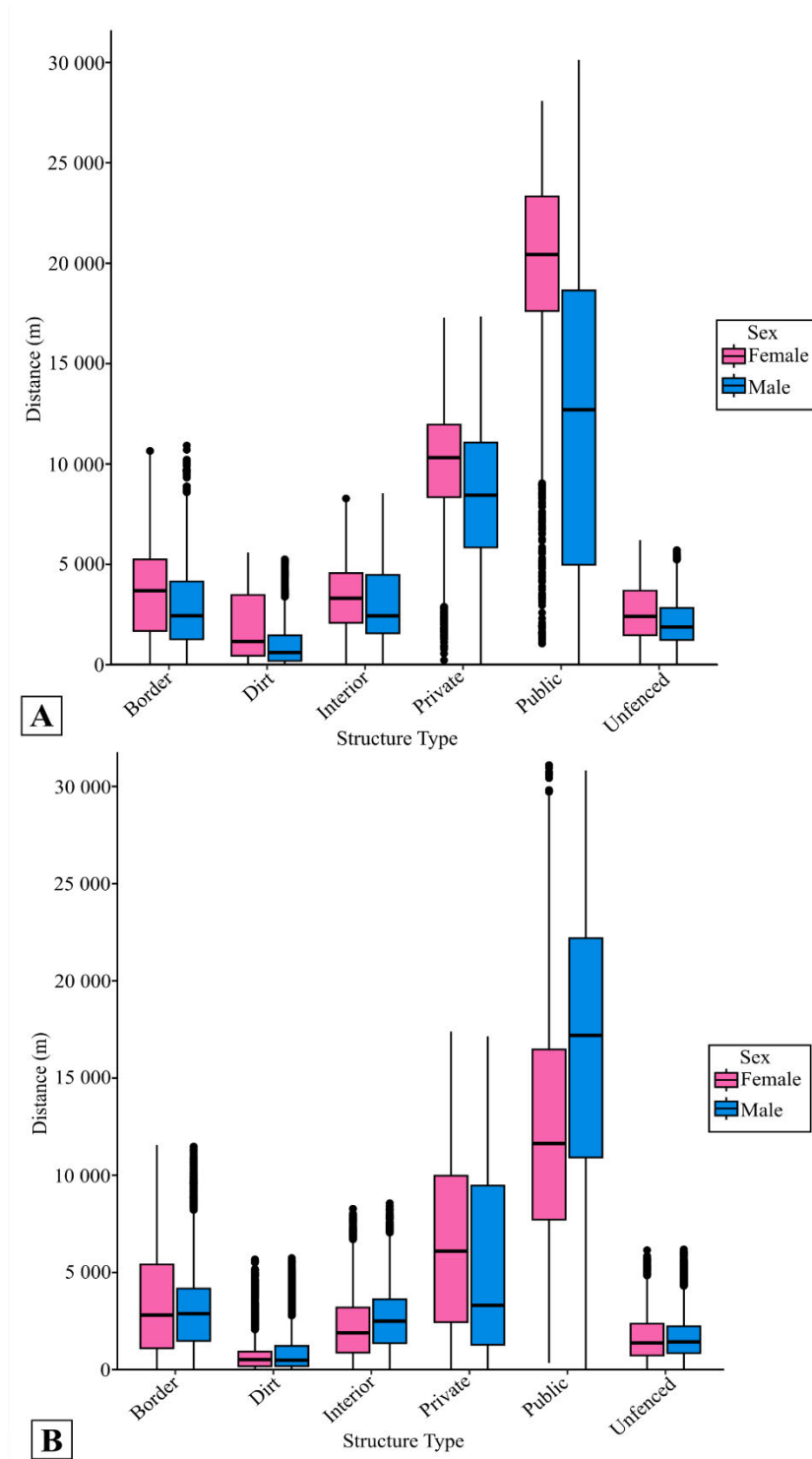


Figure 5.7: Distance kept by male and female A) cheetahs (*Acinonyx jubatus*) and B) wild dogs (*Lycaon pictus*), from types of anthropogenic structure in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

