What makes a snake bite? A South Africa perspective

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DECLARATION

I, Hiral Naik (Student number: 452805), declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other University.

Research protocols were cleared by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance number: 2019/04/29/B) and the Human Research Ethics Committee (Non-Medical; clearance number: M21/11/94). Collection of data from the Poison Information Helpline of the Western Cape (PIHWC) was also approved. The AfriTox TeleLog database is registered with the University of Cape Town's Faculty of Health Science Human Research Ethics Committee (R014/2014). Permits to conduct research within Limpopo province of South Africa were received from the Department of Economic Development, Environment and Tourism (permit number: 01777).

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ABSTRACT

Increasing interactions between humans and snakes have raised the profile of human-snake conflict as a primary contributor to the public health challenge of snakebite. In 2017, the World Health Organisation declared snakebite as a neglected tropical disease as human snakebite incidences were reported to reach over 5 million per year globally, with between 81 000 and 138 000 deaths. The tropics have the highest prevalence of snakebite incidences as they have a high diversity of venomous snake species and the presence of more vulnerable human populations. Temperate regions such as South Africa also have a high diversity of snake fauna and although the number of venomous snake species does not outweigh the number of non-venomous snake species, snakebite incidences are reported to occur frequently. My research aimed to increase the knowledge of the driving factors of snakebite incidences in South Africa and investigate the behavioural ecology of some of the medically important snake species responsible for these incidences.

Snakebite appears to be generally well managed in South Africa and assessed datasets show that there are few recorded deaths. This is partly due to the fact that the current healthcare index is ranked highest in Africa. However, snakebite incidences are relatively high and underreported. The available datasets show a prevalence of 5.63 per 100 000 individuals, but this is likely higher due to lack of reporting from the majority of healthcare facilities. As predicted, most incidences take place during warmer months (November – March) in the evening, with males under the age of 30 accounting for the majority of these incidences. Key information regarding the circumstances of incidences is not reported but based on available information, most incidences take place when people are walking and interacting with a snake, as indicated by the large number of bites on hands. Snakebite incidences are also distributed widely across the country, but hotspots occur in areas of high population density such as Johannesburg, Durban and Cape Town, with the vast majority taking place outside of protected areas. The higher numbers of incidences in human populated areas indicate that interactions between people and snakes occur frequently.

During these interactions, different snake species exhibit a range of defensive behaviours. As interactions between humans and snakes increase, gaining insight into the factors that influence these behaviours is crucial. Behavioural experiments on puff adder (*Bitis arietans*), black mamba (*Dendroaspis polylepis*) and snouted cobra (*Naja annulifera*) showed that

snakes are most likely to strike and bite when a threat is in very close proximity (< 50 cm) and although generally quite tolerant of harassment, bites are likely if they are repeatedly provoked. Even *D. polylepis* with its long body length will flee rather than strike when provided the opportunity. However, strike velocities were high for all three species (< 0.4 seconds) indicating that bites can occur quickly. The Mozambique spitting cobra (*Naja mossambica*) relies primarily on its ability to spit rather than strike as its defence mechanism. Since most bites from *N. mossambica* occur in sleeping humans, it is likely that while actively foraging at night, they mistakenly bite people. The behavioural results provide perspective into the activity of African snakes and the human-snake interactions that lead to snakebite incidences.

Evaluating the extent of overlap between people and wildlife is crucial for the conservation of biodiversity and ecological services. Snakebite prevention should be the main priority over treatment, and management procedures can be beneficial in reducing the risk of interactions between humans and snakes. This thesis provides a framework that ranges from broad to specific, focusing on the ecological drivers of snakebite incidences in South Africa and highlighting important considerations for future research. An ecosystem approach is needed to address snakebite, and I propose that large-scale education campaigns should be implemented in South Africa to mitigate snakebite incidences. These findings provide insight into creating protocols for snakebite management and implementation of education and prevention measures.

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THESIS STRUCTURE

This thesis has been divided into six chapters; a general introduction, four data chapters and a conclusion chapter. To facilitate the publication process, the four data chapters have been written in the format of scientific papers. Consequently, repetition of information has been unavoidable but minimised as much as possible. The details of each chapter are provided below:

Chapter 1 provides a general introduction to snakebite as a socio-ecological challenge including a brief overview of past research on the topic and current gaps that will be addressed through the thesis.

Chapter 2 focuses on assessing snakebite incidences in South Africa by collating data from past literature, hospital records, the National Snakebite Database and the AfriTox Telelog database established through the Poison Information Helpline of the Western Cape (PIHWC). The aim of this was to contribute to our knowledge of the actual number of snakebite incidences that are reported in South Africa.

Chapter 3 investigates the spatial patterns of snakebite incidences from the available records in Chapter 2 in relation to the distribution of nine medically important snake species. I further assessed the distribution of snakebite incidences with data on human population density in South Africa and current protected areas.

In Chapter 4, I assess the defensive behaviours of four medically important snake species: puff adder (*Bitis arietans*), black mamba (*Dendroaspis polylepis*), snouted cobra (*Naja annulifera*) and Mozambique spitting cobra (*Naja mossambica*), to investigate factors contributing to their biting behaviours.

In Chapter 5, I investigate factors that might contribute to bites in sleeping humans from *N. mossambica*. Individuals were presented with various chemical cues in a testing arena to assess whether behaviours were predatory or defensive.

Chapter 6 provides an overarching summary of the thesis and recommendations of how this research contributes towards mitigating snakebite incidences and human-snake conflict.

PUBLICATIONS AND PRESENTATIONS

Manuscripts published:

Chapter 2

Naik, H. and Alexander, G.J. 2025. The incidence of snakebite in South Africa and the challenges associated with lack of reporting. Transactions of the Royal Society of Tropical Medicine and Hygiene, trae109, 1–8.

Manuscripts in preparation:

Chapter 3

Naik, H. and Alexander, G.J. The spatial distribution of snakebite incidences in South Africa for nine venomous snake species. (In preparation).

Chapter 4

Naik, H. and Alexander, G.J. Determinants of striking behaviour in four South African medically important snake species. (In preparation).

Chapter 5

Naik, H. and Alexander, G.J. Unveiling the allure: Chemical cue investigation in Mozambique spitting cobra (*Naja mossambica*). (In preparation).

Other publications during candidature:

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Barends, J.M. and Naik, H. 2023. Body size predicts prey preference but not diet breadth in pythons. Journal of Zoology. 321(1): 50–58

Bickford, D., Garda, A., K.S. Seshadri, Arifin, U., Naik, H., Ghaffari, H. and Safaei-Mahroo, B. 2024. Amphibians and reptiles of forest ecosystems, in Peh, S.H.K., Corlett, R.T. and Bergeron, Y. (Eds), *Routledge handbook of forest ecology*. Taylor and Francis, Routledge, New York, USA. Pp. 314–326.

Conference presentations

Naik, H. and Alexander, G.J. 2023. What makes a snake bite? A South Africa perspective. 15^{th} Conference of the Herpetological Association of Africa. Hoedspruit, South Africa.

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Chapter 1: Snakebite: A socio-ecological challenge

1.1 What is snakebite?

People and wildlife have lived in close proximity to each other for millennia, however as human populations rapidly increase, adjacent to natural spaces, there is a higher risk of increased conflict between people and wildlife (Hampson et al. 2015; Mekonen 2020). Extensive efforts have been made to address and manage conflict situations, but these tend to be dynamic and complex and require an in-depth knowledge of animal behaviour. Venomous snakes are among the few wildlife groups that are most feared and pose a direct threat to humans (Glaudas et al. 2005). Within the context of human-snake conflict, snakes will typically feel threatened and respond by fleeing or directly confronting humans and defending themselves (Swaisgood et al. 1999). When these confrontations occur between humans and venomous snakes, there is a potential outcome of envenomation.

Envenomation results from the injection of venom (consisting of many toxins) from a snake into another living organism (Gutiérrez et al. 2017), usually through fangs. Venoms are complex and when they enter the human body through puncture wounds, they can lead to serious health problems and can be life threatening (Chippaux 2017). As human populations have increased, conflicts with snakes have increased and as a result, snakebite incidences have been on the rise. In 2017, the World Health Organisation declared snakebite as a neglected tropical disease after being removed from this list in 2013. This came after various stakeholders from around the world advocated for the need to address challenges regarding the accessibility of antivenom, education of at-risk populations and training of healthcare professionals in regions that are most affected by snakebite (Chippaux 2017).

Snakebite affects millions of people globally (Chippaux 2017; WHO 2017), hundreds of thousands of people in sub-Saharan Africa (Chippaux et al. 2011; Halilu et al. 2019) and has significant socio-economic implications on vulnerable sectors of society. The first worldwide assessment of snakebite was conducted by Swaroop and Grab (1954) estimating that deaths from venomous snake species were between 30 000 and 40 000 annually. This was a gross underestimate due to lack of information, and it was only in 1998 when a second assessment was done showing that the actual number is closer to 81 000 – 138 000 deaths per year (Gutiérrez et al. 2017). Seventy years later, we are still facing challenges with regard to collecting more accurate information on snakebite, while interactions between people and snakes have significantly increased (Gutiérrez et al. 2017).

