UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Factors affecting mammal utilisation of non-wildlife railway underpasses within the Greater Kruger, South Africa

by

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the Master of Science degree in Resource Conservation Biology at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree for examination at any other university.

(Signature of candidate)

17 September 2023

ABSTRACT

Railways are an integral component of sustainable transport systems, but cause significant impacts to biodiversity, primarily through wildlife-train collisions and the habitat fragmentation created in the landscape. Crossing structures (CSs) for wildlife are the key mitigation measure for these adverse impacts, which will be critical throughout Africa, where 55,000 km of new railway lines are predicted to cut through hundreds of protected areas. For the first time in southern Africa, mammal usage of railway underpasses that were not designed to facilitate wildlife movement was studied using camera traps in the Greater Kruger National Park, South Africa. This study demonstrates that 70% of the terrestrial medium and large mammals species present in the area used at least one non-wildlife CS during the study period, with a total of 1,823 usage events by 33 species. Twelve medium to large African mammal species used underpasses regularly, improving the permeability of the railway line for these species. A viaduct design was characterised by almost four times the usage rate of a typical box culvert and was used by twice as many species, including megaherbivores and mesoherbivores, whereas box culverts supported a smaller number of species, of which the majority were predators. Species most affected by rail collisions in the study area were mostly herbivorous and seldom used non-wildlife underpasses. These results indicate that a railway line with only drainage culverts and the occasional viaduct is not effective in providing adequate safe crossing structures for megaherbivores and ungulate mesoherbivores. Contrary to expectations, vegetation and environmental factors influenced mammal usage of box culverts more than structural dimensions. Ensuring that there is high visibility through the structure, with less herbaceous cover and more woody cover outside of entrances, is likely to enhance culvert usage by most trophic groups. In a savanna context where faunal diversity is high and no single species is a target for mitigation, a range of underpass designs including larger viaducts, located in different types of vegetation cover, thereby combining the range of requirements of different guilds, is recommended.

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CHAPTER 1

1.1 STUDY AIM

The aim of this study is to understand the factors influencing large and medium-sized mammal use of non-wildlife underpasses below a railway which runs through a big five protected reserve in South Africa. The objectives are 1) to assess the influence of underpasses' structural, vegetation and environmental characteristics on the use of these passages by mammals; 2) to assess how usage of underpasses differs based on mammal characteristics (such as species, trophic level, group size, nocturnal vs diurnal habits), and 3) to determine how mammals use underpasses.

1.2 MOTIVATION

Linear infrastructure, consisting of transport infrastructure such as roads and railways, as well as power lines, fencing and pipelines, is extensive and integral to modern society. Transport infrastructure in particular plays a pivotal role in economic and social development through the improved access to resources and therefore livelihood it provides. However, with its 65 million kilometre footprint it dominates landscapes worldwide (Dulac 2013, van der Ree *et al.* 2015). Furthermore, road and rail networks are expected to undergo rapid global expansion, increasing 39% and 45% respectively by 2050, largely in developing nations (van der Ree *et al.* 2015, Popp and Boyle 2017).

In Africa, 33 massive development corridors involving transport infrastructure are planned or progressing across the continent, which will threaten some of Earth's most diverse ecosystems by cutting through over 400 protected areas and degrading a further ~1,800 (Laurance *et al.* 2015). Africa's railway network of over 65,000 km was largely built by European colonial powers in the early 1900s for military purposes and for transporting mined or farmed resources to ports (African Development Bank 2015, World Bank 2022). This rail network is expected to expand significantly by another 55,000 km (+85%), with the value of all current rail projects across Africa estimated at USD495 billion (Norton Rose Fulbright 2016, GlobalData 2019). These projects have the potential to have major socio-economic benefits, if managed effectively (Bouraima *et al.* 2023), and are largely being funded by Chinese government and the World Bank (Marson *et al.* 2021).

Railways are a critical component of development of sustainable transportation, as they are considerably more energy efficient, result in fewer greenhouse gas emissions, require less land, and have a lower cost per ton kilometre than other forms of transport (African

Development Bank 2015, IEA 2017). Despite these advantages, there are consequences for the biodiversity within railways' paths, especially when passing through natural or protected areas which encompass a variety of ecosystems (Huijser *et al.* 2012, Dorsey *et al.* 2015, Borda-de-Água *et al.* 2017). Within the transport ecology discipline, however, rail ecology is in its infancy (particularly in developing countries and continents such as Africa), with little knowledge about the impacts of railways on biodiversity as compared with the extensively researched field of road ecology (Popp and Boyle 2017). From what is known, the two most important ecological impacts of rail infrastructure are mortalities arising from wildlife-train collisions, and the barrier created in the landscape, which fragments habitats and can isolate populations (Borda-de-Água *et al.* 2017). Some of these effects are already being witnessed along Kenya's new railway development (Jiang 2020, Lala *et al.* 2022).

Wildlife mortality as a result of collisions with trains is the most visible impact from rail infrastructure. Collisions are not easily avoided, given that trains can reach speeds of over 200 km/h and cannot stop quickly or navigate around animals in their path (Dorsey *et al.* 2015, Heske 2015). Mortality has been shown to increase with higher speeds of trains, and when tracks are curved and/or closer to water (St Clair *et al.* 2020). Most railway mortality studies have focused on large mammals, as they are most likely to cause damage or delays to trains given their size and are easier to study, and have revealed that collisions with ungulates are most common (Carvalho *et al.* 2017). Seasonal movement of animals is likely to contribute to frequent collisions, with mortality hotspots along railways typically located at important habitats or along migration routes (Gundersen and Andreassen 1998, van der Grift 1999). Train collisions account for a significant proportion of overall population mortality: 24% of Alaskan moose (*Alces alces gigas*) mortality (and the second largest cause of death), and 45% of Indian elephant (*Elephas maximus indicus*) mortality (Modafferi and Becker 1997, Sarma *et al.* 2006, Santos *et al.* 2017).

Barrier effects are regarded as one of the greatest ecological impacts of linear infrastructure (Beben 2016, Barrientos and Borda-de-Água 2017). Aside from the habitat destroyed to build railways, the infrastructure creates both physical and behavioural barriers to wildlife movement, resulting in habitat fragmentation, isolated populations, increasing edge effects, and disturbance to populations living nearby (Barrientos and Borda-de-Água 2017, Roy and Sukumar 2017). This division of the landscape is especially disadvantageous for large mammals, where it can hinder migration and prevent gene flow between different groups (Beben 2016, Ito *et al.* 2017). In fact, transport infrastructure is considered to be one of the largest drivers of habitat loss and fragmentation, and therefore biodiversity loss, worldwide (Benítez-López *et al.* 2010, van der Ree *et al.* 2015).

Wildlife crossing structures (CSs) are recognised as an effective mitigation measure to reduce both collisions and barrier effects, through facilitating the safe passage of animals over or under rail or road infrastructure (Dorsey *et al.* 2015, Carvalho *et al.* 2017). Whilst specifically designed wildlife passages are most effective for reducing barrier effects, they are also the most expensive, costing up to millions of dollars (Barrientos and Borda-de-Água 2017, Denneboom *et al.* 2021). Underpass structures that are built for drainage or engineering purposes (e.g. culverts or viaducts) can easily be modified to improve their use by wildlife; this approach is regarded as the most economical and feasible form of mitigation of both wildlife mortality and barrier effects (Glista *et al.* 2009, Kintsch and Cramer 2011). Understanding the factors affecting use of these non-wildlife underpasses is therefore critical, especially throughout Africa where biodiversity conservation typically competes with socioeconomic needs in budget allocations (Maze *et al.* 2016, Reed *et al.* 2020).

Collisions with large mammals can be expensive, and evidence shows that CS mitigation measures make sound economic sense: the benefits (cost saving) from underpasses with wildlife fencing easily outweigh the implementation costs (Huijser *et al.* 2013, 2022, Ascensão *et al.* 2021, Lee *et al.* 2021). Whilst the business case for CSs along railways is not yet fully understood, wildlife-train collisions can be extremely expensive. In Sweden, the overall socio-economic cost of wildlife-train collisions was estimated at EUR100-150 million per year, close to the EUR250 million from road collisions even though the railway footprint is below 2% of the road network, meaning a much greater cost per kilometre (Seiler and Olsson 2017). In the UK, the annual cost of animals on rail tracks amounted to GBP4.9 million in 2013, arising from 346 collisions resulting in equipment damage, long delays and compensation payments (Grey 2015). In India, train collisions with elephants invariably damage and derail trains, leading to large-scale service disruptions and high costs imposed on treasury (Mazoomdar 2016). Modern lighter-weight trains also face more expensive repair costs than older models (Seiler and Olsson 2017, Nezval and Bíl 2020). Further, with increasing rail traffic (road to rail migration) and more high-speed trains, mammal collisions will surely increase.

Despite the potential of CSs in mitigating rail impacts, there is little knowledge of their use by wildlife or effectiveness along rail infrastructure, with relatively few published studies mostly from Europe and North America (Hunt *et al.* 1987, Yanes *et al.* 1995, Rodríguez *et al.* 1996, 1997, Popp and Boyle 2017, Wang *et al.* 2018). Research on wildlife CSs has scarcely been conducted in areas with high diversity of large mammal species, such as Africa (Collinson *et al.* 2019), which is home to the world's largest intact assemblage of large mammals and a quarter of global biodiversity (UNEP-WCMC 2017). However, three recent railway CS studies were conducted along the new Standard Gauge Railway in Kenya (which has purpose-built wildlife underpasses), one of which was a multi-species mammal study (Lala *et al.* 2022) whilst the other two were focused on elephants*, Loxodonta africana* (Okita-Ouma *et al.* 2021, Koskei *et al.* 2022).

My study focuses on usage of CSs by large and medium-sized African mammals, as they are most affected by the mortality and barrier effects of railways, are most susceptible to structural dimension limitations given their body size, and are most likely to incur material costs through collisions. The findings of this study, the second of its kind in Africa, adds to that of Lala *et al.* (2022) in helping inform the design of wildlife CSs along new railways, as well as aiding reserve management in decisions regarding economically feasible modifications to drainage culverts along existing railway lines that would make them more attractive for mammal use.

1.3 LITERATURE REVIEW

A review of the literature reveals that there are three main characteristics of CSs which affect their use by mammals, namely their structural dimensions, the nature of habitat close to the CS, and the surrounding environment. The literature also shows that use of CSs depends on the characteristics of particular mammal species. These findings are addressed separately below.

STRUCTURAL CHARACTERISTICS

The physical dimensions of culverts or underpasses are considered as one of the most important factors in determining mammals' use of these structures. There is a wide variety in types of CSs, which either form part of the existing engineering design (**Table 1**) or are purpose-built for wildlife.

Table 1*: Types of non-wildlife underpasses below linear infrastructure (Jackson and Griffin 2000, van der Ree* et al. *2007, Glista* et al. *2009, Carvalho* et al. *2017)*

Type of non-wildlife CS	Description	Effectiveness
Pipe culverts	Round tunnel <2 m in diameter, designed to carry water	Used occasionally by small mammals, but sometimes partially filled with water and made from man-made materials which are both strong deterrents for entry.
Box culverts	Rectangular tunnels that are larger than pipe culverts; designed to allow water to pass but remain dry except in heavy rainfall	Generally regarded as better wildlife crossing structures than pipe culverts; allow for a wider diversity of species given larger size.

Viaducts	Relatively unconfined passages that exist where a road or railway passes over a river, water course or road	Provide a crossing point for many species (especially those that commonly use riparian corridors). Advantage of featuring natural terrain and more light.

Studies in the Northern hemisphere that analysed the effectiveness of CSs for wildlife suggested that drainage culverts do not solve connectivity problems alone, but may be effective for carnivores (Ascensão and Mira 2007, Seiler and Olsson 2009, Longcore *et al.* 2018). Culverts combined with barrier fencing have proved effective at mitigating wildlife-vehicle collisions (Taylor and Goldingay 2003, Dodd *et al.* 2007, Huijser *et al.* 2016, Rytwinski *et al.* 2016).

In analysing the influence of structure dimensions on wildlife usage of CSs, the "openness" of underpass structures (width * height / length) is often a key variable, providing a single measure of how constricted a passage is (Wang *et al.* 2018). Although structural dimensions are of different importance to different species groups, with carnivores, ungulates and small mammals being the key groups analysed, evidence shows that a higher level of openness (Seiler and Olsson 2009, Wang *et al.* 2018), with a wider width (Ford *et al.* 2022, Saxena and Habib 2022a) and a shorter length (Denneboom *et al.* 2021), are strong predictors for use by most species. In a savanna setting, height was found to be a more important predictor for railway underpass use by African large mammals than width (Lala *et al.* 2022).

The majority of studies suggest that ungulates have the highest sensitivity to underpass dimensions, preferring structures that are wide and tall with a high level of openness (Ng *et al.* 2004, Donaldson 2005, Clevenger *et al.* 2009, Seiler and Olsson 2009). Migratory moose (*Alces alces*) in Sweden crossed road fences and the highway instead of using the narrow underpasses below (Seiler *et al.* 2003). In Banff National Park, a lengthy passage was the most significant attribute that deterred elk (*Cervus elaphus*) (Clevenger and Waltho 2000). In India, even at some of the largest road CSs in the world, ungulates still preferred structures wider than 80 m (Saxena and Habib 2022a). Studies have suggested minimum structure dimensions to be attractive for ungulates, with limits set to between 3.5-5 m height and 11 m wide (Donaldson 2005, Seiler and Olsson 2009, Bhardwaj *et al.* 2020). However, in areas with high road traffic, ungulates used more constricted box culverts of 1.5-2 m in height (Kintsch and Cramer 2011). A few studies have noted that ungulates prefer overpasses instead of underpasses (Clevenger *et al.* 2009, Simpson *et al.* 2016), but others found that viaducts can be as effective as, or even preferred over, overpasses (Dodd *et al.* 2007, Denneboom *et al.* 2021).

Carnivores show conflicting reactions to structural characteristics. In North America, grizzly bears (*Ursus arctos*) seem to show a preference for more open crossing structures, whilst cougars (*Puma concolor*) and black bears (*Ursus americanus*) prefer smaller, more constricted structures (Clevenger and Waltho 2005, Sawaya *et al.* 2013). In Europe, longer passages were used more often by carnivores than other species (Ascensão and Mira 2007), and medium-sized carnivores such as badgers (*Meles meles*), red fox (*Vulpes vulpes*) and wildcat (*Felis silvestris*) used all sizes of drainage culverts in areas of undisturbed natural habitat (Rodríguez *et al.* 1997, Seiler and Olsson 2009).

Smaller mammals (generally preferred more constricted crossing structures, and were less likely to use lengthy passages (Yanes *et al.* 1995, Ascensão and Mira 2007). Their avoidance of larger tunnels is suggested to be due to risk of predation (Hunt *et al.* 1987, Rodríguez *et al.* 1996), but this has not been proven.