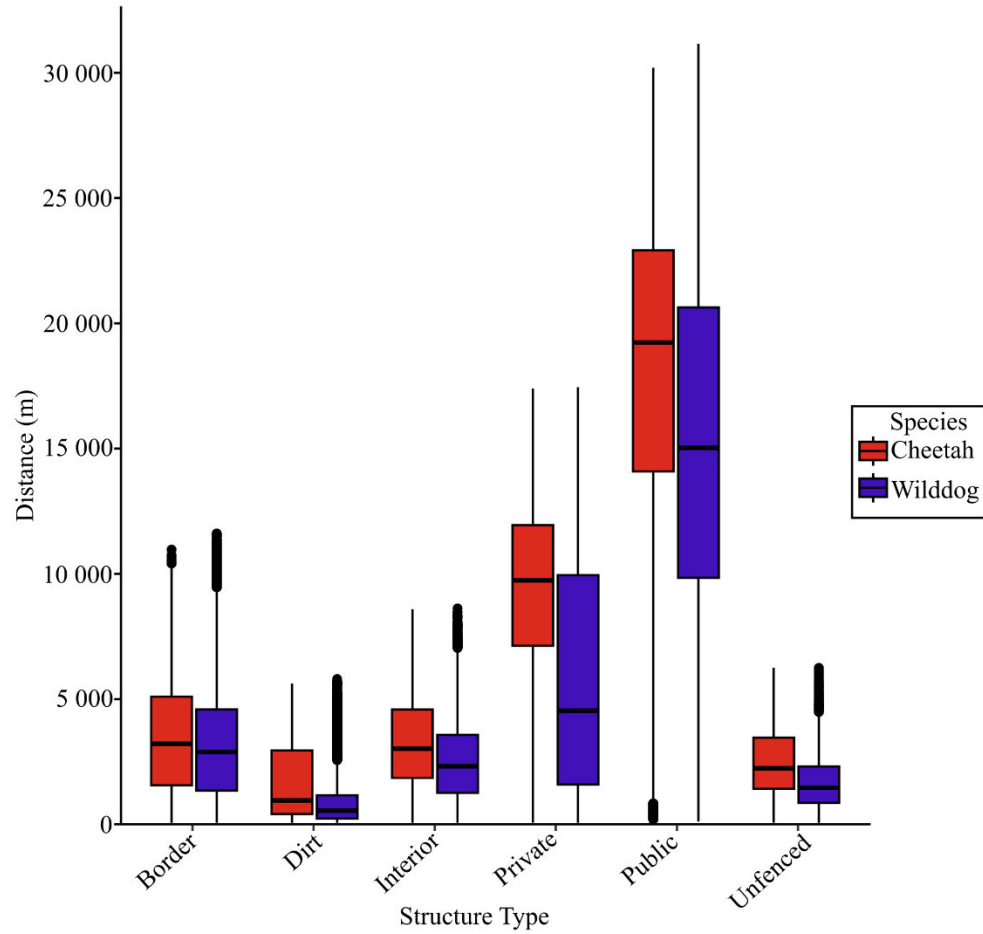


Figure 5.8: Distance kept by cheetah (*Acinonyx jubatus*) and wild dog (*Lycaon pictus*), from types of anthropogenic structure in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Table 5.3: Generalised linear mixed-effects model of factors driving maintained distance from anthropogenic structures by cheetah (*Acinonyx jubatus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Random effects	Variance	Standard Deviation	
Month	0.0003543	0.01882	
Year	0.0001318	0.01148	
Season	<0.001	<0.001	
Residual	0.0106765	0.10333	
Fixed Effects	Estimates	Standard Error	t-value
Intercept	0.0007964	0.0081416	0.098
Dirt road	0.9469035	0.0016174	585.452
Private road	0.0531328	0.0019564	27.158
Public road	0.0093483	0.0013805	6.772
Border	0.0596073	0.0017071	34.917
Interior	0.0066238	0.0012451	5.320
Unfenced	0.0002303	0.0012457	0.185
Elevation	-0.0449325	0.0011429	-39.313
Water	0.0080934	0.0009077	8.917
Wild dog	-0.0035021	0.0009730	-3.599
Lions	0.0050144	0.0011306	4.435
Sex	-0.0087510	0.0029248	-2.992

Table 5.4: Generalised linear mixed-effects model of factors driving maintained distance from anthropogenic structures by wild dog (*Lycaon pictus*) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Random effects	Variance	Standard Deviation	
Month	0.0006572	0.02564	
Year	0.0004410	0.02100	
Season	<0.001	<0.001	
Residual	0.0441108	0.21003	
Fixed Effects	Estimates	Standard Error	t-value
Intercept	0.011241	0.013365	0.841
Dirt road	0.887551	0.002338	312.954
Private road	0.016634	0.002634	6.315
Public road	0.026898	0.002836	11.503
Border	0.055102	0.002357	23.374
Interior	0.029347	0.002308	12.713
Unfenced	0.055882	0.002799	19.968
Elevation	-0.052682	0.002118	-24.869
Water	-0.007814	0.002049	-3.813
Cheetah	0.022922	0.002168	10.574
Lions	0.009858	0.002146	4.593
Sex	-0.021939	0.004589	-4.780

Table 5.5: Generalised linear mixed-effects model of factors driving maintained distance from anthropogenic structures by secondary carnivores (cheetah (*Acinonyx jubatus*) and wild dog (*Lycaon pictus*)) in Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa.

Random effects	Variance	Standard Deviation	
Month	0.0002374	0.01541	
Year	0.0006787	0.02605	
Season	<0.001	<0.001	
Residual	0.0199649	0.14130	
Fixed Effects	Estimates	Standard Error	t-value
Intercept	-0.0068170	0.0138454	-0.482
Dirt road	0.9237108	0.0014349	643.727
Private road	0.0307344	0.0013224	23.242
Public road	0.0230506	0.0010814	21.316
Border	0.0436364	0.0011499	37.948
Interior	0.0185860	0.0011361	16.360
Unfenced	0.0170696	0.0012712	13.428
Elevation	-0.0443606	0.0010127	-43.805
Water	-0.0018900	0.0008885	-2.127
Lions	0.0050298	0.0010409	4.832
Species	-0.0077115	0.0020889	-3.692