Although snakebite incidences occur around the world, they vary geographically and temporally, with vulnerable humans living in the tropics and subtropical regions of the globe being most affected (Feitosa et al. 2015) due to lack of resources rather than the diversity of venomous snakes (Luiselli et al. 2020). In Africa, the most vulnerable populations reside in Central and West Africa (Longbottom et al. 2018) where hundreds of thousands of people are bitten per year, particularly those living in rural areas. The prevalence and severity of snakebite are largely related to anthropic factors (poverty level, agricultural activities, fluctuations in the population at risk) and the biology of snakes (the snake species, population ecology, distribution, activity patterns, behaviour, and movement ecology) (Pandey 2016). Historically, there has been a disconnect between studies assessing snakebite epidemiology and those evaluating snake behaviour and ecology. In Africa, studies on both anthropic factors and the biology of snakes in relation to snakebite are rare (Akani et al. 2013). Thus, no clear patterns of incidences exist to create mitigation measures or treat snakebite incidences.

Timely administration of appropriate antivenom is the most cost effective treatment for snakebite envenomation (Winkel et al. 2006). It is manufactured by collecting hyperimmune plasma from domestic animals, typically sheep or horses, that have been immunised with the venom from either a single species (monospecific) or multiple species (polyspecific) (Bénard-Valle et al. 2015; Fitzsimons 1912; Gutiérrez 2019). Although approximately 46 laboratories globally produce antivenoms extracted from animals by one of three purification techniques (Gutiérrez 2019), the inadequate availability of accurate epidemiological data in many regions leads to an underestimation of the challenge of snakebite and subsequently limits the attention it receives (Gutiérrez 2019). Trained medical professionals can assist in the recovery of patients who have been envenomated by symptomatic treatment but in severe cases, if antivenom is not administered within several hours of a bite, the likelihood of death increases (Ralph et al. 2022).

While the symptomatic effects of different snake venoms vary, the typical protocol is to immediately get to a hospital. Antivenom exists for most medically important snake species but access to antivenom is often limited in most remote and poor areas with insufficient healthcare systems (Hansson et al. 2013). The tragedy of snakebite is not only the resultant deaths but rather the contingencies of scenarios leading up to the snakebite which relate more to the behaviours of snakes and humans. For many years, the focus on mitigating snakebite has been on the medical management of snakebite, including antivenom development and assessing the properties of various snake venoms. However, it is becoming apparent that to reduce snakebite incidences, the focus should shift to collating high-quality information on the distribution of venomous snake species, their behaviour, abundance, and habitat preferences, to create targeted prevention measures through education and outreach initiatives, improve burden estimation, and monitor progress (Malhotra et al. 2021; Murray et al. 2020).

1.2 Snakebite through the ages

Much of the research in the past has focused on improving knowledge of snakebite epidemiology (Chippaux 2011), while few studies have focused on the ecological aspects of snakebite. Only in the last five years has more awareness shifted to identifying factors that might contribute towards snakebite. Due to the listing (2009), delisting (2013) and relisting (2017) status of snakebite on the World Health Organisation's (WHO) list of neglected tropical diseases, priorities given to snakebite research have changed. Since Swaroop and Grab's (1954) first review on snakebite epidemiology on a global scale, it took over forty years to shed light on snakebite from a global perspective again. Chippaux (1998) highlighted the disparity in the incidence of snakebite in the different regions of the world and indicated that the true number exceeded 2.5 million per year, most likely because human populations and habitat transformation have increased.

Since 2017, there has been an increased interest in overcoming the challenges of snakebite incidences and thus more research on snakebite epidemiology has been conducted in regions such as the USA (Ruha et al. 2017), Brazil (Tavares et al. 2017), Costa Rica (Sasa and Cano 2020), Cameroon and Nepal (Alcoba et al. 2021) and other parts of the world (Musah et al. 2019). It is evident from this research that some countries are more effective than others when reporting snakebite incidences, largely due to better healthcare systems to record and treat incidences. Countries in Asia and Central Africa, which often have the greatest snakebite mortality, have poor health system capacity and experience socioeconomic vulnerability. Thus, they have poor capabilities to report snakebite incidences (Roberts et al. 2022). Although some recent research from Eswatini (Padidar et al. 2023), Mozambique (Farooq et al. 2022) and Rwanda (Hakizamana et al. 2024) have been significant contributions towards the field. The consensus has been to work towards creating large global health repositories to better record the incidence of snakebite at the regional and global scale (Pintor et al. 2021) but additional environmental and social factors also need to be considered.

Ecological niche modelling has proven to be very useful in wildlife research to predict suitable habitats for species under various environmental conditions and to estimate their true geographic distributions (Peterson et al. 2015). Various studies have attempted to assess the impact of snakebite through species distribution modelling (Longbottom et al. 2018; Yousefi et al. 2020), risk mapping (Ediriweera et al. 2016; Ediriweera et al. 2018; Padidar et al. 2023), mathematical modelling (Bravo-Vega et al. 2019; Goldstein et al. 2021) and climatic modelling on venomous snakes (Zacarias and Loyola 2019). These studies have provided comprehensive approaches to assessing the attributes of the incidence of snakebite. Yousefi et al. (2020) modelled the distributions of venomous snakes and human populations in Iran, an exercise which was shown to be useful in snakebite management. Such model-based approaches have also been used to create risk maps for an entire country (Ediriweera et al. 2016). Model-based studies have also shown close associations between the incidence of snakebite and environmental variables (Yañez-Arenas et al. 2016; Zacarias and Loyola 2019) and the implications of this for the future. As environmental factors drive the distribution and location of suitable habitats for species and species occurrence, it is important to consider the effect of this on the incidence of snakebite, particularly at a regional level. Many of the model-based approaches can be used as a foundation for identifying high-risk areas, however, these should be confirmed by incidence records from healthcare systems and more importantly improved ensembled models that are supported by fieldwork data (Entiauspe-Neto et al. 2024).

Community-based household surveys have recently been conducted in various parts of the world including India (Suraweera et al. 2020), Sri Lanka (Ediriweera et al. 2016), Nepal (Alcoba et al. 2022) and Mozambique (Farooq et al. 2022) to assess the burden of snakebite through targeted data collection. Such studies have provided insight into the pitfalls that can exist when collecting data on the incidence of snakebite and creating methods to better predict the incidence in the future. One of the most useful papers on snakebite (Longbottom et al. 2018) sought to highlight the world’s most vulnerable populations in relation to snakebite, with a specific focus on the value of data collection to make informative predictions. Longbottom et al. (2018) were the first to produce maps that show countries and regions that are most affected by snakebite and the factors that contribute to making these regions vulnerable. Robert et al. (2022) provide further support and show similar trends in the estimations of where the most vulnerable populations exist and their risk to snakebite. However, few have researched the relationship between snakes and people within these vulnerable regions.

While environmental factors and biogeographic history dictate where snakes are found, identifying the morphological and functional traits that influence the probability of snakebite incidences are as important. Our knowledge of the composition of venoms of different snakes has advanced our ability to manufacture effective antivenoms (Williams et al. 2011). The primary function of snake venoms is the immobilisation of prey (Barlow et al. 2009) but besides foraging, snakes also use venom to defend themselves against predators (Casewell et al. 2020). Research has focused on venom variation as it directly affects the efficacy of antivenoms and the management of bites. However, many recent studies have also shown that within a species, venoms can vary across the geographic distribution and age of a snake (Daltry et al. 1996), adding further complexity to the manufacture of antivenoms (Casewell et al. 2020; Gutiérrez et al. 2017). The mixture of proteinaceous components that make up snake venoms exert a variety of effects on the body of an envenomed organism. Thus, snakebite victims experience a variety of effects from the toxins on their body (Slagboom et al. 2017). This complexity results in the impact of envenomation being complex and difficult to manage effectively.

Venom and the development of the venom system has played a crucial role in the diversity and evolutionary success of snakes, and subsequently their feeding ecology (Scanlon and Lee 2011). Several authors have shown that adaptation to prey specific diets can cause geographic variations in snake venoms. Some of these variations are due to selection for feeding on local prey (Daltry et al. 1996; Jackson et al. 2019) and other variations are influenced by various environmental factors such as climate and elevation (Holding et al. 2021). By investigating the evolutionary pressures that shape the activity of snake venoms and venom phenotype in different parts of the world (Panagides et al. 2017), we are likely to gain insight into the relationship of the behaviour of snakes, particularly snake feeding ecology and venom activity. Integration of this knowledge could contribute to creating more effective antivenoms. When considering the role of defence in venom evolution, several studies have shown that specific toxins that cause pain can suggest selection for defence in some snakes (Bohlen et al. 2011; Zhang et al. 2017) but this can be ineffective at the onset of an envenomation (Ward-Smith et al. 2020). In African spitting cobras (*Naja*: subgenus *Afronaja*), Asian spitting cobras (*Naja*: subgenus *Naja*) and rinkhals (*Hemachatus haemachatus*), the three independent instances of the evolution of defensive spitting were accompanied by identical shifts in venom composition and increased pain-inducing activity (Kazandjian et al. 2021). Thus far, these are the only instances of defensive venom traits in the form of fangs adapted to spray the eyes of potential aggressors (Panagides et al. 2017).

Although studies on the role of venom and defence in snakes is relatively limited, several studies have investigated the defensive striking behaviour of snakes (Glaudas 2004; Glaudas et al. 2005; Herzog et al. 1989; Whitaker et al. 2000) and how this may relate to humans as potential 'predators' to snakes. Whitaker and Shine (1999) showed that Australian brown snakes (*Pseudonaja textilis*) would avoid encounters with humans by slithering away to reduce their risk of being detected. Their findings also suggested that when observers wore darker shades of clothing, which more strongly contrasted the sky and surrounding environments, encounters with snakes were also reduced as the snake had a higher probability of moving away. Defensive behaviour has also been studied in cottonmouths (Gibbons and Dorcas 2002; Glaudas 2004; Glaudas and Gibbons 2005; Glaudas and Winne 2007; Glaudas et al. 2005; Roth and Johnson 2004), rat snakes (Penning et al. 2020), and prairie rattlesnakes (Goode and Duvall 1989), each providing insight into behavioural displays that snakes exhibit to warn off predators. Studies have shown that striking ability does not differ significantly between non-venomous and venomous snakes (Penning et al. 2020) and higher body temperatures can cause more intensive defensive striking behaviour (Whitford et al. 2020) but does not impact predatory striking (Whitford et al. 2019). These studies provide insight into the factors that influence the danger encounters with snakes present to humans.