A range of underpass sizes is recommended to enhance habitat continuity in areas with a high diversity of mammals and other terrestrial vertebrates, which have different behavioural responses to CSs (Clevenger and Waltho 2005, Kintsch *et al.* 2015, Murphy-Mariscal *et al.* 2015, Denneboom *et al.* 2021, Ford *et al.* 2022, Lala *et al.* 2022, Saxena and Habib 2022a). In Europe, the recommended dimensions of adapted culverts or multi-use underpasses in order to be suitable for ungulates, large carnivores and other terrestrial species include a width of 10-20 m, a minimum height of 3-4 m and an openness index of at least 1.5 (IENE 2022). In the African context, it is expected that herbivores which prefer open spaces will avoid smaller constricted structures, and that megaherbivores (more than 1000 kg, Owen-Smith 1988) will be precluded from smaller structures due to their large body size, whilst carnivores will be frequent users of all underpass designs. Nevertheless, animals can become habituated to CSs after multiple years, which may contribute to a reduced influence of structural dimensions on use of these passages (Clevenger and Waltho 2000).

VEGETATION AND HABITAT

Approaches to wildlife CSs that closely resemble species' natural habitats tend to increase their use by wildlife (Denneboom *et al.* 2021). Studies have found that the presence of woody vegetation cover around the entrances of CSs improved use of these structures for most mammal species (Ng *et al.* 2004, Ascensão and Mira 2007, Denneboom *et al.* 2021). Indeed, vegetation cover near underpasses was seen to improve usage by prey species, probably minimising the perceived risk of ambush at structures (Saxena and Habib 2022a). Newly built underpasses are not used immediately, but once revegetation occurs the usage becomes more frequent (Hunt *et al.* 1987, Gagnon *et al.* 2011). Whilst most studies examined variables related to woody cover, only three studies looked at the presence of herbaceous cover at entrances to underpasses. Herbaceous cover was found to be attractive for all faunal groups (Smith 2003) and unattractive for small-medium carnivores as the tall grasses hindered culvert discovery (Craveiro *et al.* 2019), whereas Ford *et al.* (2022) found it had no effect on CS usage for any species. Little else is known about how this seasonal cover affects CS usage, especially in a savanna context.

If vegetation density at CS entrances obstructs or restricts visibility directly through to the habitat on the other side, it can deter ungulates and smaller mammals from using the underpass (Foster and Humphrey 1995, Donaldson 2005, Glista *et al.* 2009). This restricted line of sight through tunnels did not affect carnivores' likelihood of using structures, and therefore might be linked to a perceived risk of ambush for ungulate and small mammal species (Jackson and Griffin 2000).

A ground cover resembling natural habitat, such as soil or vegetation, is recommended to improve usage (IENE 2022). Crossing structures that did not have an artificial bottom surface were significantly more attractive for animal use and acted to provide continuity of habitat (Donaldson 2005, Glista *et al.* 2009, Brunen *et al.* 2020). Viaducts were often most favoured by a wide range of species, perhaps as a result of more light promoting natural vegetation (Mysłajek *et al.* 2020, Denneboom *et al.* 2021).

Clearing vegetation to enable line-of-sight, planting new vegetation or ensuring culvert bottoms are covered with soil all represent easy and low-cost modifications for reserve management to improve culvert usage. Furthermore, cover within passages for prey species can be created through strategic placement of logs and rocks (Mata *et al.* 2020, Saxena and Habib 2022a).

ENVIRONMENTAL FACTORS

Whilst many studies have focused on underpass characteristics, others have argued that the underpass location within the landscape can be most important (Rodríguez *et al.* 1996, Clevenger and Waltho 2000, Andis *et al.* 2017). Studies that highlighted landscape variables were based on transport corridors that passed through diverse land-use areas with varying levels of anthropogenic influence, and found that CSs were used more frequently in areas of natural habitat with minimal development (Ng *et al.* 2004, Gagnon *et al.* 2011, Andis *et al.* 2017). This finding links closely to the strong preference by carnivores and sometimes ungulates for CSs with minimal human disturbance (Longcore *et al.* 2018, Bhardwaj *et al.* 2020, Denneboom *et al.* 2021), although may simply be because those structures are located where wildlife is more abundant. In apparent contradiction, human presence within CSs located in farmland or suburbia did not deter red fox and racoons (*Procyon lotor*), which are common residents of more densely populated habitats (Sparks and Gates 2017, Ważna *et al.* 2020).

Vehicle traffic on roads has been found to negatively impact underpass use, which links to human disturbance. For example, deer and elk responded to cars and other vehicles by foraging less and displaying more vigilance and flight behaviour, and used underpasses more when there was no traffic (Nojoumi *et al.* 2022). Results also suggested, however, that animals can become habituated to vehicle traffic over time (Barrueto *et al.* 2014, Bhardwaj *et al.* 2020, Nojoumi *et al.* 2022) and that more traffic could discourage road crossings and encourage animals to use an alternative method of crossing in the form of CSs (Ascensão and Mira 2007). The impact of rail traffic is not known, but is very different in nature to that of roads, often having long periods without a train (Borda-de-Água *et al.* 2017).

Underpasses located near to a riparian corridor or drainage line experienced higher usage, mostly by carnivores (Clevenger and Waltho 2005). However, the presence of flowing or stagnant water underneath viaducts or inside of drainage culverts is often a strong deterrent for use by most mammals (Brunen *et al.* 2020, Ważna *et al.* 2020, Denneboom *et al.* 2021), but did not deter use of pipe culverts or bridges in Brazil (Alves *et al.* 2021). Carnivores were only deterred from using culverts when the structure was completely flooded, but a dry pathway next to a stream enabled crossings (Craveiro *et al.* 2019).

The density of crossing structures in the landscape is also important in determining use (Ford *et al.* 2022). One study, for example, recommended that the maximum distance between CSs is 325 m (Smith 2003), whilst others recommend 500 m for smaller vertebrates and 1 km for large mammals (IENE 2022). Unfortunately, larger underpasses carry a higher price tag for transport operators, as would a high density of CSs along linear infrastructure, and so there is often a dilemma over whether a single large or several small underpasses is preferable – the SLOSS ("Single Large or Several Small") dilemma of transport ecology (Helldin 2022). The density of structures will depend on mobility of target species, but generally it is suggested that several small CSs are ecologically better and diversifies the risk, provided they are above some minimum size relevant to the target species (Ford *et al.* 2022, Helldin 2022, IENE 2022). In an African context, given the diversity of mammal species coupled with constraints on transport capital allocated towards wildlife conservation, frequent smaller culverts could be combined with a few larger underpasses to achieve a range of underpass designs.

MAMMAL CHARACTERISTICS

Most research regarding usage of crossing structures has been conducted in North America and Europe. Additionally, multi-species studies were at most focused on a handful of large mammal species (Clevenger and Waltho 2005, Donaldson 2005, Andis *et al.* 2017). There is a large gap in the literature looking at areas with high diversity of large mammal species (however, see Lala *et al.*, 2022; Saxena & Habib, 2022).

Species respond differently to the attributes of various crossing structures, and in areas without significant human disturbance, structural characteristics have best explained usage of underpasses by both large predator and prey species (Clevenger and Waltho 2005, Denneboom *et al.* 2021). However, there are some temporal and behavioural usage patterns that emerge relating to species' characteristics.

The highest usage of CSs for mammal species studied was in summer and spring in the Northern hemisphere due to high foraging or mating driving greater movement, or offspring becoming more mobile in early summer (Dodd *et al.* 2007, Sawaya *et al.* 2013, Ważna *et al.* 2020). Usage of CSs was lowest in winter for small mammals due to highest breeding activity (Rodríguez *et al.* 1996, Sparks and Gates 2017), and for other mammals due to reduced migratory movements and hibernation (Ważna *et al.* 2020). In savanna ecosystems, alternatively, highest mobility is instead likely to occur in the dry season (winter) as mammals travel further to find food and water, and in search of breeding partners.

In areas where there was high human or domestic animal disturbance during the daytime, wildlife primarily used passages at night (Longcore *et al.* 2018, Mysłajek *et al.* 2020) or during crepuscular periods (Donaldson 2005, Gagnon *et al.* 2011). Elephants in Kenya's Tsavo National Park restricted underpass activity to night time (78% of crossings) when the train stopped running, and even whilst crossing showed typical behaviour of being in risky landscapes or under stress (Okita-Ouma *et al.* 2021). Whilst this railway is new and elephants may still be habituating to underpasses, this behaviour could indicate that the activity of the train itself, even in a nature reserve context without other human disturbance, may deter wildlife from crossing.

There are few studies of different trophic levels (herbivore, carnivore, apex predator) and their usage of underpasses. One study suggested that predators may use culverts as funnels for their prey (Hunt *et al.* 1987), but later studies found no evidence of prey species avoiding structures that had a high concentration of predators, or of predators using these structures to trap prey (Ford and Clevenger 2010, Andis *et al.* 2017, Saxena and Habib 2022a). Other studies found that whilst predators and prey species used the same structures, predator-prey use of CSs is not independent, and that prey sometimes avoided crossings in the presence of predators or which had frequent predator use, and that predators may sometimes follow prey (Caldwell and Klip 2020, Mata *et al.* 2020). Different predators have also been seen to avoid using the same crossing structures at similar times, suggesting a temporal segregation of competitor species (Clevenger *et al.* 2009). It is, of course, possible that these segregations or attractions may simply be a reflection of wider landscape-level interactions (Little *et al.* 2002).

Only one study looked at sociality and concluded that for groups of mammals (white-tailed deer, *Odocoileus virginianus*, mule deer, *O. hemionus*, and black bear), either all or none of the individuals in a group would choose to cross through an underpass (Allen 2011). Other studies have not mentioned sociality in relation to usage of crossing structures, perhaps because of the use of track beds to collect data or the analysis being conducted at a species-occurrence level.

Kintsch *et al.* (2015) proposed an important framework related to the behavioural and physiological characteristics of terrestrial wildlife that help them to maximise survival, which affect their willingness to use underpasses. The proposed 'wildlife crossing guilds' are based on five factors, namely anti-risk behaviour, need for specialised habitat conditions, movement capacity, need for openness or enclosure, and body size restrictions. These guilds go beyond taxonomic groups or simple trophic levels, where, for example, different antelope species have displayed remarkably different responses to CSs. In an African context, the high diversity of large herbivores and carnivores of wide-ranging body size and different behavioural adaptations to minimising mortality threats, coupled with minimal human disturbance in a nature reserve setting, could potentially lead to new findings being uncovered on how mammal characteristics impact CS usage. In particular, certain African herbivores of smaller body size, and which are therefore more vulnerable, tend to avoid low-visibility habitats due to high predation risk within their "landscape of fear" (Laundre *et al.* 2010, Pringle 2018), and may therefore avoid culverts. Others may be reliant on vegetation cover to minimise the perceived risk of predation, so may require sufficient cover in or around underpasses.

HOW MAMMALS USE CROSSING STRUCTURES

Evidence shows that both carnivores and ungulates are often detected near CS entrances, but choose not to enter or cross them (Gagnon *et al.* 2011, Brunen *et al.* 2020). The refusal to enter is likely driven by unfavourable characteristics of the structure for the species approaching, the nuances of which are discussed above. Black bears, for example, which are known to be sensitive to constricted structures, remained at the entrance of an underpass for up to 38 minutes without entering (Donaldson 2005). Generally, ungulates demonstrated this refusal behaviour more so than carnivores (Longcore *et al.* 2018), which is likely linked to their sensitivity to structural dimensions. 30% of elephants monitored in Tsavo National Park did not use underpasses despite ranging in their vicinity (Okita-Ouma *et al.* 2021). Overall, between 50-80% of species detected near underpass approaches entered them (Gagnon *et al.* 2011, Brunen *et al.* 2020), whilst between 60-98% of those that entered underpasses actually crossed through (Clevenger and Waltho 2000, Simpson *et al.* 2016, Ważna *et al.* 2020). With the focus of this planned study on drainage culverts, it is expected that unsuitable structural characteristics may deter some mammals from entering.

A study of road culverts in Portugal suggested that the peak in seasonal use of crossing structures in summer may actually be due to thermoregulatory behaviours of mammals, where culverts were shown to consistently have a lower temperature than the area outside (Ascensão and Mira 2007). The cooler CSs may therefore be more attractive for wildlife use during the hottest months of the year. Indeed, bobcats have been seen to use drainage culverts as rest sites (Cain *et al.* 2003). Given the semi-arid climate in a southern African context, it is likely that certain animals will use the culverts as shaded rest spots.

1.4 REPORT STRUCTURE

This report consists of two chapters. Chapter 1 contains a general introduction and motivation for the study with a review of existing literature. Chapter 2 elaborates on the research conducted including details on methods used and data analyses. Chapter 2 is written in the form of a standalone research article with its own introduction, methods, results and discussion sections. As such, there is some inevitable repetition. The list of references for both chapters are combined and provided at the end of the report.

CHAPTER 2

FACTORS AFFECTING MAMMAL UTILISATION OF NON-WILDLIFE RAILWAY UNDERPASSES WITHIN THE GREATER KRUGER, SOUTH AFRICA

2.1 INTRODUCTION

New rail developments are planned or progressing across the African continent, involving both upgrades and the addition of 55,000 km to the existing railway network (+85%) and driving potentially substantial socio-economic benefits for host countries (GlobalData 2019, Bouraima *et al.* 2023). Rail is an integral component of sustainable and efficient transportation (IEA 2017). However, rail expansion within planned transport corridors in Sub-Saharan Africa is expected to cut through more than 400 protected areas, which support the world's largest intact assemblage of large mammal species (Laurance *et al.* 2015, UNEP-WCMC 2017). Railways pose major risks to biodiversity, with the two most important ecological impacts being mortalities arising from wildlife-train collisions (WTC), and the barrier-effect, which fragments habitats and can isolate populations (Borda-de-Água *et al.* 2017). This division of the landscape is especially disadvantageous for large mammals, where it can hinder migration and prevent gene flow between different groups (Beben 2016, Ito *et al.* 2017). Further, WTC accounted for a significant proportion (24% - 45%) of ungulate population mortality in affected areas (Modafferi and Becker 1997, Santos *et al.* 2017), and WTC are expected to increase with higher speed trains and road-to-rail migration driving more rail traffic (St Clair *et al.* 2020). As such, there is an urgent need to understand and mitigate the negative effects of rail development on Africa's biodiversity.

Wildlife crossing structures (CSs) are recognised as an effective mitigation measure to reduce both collisions and barrier effects, through facilitating the safe passage of animals over or under transport infrastructure (Dorsey *et al.* 2015, Carvalho *et al.* 2017). In road systems, the cost saving from avoided collisions with wildlife outweigh implementation costs of CSs (Huijser *et al.* 2013, 2022, Ascensão *et al.* 2021, Lee *et al.* 2021), and whilst the business case for railway mitigation is not fully understood, WTC can be very expensive (Grey 2015, Mazoomdar 2016, Seiler and Olsson 2017). Whilst passages specifically designed to facilitate wildlife crossing are most effective for reducing barrier effects, they are also the most expensive (Barrientos and Borda-de-Água 2017, Denneboom *et al.* 2021). Underpasses that are designed for hydrological or engineering purposes (e.g. culverts or viaducts) can be modified to improve wildlife usage, which is regarded as the most economical form of mitigation as they are a common feature of transport infrastructure (Glista *et al.* 2009, Kintsch and Cramer 2011).