5.6 Discussion

Our study aimed to determine how anthropogenic structures affected cheetahs and wild dogs' human avoidance patterns in HiP. We found that most recorded GPS-fixes for wild dogs and male cheetahs were within 1000 m of anthropogenic structures in HiP, with the upper quartile of the distance kept from structures by female cheetahs extending up to ~3,500 m. Only GPS-fixes with outlier distances from anthropogenic structures showed distances from structures greater than the daily movement capabilities of either species (~ 4,000 m for both cheetah and wild dog (Hayward et al., 2008)). Significant differences in the distance kept from each structure type were found between species and between males and females of each species, with wild dogs maintaining smaller distances from all anthropogenic structures. However, male wild dogs maintained larger

distances to all anthropogenic structures with the exception of the private paved road in HiP. In contrast to wild dogs, male cheetahs maintained smaller distances to all anthropogenic structures within HiP. Distances to anthropogenic structures except paved roads were all within a single day's movement, while the maintained distance to the private paved road was double the daily movement capabilities of cheetahs, while the distance to the public paved road was four to five times higher than the daily movement capabilities of both species. The most influential factors included the distance to dirt roads, border fences, unfenced structures and paved roads, with maintained distances all being lower in areas where these structures were present, except for the paved roads, where distances were typically higher, with avoidance (i.e., greater maintained distance) of paved roads higher for cheetahs. The density of lions, although not the most influential factor, still showed a significant effect on the maintained distances to anthropogenic structures, thereby heavily influencing the habitat selection and movement capabilities of both cheetah and wild dog in HiP. This was expected as the presence of lions has been previously recorded affecting the time of movements and hunting site selection within HiP, with wild dogs actively avoiding lion territories (Somers et al., 2017). Additionally, cheetahs and wild dogs are direct competitors for food resources in HiP, with both species favouring nyala and impala as prey. However, Somers et al. (2017) found that wild dogs were selecting nyala in the Hluhluwe portion and impala in the iMfolozi portion, although they were most successful when hunting nyala. Cheetahs in Phinda Game Reserve were found to select habitats through a trade-off of resource acquisition and lion avoidance (Rostro-Garcia et al., 2015).

Avoiding interspecific conflict may also be the driver of the different movement patterns around the various structure types, with cheetahs remaining further from anthropogenic structures, while wild dogs remained nearer to human-made structures. Additionally, wild dogs may actively

use interior fences during hunting activities, as individual wild dogs have been recorded using fences to improve hunting success in multiple protected areas across Africa (van Dyke and Slotow, 2003; Romañach and Lindsey, 2008; Somers et al., 2017). The difference in use of the anthropogenic structures in HiP is akin to niche partitioning, which is further supported by previous findings by Saleni et al. (2007) and Darnell et al. (2014) in relation to differences in temporal activity and habitat utilisation by cheetah and wild dog in relation to the presence of lions. The general trend of cheetah and wild dog more heavily utilising areas with lower lion presence is repeated here, with cheetahs still utilising areas where lions had a stronger presence and wild dog utilising areas that lions avoided, as seen in patterns of road network usage by lions in HiP during the same period (Cormac et al. in review). However, the selective use of anthropogenic structures in HiP may also be linked to high levels of human habituation because of lingering effects on behaviour from rehabilitation methods (such as feeding carnivores in temporary housing facilities, i.e., bomas) and post-release effects of unfamiliar surroundings. Although this is likely secondary to the avoidance of interspecific conflict, it still holds importance for species management activities within HiP. In Dinokeng Game Reserve, South Africa, lions released into areas where all historical individuals were extirpated varied but, on average, followed a pattern of increasing dispersal from the release site, however the dispersal of lion individuals from release sites in unfamiliar locations reduced with each subsequent release, with movement reductions increasing with the increase of inter- and intraspecific territory conflict (Yui et al., 2015). A study on the rehabilitation of orphaned white rhinos also found that hands-on rehabilitation led to significantly higher levels of habituation and reductions in alert or defence behaviours (Fàbregas et al., 2020). Sustained management and veterinary needs during capture and rehabilitation may also inhibit the development of natural behaviours in translocated predators (Goldenberg et al., 2022). This was

evident in the numerous returns to the temporary housing facilities within HiP during dry periods when resources are scarcer. Further evidence of potential habituation to human structures in HiP can be seen in the lack of change in density of recording and mean average distance kept from anthropogenic structures in the park during the drastic changes to human activity in the park during lockdown procedures implemented during the 2020 COVID-19 pandemic, with only minimal variation in distance kept from roads across years. These results also differ from changes in wildlife movement patterns seen during the global “lockdown”, which restricted human movement and protected areas were closed to tourist activities (Procko et al., 2022; Tucker et al., 2023).

This apparent habituation to human activity has both positive and negative effects. The most prominent positive effect does not directly benefit the wildlife, but tourists who visit HiP are more likely to observe individuals, which increases visitation by tourists seeking to observe these iconic African species. This, in turn, increases revenue from ecotourism and subsequently increases funding for conservation (Higginbottom, 2004). However, the negative effects do directly hinder cheetah and wild dog, these negatives include increased risk of mortality because of proximity to roads (Moore et al., 2023), decreased avoidance of humans leading to direct conflict because of predation on livestock (Woodroffe et al., 2005), and increased permeation of protected areas' boundary fences (van Dyk and Slotow, 2003). Despite the lack of avoidance of most anthropogenic structures in HiP, significant avoidance of the public paved road (R618) was observed in wild dogs, forming a nearly impassable boundary, with only individuals from a single pack crossing this road. This avoidance of the R618, a road that separates the Hluhluwe and iMfolozi portions of HiP, coupled with the recorded avoidance of inbreeding by wild dogs in HiP (Spiering et al., 2011; Somers et al., 2017), increases the likelihood of wild dogs' dispersion to areas outside of HiP, which has been observed on multiple occasions in wild dog individuals

collared in HiP (Whittington-Jones et al., 2014; Somers et al., 2017). This increases the threat to species persistence within the park because of reductions in genetic diversity, increased human-wildlife conflict in rural communities surrounding the reserve, as well as wasted funds used on the translocation and recapture of escaped individuals (Higginbottom, 2004).