Radio-telemetry studies have been used to study the behaviour and ecology of snakes in the field for many years (Boback et al. 2020; Reinert and Cundall 1982; Újvári and Korsós 2000). These studies have also been proposed to be useful in evaluating human-snake interactions and preventing conflict situations (Devan-Song et al. 2016; Glaudas 2021; Maida et al. 2020). Spatial ecology studies have also investigated the temporal activity patterns of venomous snake species to identify when human-snake encounters would increase (Waldron et al. 2013; Whitaker and Shine 2002). Other studies have shown that improving knowledge of the habitat use and selection by snake species, may help reduce the number of encounters between people and snakes, either by deterring snakes from human inhabited areas (Carter et al. 2014) or keeping humans away from snakes (Fraga et al. 2013). There is still a need for more studies to evaluate conflict prevention strategies through radio telemetry, some of which are taking place in Thailand (Hodges et al. 2021). As radio-telemetry studies can be expensive to implement, other avenues to study these interactions and to collect information on these encounters are necessary.

As human populations continue to grow, encounters with snakes increase and thus bites are more likely to occur. Snakebite case reports for different venomous snake species globally have been reported for countries such as Vietnam (Patra and Mukherjee 2021), India (Suraweera et al. 2020) and the Americas (Bravo-Vega et al. 2019; Chippaux 2017). In Africa, reports cover snakebite incidence in Kenya (Erulu et al. 2018), Ghana (Musah et al. 2019), Uganda (Lang et al. 2020) and Zimbabwe (Nhachi and Kasilo 1994). Within South Africa, case studies of snakebite have also been reported by several authors (Blaylock 2004; Chippaux 2011; Krüger and Lemke 2019; Ogunbanjo et al. 2016; Pattinson et al. 2017; Verburgt et al. 2018; Wagener 2016; Wood et al. 2017). These reports suggest that there are only a few accounts that are published in various journals and many other accounts of snakebite are likely not published.

1.3 Snakebite in South Africa

Although snakebite is not a reportable cause of death in South Africa (Wood et al. 2017) anecdotal evidence suggests that it is the cause of at least a few deaths annually (Govender and Tumbo 2019). Several reported cases of snakebite incidences have been published in the past (Blaylock 2004; Coetzer and Tilbury 1982; McNally and Reitz 1987; Tilbury 1982) and a few more recent publications since snakebite first became a neglected tropical disease (Ogunbanjo 2009; Pattinson et al. 2017; Wagener 2016; Wood et al. 2009; Wood et al. 2016). Overall, the literature on snakebite is scant. These studies have provided insight into some of the snake species that are commonly featured in snakebite in South Africa and the hospitals where snakebite patients have received treatment. However, there is little or no content on the circumstances that caused or led to the bite. This is likely because of the historical disconnect between the disciplines of medicine and the natural sciences and the limited knowledge that medical practitioners have of snakes in South Africa.

There are 114 snake species in South Africa (Tolley et al. 2023), of which not more than 11% are potentially dangerous to humans (Marais 2022). The WHO has classified highly venomous snakes into two categories. Category one includes species of high medical importance, as they are highly venomous, common and/or widespread, and cause numerous incidences of snakebite that result in death or disability. Category two includes medically important snake species that can cause morbidity, death, or disability but for which: 1) there is a lack of robust epidemiological data, 2) feature less in snakebite incidences due to their behaviour and activity, small distribution range or specific habitat preferences (WHO 2017). In South Africa, the medically important Category 1 species include puff adder (*Bitis arietans*), eastern green mamba (*Dendroaspis angusticeps*), black mamba (*Dendroaspis polylepis*), snouted cobra (*Naja annulifera*), Mozambique spitting cobra (*Naja mossambica*), cape cobra (*Naja nivea*) and Category 2 species include Bibron's stiletto snake (*Atractaspis bibronii*), gaboon adder (*Bitis gabonica*), boomslang (*Dispholidus typus*), rinkhals (*Hemachatus haemachatus*), forest cobra (*Naja subfulva*), black spitting cobra (*Naja nigricincta*) and southern vine snake (*Thelotornis capensis*) (WHO 2017).

Of the medically important snake species, two commonly featured species in snakebite incidences in South Africa are *B. arietans* and *N. mossambica* (Wood et al. 2017). The former is a viperid and the latter an elapid, both of which have potent cytotoxic venom (Wood et al. 2017). Medically important snake species that are most capable of causing human fatalities include *D. polylepis* and *N. nivea*, both of which have potent neurotoxic venoms (Marais 2022). South Africa produces a polyvalent antivenom to treat bites from 10 of the medically important snake species (*B. arietans*, *B. gabonica*, *H. haemachatus*, *D. angusticeps*, *D. jamesoni*, *D. polylepis*, *N. nivea*, *N. melanoleuca*, *N. annulifera*, *N. mossambica*) (Pantanowitz et al. 1998). One snake that can cause fatal bleeding from bites that is not included in the polyvalent antivenom is *D. typus* (Pantanowitz et al. 1998). It is a colubrid snake with a haemotoxic venom, which has coagulotoxic effects but rarely is it featured in snakebite related deaths (Wood et al. 2017). Due to the potency of its venom, a monovalent antivenom is used to treat bites from *D. typus*. Another medically significant colubrid snake is *T. capensis*. Although its venom composition is similar to that of the *D. typus*, bites from *T. capensis* cannot be treated with the same monovalent antivenom used *D. typus* (Debono et al. 2017). Thus, the variation in snake venoms can cause complications for snakebite treatment. However, the availability of antivenom in South Africa has been useful to treat snakebite incidences.

The development and production of snakebite antivenom was first pioneered by Mitchell and Watkins-Pitchford in 1916 after Albert Calmette's international contribution in 1895. However, several contributors including F.W. FitzSimons contributed towards an evolved and improved antivenom in South Africa (Pantanowitz et al. 1998). Despite the limited knowledge at the time, these early contributions towards antivenom development created the protocols that are still used today including the initial injection of a high dose of serum, estimated danger periods from bites (24 – 48 hours), allergy to snakebite serum and the assistance of artificial respiration in treatments (Fitzsimons 1912). Antivenom production began at the South African Institute for Medical Research (SAIMR) in 1928, and several domesticated animals were used as test subjects. After the improvement of antivenom serum over the years and the incorporation of new species, the South African Vaccine Producers (SAVP), a subsidiary of the SAIMR, became best known for their efficacy in antivenom efficacy (Pantanowitz et al. 1998). SAVP currently produces the only antivenoms to treat incidences of snakebite in South Africa and recently, there has been a shortage of antivenom in South Africa resulting in the use of other snake antivenoms produced by other manufacturers receiving approval for use in South Africa through the Section 21 of the Medicines and Related Substances Act of 1965. Thus, to reduce the reliance on antivenom and treatment, there is a need to focus more on the ecological factors that contribute towards the distribution of medically important snake species in South Africa and the contributing factors towards snakebite incidences.

1.4 Current knowledge gaps

Many of the current themes in the snakebite literature focus on broadscale modelling in an attempt to gain a global perspective on the incidence of snakebite. The challenge with global studies is that the approach is often generalised and is useful only in a foundational context. While Longbottom et al. (2018) provides useful statistics on the distribution of venomous snakes in relation to healthcare systems globally, consideration of the ecological and behavioural traits of snake species that are responsible for the incidence of venomous bites is missing from the analysis. Goldstein et al. (2021) overcame these shortfalls as they used agent-based models to integrate data on both snake ecology and human behaviour in high-risk landscapes in Sri Lanka, and they demonstrated the importance of focusing on both environmental and human factors. Thus, by evaluating current knowledge about snake distributions and ecology, it is possible to better mitigate and avoid snakebite incidences. However, this needs to be done for all countries where the snakebite burden is high.

Studies that have focused on identifying the properties of venom from various medically important snake species (Panagides et al. 2017; Bittenbinder et al. 2018; Bittenbinder et al. 2019; Harris et al. 2020; Oh et al. 2017; Williams et al. 2011) have been limited to only providing knowledge from a functional perspective. This information is very important in the use of venom for medicinal purposes, but there is a requirement for more studies on the general ecology and defensive behaviour of snakes. Some ecological studies have been conducted on medically important snake species such as *Bothrops* (Bisneto et al. 2019; Martins et al. 2001; Monteiro et al. 2006), but equivalent species-specific studies in Africa and Asia are limited. For example, there are few ecological studies on *Bungarus* species in Asia (Goldstein et al. 2021; Pandey et al. 2020) with one recent study on *Daboia russelii* (Glaudas 2021). While in Africa, the only ecological studies are on *Bitis arietans* (Glaudas and Alexander 2016; Glaudas and Alexander 2017; Glaudas et al. 2017). There are also knowledge gaps of how the behavioural and ecological traits of snakes may differ across the ranges of those that span several regions (e.g., southern Africa vs. Central Africa). It is evident that efforts need to be placed on improving our knowledge of snake ecology and prevention to alleviate the pressure on current health systems, particularly in areas with the most vulnerable populations to snakebite which are already under pressure to mobilise expensive antivenoms.