Understanding the factors affecting wildlife use of these culverts or viaducts along railways is therefore critical, especially throughout Africa where biodiversity conservation typically competes with socioeconomic needs in budget allocations (Maze *et al.* 2016, Reed *et al.* 2020).

Despite the potential of CSs in mitigating rail impacts, there is scarce knowledge of their use or effectiveness along rail infrastructure, and CS research for roads have to date been highly concentrated in Europe and North America (Popp and Boyle 2017). Research on CSs is scant in areas with high diversity of large mammal species, such as Africa (Collinson *et al.* 2019), although recent studies have been conducted along Kenya's new Standard Gauge Railway which has purpose-built wildlife underpasses (Okita-Ouma *et al.* 2021, Koskei *et al.* 2022, Lala *et al.* 2022). Only one of these Kenyan studies collected data on multiple mammal species (Lala *et al.* 2022), thus more baseline research in other landscapes is urgently needed.

Factors affecting wildlife CS usage include the structural, vegetation and environmental characteristics of underpasses, as well as the target species characteristics. Structural dimensions of underpasses are understood to be one of the most important factors in determining mammals' use of these structures, where a higher level of openness (Seiler and Olsson 2009, Wang *et al.* 2018), with a wider width (Ford *et al.* 2022, Saxena and Habib 2022a) and a shorter length (Denneboom *et al.* 2021) are strong predictors for use by most species. In a savanna setting, height was found to be most important for railway underpass use by African large mammals (Lala *et al.* 2022). The nature of the habitat in and around underpasses is also important (Denneboom *et al.* 2021), including ground cover (Glista *et al.* 2009, Brunen *et al.* 2020), vegetation cover (Ng *et al.* 2004, Ascensão and Mira 2007, Ford *et al.* 2022, Saxena and Habib 2022a) and the level of visibility through structures to the habitat on the opposite side (Foster and Humphrey 1995, Donaldson 2005). Other environmental factors evidenced to affect CS usage include proximity to water and riparian habitat (Clevenger and Waltho 2005, Brunen *et al.* 2020, Ważna *et al.* 2020, Alves *et al.* 2021), traffic (Barrueto *et al.* 2014, Nojoumi *et al.* 2022), density of CSs along transport infrastructure (Ford *et al.* 2022, Helldin 2022) and season (Rodríguez *et al.* 1996, Sparks and Gates 2017, Ważna *et al.* 2020). Species behavioural and physiological characteristics, and what is considered their 'landscape of fear', affect willingness to use different underpasses designs (Laundre *et al.* 2010, Kintsch *et al.* 2015, Pringle 2018, Ford *et al.* 2022), meaning there is no one-size-fits-all optimal design. Predator-prey or inter-specific interactions may also affect usage (Ford and Clevenger 2010, Andis *et al.* 2017, Caldwell and Klip 2020, Mata *et al.* 2020).

This study presents the results of camera-detected mammal usage of 12 non-wildlife underpasses along a freight railway within the Greater Kruger National Park, one of the largest and most iconic conservation areas on the African continent. This railway is responsible for a minimum of 12 – 19 fatal mammal collisions per year (Elliot *et al.* 2019, Thela 2021), but may be much higher (Lee *et al.* 2021). Species richness and diversity of mammals utilising different underpasses were analysed, and the proportion of species using underpasses out of those known to be present locally was calculated. The impact of structural, vegetation and environmental factors on underpass use were analysed across different trophic groups (apex predators, mesopredators, megaherbivores, mesoherbivores and omnivores). This study is the second in an African savanna landscape to review multi-species use of rail underpasses, and the first to focus on baseline usage of drainage culverts and viaducts rather than wildlife-designed structures. It adds to that of Lala *et al.* (2022) in helping inform the design of wildlife CS mitigation along new railways on the continent, as well as in identifying opportunities to modify structures along existing railway lines to make them more attractive for wildlife.

Based on the literature, it was expected that underpasses which are less constricted (i.e. wider and taller, with a shorter length) would see more frequent crossings and support a wider variety of species. Where plentiful woody vegetation was present in the area around underpass entrances, but where dense vegetation did not obscure visibility through the structure, it was expected that mammal use would be more frequent. Where the ground surface within a structure was exposed concrete, mammal use was expected to be less frequent, and where underpasses are located closer to riparian corridors or water sources, more frequent usage was expected. It was expected that there would be more frequent observations of meso- and apex predator species using underpasses than that of herbivore species, and that usage by a single unaccompanied animal will be more frequent than larger groups or herds. Timing of underpass usage was expected to reflect species' diel activity patterns. Lastly, it was expected that most mammals detected approaching an underpass entrance would proceed to enter it, and that some animals would remain inside structures to use them as refugia or shaded rest sites.

2.2 METHODS

STUDY AREA

The study was conducted within a section of the Balule Nature Reserve (BNR), part of the Greater Kruger National Park and within the Kruger to Canyons Biosphere Reserve (Figure 1). The BNR spans an area of 56,000 ha within the Limpopo province of South Africa, having been established in 1993 and officially recognised as a nature reserve in 2005 (Elliot *et al.* 2019). There is no fence between the BNR, the Kruger National Park (KNP) and other private nature reserves between them, meaning that wildlife can move freely between these protected areas. It is an area of high mammal diversity with over 145 species present (SANParks 2021), including megafauna and apex predators, of which a number are threatened (Table A7). The BNR lies within the Savanna biome, and is largely of the Granite Lowveld vegetation type (SANBI 2018). This vegetation is characterised by tall shrubland with a few trees, moderately-dense low woodland on deep sandy uplands, and irregular plains with a number of prominent hills (Mucina and Rutherford 2006). The climate is classified as hot semi-arid or steppe (Beck *et al.* 2018). Average daily temperatures typically ranging between 12-30ºC, with minimums reaching as low as 2.5ºC and maximum temperatures exceeding 40ºC (The Weather Company 2023). The wet season spans November to April, with mean annual rainfall of 489 mm and little to no rainfall between May and October (Schulze 1997, Olifants River Game Reserve 2021). The perennial Olifants River runs through the BNR.

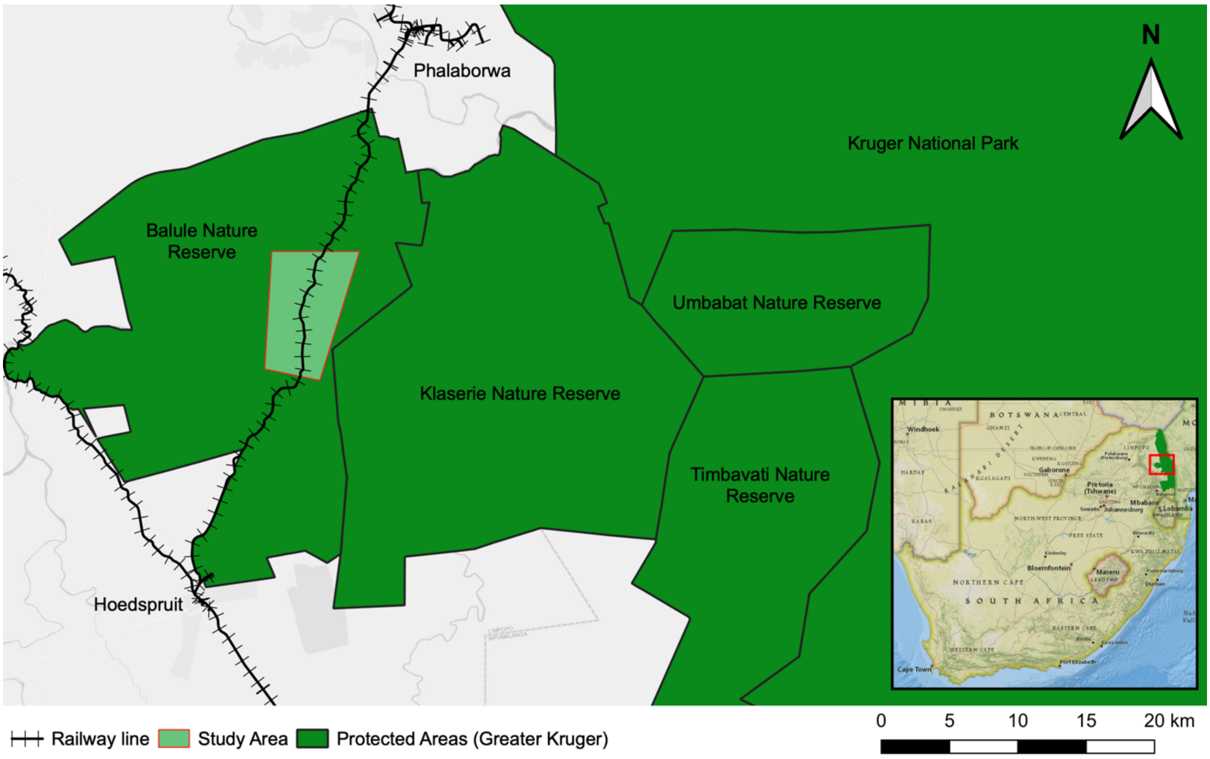


***Figure 1**: Balule Nature Reserve and Study Area within it relative to the Phalaborwa-Hoedspruit railway line and other protected areas that form part of the unfenced Greater Kruger conservation area, South Africa (inset).*

A single-track freight railway runs through the BNR between the towns of Phalaborwa and Hoedspruit, spanning a length of 45 km within the reserve (Figure 1). This railway was built in 1963, before the area became a protected wildlife area, to transport phosphate, magnetite and copper from mines located in Phalaborwa (Limpopo Province Freight Transport Data Bank 2012). An average of 12 trains per day were observed passing through the BNR during this study. The majority (31 km) of the track has no fencing preventing wildlife crossing, although 14 km of the southernmost section of the track near Hoedspruit is fenced on both sides due to landowner boundaries. The railway line is level with the ground, allowing animals an easy passage across it (Figure 2), except in a small number of undulating sections where the line runs through a cutting or crosses over a watercourse. The study area is located along a 9 km section of the rail without fencing, where an unpaved road runs parallel to the track.

Figure 2*: Photos of the railway line within the study area in Balule Nature Reserve, South Africa. The railway is unfenced and level with the ground, allowing wildlife an easy passage across it. These photos demonstrate railway usage by African bush elephant* (Loxodonta africana) *and African wild dog* (Lycaon pictus)*, as well as giraffe* (Giraffa camelopardalis) in close proximity to the track.

UNDERPASS USE

There are a number of long-established underpasses (culverts and viaducts) along the length of the railway line, designed for hydrological reasons rather than wildlife movement. Along the 9 km section of rail in the study area, there are 12 underpasses, namely 10 box culverts, one larger double box culvert, and one viaduct (Figure 3), at varying distance from water sources and riparian corridors (Figure 4). The box culverts varied in size, with widths of 1.2 – 3.5 m and heights of 0.7 – 2.5 m, whilst the double box culvert and viaduct had a width of 11 m and 30 m and a height of 4.6 m and 6.1 m respectively (Table 2). The mean distance between sites is 506 m, although two of the most northern underpasses were 92 m apart. Small pipe culverts in the study area were not included in the scope due to a focus on larger mammals.

Figure 3: *Examples of underpass structures in study area, in Balule Nature Reserve, South Africa: [A] box culverts [B] double box culvert [C] viaduct.*

Table 2: *Dimensions of underpass structures located in study area within the Balule Nature Reserve, South Africa.*

Site ID	Structure Type	Width (m)	Height (m)	Length (m)
Site 1	Box culvert	1.8	1.6	12.5
Site 2	Box culvert	3.5	1.8	13.4
Site 3	Box culvert	1.8	1.8	18.5
Site 4	Double box culvert	11.1	4.6	23.9
Site 5	Box culvert	2.7	1.6	15.7
Site 6	Box culvert	2.7	0.9	15.5
Site 7	Box culvert	1.8	0.7	14.1
Site 8	Viaduct	29.9	6.1	7.0
Site 9	Box culvert	1.8	1.5	6.4
Site 10	Box culvert	1.8	1.9	19.0
Site 11	Box culvert	1.2	1.0	9.1
Site 12	Box culvert	2.8	2.5	39.7

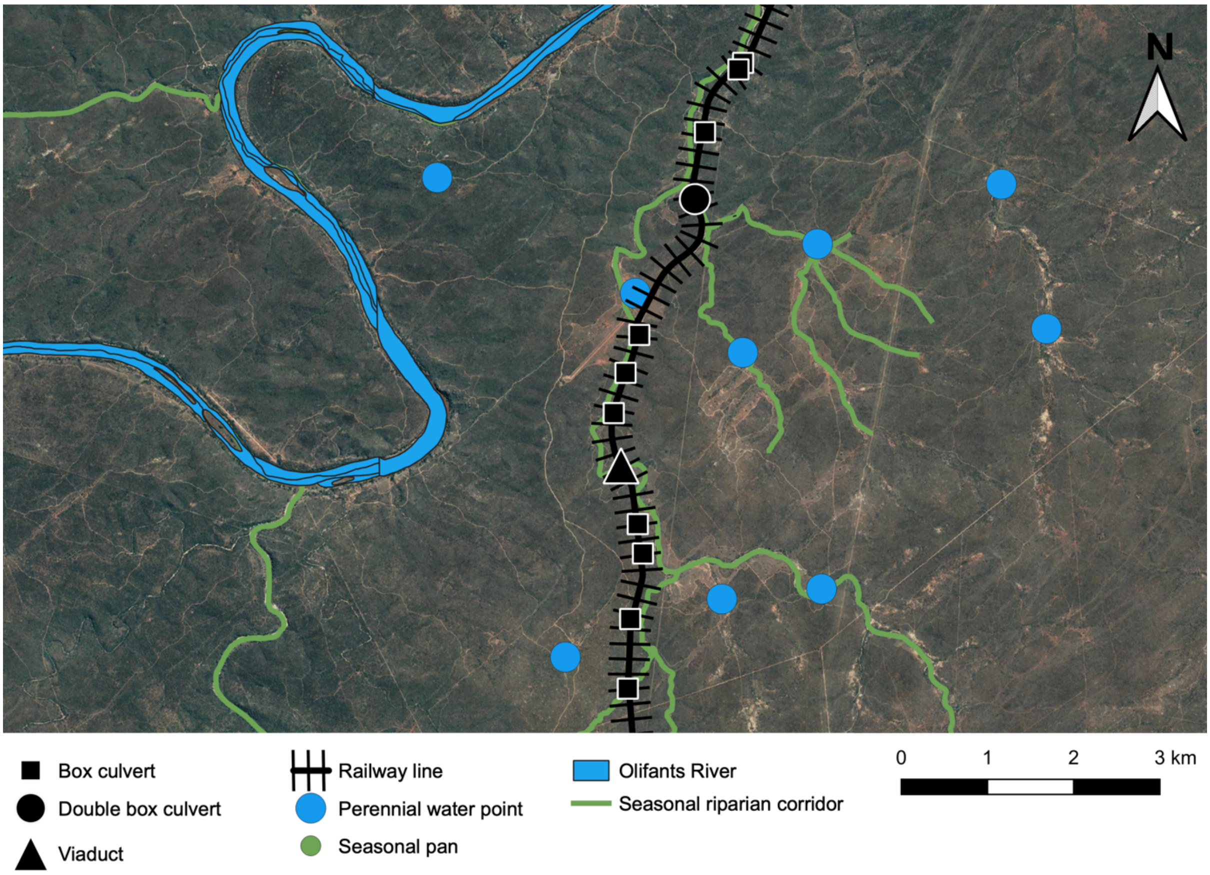


Figure 4: *Detailed map of study area and underpass locations along 9 km stretch of the railway line in the Balule Nature Reserve, South Africa. Underpass sites are numbered 1-12 from north to south, i.e. Site 1 is the northermost underpass and Site 12 is the sourthernmost underpass.*

Animal use of these structures was monitored through the setup of 29 Bushnell low-glow motion-triggered camera traps, for a total period of 16 weeks that spanned across four separate four week sampling periods. Two of these sampling periods were in the dry season, in July and September 2021, and another two were during the wet season in January and March 2022. Typically, two cameras were positioned at the entrance and exit of each underpass and directed to face the entrance, so that animals were detected approaching or exiting the culvert. At one box culvert (Site 1), thick vegetation completely obscured one entrance and so only one camera monitored this site for wildlife at the opposite end, although tracks from inside the culvert were also recorded during site checks to supplement camera detections. At the double box culvert and viaduct sites, additional cameras (4 and 6 cameras in total, respectively) were required to monitor the full width of the structure. Cameras were affixed to trees or concrete structures, or mounted on a steel post, positioned between 1.8 m to 13 m from the underpass entrance and at a height of approximately 0.6 m above ground. Cameras were enclosed in a metal case for protection from animal interference. The camera settings were standardised at all sites, set up to trigger as quickly as possible (0.3 seconds) with an interval between triggers of 3 seconds and with 2 successive images captured per trigger event. Herbaceous vegetation was cleared in front of the cameras to minimise false-triggers, but care was taken to not disturb the site beyond this. As recommended by a recent wildlife CS study (Denneboom *et al.* 2021), the camera setup attempted to monitor the area outside of the entrance to underpasses and record animal approaches as well as crossings.