Our study was limited by the resolution of the available data, as the period between recordings in the presently available data did not allow for finer-scale non-assumptive spatial analyses to determine the per-hour scale of time spent near anthropogenic structures. Additionally, as no accelerometer data were available, no analyses could be conducted to determine activity patterns near anthropogenic structures. In addition to the lack of fine-scale GPS-linked movement data, the scarcity of fine-scale habitat data limited the secondary information available to investigate the factors driving cheetahs and wild dogs' activity near anthropogenic structures. Future work should include the collection of GPS records taken every hour. It should also include accelerometer data, allowing questions regarding time spent near structures, direction of movement, speed of movement, and activity patterns near structures to be addressed. Future studies should also investigate the correlation to other known factors of habitat selection, such as tourist movement patterns and other non-consumptive effects. Additionally, studies should be expanded to include investigations of the effect of prey movement and availability as well as conflict with larger predators such as lion, leopard, and spotted hyena (*Crocuta crocuta*) in multiple protected areas, in particular to protected areas that have historically present lineages and to protected areas that have a mixture of historically present and translocated lineages of these species. This should be done to determine if translocated individuals (particularly those kept in boma structures) are less likely to avoid human structures and to inform the development of species-specific management plans.

5.6.1 Conclusions

Minimal avoidance of anthropogenic structures by wild dogs was observed, indicating potential habituation to anthropogenic structures in HiP. However, despite the species' activity of both cheetahs and wild dogs covering most of HiP, both species made heavy use of less than 3% of the park's area. This is of particular concern as cheetahs and wild dogs are both species whose persistence is under threat in HiP. Therefore, this study's results provide key insights into areas of conservation concern for both species and further indicate the need for more effective species management to reduce human-wildlife conflicts in the rural communities surrounding HiP.

Author Contributions

Cameron Cormac: Writing — review & editing, Writing — original draft, Visualisation, Methodology, Formal analysis, Data curation, Conceptualisation. Suveena Govender: review & editing, Visualisation, Formal analysis. Cormac Price: Writing — review & editing, Supervision. Jarryd Streicher: Writing — review & editing, Supervision. Dave Druce: Writing — review & editing, Data curation, Conceptualisation. Chris Kelly: Writing — review & editing, Data curation, Conceptualisation. Colleen T. Downs: Writing — review & editing, Methodology, Conceptualisation, Supervision, Funding acquisition.

Data Availability

The data used in this study do not belong to the authors. They received special permission to use it.

Declaration of Interest

The authors declare that they have no conflicts of interest.

5.7 Acknowledgements

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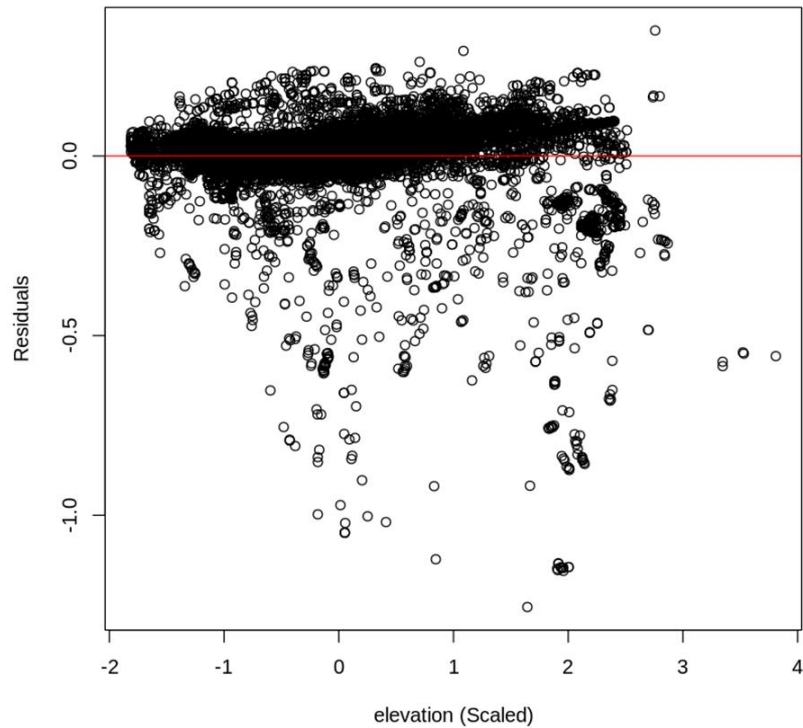
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5.9 Supplementary information