A crucial aspect of dissecting the factors leading to snakebite is evaluating the relationship between snake and human activities in areas of overlap (Ediriweera et al. 2018; Goldstein et al. 2021; Longkumer et al. 2016) especially on farms which are hotspots for human-snake conflict and snakebite incidences (Gutiérrez et al. 2017; Malhotra et al. 2021). However, very few studies have assessed the risk factors of snakebite by including information on snake ecology (Akani et al. 2013; Angarita-Gerlein 2017; Nori et al. 2014; Yañez-Arenas et al. 2014; Yañez- Arenas et al. 2016) and the socio-ecological implications to at-risk communities. Studies that focus on the themes of activity, space use and foraging ecology can be particularly useful in learning about human-venomous snake conflict (Glaudas 2021) and these data can be collected through snakebite reports that indicate the snake taxa responsible for the bite. It is also unknown why certain species of cobras are renowned for unprovoked bites in sleeping humans (Natusch et al. 2021) indicating that there is a need for improved data on specific behaviours.

1.5 Problem statement

Snakebite incidences occur when there is an interaction between two different species, humans and snakes. Any process or factor that influences human-snake encounters including incidence and outcomes, will affect its epidemiology (Martín et al. 2022). Much of the previous research has focused on creating antivenoms, snakebite treatment and reducing the burden on current health systems. However, the WHO's goal of reducing snakebite mortality and disability by half by 2030 will only be attainable by improving knowledge on the ecology of venomous snakes including their geographic variation, the relationship between humans, snakes and snakebite frequency and the collection of high-quality data on the behaviour of snakes in relation to snakebite (Glaudas 2021). Some recent studies have attempted to gain perspective on the circumstances of biting incidences, but this is likely to differ in different countries and regions. To mitigate the multifactorial snakebite health crisis, a biological lens is necessary. Sub-Saharan Africa has been reported to face challenges such as shortcomings in the capacity of the current health systems and inaccessibility to medical facilities in rural community and these challenges also exist in South Africa (Roberts et al. 2022). My thesis attempts to address some of the current gaps in knowledge of snakebite within a South African context with the goal of creating prevention measures that can be implemented not only in South Africa but throughout Africa.

1.6 Aims and Objectives

In this thesis, I aimed to investigate the spatial and temporal occurrence of snakebite in South Africa and identify factors that impact the current patterns, and associated challenges and risks. I also investigated the behavioural responses exhibited by various medically important snake species when they are presented with chemical and visual stimuli and interpreted these observations in the context of mitigation and prevention measures for snakebite. Below are my specific aims and objectives:

1.6.1. Assess the incidence of snakebite in South Africa

1. Assess and synthesize data from available records on incidence of snakebite
2. Investigate the conditions under which snakebite incidences have taken place including when and how they occurred

1.6.2. Map the spatial distribution of snakebite incidences from nine medically important snake species in South Africa

1. Produce geographic distribution maps of snakebite incidences and species occurrence records
2. Identify factors contributing towards spatial distribution
3. Compare the spatial distribution of incidences in relation to human population density and protected areas
4. Describe as precisely as possible the ecological characteristics of incidences of snakebite in South Africa to assist in creating prevention strategies for snakebite

1.6.3. Investigate the defensive behaviour of various South African venomous snake species

1. Assess and compare the defensive striking behaviour of four venomous snake species commonly featured in snakebite incidences
2. Investigate the attraction of Mozambique spitting cobra (*Naja mossambica*) to various chemical cues that elicit biting
3. Identify the importance of snake behaviours in snakebite prevention

My thesis provides the first country-level assessment of snakebite in South Africa that places considerable focus on the ecological driving factors of snakebite incidences. It significantly advances knowledge of the behaviour of medically important venomous snake species in South Africa and the implications of this for snakebite management in Africa and globally. This thesis aims to use the evidence from the data to create effective prevention measures by improving knowledge of snakes and snakebite incidences. These prevention measures will be implemented in existing education and outreach initiatives to promote snake conservation. This is a unified body of work, and each chapter works coherently with the other.

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Chapter 2: The incidence of snakebite in South Africa and the challenges associated with lack of reporting

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2.1 Abstract

Snakebite is a public health challenge that has a substantial impact on humans and snakes. Globally, millions of people every year are affected by snakebite but there is a paucity of detailed data on snakebite incidences. The aim of this study was to assess the patterns of snakebite incidences in South Africa. I collected data from hospital records, information from the National Snakebite Database and records from the AfriTox Telelog database from 2011 to 2024. The combined dataset showed that 3496 snakebite incidences have been recorded over 12.5 years. The prevalence for the 12.5 years was calculated to be 5.63 per 100 000 individuals in the population of South Africa, with the highest prevalence in KwaZulu Natal (8.83). Males made up 65.9% of snakebite incidences and most incidences were recorded between November and March. The snake species responsible for most snakebite incidences was *Bitis arietans*. Snakebite is generally well managed in South Africa with few recorded deaths despite the relatively high number of bites. However, incidents are often not recorded or have missing information particularly regarding the circumstances of the bite. This study provides insight into the snake species responsible for incidences in South Africa and can be used to create prevention measures.

2.2 Introduction

Snakebite is a public health issue which is currently estimated to affect 2.5 million people globally each year (Gutiérrez et al. 2017; Kasturiratne et al. 2008). Negative interactions between humans and snakes increase the probability of snakebite incidences, especially in cases involving venomous species, which can result in envenomation (Pintor et al. 2021). Envenomation can lead to death, but if snakebite victims receive medical care and antivenom, they are likely to survive (Kasturiratne et al. 2008). Besides death, permanent disability can result from necrosis, nerve damage and venom ophthalmia, leading to long-term psychological consequences of snakebite (Kasturiratne et al. 2021). Having declared snakebite as a neglected tropical disease, the World Health Organisation (WHO) has developed a strategy for the prevention and control of envenoming and to reduce deaths and disabilities due to snakebite by 50% by 2030 (WHO 2017). This has raised the profile of snakebite as a One Health challenge that needs to be addressed from a transdisciplinary approach (Martins et al. 2022).

One Health approaches require the compilation of reliable information on the incidence of snakebite and resulting rates of mortality (Gutiérrez et al. 2010), information on the behaviour and ecology of medically important venomous snake species and prevention measures implemented at the community level (Gutiérrez et al. 2021; Malhotra et al. 2021). However, past research has focused mostly on the treatment of snakebite and not the ecology of snakes in relation to snakebite (Gutiérrez et al. 2010; Kasturiratne et al. 2008; Kasturiratne et al. 2021). The complexity and context of snakebite vary across global and regional landscapes due to the diversity and distribution of venomous snakes and this can have several implications for rural populations (Gutiérrez et al. 2021). Only recently have studies showcased the snake species involved in snakebite incidences and the impact on the demographics of the population (Gutiérrez et al. 2021, Suraweera et al. 2020). Various studies have also highlighted regional hotspots for snakebite, including India (Suraweera et al. 2020), Central Africa (Chippaux 2011), south-east Asia and parts of Central and South America (Longbottom et al. 2018). However, no studies have focused exclusively on southern Africa.

Snakebite incidents in South Africa are relatively common (Wood et al. 2016). However, snakebite-related deaths are lower than in tropical, low GDP countries (Buitendag et al. 2022). This is due largely to the availability of antivenom that has been produced by the South African Institute for Medical Research (SAIMR) and manufactured by the South African Vaccine Producers (SAVP) (Pantanowitz et al. 1998). Antivenom in South Africa has been developed and improved since 1916 (Govender and Tambo 2019), and thus there has been a long history of effective treatment of snakebite in South Africa. Despite this long-standing investment in treatment, only some provincial studies on epidemiology have been conducted in the past (Blaylock 2004, Coetzer and Tilbury 1982; Wood et al. 2016), but the actual patterns of snakebite at the national scale have remained undocumented. Of the 114 snake species in South Africa (Tolley et al. 2023), approximately 16 can cause significant harm to humans through envenomation and have been categorised by the WHO (WHO 2017) according to the impact of their venom. Medically important Category-1 South African snake species include *Dendroaspis angusticeps*, *Dendroaspis polylepis*, *Naja annulifera*, *Naja nivea*, *Naja mossambica* and *Bitis arietans*. Most of these snake species are widespread, occurring over large parts of southern Africa and feature commonly in biting incidents (WHO 2017). Category-2 species include *Atractaspis bibronii, Dispholidus typus, Thelotornis capensis*, *Hemachatus haemachatus*, *Naja subfulva*, *Naja nigricincta woodi* and *Bitis gabonica*. These species are featured less often in snakebite incidences, and some have smaller distributions than Category-1 snakes (WHO 2017).

Snakebite in South Africa occurs in two scenarios, which are categorised as ‘legitimate’ and ‘illegitimate’ (Muller et al. 2012). Legitimate bites are defined as incidents where a person unknowingly and unintentionally provokes a snake, while illegitimate bites are defined as incidents where an individual sees a snake and attempts to catch or kill it, or otherwise interact with it, resulting in a bite (Muller et al. 2012). Illegitimate bites typically occur in snake keepers, breeders or snake-removers who are intentionally interacting with venomous snakes (Curry et al. 1989). The circumstances under which legitimate bites occur are often unknown and the snake species responsible is also often not identified. Thus, medical experts use a syndromic approach to diagnose and manage snakebite in South Africa (Blaylock 2005; Wood et al. 2016), instead of relying on snake identification.

The activity of snakes and their movement patterns primarily impact the circumstances leading to a bite and spatial variability in incidences (Malhotra et al. 2021). These incidences of snakebite are highly dependent on environmental factors such as climate and habitat, and human demographics (Martín et al. 2022), all of which are undergoing change. This study aimed to assess the current incidence of snakebite in South Africa and evaluated the differences across a spatial and demographic landscape with the goal of identifying the snake species responsible for the highest number of bites and creating targeted prevention measures.