All mammal species detected by the camera traps and identifiable in images were recorded, except for flying bats (order *Chiroptera*). Image processing was performed using Camelot version 1.6.14 (Hendry and Mann 2018), to create a sightings database from all images captured, helped by its Animal Detection feature which flagged images that contained an animal (90% confidence threshold). The accuracy of this detection feature was close to 100% for medium and large mammals, whilst very small mammals, birds and reptiles were occasionally undetected, especially if behind vegetation. Nonetheless, manual checks of non-tagged images were conducted to record any missed sightings from false negatives.

An Independent Observation (IO) was defined as a sighting of one species (solitary or group of individuals) in the detection-vicinity of one underpass site, as captured by the camera(s). Observations of the same species at the same site were considered independent if two photos were taken at least 20 minutes apart (Alves *et al.* 2021). All photos of the same species at the same IO were grouped. In the event of an animal remaining inside a structure for longer than 20 minutes, that animal's exit from the structure was grouped with that same animal's entrance event IO. For each IO, information regarding the species, the trophic level (megaherbivore, mesoherbivore, apex predator, mesopredator, omnivore), the typical diel activity (diurnal, crepuscular, nocturnal), the number of individuals present (solitary, small groups, large groups) and the species activity around the underpass (crossed, entered, refused, pass by) was recorded (Table 3). Information regarding species' characteristics was sourced from Skinner & Chimimba (2013).

Table 3: *Information regarding mammal species, group size and underpass activity recorded with each Independent Observation (IO: sighting event of one species in the detection-vicinity of one underpass site, as captured by the cameras).*

Variable name	**Description**
Date and Time	Date and time of the first image captured in the sequence assigned to an IO
Species	Mammal common and vernacular species name (e.g. Leopard; *Panthera pardus*)
Trophic level	Megaherbivore (over 1,000 kg body mass, Owen-Smith, 1988), mesoherbivore (body mass less than 1,000 kg), apex predator, mesopredator (any carnivore not classified as an apex predator), omnivore
Typical diel activity	Expected diel activity for each species: Diurnal, Crepuscular, Nocturnal, Crepuscular & Nocturnal, Irregular (any time of day or night)
Group size	Solitary individual, small group (<=5), large group (6+); exact number of individuals per IO was also recorded

Underpass activity	*Crossed (*Figure A12a): where photos of the species are captured at both ends of the structure moving in the same direction, or where one camera has captured an animal obviously entering a structure without returning out the same side, and the second camera has not detected the exit. Appendix Figure A12 *Entered*: where the species is captured entering and exiting from the same entrance. *Refused (*Figure A12b): where the species clearly approaches the structure and pauses to look inside or investigate, but then turns around and does not enter. *Pass by (*Figure A12c): where the species walks past or is eating vegetation or drinking water between the camera and the entrance, with no apparent interest in the structure.
Remain inside	Minutes that the species remains inside the structure (if enter and exit times are separated by more than two minutes)

Underpass usage was defined, for the purposes of this study, to include both “crossed” and “entered” events. This decision is supported by findings in Brunen *et al.* (2020), where underpass characteristics explained an animal’s entrance into an underpass, but no factors influenced whether it crossed through after the initial decision to go inside. An event where an animal entered but did not cross through could be due to it intending to cross but being deterred by something within or outside the structure, or it could be due to it exploring or foraging inside, or using the underpass as a shady resting spot, all of which was evidenced in the images captured. “Pass by” events were ignored for the purposes of this study, as the animals clearly showed no interest in the underpass, and were often simply walking past along the road parallel to the railway line. “Refused” events constitute valuable data, but because there was unavoidable variation in camera positioning at each site (due to vegetation or topographical obstructions), the area that was monitored for potential approaches varied significantly between sites. The number of approaches and “refused” events recorded was thus not like-for-like across sites and could not be used as a response variable in testing the effect of underpass characteristics, or for site-by-site comparisons. As such, the response variable selected for regression analysis was the usage rate (count of usage events) per operative day (following Brunen *et al.*, 2020; Ford *et al.*, 2022; Mysłajek *et al.*, 2020; Saxena & Habib, 2022b).

EXPLANATORY VARIABLES

For each underpass site, various explanatory variables were measured and recorded. These included structural characteristics, vegetation cover measurements as well as environmental characteristics (Table 4). All vegetation-related variables were measured for each sampling period, due to the major changes that occur to vegetation cover between the dry and wet seasons. All spatial distance measurements were performed using QGIS version 3.28 (QGIS.org 2022). Weather data was obtained from recordings taken at the closest weather station to the study area located inside the BNR, Lengau Lodge (IBAPHA1), and accessed online via Weather Underground (The Weather Company 2023). Train traffic was measured by manually counting the number of individual trains passing in either direction through the reserve on each day in the study period, from images captured using a single motion-detected camera trap mounted next to the railway line in the middle of the study area.

***Table 4**: Structural, vegetation cover and environmental explanatory variables for each of the 12 monitored underpass sites in the Balule Nature Reserve, South Africa, with details on how measurements were taken or data captured. These explanatory variables are used in GLMM with underpass usage rate as the response variable.*

Category	Variable name	Type	Details
Structural	Width	Continuous	Horizontal width of structure entrance
	Height	Continuous	Vertical height of structure entrance
	Length	Continuous	Horizontal distance between both entrances, perpendicular to railway line
	Openness index	Continuous	Width x height / length
	Ground cover within underpass	Categorical	Mostly concrete (fully concrete or only slight covering from accumulated substrate), Mostly natural (majority or full coverage of natural accumulated substrate)
Vegetation	Woody cover	Continuous	Measured within 5 m radius outside of both entrances. Diameter of individual woody plant canopies or closely intertwined individual clumps of woody plants at 1 m height above ground, or at its maximum height if shorter than 1 m. The canopy diameters were used to calculate the percentage ground cover by woody plants. Woody plants with a height of less than 30 cm above ground were excluded. Measurement taken for each sampling period.
	Herbaceous cover	Continuous	Percentage of herbaceous ground cover within 5 m radius outside of both entrances, measured using quadrat sampling and visual estimation. Measurement taken for each sampling period.
	Visibility through	Continuous	Proportion of visibility through structure (i.e. not obscured by vegetation/logs/rocks) at each entrance. Measured using a photographic digital grid overlay method (Figure A13) from a photograph taken 2 m outside the centre of the entrance looking in, at a height of 1 m above the ground, or where the culvert height was less than 1 m, the mid-height of the structure. Measurements taken for each sampling period.
Environmental	Distance to riparian corridor	Continuous	Straight-line distance to nearest riverbed, watercourse or drainage line
	Distance to water	Continuous	Distance to nearest perennial water source (including artificial water points and the Olifants river)
	Ambient temperature	Continuous	Average daily temperature recorded at the nearest weather station in BNR (ºC)
	Rainfall	Continuous	Millimetres of precipitation per day recorded at the nearest weather station in BNR
	Train traffic	Continuous	Count of trains passing through the reserve in either direction per day, as recorded by a single camera trap mounted along the railway line in the middle of the study area
	Season	Categorical	Dry season (July and September-October 2021) and wet season (January-February and March 2022)

DATA ANALYSES

All data analyses were performed in R version 4.2.2 (R Core Team 2022). For each underpass site, summary statistics were calculated, including the usage rate per operative camera day, overall species richness and the Shannon diversity index (using package 'vegan', Oksanen *et al.*, 2022). The number of species recorded using underpasses was compared to the proportion of ground-based mammals known to be present in the study area (not including rodents or other mammals smaller than that able to be detected or identified from camera traps), which was sourced from the reserve warden.

To determine what affected mammal usage rates of these structures and could explain the difference in usage rates across underpass sites, I performed multivariate regression to investigate whether explanatory variables (structural, vegetation and environmental characteristics) had an effect on underpass usage (response variable). Each monitored underpass was assumed to be independent from the other underpass sites. Based on its major physical differences to box culverts (Table 2), the viaduct was considered to be a structural outlier, and was excluded from all regression analyses.

Generalised linear mixed models (GLMM) using the 'glmmTMB' package (Brooks *et al.* 2017) were used for each trophic level group, with the usage count per day as the response variable. The model fit and choice of distribution were assessed using diagnostic plots from package 'DHARMa' (Hartig 2022) and tests from package 'performance' (Lüdecke *et al.* 2021), checking for overdispersion, excess zeros, outliers, and a Kolmogorov-Smirnov test for deviance of residuals. Poisson, quasi-Poisson, Poisson-lognormal (with an observation-level random effect) and negative binomial distributions were all fitted, due to overdispersion present in the data (Harrison 2014, Bolker 2015), with the negative binomial distribution favoured for each trophic level group. Multicollinearity was tested for using variance inflation factors (VIF) and no variables in any of the models were found to have a VIF threshold of above 5 (James *et al.* 2013). Autocorrelation was also tested for and did not prove a major factor. For each model the Akaike Information Criterion scores corrected for small sample bias (AICc) were calculated using the 'AICcmodavg' package (Mazerolle 2020).

The underpass site ID was used as a random effect in order to account for lack of independence between daily observations (repeated measures) at a specific underpass sites. All continuous variables were centred and scaled. Width, Height, Length variables exhibited high correlation and were removed from the model, leaving the Openness Index parameter (Table 4) which captures all three dimensions. Interactions between Season and vegetation-related variables (Visibility, Herbaceous cover and Woody cover) were considered due to the change in appearance of vegetation in wet and dry seasons, meaning that the effect of these variables on underpass usage may differ based on the season. An interaction between Season and Distance to water was also considered, given the abundance of water in the wet season and scarcity of it in the dry season, again meaning its effect on underpass usage may differ based on the season.

Including the underpass site ID as a random effect resulted in a singular fit for apex predator, mesoherbivore and omnivore models, meaning that the random effect had no impact in the models. Because the simplest random effect structure was used, and there were 11 grouping levels of the random effect, this is likely because there was insufficient variation between sites (Bolker 2015). Models were refit without the random effect as a check, confirming that fixed effect estimates remained the same and that the model had not run into any problems. For all analyses, the random effect was nevertheless included to satisfy the requirement for independence of observations, due to repeated measures per site.

Model selection to identify the best models for each trophic level group was conducted using AICc, with the best model having the lowest AICc value (Anderson 2008, Burnham *et al.* 2011). For each trophic level group, a global model containing all 11 fixed effects and the selected interactions was built, as well as a null model without any fixed effects, to ensure that fixed effects add value to the model. A further 28 models were built for each trophic level group with combinations of fixed effects and interactions between them that were considered biologically sound. Stepwise selection was also performed as a final check, given the large number of variable combinations possible, using the 'buildmer' package (Voeten 2023) to ensure that any fixed effect combinations with lower AICc values were not missed. A difference (delta) of 2 in AICc was considered as the cut-off for model selection (Burnham and Anderson 2002). The nesting selection rule (Richards *et al.* 2011, Harrison *et al.* 2018) was applied where the top model set consisted of more complex versions of nested models which had better AICc support, to improve accuracy of inference and prevent overfitting. Model averaging was not conducted due to complexities with interaction terms, which were included in all top model sets. Accordingly, the single best model was selected for inference for each trophic level group.