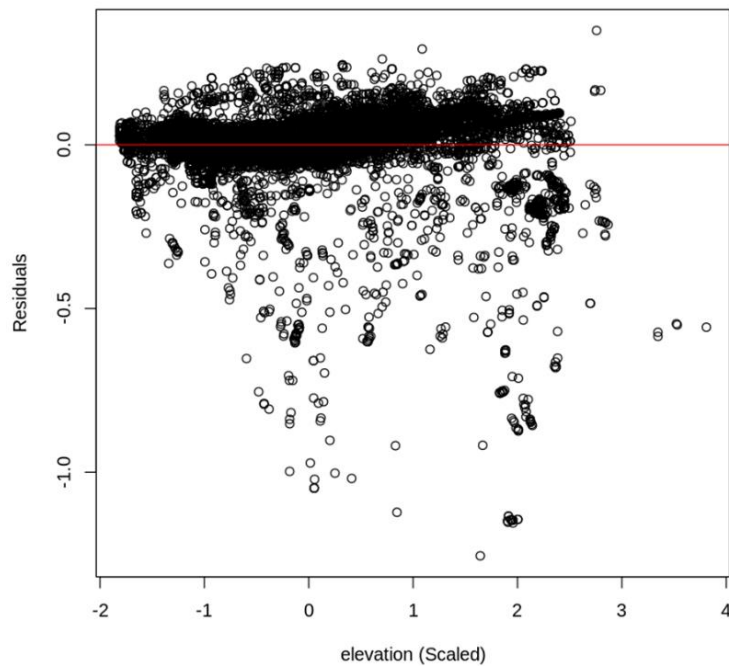
Supplementary information Table S5.1: Variables used in generalised linear mixed-effects

models

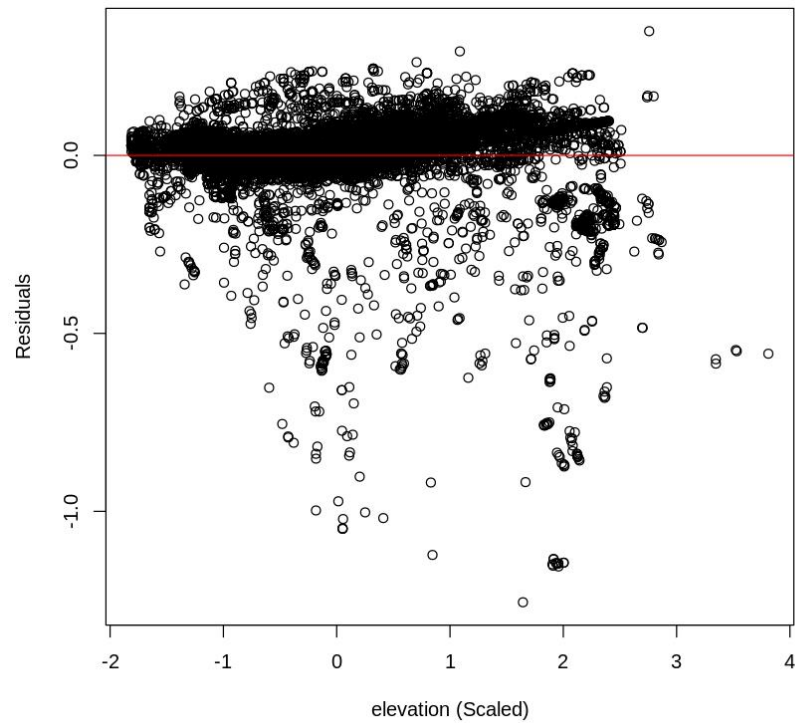
Variable	Effect type	Data type
Distance to roads (Roads) (m)	Dependant	Scale
Distance to public paved road (Public) (m)	Fixed	Scale
Distance to private paved road (Private) (m)	Fixed	Scale
Distance to unpaved roads (Dirt) (m)	Fixed	Scale
Distance to border fence (Border) (m)	Fixed	Scale
Distance to interior fences (Interior) (m)	Fixed	Scale
Distance to unfenced structures (Unfenced) (m)	Fixed	Scale
Distance to water source (Water) (m)	Fixed	Scale
Elevation (m a.s.l)	Fixed	Scale
Vegetation	Fixed	Nominal
Biome	Fixed	Nominal
Lion (<i>Panthera leo</i>) density	Fixed	Scale
Conflicting species density (cheetah/wild dog presence)	Fixed	Scale
Sex	Fixed	Binomial
Species	Fixed	Binomial
Year	Random	Nominal
Season	Random	Binomial
Month	Random	Nominal
Time of Day	Random	Binomial



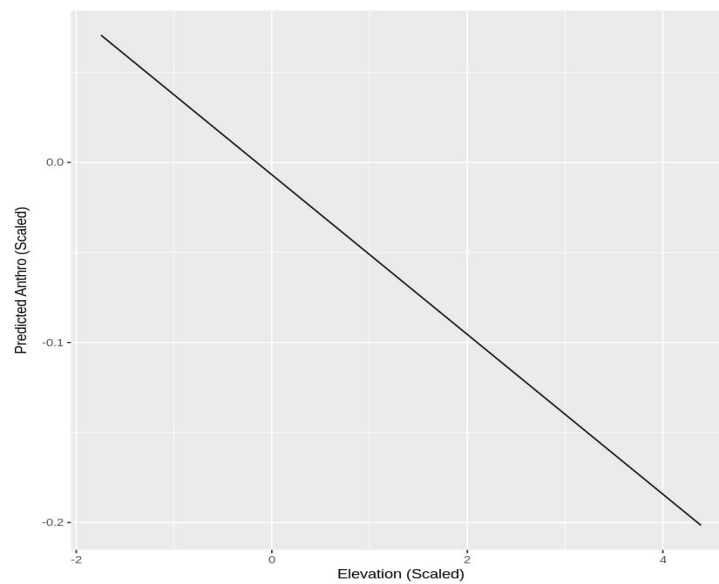
Supplementary information Figure S5.1: Distance maintained from anthropogenic structures by cheetah (*Acinonyx jubatus*) in response to increasing elevation in Hluhluwe-iMfolozi Park.