Three data sources were compared to provide insight into provinces that are at the highest risk for snakebite incidences and how these incidents might occur.

2.3 Materials and Methods

Three data sources were used to assess the incidence of snakebite in South Africa: hospital records from 36 of 754 private and public hospitals (Niohuru 2023), information from the recently established National Snakebite Database (NSBD) and records from the AfriTox Telelog database established through the Poison Information Helpline of the Western Cape (PIHWC) (Stephen et al. 2016). Only a subset of the hospitals provided data on snakebite incidences as some hospitals that were approached did not have the required data, did not respond to my request or did not grant access to their data.

2.3.1 Hospital Data

A retrospective, cross-sectional study was conducted on data from 36 hospitals across South Africa from August 2011 to December 2022. The following variables were extracted from records: date, time of day when person was bitten, province, patient age, sex, ethnic group, snake species responsible, site of the bite on the body, and patient activity at the time of the bite (e.g., walking). Patient age was categorised into ten bins: 0–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90 and 91–100. Dates were grouped by month, and time of day was grouped into early morning (midnight to 6:00 am), mid-morning (6:00 am to midday), afternoon (midday to 6:00 pm) and night (6:00 pm to midnight). Ethnic groups were categorised according to African (defined as Black African), Coloured (multiracial group), White and Indian (Include other Asian groups).

2.3.2 AfriTox Telelog database

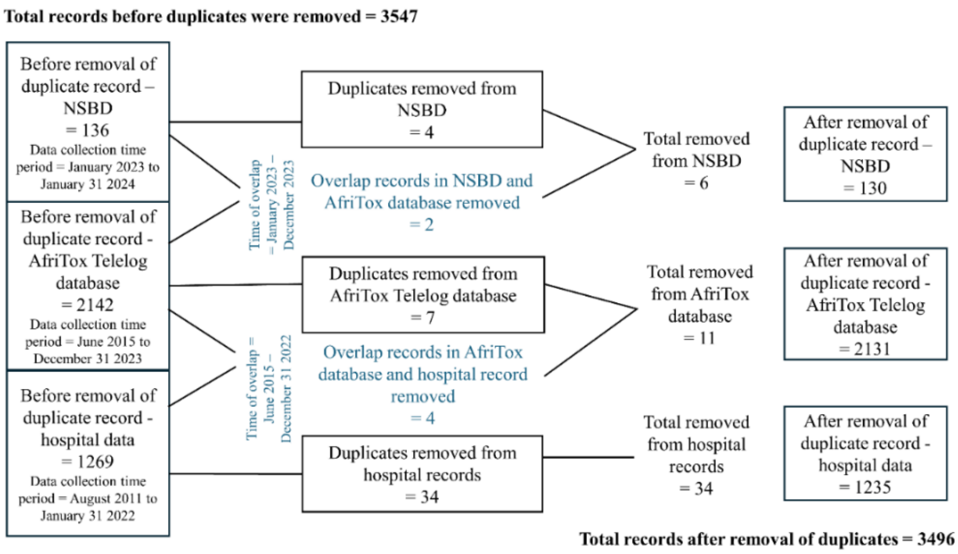
A retrospective, cross-sectional study was conducted on data downloaded from the AfriTox Telelog database consisting of calls received by the PIHWC over a nine-year period from 1 June 2015 to 31 December 2023. Data were received on January 12, 2024 for analysis. The PIHWC is a 24-hour helpline service for the public to report on poisoning or envenomation events. The centre is part of the University of Cape Town's Department of Paediatrics and Child Health, and it is currently situated at the Red Cross Children's Hospital. The PIHWC telephone service was established on 1 June 2015 and the information has since been captured on the AfriTox database. Data categories included on snakebite were the date and time of the incident, calls from medical personnel and the public, the province where the bite was recorded, sex and age of patients and the snake species responsible for the bite. Calls listed under the unidentified snake species category were also included. Any additional notes were consulted to further extract information regarding the site of the bite on the body and the activity when the incident occurred. Only human-related calls were included and repeated calls were not included.

2.3.3 National Snakebite Database

The National Snakebite Advisory Group has existed in South Africa since 2018 to provide clinical advice, medical support and manage snakebite in both humans and animals (Hardcastle 2023). The group is a voluntary team of experts from the medical, veterinary and herpetology profession and through the organisation of the first South African Snakebite Symposium, the group identified the need for a snakebite database. Thus, a National Snakebite Database was established in January 2023 to collect data on snakebite incidences across South Africa. The analysis included data collected from the NSBD from 1 January 2023 to 31 January 2024. The variables extracted were the same as those from the hospital records.

2.3.4 Combined dataset

All three datasets were combined and analysed using descriptive statistics to provide a more complete perspective of incidences in South Africa, regardless of the source used to collect the data. The combined dataset included data on the date and time of the incident, province (where available), patient age, sex, snake species responsible for the bite, site of the bite on the body and the activity when the incident occurred. To avoid double-counting incidents, duplicates were removed from the combined dataset (Box A).



Box A. Process of removal of duplicate records from the datasets. The only overlaps in data were across the AfriTox Telelog database and the NSBD, and the AfriTox data and the hospital data. The records with the most information about the circumstances of the bite were chosen.

2.4 Results

2.4.1 Hospital Data

Data from the hospital records indicates that over the 11 years and five months of data capture (August 2011 to December 2022), 1235 snakebite incidences were recorded. Of these, there was only one recorded death in 2018. Overall, the only categories with no missing data were age, time of bite and month. All additional categories had cases of missing or unrecorded data. Thus, results were based on available data and the rest were considered as unknown in the assessment. From the total bites, more males (65.1%) were bitten than females (34.8%), and this pattern was generally the same for each year. One record did not report the sex (0.1%) of the bite recipient. The highest recorded incidences in the ethnic group were White (47.8%) and the fewest were Coloured (3.7%, Table 1). Incidences were highest in the 21–30 age demographic compared to the age categories. The majority of snakebite incidents occurred during summer/rainfall months (October to March) and the fewest occurred during cooler months (April to September, Table 2). Most cases occurred between 6:00 pm and midnight.

Provincial locations were only recorded for 1057 of the 1235 incidences (85.6%). The province with the highest record of snakebite incidences was KwaZulu Natal (40.9%), followed by Gauteng (30.7%), and the fewest incidences were recorded in the Northen Cape (0.1%, Table 2). From the available data (only 2.4% of the reported incidents included the snake species), the snakes responsible for snakebite incidents included *Naja* spp (n = 12) (species not specified), *B. arietans* (n = 6), *Causus rhombeatus* (n = 4), *A. bibronii* (n = 3), *D. polylepis* (n = 2), *H. haemachatus* (n = 2), and *B. atropos* (n = 1). The most common part of the body recorded to be bitten was the foot (4.5%), followed by the finger (3.2%) and the hand (3.1%). However, in 83.1% of cases, the site of the bite on the body was not recorded.

Table 1. Snakebite incidents and the demographics of the individuals in South Africa recorded from August 2011 to January 2024 from three data sources. (Total for Hospital records = 1235, National Snakebite Database = 130, AfriTox Telelog Database = 2131)

Category		Hospital records (%)	National Snakebite Database (%)	AfriTox Telelog Database (%)	Total (%)
Sex	Male	65.1	70.0	66.1	65.9
	Female	34.8	29.2	33.6	33.9
	Unknown	0.1	0.8	0.3	0.2
Ethnicity	White	47.8	33.1	NA	18.1
	African	39.4	54.6	NA	15.9
	Coloured	3.7	2.3	NA	1.4
	Indian	7.6	3.1	NA	2.8
	Unknown	1.5	6.9	NA	0.8
Age	0-10	11.4	15.4	22.0	18.0
	11-20	13.0	16.9	18.0	16.2
	21-30	19.6	16.9	20.1	19.8
	31-40	19.4	15.4	16.5	17.5
	41-50	16.9	16.2	11.0	13.3
	51-60	11.0	12.3	6.5	8.3
	61-70	6.2	3.8	4.0	4.7
	71-80	2.3	0.8	1.6	1.8
	81-90	0.2	NA	0.3	0.3
	91-100	NA	0.8	NA	0.1
	Unknown	NA	1.5	NA	0.1

Table 2. Characteristics of snakebite incidents in South Africa recorded from August 2011 to January 2024 from three data sources. (Total for Hospital records = 1235, National Snakebite Database = 130, AfriTox Telelog Database = 2131)

Category		Hospital records (%)	National Snakebite Database (%)	AfriTox Telelog Database (%)	Total (%)
Time of day	midnight to 6:00 am	7.0	6.2	9.7	8.6
	6:00 am to midday	20.0	23.8	19.5	19.9
	midday to 6:00 pm	34.2	33.1	36.6	35.6
	6:00 pm to midnight	38.9	35.4	34.2	35.9
	Unknown	0.0	1.5	0.0	0.1
Time of year	January	14.9	20.0	14.7	15.0
	February	11.2	2.3	11.9	11.3
	March	15.1	9.2	13.6	13.9
	April	8.3	15.4	8.1	8.4
	May	4.7	10.0	4.4	4.7
	June	1.7	2.3	2.8	2.4
	July	2.8	1.5	1.7	2.1
	August	3.4	1.5	1.6	2.2
	September	4.9	3.1	5.7	5.3
	October	8.6	5.4	9.6	9.1
	November	12.6	10.0	12.0	12.2
	December	11.9	19.2	14.0	13.5
Province	Eastern Cape	2.5	4.6	9.2	6.7
	Free State	0.6	2.3	2.5	1.8
	Gauteng	30.7	32.3	11.8	19.2
	KwaZulu-Natal	40.9	39.2	25.4	31.4
	Limpopo	3.9	7.7	9.6	7.5
	Mpumalanga	0.9	0.0	11.5	7.3
	North-West	4.1	4.6	12.3	9.2
	Northern Cape	0.1	0.8	2.7	1.7
	Western Cape	1.9	2.3	14.9	9.8
Site of bite on the body	Ankle	0.9	11.5	7.4	5.3
	Foot	4.5	20.0	23.3	16.5
	Hand	3.2	19.2	11.8	9.0
	Finger	3.2	15.4	13.5	9.9
	Knee	0.0	1.5	2.7	1.7