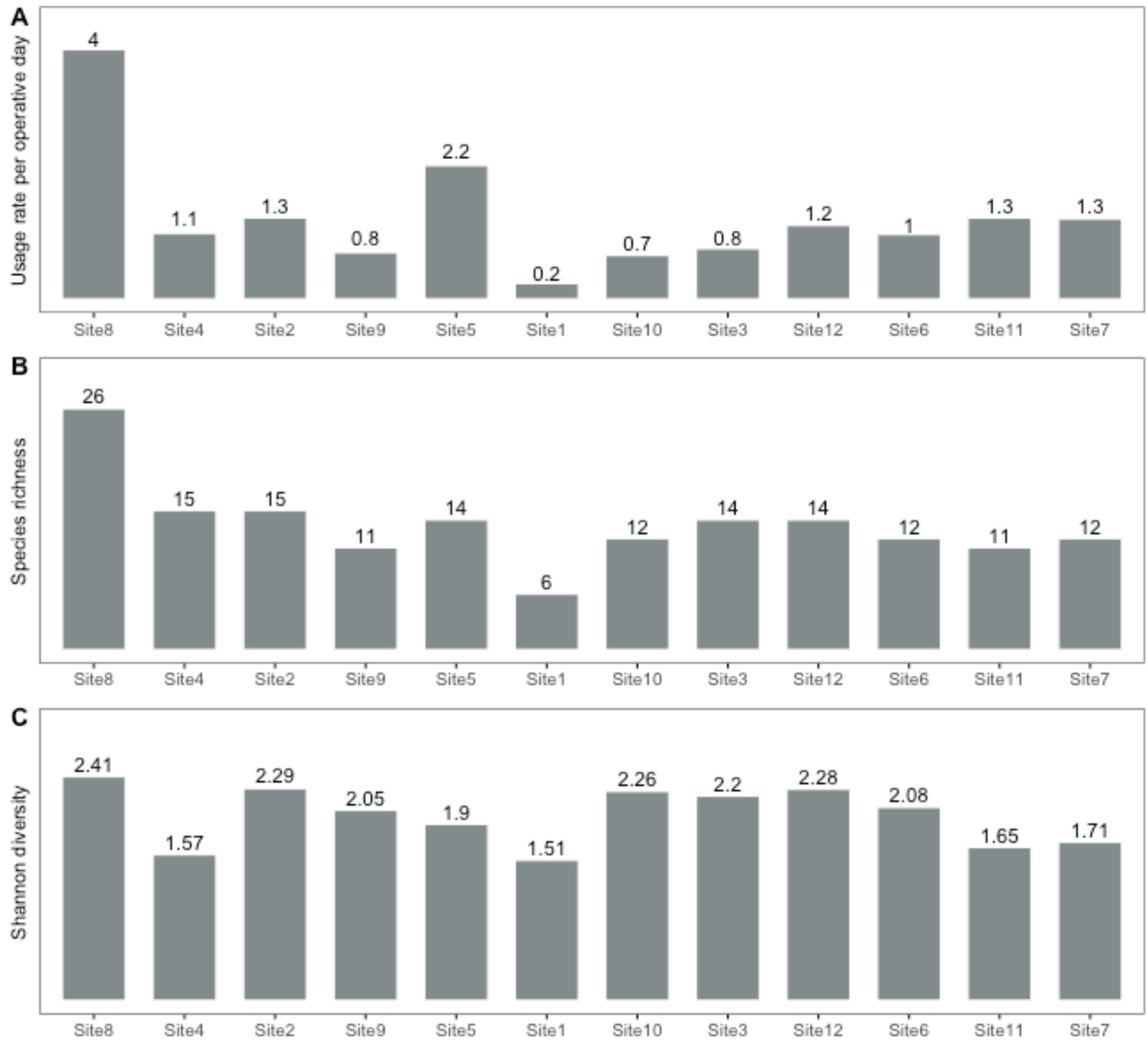
Marginal and conditional pseudo-R^2 values (Nakagawa and Schielzeth 2013, Nakagawa *et al.* 2017) were also reported as a secondary measure of variation explained by the best model, in addition to the AICc delta between the best and the null model. These pseudo R^2 metrics have gained some support in recent years, but have limitations versus their linear model counterpart, which is well-defined (Harrison *et al.* 2018). Incidence Rate Ratios (IRR) and their 95% confidence intervals (CI) were calculated for each parameter estimate. Significance was determined by whether confidence intervals cross the line at 1.0 IRR. Model plots were made with the sJPlot package (Lüdecke *et al.* 2022).

2.3 RESULTS

UNDERPASS USE OVERVIEW

Camera traps positioned at underpass sites recorded 394,074 images over the 16 week monitoring period, of which 151,013 were from the dry season and 243,061 from the wet season. From these images, a total of 3,029 IOs of mammal species near an underpass site were recorded, of which 1,002 were ignored due to classification as a “pass by” event. The remaining 2,027 IOs constituted a total of 1,823 usage events (1,518 “crossed” events, accounting for 83% of usage events, and 305 “entered” events accounting for the remaining 17%), or an average of 1.33 underpass usage events per operative camera day per site, as well as 204 “refused” events. From the 1,823 usage events, a minimum of 2,787 individual mammals used underpasses (those captured on camera), or an average of 1.5 individuals per visit. A total of 37 terrestrial mammal species were captured on cameras near underpasses, of which 34 species were seen to approach underpasses and 33 species were recorded using at least one structure.

Underpass usage varied considerably across different sites (Figure 5A). The viaduct (Site 8), which was the largest and most open of the underpasses sampled, had 3.7 times the usage of all other sites, as well as 1.9 times the number of species that visited it, as well as the highest overall Shannon diversity index (Figure 5A, B, C). Whilst the large size of this viaduct probably explains its more frequent usage and the wider variety of mammal species that utilise it, there is no clear pattern of declining usage, richness or diversity with smaller, more constricted structures. The three least open underpasses (Site 7, Site 11 and Site 6) all had a height of 1 m or less, but did not have the lowest species richness or diversity (Figure 5B, C). Site 1 ranked lowest on all three usage metrics, despite being of medium openness. Whilst some of the culverts (e.g., Site 2, Site 10, Site 12) had a fairly even distribution of species abundance using them (Shannon diversity index was close to the maximum based on richness, Figure 5C), the viaduct had a comparatively less even distribution of species using it, largely skewed by the abundance of common duiker *(Sylvicapra grimmia)* that visited it.



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	Site8	Site4	Site2	Site9	Site5	Site1	Site10	Site3	Site12	Site6	Site11	Site7
Openness Index	26.28	2.12	0.48	0.43	0.26	0.23	0.18	0.18	0.17	0.16	0.14	0.1

Figure 5: *Overview of underpass site usage, species richness and diversity, with underpass sites ranked from left to right in order of descending Openness Index in the study area within Balule Nature Reserve, South Africa. Site 8 is the viaduct, whilst Site 4 is the double box culvert. [A] Usage rate per operative camera day for each site, for all mammal species, across the full monitoring period. [B] Species richness for each underpass site across the full monitoring period. [C] Shannon diversity index calculated for each underpass site across the full monitoring period. [D] Openness Index of each site (width x height / length).*

Mesopredators were the most frequent users across all sites, accounting for 52% of all underpass visits (Figure 6). Apex predators (consisting of just 3 species) had almost two thirds the number of usage events of mesoherbivores (consisting of 12 species), and were present at all sites (Figure 6). For all standard box culverts (excluding Site 8 and Site 4), predator guilds accounted for the majority of usage events. Combined predator guild Mesoherbivores, whilst being the second most frequent users of underpasses in this reserve overall, used the viaduct (Site 8) disproportionately (51% of all mesoherbivore usage events), with relatively few visits across other sites (Figure 6). Mesoherbivores were the most frequent users of both the viaduct (46% of Site 8 usage events) and the larger double box culvert (39% of Site 4 usage events). Megaherbivores did not use any standard box culverts, and used the two larger underpasses very infrequently, with only 2 visits (1.7% of total) to the double box culvert (Site 4) and 15 visits (3.2% of total) to the viaduct across the 16 week period (Figure 6). Megaherbivores did not use underpasses sufficiently for further statistical modelling.

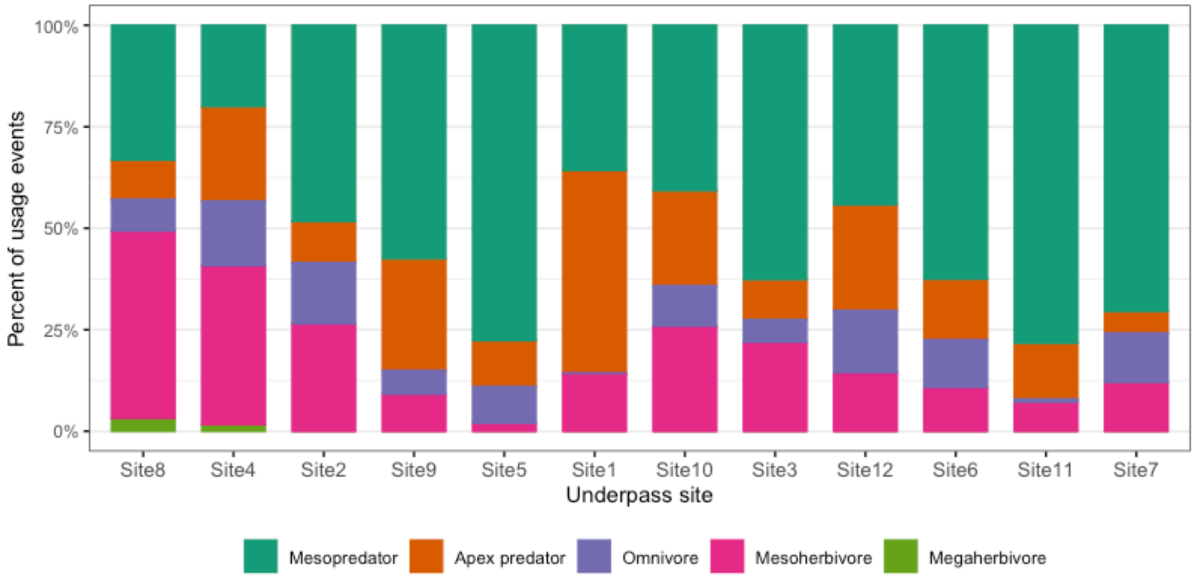
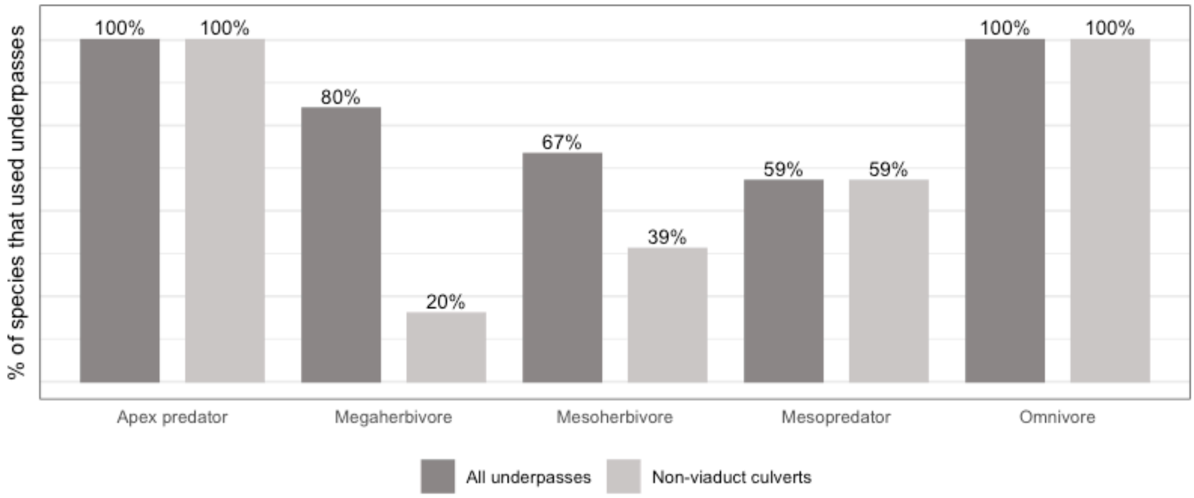


Figure 6: *Percentage of total usage events across the full study period by trophic level group for each underpass site. Underpass sites are ranked left to right in descending order of size (Openness Index).*

Of the 46 relevant mammal species (excluding bats and small rodents) known to be present in the reserve (Table A7), 14 species were not detected using any of the 12 underpasses at all, and 22 species did not use any of the 11 non-viaduct culverts (Figure 7). This gap of 8 species that used the viaduct but did not use any box culverts is solely attributed to the herbivore guild. Two thirds of mesoherbivore species present in the area were recorded using underpass structures during the period, which decreases to 39% if the viaduct is excluded (Figure 7). Only one megaherbivore species used the double box culvert (Site 4, used by a hippopotamus, *Hippopotamus amphibius*), whilst 4 of the 5 megaherbivore species present in the study area used the viaduct. A total of 7 mesopredator species did not use any underpasses in the study area (Table A7).



	Apex predator	Megaherbivore	Mesoherbivore	Mesopredator	Omnivore
Total spp present	3	5	18	17	3

Figure 7: *Percentage of the total mammal species known to be present in the study area that were recorded using underpasses, for all 12 underpass sites combined and for the 11 non-viaduct culvert sites. The total number of species known to be locally present in the study area within each trophic group is shown below the chart, with the full species list presented in Table A7.*

The three species most frequently using underpasses were all mesopredators, namely slender mongoose *(Herpestes sanguinea)*, African civet *(Civettictis civetta)* and the common large-spotted genet *(Genetta maculata)*, each of which visited all 12 underpass sites during the sampling period (Table 5). These 3 species used underpasses between 2 – 3.5 times per day, on average. The common duiker was the fourth highest user, but only of two underpasses, of which 98% of visitations were at the viaduct (Table 5). The second underpass that common duiker used just 3 times was Site 3, which is notably the least open structure of the 12 monitored. Spotted hyaena *(Crocuta crocuta)*, leopard *(Panthera pardus)*, tree squirrels *(Paraxerus cepapi)* and Cape porcupine *(Hystrix africaeaustralis)* all used underpasses more than 100 times during the sampling period, equating to an average of at least one visit per day to an underpass in the study area (Table 5). Spotted hyaena and leopard also were present at all 12 underpasses, whereas tree squirrel and Cape porcupine visited 10 and 11 of the sites respectively. Lion *(Panthera leo)* were remarkably infrequent underpass users, given the frequency of other apex predator visits (Table 5). Megaherbivores seldom visited underpasses during the study period.

Refusals, which may be understated based on detection vicinity of cameras, are a small proportion of overall usage events recorded, with 90% of all approaches resulting in the mammal(s) entering the structure (Table 5). Black rhinoceros (*Diceros bicomis*) was the only species that was captured refusing to enter an underpass but never captured using an underpass. Steenbok *(Raphicerus campestris)* and lion were the only species with usage rates as a proportion of total approaches less than 50% (Table 5), however the number of data points for these species is low. Other species, such as birds, bats and reptiles (most frequently monitor lizards, *Varanus albigularis*, and occasionally smaller lizards and snakes) were also detected using underpasses but are outside of the scope of this study.