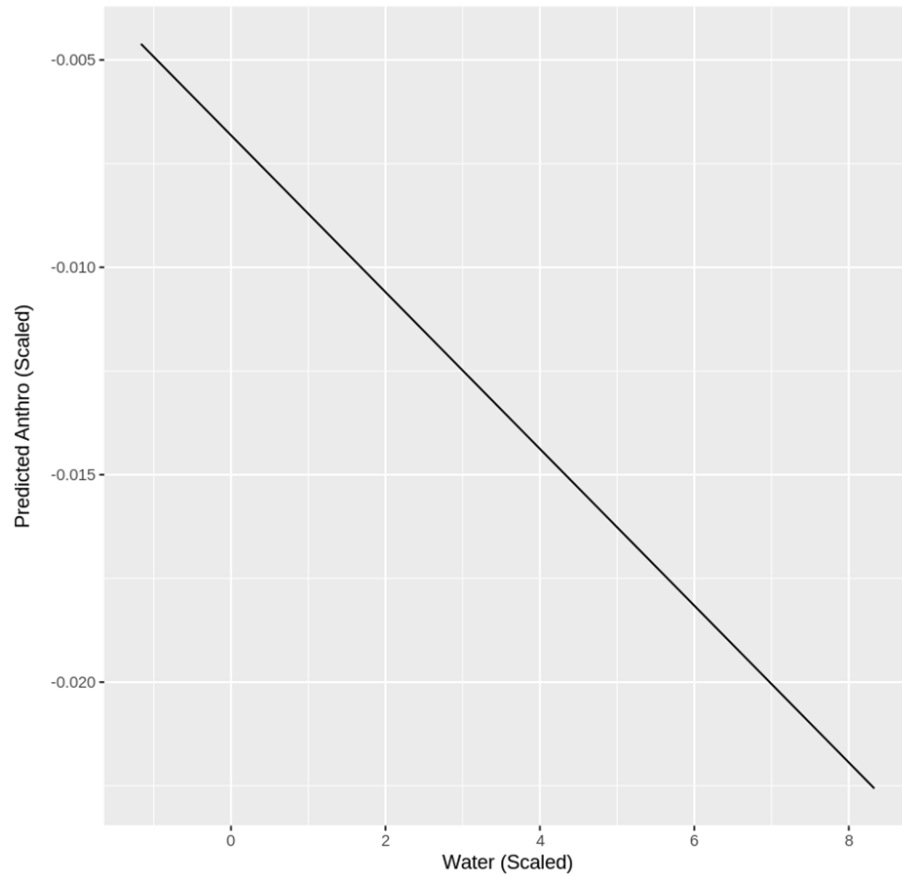
Supplementary information Figure S5.2: Distance maintained from anthropogenic structures by wild dog (*Lycaon pictus*) in response to increasing elevation in Hluhluwe-iMfolozi Park.



Supplementary information Figure S5.3: Distance maintained from anthropogenic structures by wild dog (*Lycaon pictus*) in response to distance from water in Hluhluwe-iMfolozi Park.



Supplementary information Figure S5.4: Distance maintained from anthropogenic structures by both cheetah (*Acinonyx jubatus*) and wild dog (*Lycaon pictus*) in response to an increase in elevation in Hluhluwe-iMfolozi Park.



Supplementary information Figure S5.5: Distance maintained from anthropogenic structures by both cheetah (*Acinonyx jubatus*) and wild dog (*Lycaon pictus*) in response to distance from water in Hluhluwe-iMfolozi Park.

CHAPTER 6

General discussion, conclusions and recommendations

6.1 Background

Roads are key to the operation of modern human society, connecting communities to hubs of resource trading and service delivery (Weng et al., 2013; Freire et al., 2020). However, roads are predominantly beneficial to humans but negatively impact natural ecosystems in their surroundings (Van der Ree et al., 2015; Barbosa et al., 2020). The negative impacts caused by roads include restrictions on movement patterns and gene flow (Forman and Alexander, 1998; Strasburg, 2006), fragmentation and degradation (Fahrig and Rytwinski, 2009; Hlatshwayo et al., 2024). Roads' largest impact on ecosystems is a direct cause of mortality through wildlife-vehicle collisions (WVCs) (Grilo et al., 2009; Collinson et al., 2014). Roads may attract wildlife as well (Hill et al., 2020), providing a source of carrion and heat sources (Antworth et al., 2005; Sisson and Roosenburg, 2023). The threat that roads pose to natural environments is predicted to rise as anthropogenic land change increases, with African road networks projected to double (Dulac, 2013).