Calf	0.0	3.8	1.5	1.1
Face	1.0	1.5	1.4	0.9
Eyes (spat)	NA	5.4	5.7	4.0
Shoulder	0.0	0.0	0.2	0.1
Abdomen	0.0	0.0	0.2	0.1
Lower arm	0.8	3.1	1.9	1.6
Upper arm	0.5	0.8	0.4	0.5
Lower leg	2.3	9.2	3.9	3.5
Upper leg	0.4	1.5	1.0	0.8
Buttocks	0.0	0.0	0.3	0.6
Unknown	83.1	8.5	24.7	44.7

2.4.2 AfriTox Telelog database

A total of 2131 incidences of snakebite were recorded from the AfriTox Telelog database. Of these, 2009 were bites and 122 were spitting events. Only 26% of the reported incidents included the snake species. *Bitis arietans* was most commonly responsible for bites, while 38 other species were responsible for at least one bite. Most bites were recorded in 2023, mainly by *B. arietans* and *N. mossambica*. The majority of bites were on the foot (23.3%), the finger (13.5%) and the hand (11.8%, Table 2). Twenty-five percent of the records did not include information on the site of the bite on the body. The greatest number of snakebite incidents were recorded in KwaZulu Natal (25.4%) and the fewest in the Northern Cape (2.7%, Table 2). Males made up 66.1% of all snakebite incidents and most were recorded in people aged 0–10 (22.0%, Table 1). The majority of incidents were recorded between October and March and between midday and 6:00 pm (36.6%, Table 2). Of the known activities (173/2131, ~8.1%), walking ranked highest (3.1%) followed by interacting with a snake (1.4%) (interactions that did not involve relocations), snake catching for relocations (1.2%) and sleeping (1.2%).

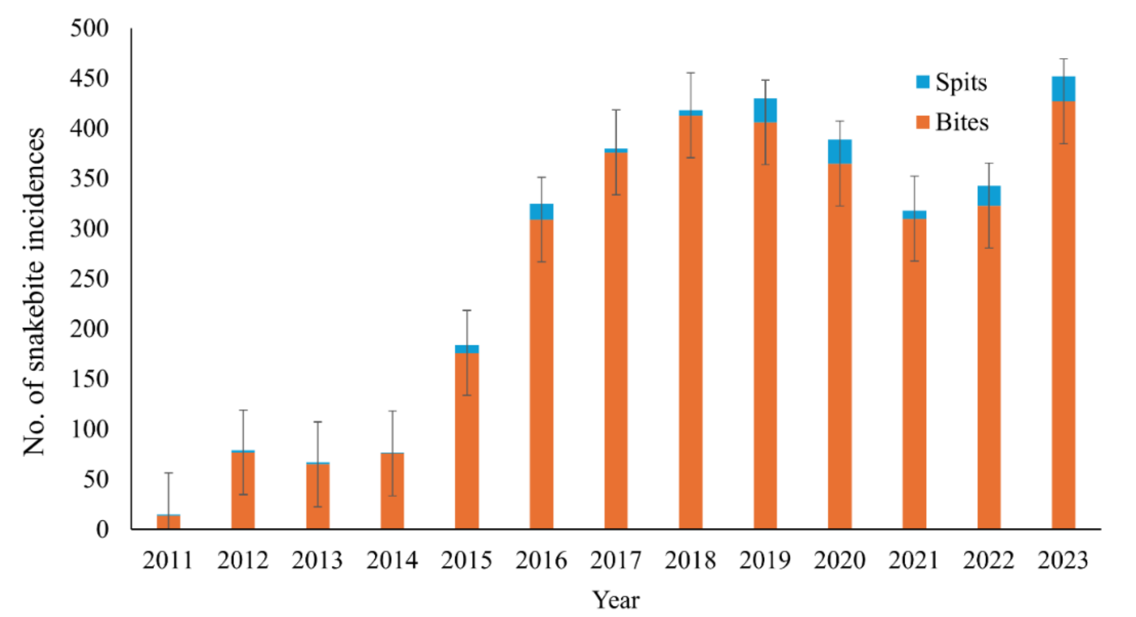


Figure 1. The number of snakebite incidences recorded in South Africa each year from August 2011 to December 2023. Spitting incidents (blue) were separated from biting incidents (orange). 2011 did not include datasets for the full year. Standard bars represent the standard statistical error.

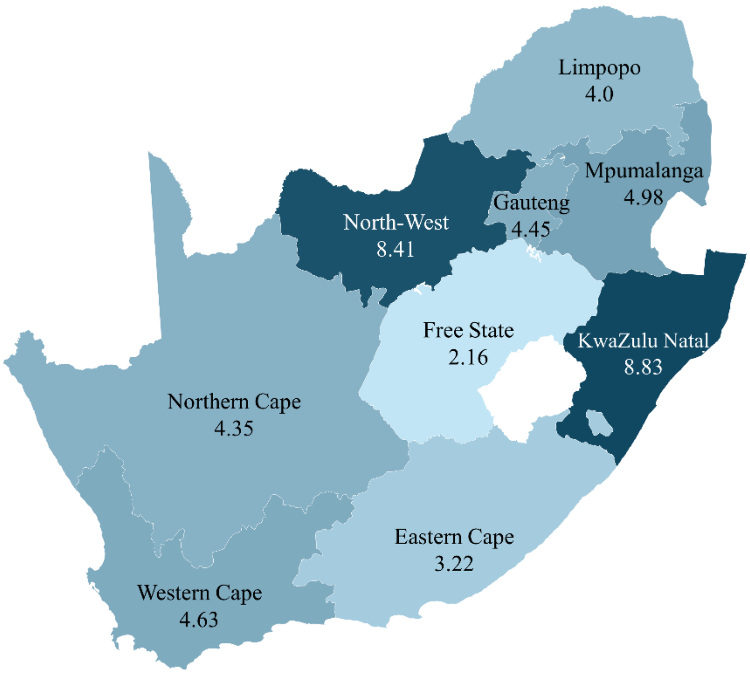


Figure 2. The prevalence of snakebite from all the available datasets over 12.5 years was calculated per 100 000 individuals of the population in each province in South Africa with the highest rate in KwaZulu Natal (8.83).

2.4.3 National Snakebite Database

One hundred and thirty incidences of snakebite were recorded between the beginning of January 2023 and the end of January 2024 on the National Snakebite Database. Males made up 70.0% of people bitten. One entry in the database did not record the sex. The ethnic group recorded with the highest recorded incidences was African (54.6%) and lowest was Coloured (2.3%, Table 1). The ethnicity of 6.9% of people were not recorded. The age category with the most snakebite incidences recorded was 11–20 (16.9%) and 21–30 years (16.9%). The majority of incidents were recorded from November to April, and between 6:00 pm and midnight (35.4%). The majority of incidents were recorded in KwaZulu Natal (39.2%) and fewest in Northern Cape (0.8%, Table 2). The NSBD listed 17 species responsible for bites and these were recorded for 68% of incidences. *Bitis arietans* was responsible for the greatest number of bites. Of the known activities (105/130, ~80.7%), walking ranked highest (77.1%) followed by other activities such as gardening (10.5%), camping (5.7%) and sleeping (3.8%).

2.4.4 Combined dataset

The combined dataset showed that 3547 snakebite related incidences were recorded between 2011 and 2024 from the three data sources. Fifty-one duplicates across the three sources were removed and a total of 3496 incidences were included in the study. Of the total incidences, 141 were spitting incidences that did not lead to bites. Based on the three available datasets, an average of 280 ($\bar{x} \pm SD = 167.5$) incidents of snakebite were recorded per year over 12.5 years. The majority of incidences were logged during 2023 (427 bites and 25 spitting incidences, Fig. 1). Snakebite records were low from 2011 to 2015, however, since 2015, more than 150 bites were recorded per year (Fig. 1). The prevalence over 12.5 years was calculated as 5.63 for South Africa per 100 000 individuals of the population and provincially the rate was highest in KwaZulu Natal (8.83, Fig. 2).

More males (65.9%) were bitten than females (33.9%, Fig. 3a) and most records of incidences were in individuals aged 21–30 (19.8%). The majority of incidents were recorded between midday and midnight (71.5%, Fig. 3b) and between November and March (Fig. 3c). For 91.9% of incidences, the activity at the time of the incidences was not recorded. Of the 8.1% of bites where the activity was recorded, people were bitten most often while they were walking, followed by other high ranked activities such as interacting with a snake, snake catching for relocations and sleeping (Fig. 4 – Fig. 6). Bites in sleeping people were recorded from six snake species (Fig. 5; Fig. 6) and *N. mossambica* was responsible for most bites. All snake species that bit sleeping humans are active foragers. In 55.3% of the known records for the site of the bite on the body, most occurred on the foot (16.5%, Fig. 3d). Overall, snake species were listed for 32.1% of snakebite incidences and the top ten were *B. arietans*, *N. mossambica*, *A. bibronii*, *N. nivea*, *Causus* spp, *H. haemachatus*, *D. polylepis*, *Boaedon capensis* (a non-venomous snake), *D. typus* and *Bitis atropos*.