Table 5: *Usage and refusal events by mammal species across all twelve underpass sites. Species are grouped by trophic level.*

Species	Common Name	Crossed	Entered	Total usage	Refused	Usage proportion
Apex predator		**211**	**54**	**265**	**47**	**85%**
Crocuta crocuta	Spotted hyaena	103	26	129	29	82%
Panthera pardus	Leopard	100	27	127	8	94%
Panthera leo	Lion	8	1	9	10	47%
Mesopredator		**820**	**130**	**950**	**73**	**93%**
Herpestes sanguinea	Slender mongoose	352	44	396	31	93%
Civettictis civetta	African Civet	201	33	234	17	93%
Genetta maculata	Common large-spotted genet	198	19	217	11	95%
Helogale parvula	Dwarf mongoose	20	25	45	5	90%
Mungos mungo	Banded mongoose	25	5	30	2	94%
Mellivora capensis	Honey badger	13	1	14	-	100%
Genetta genetta	Small-spotted genet	9	1	10	5	67%
Canis mesomelas	Black-backed jackal	1	1	2	1	67%
Felis silvestris	African wild cat	1	-	1	1	50%
Ichneumia albicauda	White-tailed mongoose	-	1	1	-	100%
Megaherbivore		**15**	**2**	**17**	**4**	**81%**
Loxodonta africana	African elephant	9	2	11	3	79%
Hippopotamus amphibius	Hippopotamus	4	-	4	-	100%
Ceratotherium simum	Southern white rhinoceros	1	-	1	-	100%
Giraffa camelopardalis	Giraffe	1	-	1	-	100%
Diceros bicornis	Black rhinoceros	-	-	-	1	0%
Mesoherbivore		**336**	**84**	**420**	**60**	**88%**
Sylvicapra grimmia	Common duiker	98	43	141	5	97%
Hystrix africaeaustralis	Cape porcupine	101	11	112	6	95%
Lepus saxatilis	Scrub hare	83	6	89	27	77%
Phacochoerus africanus	Common warthog	26	14	40	2	95%
Raphicerus sharpei	Sharpe's grysbok	13	7	20	8	71%
Tragelaphus strepsiceros	Greater kudu	5	2	7	1	88%
Aepyceros melampus	Impala	6	-	6	3	67%
Oreotragus oreotragus	Klipspringer	1	-	1	-	100%
Potamochoerus larvatus	Bushpig	1	-	1	-	100%
Raphicerus campestris	Steenbok	-	1	1	8	11%
Syncerus caffer	African buffalo	1	-	1	-	100%
Thryonomys swinderianus	Greater cane-rat	1	-	1	-	100%
Omnivore		**136**	**35**	**171**	**20**	**90%**
Paraxerus cepapi	Tree squirrel	100	21	121	10	92%
Papio ursinus	Chacma baboon	26	13	39	8	83%
Chlorocebus pygerythrus	Vervet monkey	5	1	6	1	86%
Unidentified mouse	Unidentified mouse	5	-	5	1	83%

Timing of underpass usage across all sites was consistent throughout the day, with a slight peak during the middle of the day, and closely reflected the diel activity patterns of the species using the sites (Figure 8). 88% of usage events were by an individual mammal, with just 9% by small groups and 3% by large groups (Figure 9). Similarly, this pattern reflects the normally solitary nature (or occasionally moving around in pairs or small groups) of the species commonly using underpasses. Large group visits consisted mainly of Chacma baboon *(Papio* *ursinus)*, dwarf mongoose *(Helogale parvula),* banded mongoose *(Mungos mungo)* and occasionally elephant. For 12% of total usage events, the species visiting remained inside the underpass for longer than 2 minutes (Figure 10). Aside from duiker frequently foraging beneath the viaduct, the majority of these extended visits were by mongoose species, genet, leopard, baboon, hyaena, civet, warthog *(Phacochoerus africanus)* and tree squirrels. 51% of these extended visits were for 10 minutes or less, and 78% were less than 30 minutes, although 7% were longer than 2 hours, most commonly by leopard or spotted hyaena (apex predators), which on a few occasions remained at an underpass for longer than 6 hours throughout the heat of the day (Figure 10).

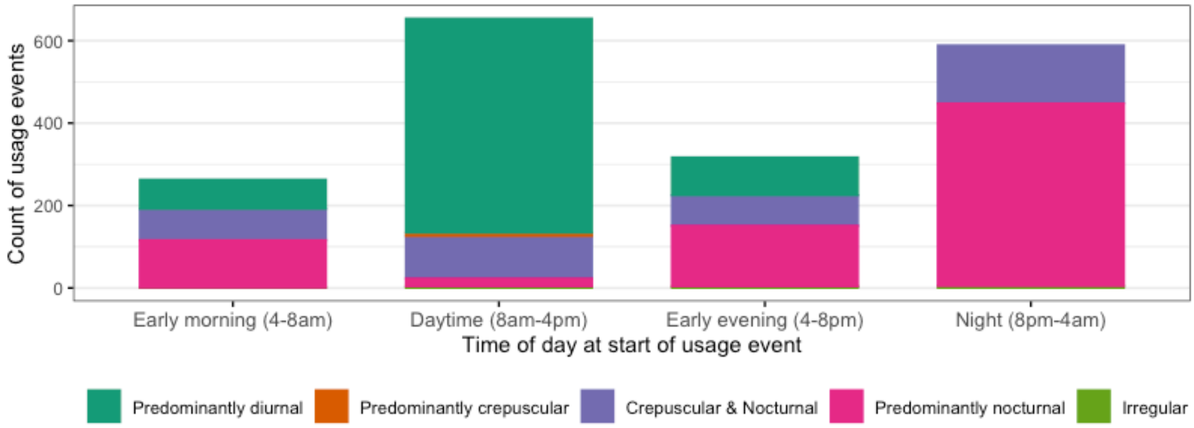


Figure 8*: Underpass usage events by time of day, broken down by the typical diel activity pattern of species using the structures.*

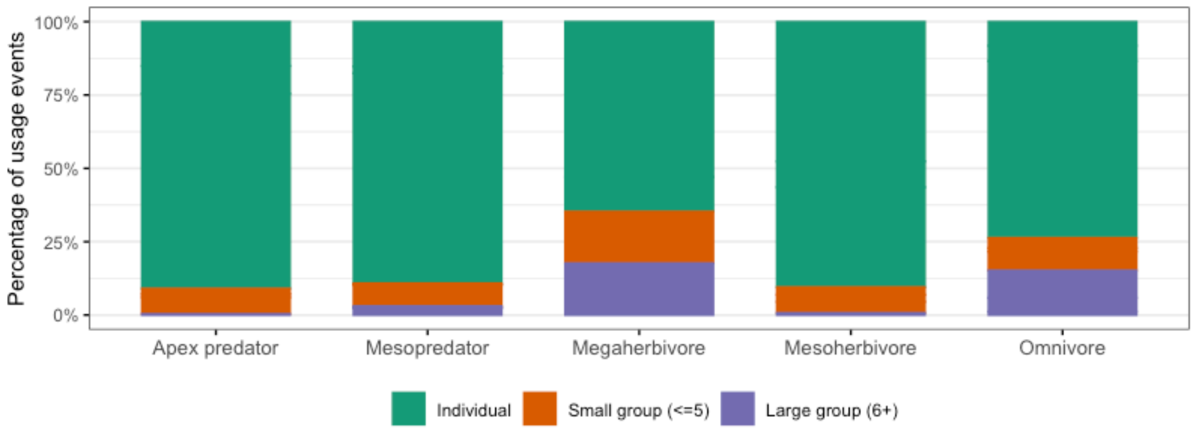


Figure 9*: Trophic level usage of underpasses proportioned by the number of species individuals per usage event*

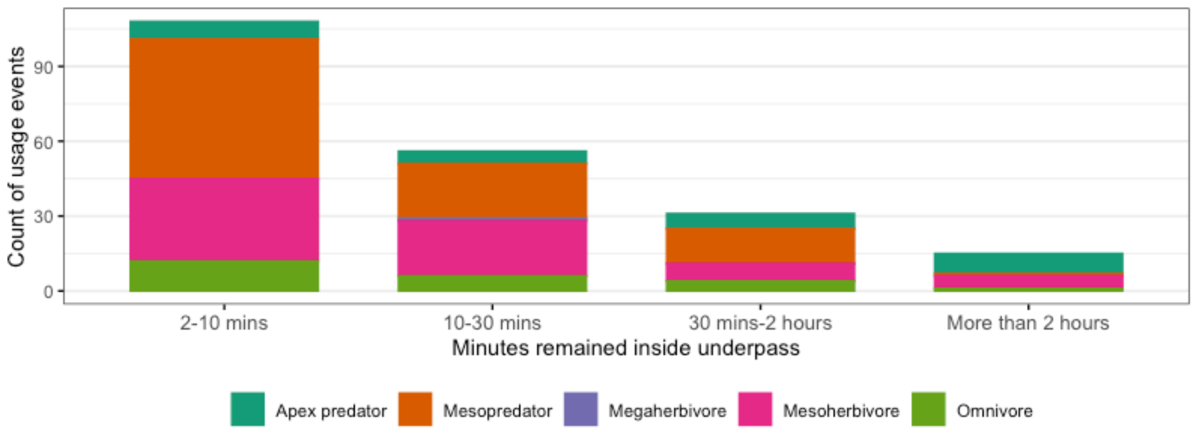


Figure 10*: Underpass usage events where mammals remained inside the structure for longer than 2 minutes, showing the length of visit broken down by trophic groups.*

FACTORS AFFECTING CULVERT USE

The top five models for each trophic group had an AICc delta of less than or very close to 2 (Table 6), but the single best model is used for inference based on the nesting rule, as model numbers 2 – 5 are all variations of model 1. Whilst marginal pseudo-R^2 values for GLMMs ranged from 0.15 to 0.49 depending on trophic level group, the AICc from the null models as compared to the best models (AICc delta of between 51 and 119) indicates the models for each trophic group are significantly improved by the inclusion of fixed effects, which explain variability in the response.

__Table 6__: AICc results of the five best models for each trophic level group for predicting underpass usage, with n(1262) for each model. The nesting rule was applied to select the single best model for inference.

Model No.	Model	K	AICc	Delta_AICc	AICcWt
Apex predators					
1	use ~ Visibility + HerbaceousCover * Season + DistanceWater + TrainCount + Temp + (1 | Station)	10	1201.07	0.00	0.25
2	use ~ Openness + Visibility + HerbaceousCover * Season + DistanceWater + TrainCount + Temp + (1 | Station)	11	1201.34	0.27	0.22
3	use ~ Visibility + HerbaceousCover * Season + DistanceWater + WoodyCover + TrainCount + Temp + (1 | Station)	11	1201.41	0.34	0.21
4	use ~ (Visibility + HerbaceousCover) * Season + DistanceWater + TrainCount + Temp + (1 | Station)	11	1201.68	0.61	0.19
5	use ~ HerbaceousCover * Season + DistanceWater + TrainCount + Temp + (1 | Station)	9	1202.57	1.50	0.12
Mesopredators					
1	use ~ Visibility + WoodyCover + DistanceWater * Season + TrainCount + Temp + (1 | Station)	10	2472.70	0.00	0.38
2	use ~ Openness + Visibility + DistanceWater * Season + WoodyCover + TrainCount + Temp + (1 | Station)	11	2474.26	1.57	0.17
3	use ~ (Visibility + DistanceWater) * Season + WoodyCover + TrainCount + Temp + (1 | Station)	11	2474.37	1.67	0.16
4	use ~ Visibility + HerbaceousCover + WoodyCover + DistanceWater * Season + TrainCount + Temp + (1 | Station)	11	2474.44	1.75	0.16
5	use ~ Openness + Visibility + HerbaceousCover + DistanceWater * Season + WoodyCover + TrainCount + Temp + (1 | Station)	12	2474.82	2.12	0.13
Mesoherbivores					
1	use ~ Openness + (Visibility + DistanceWater) * Season + HerbaceousCover + WoodyCover + TrainCount + (1 | Station)	12	1099.72	0.00	0.37
2	use ~ Openness + (Visibility + DistanceWater) * Season + HerbaceousCover + WoodyCover + DistanceRiparian + TrainCount + (1 | Station)	13	1101.25	1.52	0.17
3	use ~ (Visibility + DistanceWater) * Season + HerbaceousCover + WoodyCover + TrainCount + (1 | Station)	11	1101.33	1.61	0.16
4	use ~ Openness + (Visibility + DistanceWater) * Season + HerbaceousCover + TrainCount + (1 | Station)	11	1101.44	1.71	0.16
5	use ~ Openness + (Visibility + DistanceWater + HerbaceousCover) * Season + WoodyCover + TrainCount + (1 | Station)	13	1101.70	1.98	0.14
Omnivores					
1	use ~ Openness + Visibility * Season + HerbaceousCover + WoodyCover + TrainCount + Temp + (1 | Station)	11	766.88	0.00	0.28
2	use ~ Visibility * Season + HerbaceousCover + WoodyCover + +TrainCount + Temp + (1 | Station)	10	767.33	0.45	0.22
3	use ~ Openness + (Visibility + HerbaceousCover) * Season + WoodyCover + TrainCount + Temp + (1 | Station)	12	767.44	0.56	0.21
4	use ~ Openness + Visibility * Season + HerbaceousCover + WoodyCover + TrainCount + Temp + DistanceWater + (1 | Station)	12	767.86	0.97	0.17
5	use ~ Openness + Visibility * Season + HerbaceousCover + WoodyCover + TrainCount + Temp + DistanceWater + GroundCover + (1 | Station)	13	768.59	1.71	0.12

Apex predators

Apex predator use of underpasses was strongly positively related to higher temperatures (IRR = 1.55, 95% CI of 1.29 – 1.88) (Figure 11). Underpasses located further from permanent water sources were used significantly less (IRR = 0.76, 95% CI of 0.63 – 0.9). Apex predators used underpasses much less frequently in the wet season (IRR = 0.48, 95% CI of 0.33 – 0.7). More grass cover outside of underpass entrances significantly reduced apex predator usage in the wet season (IRR = 0.56, 95% CI of 0.4-0.78), when grass was likely to be much taller, but in the dry season the effect was not significant. There was no evidence that apex predator use of underpasses was significantly affected by either the level of visibility through the structure and the number of trains passing through the reserve, although both parameters were included in the best model.

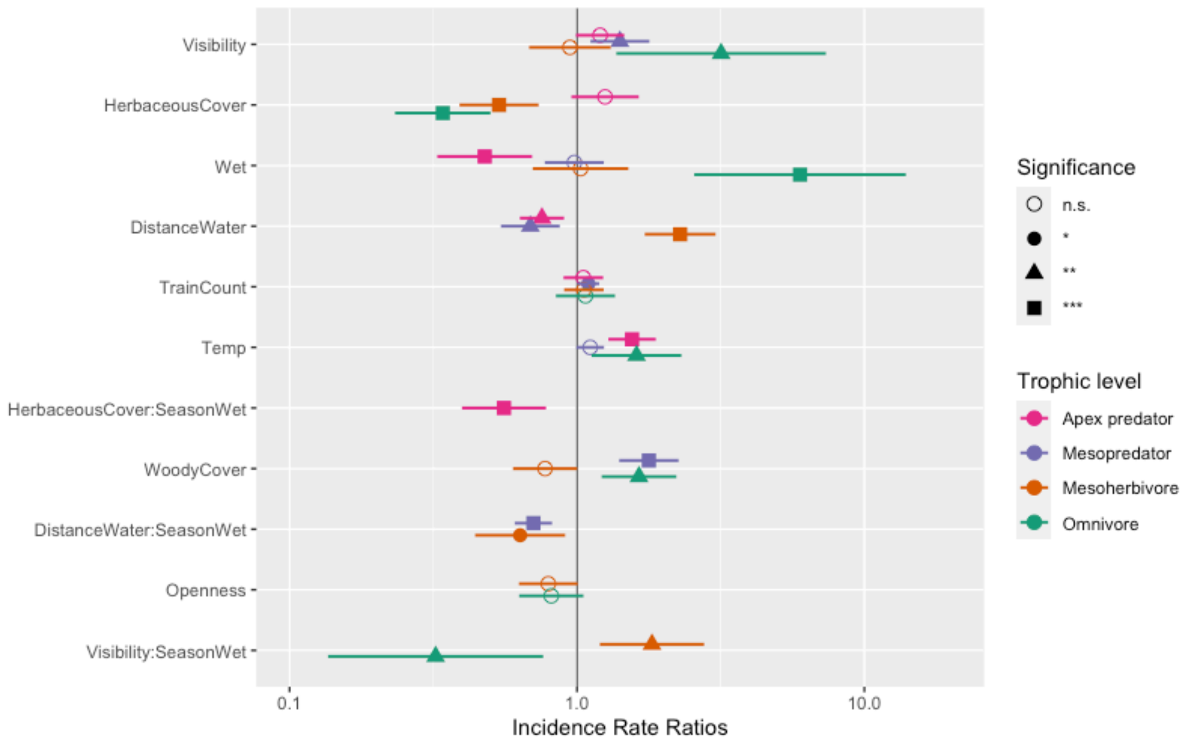


Figure 11: *Incidence Rate Ratios (IRR, ± 95% confidence interval) from the best, most parsimonious model (GLMM) for predicted underpass usage of each trophic level group. Significance is determined by whether confidence intervals cross the line at 1.0 IRR.*

Mesopredators

Mesopredators showed a strong preference for underpasses with more woody plant cover with a 5 m radius outside of the entrance (IRR = 1.78, 95% CI of 1.40 – 2.26), but at the same time favoured underpasses through which there was higher visibility or where vegetation did not obscure the line of sight through the entrance itself (IRR = 1.41, 95% CI of 1.11 – 1.79) (Figure 11). Mesopredators used underpasses more frequently on days when a higher number of trains passed through the reserve (IRR = 1.1, 95% CI of 1.01 – 1.19). These smaller predators were significantly more likely to use underpasses located closer to permanent water sources, more so in the wet season (IRR = 0.71, 95% CI of 0.61 – 0.82) but also in the dry season (IRR = 0.69, 95% CI of 0.54 – 0.87). Whilst ambient temperature was included in the best model, there was no evidence that mesopredator use of underpasses was significantly affected by this parameter.