Herpetofauna are among the taxa whose populations are most impacted by roads (Colino-Rabanal and Lizana, 2012). Morphometric and behavioural characteristics make herpetofauna particularly susceptible to vehicle collisions (Heigl et al., 2017). Additionally, herpetofauna roadkill may be higher because of persecution by humans (de Resende Assis et al., 2022). Large roads are particularly difficult barriers for smaller amphibian species to cross (Heigl et al., 2017). This strong barrier effect creates habitat fragmentation, leading to reductions in genetic diversity (Lukanov et al., 2014). Additionally, because amphibians are highly sensitive to habitat degradation, further reductions in amphibian populations and genetic diversity may occur (Hopkins and Brodie, 2015). Road infrastructure is a major factor

contributing to global reptile decline (Lal and Nadim, 2021; Paterson et al., 2021). Reptiles use roads as basking sites to regulate their body temperature (Colino-Rabanal and Lizana, 2012; McCardle and Fontenot, 2016). Additionally, reptiles may attempt to traverse roads during mating seasons and when young disperse (Doherty et al., 2020; Jones et al., 2022). Consequently, roads pose a constant threat to any reptile species that uses them because of vehicle traffic (Paterson et al., 2021; Jones et al., 2022).

Additionally, within protected areas, the effects of anthropogenic structures on movement patterns are compounded by the areas being fenced. Fences, like roads, have negative impacts on vertebrate fauna, as they impair the movement and migration of animals between areas (Hepenstrick et al., 2012). Additionally, they can cause mortalities (Lee et al., 2021). Predator-prey relationships can be influenced by avoidance of roads (Ruiz-Capillas et al., 2021). Road avoidance can change habitat selection, home range size, movement patterns and reproductive rates (Dennehy et al., 2020; Proctor et al., 2020), with prey species responding to roads the same way they would respond to predator presence (Prokopenko et al., 2017).

In light of the conservation challenges associated with roads, this study's research had fourfold aims. Firstly, to identify and compare the species assemblages of vertebrate wildlife-vehicle collisions (WVCs), along the ~18 km section of the R618 road that splits Hluhluwe-iMfolozi Park (HiP) and of the R22 road that passes through the northern border of iSimangaliso Wetland Park (ISWP). Secondly, to determine the demographics of reptiles present on paved roads in HiP, ISWP and Giba rural community, an adjacent developing community on ISWP's border. Thirdly, to determine road avoidance by lions (*Panthera leo*) and blue wildebeest (*Connochaetes taurinus*) in HiP. Finally, to determine the interaction with anthropogenic structures by cheetahs (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*) in HiP.

6.2 Research findings

The study of the effect of public roads on wildlife collisions in HiP and ISWP found that the public paved roads, R618 and R22 in HiP and ISWP, respectively, had statistically similar wildlife mortality rates, and that amphibians had the highest rate of mortality (Chapter 3). It was also found that, as expected, wet seasons had higher mortality rates than dry seasons, with wet season mortalities reaching as high as 13 carcasses km⁻¹ and that 106 species were identified in the mortality record, but only 26 species had habitat change or environmental degradation listed as threats to the species' survival. The study also found that hotspots of road mortality existed in areas where animals were forced to traverse the road to reach water sources beyond 50 m away or when water sources were directly adjacent to the road, in the case of amphibians. The concentration of mortalities was further influenced by the presence of a flat road surface with sloped edges. The study of the demographics of reptiles along the R618 and R22 found no significant differences between the number of reptiles recorded within or outside protected areas. However, the considerable majority of reptiles were found dead across locations, with snakes making up the majority of the total data. Detection was highest during the wet season, and the number of reptiles detected, and therefore dying, increased with increased vehicle traffic density in areas near water with sloped road edges and flat road surfaces (Chapter 3).

The studies of the distance kept from roads by lion (*P. leo*) and wildebeest (*C. taurinus*) and the interaction with anthropogenic structures by cheetah (*A. jubatus*) and wild dog (*L. pictus*) found that lions, wildebeest and cheetah all maintained larger distances from roads or anthropogenic structures, while wild dogs maintained smaller distances from paved roads (Chapters 4 and 5). With all four species keeping the largest average distances from the large public paved road (R618) in the centre of HiP. The studies also found that the distances kept from roads and other anthropogenic structures by cheetah, wild dog and wildebeest were

smaller in areas where lion activity decreased. Female wildebeest and female wild dogs maintained closer distances to roads and anthropogenic structures, while female cheetahs maintained significantly larger distances from anthropogenic structures than their male counterparts. However, cheetahs also avoided areas with higher densities of wild dogs (Chapters 4 and 5).

The mortality rate findings (Chapters 2 and 3) represent not only the highest recorded wildlife mortality in South Africa but also the highest recorded mortality rate in Africa as a whole, where the highest recorded mortality rate before was described by Collinson et al. (2015), who found a wet season mortality rate of 4.3 carcasses km⁻¹. Additionally, other African studies have only recorded mortality rates of below 0.2 carcasses km⁻¹ (Kioko et al., 2015; Lala et al., 2021), however, crucially, these other African studies only recorded mortalities for mammals and birds, which also showed lower mortality rates in this study. The results observed in the reptile demographics and amphibian mortalities also follow trends identified in previous research that speak to the road-mortality susceptibility of herpetofauna (Crawford and Andrews, 2016), further emphasising the importance of including herpetofauna in mortality surveys (Lal and Nadim, 2021). The results of the distances kept from human structures by large mammals in HiP also follow similar trends seen globally, where roads have altered animal movement behaviours to avoid roads (Tipton et al., 2023), increasing barrier effects (Van der Ree et al., 2015; Paterson et al., 2019) and genetic isolation (Strasburg, 2006; Herrmann et al., 2017; Jones et al., 2022). But the trend of female animals remaining closer to roads in areas where apex predators avoid because of fear of humans has also been observed previously in other regions (Berger, 2007; Laundré et al., 2010; Sivertsen et al., 2016). These results provide insights into two key areas of linear infrastructure ecology, namely what wildlife are experiencing direct mortalities, along roads (Chapters 2 and 3), and how roads are affecting species survival and habitat use (Chapters 3, 4 and 5). Both of these areas provide