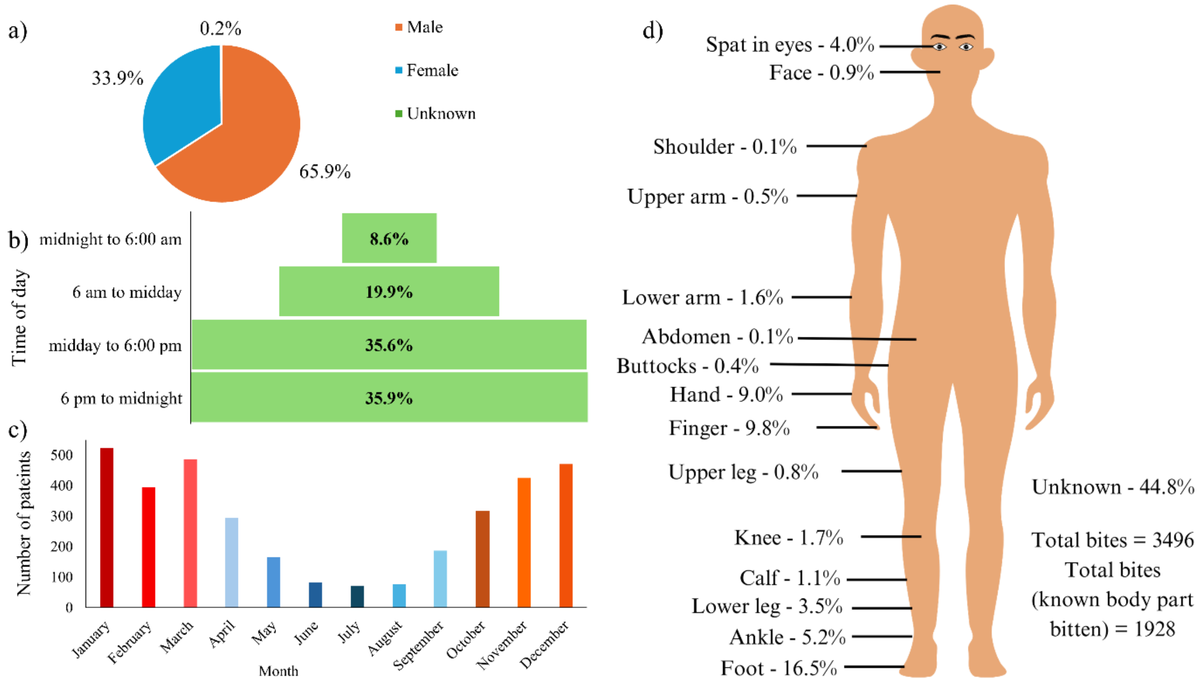


Figure 3. a) Comparison of the incidence of snakebite between males and females, b) The time of day when incidences of snakebite were recorded, c) The time of year when incidences of snakebite were recorded, d) Records of the incidence of snakebite on different parts of the body. Figures show data from the combined dataset.

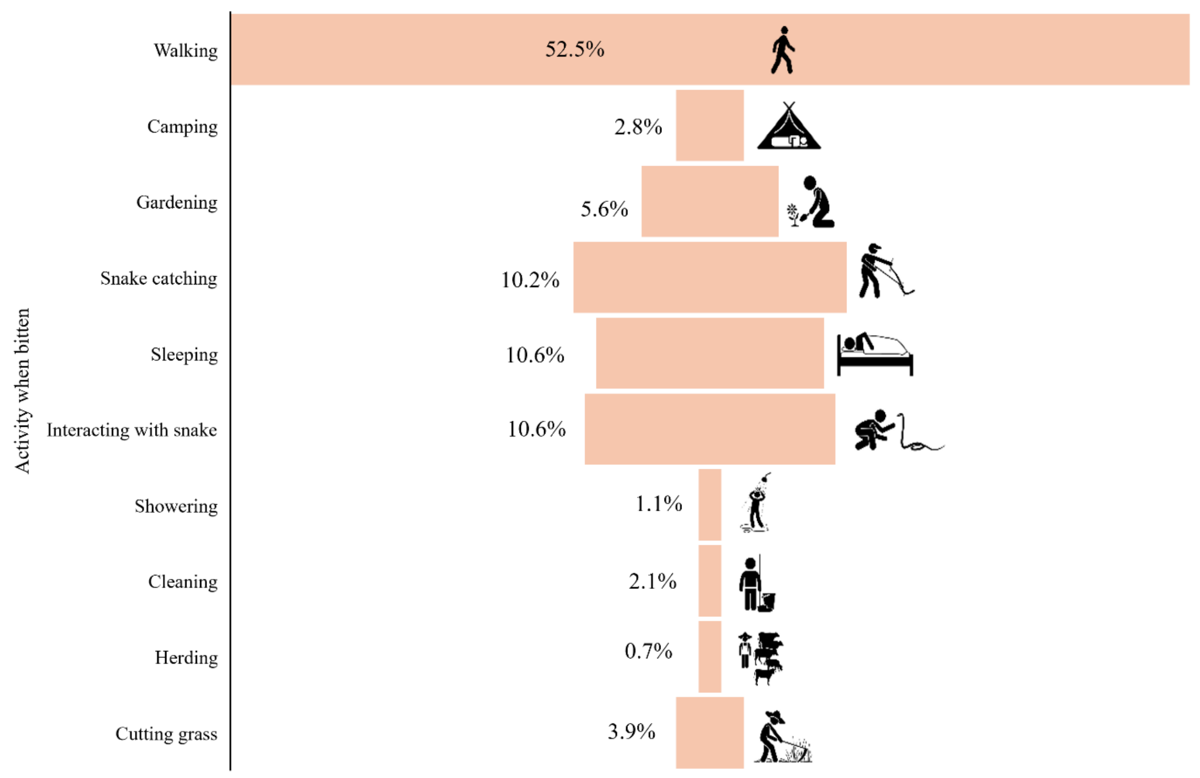


Figure 4. The activity at the time of snakebite incidents. Data are reported for only the known activities. Figure shows data from the combined dataset.

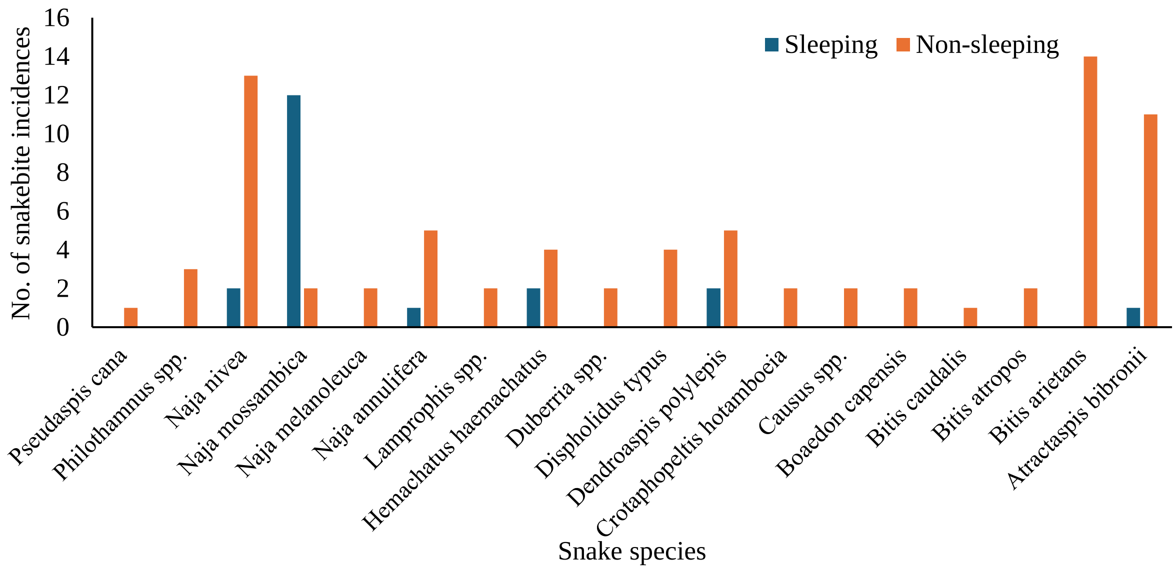


Figure 5. Snakebite incidences in sleeping people and non-sleeping people reported in the AfriTox Telelog database and the National Snakebite Database. Hospital data did not include any data on bites in sleeping people.