Mesoherbivores

Mesoherbivores were significantly more likely to use underpasses further away from permanent water sources in the dry season (IRR = 2.29, 95% CI of 1.72 – 3.03), and conversely showed a preference for underpasses closer to water sources in the wet season (IRR = 0.63, 95% CI of 0.44 – 0.91) (Figure 11). These species also showed a strong preference for underpasses which had a higher level of visibility through them in the wet season (IRR = 1.82, 95% CI of 1.20 – 2.77), and with less herbaceous cover outside of entrances (IRR = 0.54, 95% CI of 0.39 – 0.74). Whilst included in the best model, openness, woody cover, and train count had no evidence for having a significant effect on underpass usage by mesoherbivores.

Omnivores

Omnivores used underpasses much more frequently in the wet season (IRR = 5.99, 95% CI of 2.57 – 13.98) (Figure 11). In the dry season, omnivores showed a strong preference for structures through which there was with a high level of visibility (IRR = 3.17, 95% CI of 1.37 – 7.35), but in the wet season these species preferred structures with less visibility through them (IRR = 0.32, 95% CI of 0.14 – 0.76). No matter the season, omnivores showed a preference for underpasses with less herbaceous cover (IRR = 0.34, 95% CI of 0.23 – 0.5) and more woody cover (IRR = 1.64, 95% CI of 1.22 – 2.22) outside the entrance. Omnivore use of underpasses was also positively associated with higher temperatures (IRR = 1.61, 95% CI of 1.12 – 2.31). Whilst included in the best model, openness index and train count had no evidence for significantly affecting underpass usage by omnivores.

2.4 DISCUSSION

UNDERPASS USAGE

To the best of my knowledge, this is the first study in an Africa savanna landscape which demonstrates multi-species use of underpasses not designed to facilitate wildlife crossing by medium and large mammals. These passages certainly increase the permeability of the railway and can aid conservation, but not for all species: 70% of the medium-large terrestrial mammal species known to be locally present used at least one underpass in the study area, including the viaduct, whilst 52% used at least one box culvert. Of the 30% of species who did not use any underpasses during the study, half were common resident herbivorous ungulates (excluding *Hippotragus niger*), whilst the other half were rare, low-density mesopredator species (e.g., *Orycteropus afer*, *Smutsia temminckii*, *Caracal caracal, Canis adustrus, Leptailurus serval*, Table A7). Furthermore, of the 8 herbivore species that used the viaduct but did not use the box culverts, 3 species were probably precluded from using the structure based on body size (elephant, giraffe *Giraffa camelopardalis*, southern white rhinoceros *Ceratotherium simum*), and 3 were abundant ungulate mesoherbivore species (impala

Aepyceros melampus, greater kudu *Tragelaphus strepsiceros,* African buffalo, *Syncerus caffer*). These results could indicate a density-dependent pattern for mesopredator use of underpasses, where species with lower abundance may not come across a CS in their movements. However, the same pattern is not evident for mesoherbivore non-users.

The single viaduct monitored in the study was used most frequently by the widest variety of species, seeing almost four times the usage rate of a typical box culvert and almost twice the species richness, alone supporting 57% of the medium-large terrestrial mammal species known to be locally present. These results are in line with other studies, which showed that viaducts are the most effective CSs for large mammals (Mysłajek *et al.* 2020, Denneboom *et al.* 2021). Whilst the viaduct was excluded from regression analyses as an outlier, it is very likely that its more frequent and diverse usage by both predator and prey species is due to it being a larger, more open structure (65 times the mean openness of sampled culverts) that blends more into the natural environment with vegetation inside (Clevenger and Waltho 2005, Seiler and Olsson 2009, Wang *et al.* 2018, Denneboom *et al.* 2021). Indeed, in areas with similar diversity of large and medium mammal species, increasing width (Saxena and Habib 2022a) and height (Lala *et al.* 2022) resulted in higher usage and species richness.

In contrast, box culverts were seldom used by megaherbivores and most mesoherbivores, suggesting they are not effective in providing ordinary safe passage across the railway line for these species, even though there has been a long time for habituation to occur. This finding is supported by studies in the northern hemisphere that suggested drainage culverts do not solve connectivity problems alone, but may be effective for carnivores (Rodríguez *et al.* 1996, Ascensão and Mira 2007, Seiler and Olsson 2009, Longcore *et al.* 2018). Ungulates have been shown to be most sensitive to structural dimensions, having a proclivity for more open structures (Ng *et al.* 2004, Dodd *et al.* 2007, Clevenger *et al.* 2009, Bhardwaj *et al.* 2020, Lala *et al.* 2022) which could explain why many ungulates in this study did not use the box culverts. Whilst some megaherbivore species were precluded from using smaller culverts due to their large body size, the infrequent underpass use of locally abundant mesoherbivore ungulate species (e.g., impala, greater kudu, buffalo, Table A7) is remarkable, and their response to CSs must be explained by certain behavioural or physiological traits.

To understand the usefulness of these non-wildlife passages in mitigating WTC, species using underpasses can be compared with the species most affected by train collisions. In Elliot *et al.* (2019), the mammal species with highest rail mortality in the BNR over the period 1993-2019 were buffalo (98), lion (96), impala (86), giraffe (57), kudu (48) and elephant (32), whereas Thela (2021) found impala to have the highest rail mortality (7 carcasses in 90 days). Most of these species are either threatened (Table A7) or important from a tourism perspective, being charismatic. Combined underpass usage by these species accounted for only 1.9% of all visits during the study period (Table 5), suggesting there is a disconnect between species that are more likely to use non-wildlife underpasses and those most impacted by rail mortality. Furthermore, use of the viaduct by elephant and giraffe, the tallest megaherbivores, is exceptionally low given their abundance in the reserve (27 elephants and 5 giraffe per 1000 Ha, 2022 BNR census), in all likelihood because there are too few sufficiently large underpasses in the area for use to become a regular habit. It is apparent that the species most susceptible to collisions frequently cross over the railway line: a single camera positioned on the railway line (for the purpose of measuring train traffic) recorded an average of one crossing per day by 16 mammal species, a higher richness than any single box culvert (maximum of 15 species), including impala, buffalo, giraffe, elephant, and lion. Although captured from a single site, impala alone crossed the railway at this point 24 times – four times that of their six visits to the viaduct during the same period (Table 5). Threatened, low-density species, including African wild dog (*Lycaon pictus*) and cheetah (*Acinonyx jubatus*), were also occasionally involved in train collisions (Elliot *et al.* 2019, Thela 2021) but did not use underpasses, although these mammals were observed crossing over the railway during fieldwork, and wild dog were detected by cameras near underpasses on two occasions.

Different species are known to respond differently to underpass structural and environmental characteristics (Clevenger and Waltho 2005, Andis *et al.* 2017, Brunen *et al.* 2020, Denneboom *et al.* 2021, Lala *et al.* 2022, Saxena and Habib 2022a). This variation in response is thought to be due to behavioural and physiological traits that help maximise survival and thus determine a species' willingness to enter an underpass (Kintsch *et al.* 2015). There is likely to be a wide range of proclivities for using different underpasses in an area with such high mammal diversity, dependent on what a species regards as its 'landscape of fear' or the inherent predation risk at different structures (Laundre *et al.* 2010, Pringle 2018). For example, certain smaller-bodied mesoherbivores tend to avoid low-visibility habitats which may explain a reluctance to enter culverts, whilst others are reliant on cover to minimise predation risk (Anderson *et al.* 2016, Pringle 2018). This study reveals that species which frequently used all or most of the 12 underpasses in the study area (certain apex predator and mesopredator species such as hyaena, leopard, mongoose species, civet and large-spotted genet, as well as some prey species such as porcupine, tree squirrel, scrub hare and perhaps warthog) can be regarded as 'medium structure generalists' (Kintsch *et al.* 2015), for which box culverts appear to be effective at providing a safe passage across the railway. However, further research is needed to inform mitigation design for African ungulate herbivores that seldom or never used box culverts (and hence do not factor into the regression results). In Kenya, elephant, buffalo and plains zebra *(Equus quagga)* were frequent users of larger underpass structures, suggesting these species may be 'large structure generalists' or 'openness obligates', whereas giraffe and a number of antelope species used larger structures much less than expected (Lala *et al.* 2022), similar to results from this study. Interestingly, warthog were uncommon users of larger underpasses and bridges in Kenya, but more frequently used smaller, more constricted culverts in the study area and have been observed using pipe culverts in the BNR, suggesting they may prefer less open structures. Black-backed jackal (*Canis mesomelas*) used underpasses much less than expected based on their being a common resident and habitat generalist (Skinner and Chimimba 2013); this finding was also observed in Kenya (Lala *et al.* 2022).

Predator-prey interactions were not specifically considered as part of this study, due to conflicting evidence on whether they affect CS usage (Ford and Clevenger 2010, Mata *et al.* 2015, 2020, Andis *et al.* 2017, Caldwell and Klip 2020, Saxena and Habib 2022b) and because attraction or segregation at underpasses may simply be a reflection of wider landscape-level interactions (Little *et al.* 2002). However, frequent box culvert use by leopard and spotted hyaena, and common scent marking by leopards evidenced in photos, may have dissuaded mesoherbivore prey species from using these structures (Hunt *et al.* 1987, Clevenger and Waltho 2000, Apfelbach *et al.* 2005). Lala *et al.* (2022) proposed a similar avoidance pattern in Kenya, suggesting that higher-than-expected usage by apex predators may have resulted in lower-than-expected usage by their common prey species, as well as demonstrating that apex predators were more likely to use underpasses where key prey species visited. Predator-prey interactions may also explain the use of underpasses by mostly solitary mammals in BNR, where larger groups include females and juveniles which tend to be more vigilant (Owen-Smith *et al.* 2020). To address potential deleterious interactions, wider structures are recommended, as well as ensuring there is sufficient cover for prey species, including structural complexity within a passage through use of logs and rocks (Mata *et al.* 2020, Saxena and Habib 2022b).

Despite the infrequent use of non-wildlife underpasses observed by some herbivore species, a certain benefit of these structures as a tool for mitigating railway effects on wildlife is the typical high density of these structures (sampled culverts were 506 m apart on average), which are prevalent across all types of transport infrastructure and are cost effective. Indeed, the Single Large or Several Small (SLOSS) debate of wildlife underpasses concludes that several smaller structures are definitely advantageous, although a high diversity of structure designs is also important to address multiple focal species (Ford *et al.* 2022, Helldin 2022). Unfortunately, the box culverts sampled for this study were relatively small and had little variation in openness.

FACTORS AFFECTING CULVERT USE

Structural factors

Structural dimensions (openness index) did not significantly influence culvert use for any specific trophic group. This is different to most other CS studies, which found that structural dimensions are most important in determining mammal, and especially ungulate use of underpasses, but 1) reflects the adaptability of the prey species that more frequently used these structures (porcupine, scrub hare, warthog, tree squirrel), and 2) is probably due to little variation in the openness of sampled box culverts. It is worth noting that all sampled culverts are smaller than the recommended minimum for ungulates in northern hemisphere regions (Donaldson 2005, Seiler and Olsson 2009, Langbein *et al.* 2011, Bhardwaj *et al.* 2020). Even the double box culvert, whilst having a larger opening (4.6 m height and 11 m width) than other box culverts, was over 20 m in length, which has commonly been seen to deter ungulate use (Denneboom *et al.* 2021), and had a dividing wall down the middle which leads to a more constricted appearance. Only four ungulate visits to this double culvert were recorded during this study. In general, the ungulate species that were observed using box culverts, including warthog, Sharpe's grysbok (*Raphicerus sharpei*), and very occasionally duiker, klipspringer (*Oreotragus oreotragus*) and steenbok, are more cryptic species (Skinner and Chimimba 2013). In a savanna setting in Kenya, with a range of much larger underpasses and viaducts, height was found to be the most important structural predictor for use by all large mammals (Lala *et al.* 2022).

Ground cover also did not appear to influence culvert usage for any trophic group, in apparent contradiction to other studies (Smith 2003, Donaldson 2005, Glista *et al.* 2009, Brunen *et al.* 2020). This observation could be because culverts were all concrete based with varying degrees of a thin layer of substrate accumulated over the years. The viaduct was the only structure with a true natural ground cover in the study area, where more light promotes vegetation growth and could encourage use (Mysłajek *et al.* 2020, Denneboom *et al.* 2021). Covering concrete bases of culverts with a thick layer of soil or sand, as suggested by other studies, could potentially improve or increase use, based on these results.

Vegetation factors

Both guilds of prey species preferred underpasses where there was less herbaceous cover outside, which is similar to findings in Kenya for baboons and antelope species (Lala *et al.* 2022). Apex predators were more likely to use underpasses with less herbaceous cover in the wet season, when grasses are much longer and perhaps hindered culvert discovery; such lower usage of culverts with high herbaceous cover by carnivores has been demonstrated elsewhere (Craveiro *et al.* 2019).

Mesopredators and omnivores preferred underpasses with more woody cover in the area outside the underpass entrance, supporting findings from a number of other studies (Ng *et al.* 2004, Ascensão and Mira 2007, Denneboom *et al.* 2021). Mesoherbivores (mostly porcupine, scrub hare, warthog) did not have a proclivity for more woody cover, despite the potential of greater cover in minimising the risk of ambush for prey species (Saxena and Habib 2022a). Elsewhere, ungulate underpass usage was positively influenced by vegetation cover outside the entrance (Denneboom *et al.* 2021), but because ungulates only accounted for 31% of mesoherbivore usage events (primarily warthog and Sharpe's grysbok) this preference may not have been reflected in model results.