information for the foundation of linear infrastructure ecology, namely how linear structures are affecting natural environments, all of which adds to the growing evidence detailing the scale of adverse effects that linear infrastructures, particularly large, paved roads with high traffic volumes, have on surrounding habitats and the need for their inclusion in species assessments and conservation, and urban development planning.

6.3 Recommendations

The lack of high-quality geographic information systems (GIS) and climatic data was a major limiting factor in this study, as no weather monitoring stations were operational in the study area, thereby eliminating all quantitative climatic information. We recommend that existing weather stations be repaired or upgraded to collect fine-scale (≤ 30 m accuracy) climatic data to combat this lack of data. This will aid both the study of animal movement and habitat selection. Additionally, governing bodies, either at a municipal, provincial or national level, should create fine-scale maps of road structure (surface slope, side slope, curve angle, the location of substructures and superstructures) as this data will help with both future road ecology studies and road network planning. Similar to roads, fine-scale maps of other linear anthropogenic structures (fences, powerlines, railways, etc.) should also be collected, so that the effects of their design can also be quantified. If this data is not readily available, researchers should collect quantitative road design and weather data to mitigate the challenges associated with qualitative data. Additionally, to aid in modelling the use or avoidance of anthropogenic structures when wildlife movement data is collected, it should be done at a recommended rate of one GPS-fix per hour, with accelerometer data recorded at a 10 minute minimum interval, as this allows for the creation of highly accurate models of movement patterns. A team of trained observers should also conduct future studies of wildlife road mortality, as this would maximise the detection rates.

Recommended future research includes studies of scavenging rates along and surrounding the roads included in this study, as well as mortality rates on surrounding roads, both paved and unpaved, and on roads in other protected areas within the uMkhanyakude region and across South Africa. These studies would be required to fully explore the scope of the effect of roads on vertebrate mortality within and surrounding protected areas. Taxon-specific behavioural studies in response to roads and other anthropogenic structures, as well as studies on the habitat selection influencing factors and the use/ avoidance of anthropogenic structures by species of conservation concern in protected areas, should be conducted to minimise the risk of human-wildlife conflict within and surrounding protected areas. This is of particular concern when predatory species are involved, as these animals must be culled when human habituation occurs (various pers. comm.). Future research efforts should also include studies on the frequency and demographics of wildlife's use of existing road substructures in protected areas to determine the effectiveness of these structures as safe road crossing points. Studies on the perception of roadkill of different vertebrate taxa should also be conducted to better understand the human element involved in WVCs. These studies would all be useful in creating effective measures to mitigate the negative effects of roads on protected environments.

These further studies would be important for determining the full scale of the effect of anthropogenic structures on wildlife and the subsequent development of long-term mitigation strategies to reduce the negative effects of linear structures on wildlife. However, there are inexpensive, short-term measures that can be implemented rapidly to minimise the negative effects on the environments surrounding roads until long-term solutions have been identified and implemented. These short-term solutions include the placement of structures such as speed bumps, speed traps or using seasonally changing speed limits to slow traffic (Balčiauskas et al., 2025); placing slotted culverts or guide fences, as these would allow small vertebrates better opportunities to escape oncoming vehicle traffic or restrict the above road movement of

wildlife (Grilo et al., 2010). Signage warnings for areas with high wildlife collision probability could be used to improve driver awareness of wildlife presence (Collinson et al., 2019). Beyond protected areas, measures that could be implemented include increased enforcement of legislation that prioritises wildlife in protected areas and the creation of a protected area risk status database that could aid in assessing species conservation status.

6.4 Conclusions

This study serves as a starting point for future road ecology and linear infrastructure ecology studies within protected areas in South Africa. It also indicates a clear need for changes to how roads are regulated in the two protected areas surveyed, as the mortality rates recorded were the highest reported to date in South Africa. The mortality rates are particularly high during wet seasons when species are more likely to move, especially amphibians (Filius et al., 2020). Additionally, changes should be made to how species conservation assessments are conducted, with the threats faced by any particular protected area included in assessments, as not all protected areas are equally protected. As development and the amount of vehicle traffic are likely to continue to grow, the threat that roads impose on ecosystems should be included more heavily in conservation legislation, particularly for already persecuted taxa like herpetofauna as should mortality rates in herpetofauna continue to grow the highly specialized species that are found in the region that contains HiP and ISWP will likely begin to decline rapidly eventually leading to their extinction. To aid herpetofauna conservation efforts, education campaigns can be employed to increase local awareness of the importance of herpetofauna in South African ecosystems. Further investigations of the avoidance/attraction behaviour observed in large mammals in HiP are imperative so that mitigation measures can be developed to continue allowing wildlife to move freely and to curb any potential human-wildlife conflicts

that may occur in regions where tourists are permitted to exit vehicles and where large mammalian predators are frequent.

6.5 References

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