High visibility through an underpass to the habitat on the other side has been demonstrated to be important for ungulates and small mammals (Foster and Humphrey 1995, Donaldson 2005, Glista *et al.* 2009, IENE 2022). Remarkably low visibility was probably the main factor driving the lowest usage rates (especially by prey species) at Site 1, where visibility never exceeded 40% and dropped below 10% in the wet season when vegetation density is highest. Similarly, mesopredators preferred underpasses through which there was higher visibility, as did omnivores (only in the dry season). The few mesoherbivore species that used culverts did not appear to be influenced by visibility levels. In the wet season, omnivores were more likely to use culverts with less visibility, perhaps because tree squirrels were often seen to be foraging inside or near the entrances for fruit.

Environmental factors

Both apex predators as well as omnivores used culverts more frequently in higher temperatures, which may be thermoregulatory behaviour, as culverts typically are a cooler temperature inside (Ascensao & Mira 2007). On a number of occasions, mammals (74% predators) remained inside an underpass for more than 30 minutes during the heat of the day, similar to bobcats (*Lynx rufus*) which used culverts as rest sites (Cain *et al.* 2003).

Apex predators and mesopredators were more likely to use culverts closer to permanent water sources, throughout the year. Mesoherbivores preferred culverts closer to waterpoints during the wet season, whilst preferring culverts further from waterpoints in the dry season. This behaviour may seem counterintuitive, but is perhaps explained by savanna herbivores travelling further to find water or food in the dry season due to decreased forage quality (Abraham *et al.* 2019), whereas in the wet season there is little need to forage much further from permanent water sources. Overall, underpass use was higher in the dry season for apex predators, when greater mobility is typical for leopard and hyaena due to prey scarcity (Vanak *et al.* 2013, Allen *et al.* 2020). Conversely, underpass use was higher in the wet season for omnivores, when woody canopies provide more food for squirrels and nest group sizes are higher (Nyirenda *et al.* 2021). Seasonal usage fluctuations did appear to sometimes be based on random movements of animals (e.g., several hyaena visited a single culvert multiple times over the course of a few days, carrying carrion, presumably due to a nearby carcass).

Distance to riparian corridor did not appear to influence underpass usage for any trophic group. In this study, a riparian corridor was in very close proximity to most culverts (0 – 210 m, Figure 4). and underpasses located near to riparian habitat in Banff National Park experienced higher usage, mostly by carnivores (Clevenger and Waltho 2005). Perhaps the location of the sampled culverts in the landscape, with a nearby riparian corridor, provides further explanation for the frequent use by many carnivore species. Little variation in proximity to riparian habitat may explain the insignificance of this factor in model results.

Rainfall was not a significant factor affecting culvert use for any trophic group, but during the study period there were only two notable rainfall events (35 mm+ in a day) and only shallow puddles of water were seen inside a few culverts for 1-2 days after the rainfall. The presence of water or flooding of culverts in other parts of the world sometimes had a negative effect on mammal usage of these structures (Brunen *et al.* 2020, Ważna *et al.* 2020, Denneboom *et al.* 2021), but other times had little to no effect (Craveiro *et al.* 2019, Alves *et al.* 2021). Indeed, a watercourse through an underpass may encourage wildlife use (IENE 2022). In a semi-arid savanna context, occasional extreme rainfall (sometimes in excess of 100 mm in a day) can cause widespread flooding and typically dry drainage lines to flow with water. Where necessary due to regular flooding, dry ledges can be retrofitted into culverts to encourage year-round usage (Brunen *et al.* 2020, IENE 2022).

Lastly, whilst the daily train traffic volume was included in the best model for each trophic group, it was only significant for mesopredators: culvert use was significantly higher on days when more trains passed through the reserve. As mesopredators were the most frequent users of box culverts, this finding is encouraging, suggesting that these species may change behaviour to use underpasses for a safe passage across the railway when a train is in the vicinity or when there are more frequent trains throughout the day, thereby avoiding risk of collision. However, further research is needed to confirm this behaviour, with more detail on the timing of trains and the immediate response by nearby mammals. Whilst rail traffic is very different in nature to that of roads (Barrientos *et al.* 2019), vehicle traffic on roads is thought to negatively impact underpass use, although animals can become habituated to it (Nojoumi *et al.* 2022). Ungulates are known to flee from oncoming trains (Bhardwaj *et al.* 2022), but little is known about the impact of oncoming trains on underpass use.

CONCLUSIONS & RECOMMENDATIONS

This study demonstrates that:

1) seventy percent of the terrestrial medium and large mammal species known to be locally present used at least one underpass during the study period;
2) eight medium to large African mammal species used non-wildlife passages more than once a day, and a further four species used underpasses at least twice a week, improving the permeability of the railway line for these species;
3) a viaduct design was characterised by almost four times the usage rate of a typical box culvert and was used by twice as many species, including megaherbivores and mesoherbivores;
4) box culverts supported a lower number of species of which the majority were predator guilds;
5) species most affected by rail collisions (including buffalo, impala, giraffe, kudu, elephant and lion) seldom used non-wildlife underpasses;
6) contrary to expectations based on the literature, vegetation and environmental factors influenced usage of box culverts more than structural dimensions;
7) different trophic guilds had very different proclivities for culvert characteristics, but culvert usage by most trophic groups is likely to be enhanced by ensuring that there is high visibility through the structure, with less herbaceous cover and more woody cover outside of entrances;
8) predator guilds more frequently used culverts located closer to waterpoints, whilst culvert usage by mesoherbivores, omnivores and apex predators showed seasonal variation based on typical mobility patterns; and,
9) a railway line with only drainage culverts and the occasional viaduct is not effective in providing adequate safe crossing structures for megaherbivores and most ungulate mesoherbivore species.

In a savanna context where faunal diversity is high and no single species is a target for mitigation of the negative impacts of transport infrastructure, a wide range of underpass designs, including larger viaduct designs as well as structures with an open medium between the railway tracks to allow more light into the tunnel, located in a variety of different types of vegetation cover (thereby combining the range of requirements of different guilds) is recommended. This diversity in crossing structures should enable a safe passage across the railway for the maximum number of species, and will be more effective in preventing unwanted predator-prey interactions (Kintsch *et al.* 2015, Denneboom *et al.* 2021, Ford *et al.* 2022, Lala *et al.* 2022, Saxena and Habib 2022a). However, where a railway is easily traversable, some herbivore species may still not use CSs regularly, as evidenced in this study. For existing ground-level railways where only drainage culverts and occasional viaducts are currently in place, retrofitting culverts to make them wider or higher, or adding an open medium between the tracks to allow light into the centre, or increasing the number of viaduct-like designs, could aid in improving permeability for herbivore species which are more susceptible to train collisions. Furthermore, placing logs or rocks inside of structures or on the approach to underpasses may benefit usage by species that require cover to minimise predation risk (Rodríguez *et al.* 1996, IENE 2022, Saxena and Habib 2022a). Other low-cost modifications include clearing vegetation to achieve higher visibility through underpasses, and covering concrete bases with a natural substrate, which are both likely to improve usage by most species. Barrier fencing, whilst known to be effective in increasing underpass usage and reducing collisions (Huijser *et al.* 2016, Lala *et al.* 2022), is not recommended for this area due to the detrimental effect it would have in increasing the barrier effect of a freight railway line with low traffic volumes.

In designing future research on CS effectiveness in areas with a diverse community of mammals, I recommend that particular species of concern are focused on separately, a range of different underpass sizes and designs are included to assess their effectiveness, and deeper investigation is undertaken with regards to the impact of train traffic on both underpass and railway use. Whilst trophic groups are useful for inference and generalisation across many species, focusing on individual species which are most at risk can derive a deeper understanding of behavioural responses to underpass characteristics. With the forthcoming increase in rail development and train traffic across Africa, the design of pre-development CSs and retrofitting of existing CSs should carefully consider species most at risk, with the understanding that one size does not fit all.

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APPENDIX

Figure A12: *Examples of [A] "crossed" or "entered" event, [B] "refused" event and [C] "pass by" event*

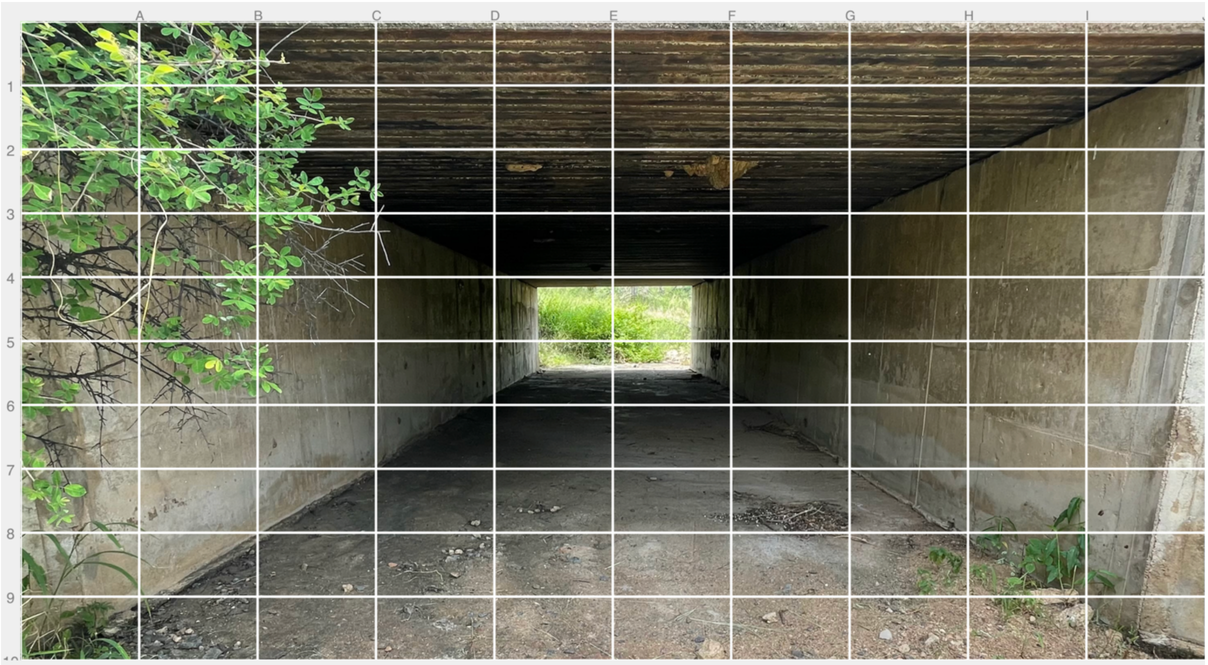

Figure A13*: Example of digital grid overlay method for recording visibility through underpass entrances. A 10 x 10 grid (100 blocks) was overlaid on a photo taken 2 m away from the centre of each underpass entrance. The blocks containing full or majority vegetation were counted as "not visible". The percentage visibility for each entrance was calculated as the number of remaining "visible" blocks out of 100. The visibility for each underpass site in each sampling period is the mean visibility across the two opposite entrances.*

Table A7*: Terrestrial medium and large mammal species known to be present in study area, excluding bats (order* Chiroptera*) and small rodents (order Rodentia) which were unlikely to be detected by cameras. Conservation status is based on the IUCN Regional (South Africa, Lesotho & Swaziland) and Global Red List, where LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN = Endangered, CR = Critical. Species that are threatened with extinction and/or near threatened are highlighted in red. The 2022 census column indicates the BNR annual game census counts for large mammal species, conducted via fixed wing and rotary aerial surveys and a predator census, where N/A means a species was not included in the count (usually due to body size or nature).*

Trophic level	Species (Vernacular)	Species (Common)	Conservation Status		2022 census	Used CSs
			Regional	Global		
Apex predator (3 species)	*Crocuta crocuta*	Spotted hyaena	NT	LC	102	Y
	Panthera leo	Lion	LC	VU	124	Y
	Panthera pardus	Leopard	VU	VU	73	Y
Mega-herbivore (5 species)s	*Ceratotherium simum*	Southern white rhinoceros	NT	NT	Un-disclosed	Y
	Diceros bicornis	Black rhinoceros	CR	CR	Un-disclosed	N
	Giraffa camelopardalis	Giraffe	LC	VU	258	Y
	Hippopotamus amphibius	Hippopotamus	LC	VU	227	Y
	Loxodonta africana	African elephant	LC	EN	1376	Y
	Aepyceros melampus	Impala	LC	LC	7665	Y

Meso-herbivore (18 species)	*Connochaetes taurinus*	Blue wildebeest	LC	LC	22	N
	Equus quagga	Plains zebra	LC	NT	149	N
	Hippotragus niger	Sable antelope	VU	LC	0	N
	Hystrix africaeaustralis	Cape porcupine	LC	LC	N/A	Y
	Kobus ellipsiprymnus	Waterbuck	LC	LC	212	N
	Lepus saxatilis	Scrub hare	LC	LC	N/A	Y
	Oreotragus oreotragus	Klipspringer	LC	LC	9	Y
	Phacochoerus africanus	Common warthog	LC	LC	111	Y
	Potamochoerus larvatus	Bushpig	LC	LC	N/A	Y
	Raphicerus campestris	Steenbok	LC	LC	52	Y
	Raphicerus sharpei	Sharpe's grysbok	LC	LC	30	Y
	Sylvicapra grimmia	Common duiker	LC	LC	37	Y
	Syncerus caffer	African buffalo	LC	NT	621	Y
	Thryonomys swinderianus	Greater cane-rat	LC	LC	N/A	Y
	Tragelaphus angasii	Nyala	LC	LC	40	N
	Tragelaphus strepsiceros	Greater kudu	LC	LC	561	Y
	Tragelaphus sylvaticus	Bushbuck	LC	LC	38	N
Meso-predator (17 species)	*Acinonyx jubatus*	Cheetah	VU	VU	N/A	N
	Canis adustus	Side-striped jackal	LC	LC	N/A	N
	Canis mesomelas	Black-backed jackal	LC	LC	16	Y
	Caracal caracal	Caracal	LC	LC	N/A	N
	Civettictis civetta	African civet	LC	LC	N/A	Y
	Felis silvestris	African wild cat	LC	LC	0	Y
	Genetta genetta	Small-spotted genet	LC	LC	N/A	Y
	Genetta maculata	Common large-spotted genet	LC	LC	N/A	Y
	Helogale parvula	Dwarf mongoose	LC	LC	N/A	Y
	Herpestes sanguineus	Slender mongoose	LC	LC	N/A	Y
	Ichneumia albicauda	White-tailed mongoose	LC	LC	N/A	Y
	Leptailurus serval	Serval	NT	LC	N/A	N
	Lycaon pictus	African wild dog	EN	EN	41	N
	Mellivora capensis	Honey badger	LC	LC	N/A	Y
	Mungos mungo	Banded mongoose	LC	LC	N/A	Y
	Orycteropus afer	Aardvark	LC	LC	N/A	N
	Smutsia temminckii	Temminck's ground pangolin	VU	VU	N/A	N
Omnivore (3 species)	*Chlorocebus pygerythrus*	Vervet monkey	LC	LC	10 troops	Y
	Papio ursinus	Chacma baboon	LC	LC	22 troops	Y
	Paraxerus cepapi	Tree squirrel	LC	LC	N/A	Y