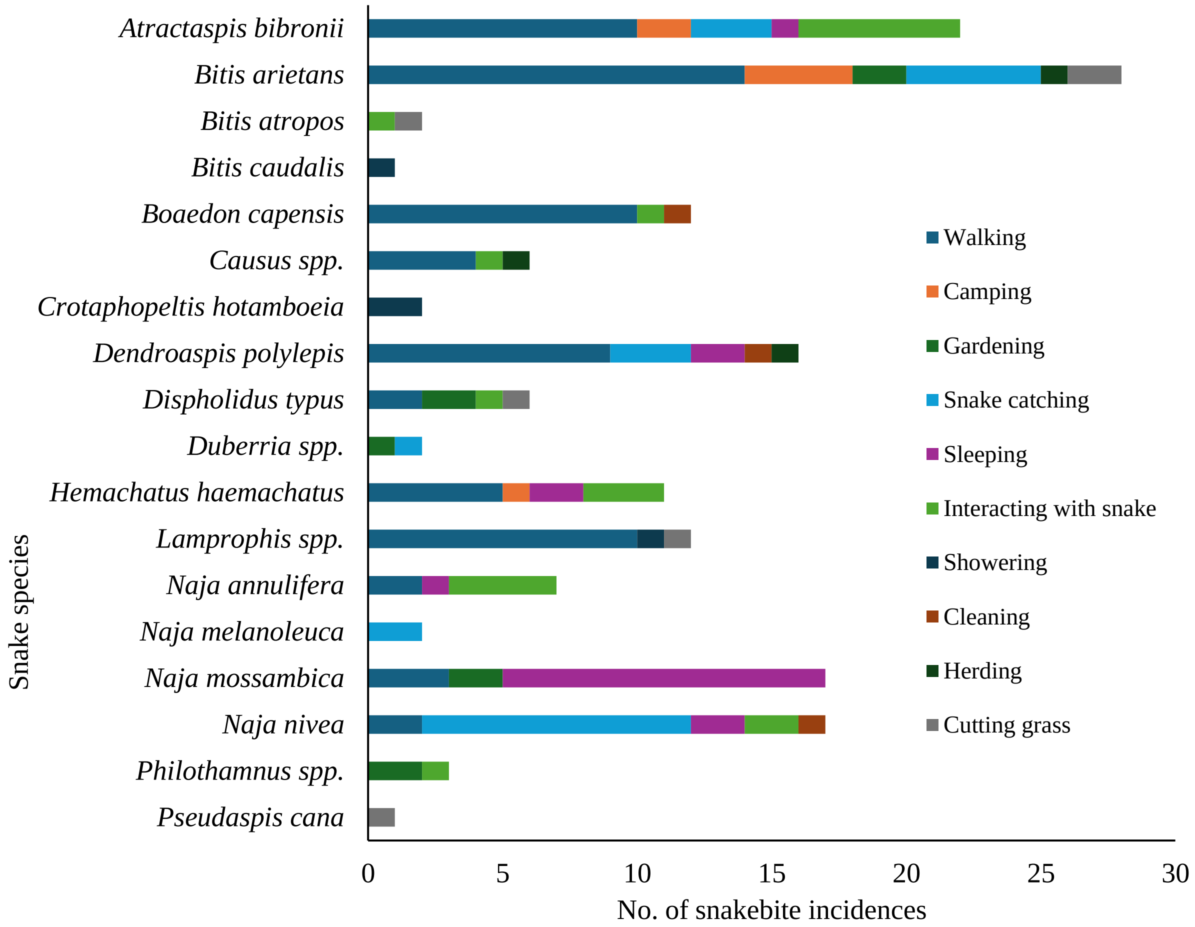


Figure 6. Snakebite incidences from various snake species and the activities reported when people were bitten.

2.5 Discussion

This is the first study to assess the incidence of snakebite in South Africa using multiple data sources. The combined data sources revealed that the incidence of snakebite was much higher than expected with 3355 cases and 141 spitting incidences recorded from August 2011 to January 2024. I also detected an increase in the number of recorded cases from 2015, with more than 150 bites recorded each subsequent year. However, it is not clear if this rise in reported cases is due to an increase in the number of snakebite incidents or improved reporting. Overall, the number of snakebite incidents reported in this study is clearly a significant underestimation of the actual snakebite incidence in the country as records were available from only 4.5% of hospitals in South Africa and only 51 of the snakebite incidents were recorded in more than one of the databases. This highlights the need for a more rigorous snakebite reporting system in South Africa.

I detected several consistent patterns in the different data sources for snakebite incidence in South Africa. For example, all datasets revealed that males made up the majority of victims in snakebite cases, and most cases occurred during the summer months when snakes are most active and searching for prey (Martín et al. 2022). KwaZulu Natal Province had the highest prevalence of snakebite in all three datasets over 12.5 years, likely due to the warm mesic climate and high snake species richness in this province. This supports the findings of previous studies that also report on high prevalence from hospitals in KwaZulu Natal (Blaylock 2004; Wood et al. 2016). *Bitis arietans* was recorded as the species responsible for most snakebite incidents, with incidences for this species peaking in March at the beginning of the mating season for *B. arietans* (Glaudas and Alexander 2017). This finding was expected given that *B. arietans* is one of the most widely distributed and common snake species in South Africa (Alexander and Marais 2007).

Some patterns varied between the datasets. For example, the results from the Telelog dataset revealed that individuals aged 0–10 years tended to be at higher risk of being bitten. However, the NSBD showed that individuals aged 11–20 and 21–30 were most at risk and hospital records recorded a higher snakebite incidence among individuals aged 21–30. Hospital records may represent a more filtered dataset of snakebite incidents since only bites resulting in hospitalisation would be recorded. Overall, all datasets showed that young people are at highest risk of being bitten. The list of snake species responsible for bites also varied depending on the data source, as hospital records usually did not list or identify the snake species. In contrast, the Telelog dataset included snakebite incidences from species that had been correctly identified by experts and thus bites were recorded from 39 species. Disparities in patterns between datasets also suggest that the recorded incidence of snakebite in South Africa significantly underrepresents the actual incidence. Thus, I recommend that systems be put in place to make the reporting of snakebite more comprehensive.

Although the majority of bites were reported to occur on the feet while walking and were most likely legitimate, bites from *A. bibronii* were generally recorded on the hand. This was likely the result of this species being misidentified as a nonvenomous species leading to irresponsible handling. Records generally did not state whether the bite was illegitimate or legitimate. Since legitimate bites are unlikely to occur on the hand while walking, bites on the hand were likely the result of catching, killing, or interacting with the snake, resulting in illegitimate bites. The relatively high number of bites on arms suggest that illegitimate bites make up a higher proportion of bites than previously thought. Bites in sleeping humans were mostly by *N. mossambica*, but several other active foraging species were also responsible for bites indicating the possibility of legitimate/accidental bites (Fig. 5). Thus, the legitimacy of a bite depends on the activity at the time of the incident. Almost half of the recorded incidents by *H. haemachatus* and *N. mossambica* were for spitting, often on the face, as spitting snakes generally defend themselves by spitting as a first line of defence (Westhoff et al. 2005). Thus, snakebite records need to include the circumstances that lead to a bite.

Many snakes in South Africa are crepuscular (Alexander and Marais 2007) and this correlates with the time of incidences in the dataset. Most snakebite incidences were recorded from midday to 6:00 pm in the Telelog dataset and from 6:00 pm to midnight in the hospital records and the NSBD suggesting that individuals should be cautious of moving snakes during the afternoon and night. Studies have shown that the use of torches at night can be useful in preventing bites (Malhotra et al. 2021) particularly in remote areas without electricity. Spatially, although the number of cases reported on snakebite in Gauteng Province were second highest in South Africa, the prevalence was higher in the North West Province (8.41 per 100 000 individuals in the population). A similar study that used data records from the PIHWC also found a high prevalence of snakebite in North West Province (Lermer et al. 2023). However, as numbers of incidents vary based on region and data records (Suraweera et al. 2019), it is recommended that the prevalence of snakebite should include information on the geographic area.

There were several challenges in the reliability and variability of information from the datasets used in the analysis. The high number of incidents recorded in the Western Cape on the AfriTox Telelog database is likely due to the location of the PIHWC in the Western Cape. The NSBD did not record any incidences of snakebite in Mpumalanga Province for 2023 while the AfriTox Telelog database reported 33 cases. The disparities in the reported incidence of snakebite are probably due to the recent establishment of the NSBD rather than the absence of bite occurrences in Mpumalanga. Biases also exist within each of the datasets as the reduced number of records from other provinces to the PIHWC may not indicate lower incidences of snakebite in those provinces. As the number of duplicate records in the study were relatively low (14%), and there were disparities in the reported cases for the same provinces, there is a high likelihood that snakebite incidences are significantly under reported in general in South Africa.

The incomplete nature of all three datasets evaluated in this study significantly impedes estimations on the actual number of snakebite incidences occurring in South Africa. The bureaucracy regarding the collection of data and access to medical records is a further impediment for investigating snakebite in South Africa and provided several limitations to the data collection process of this study. Although improved efforts have been placed into data collection for the NSBD, as evident by the reports on snake species and activities for bites, it is important that information is correctly recorded on the database. As snakebite is not a reportable incident or notifiable disease in South Africa and healthcare systems lack easily assessable data collecting protocol, the actual incidence of snakebite remains largely unknown and under reported. The high incidence of bites from *A. bibronii* and *Causus* spp also suggests that frameworks for defining categories for medically important snake species need to be reevaluated. The focus should rather be on spatial patterns of snakebite incidences within a country.

Additional studies have shown similar patterns of incidences of snakebite in other countries and the authors also propose more rigorous data collection protocols for snakebite incidences (Kasturiratne et al. 2008; Padidar et al. 2023; Rai et al. 2021). Despite the limitations in the current data collection protocols in South Africa, this study has provided important insight into situations that lead to snakebite incidences and the demographic groups most affected in the country. As young people are at higher risk of bites, there is a need for education and prevention measures to be placed at schools. Knowing the snake species responsible for bites and circumstances leading to a bite can assist in creating targeted education campaigns such as snake identification posters to prevent individuals from handling *A. bibronii* as a misidentification. Prevention measures can mitigate the conflict that occurs between humans and snakes and ultimately reduce the burden of relying on antivenom. While there are discrepancies in the data from the past and there is a means for improved collection of snakebite data (i.e., NSBD), important details regarding incidences are not included by record keepers at hospitals and on the databases. This study highlights the importance of creating standardised data collection methods and exploring data around incidences of snakebite to achieve the 2030 goals of the WHO. The insights from this study will assist in future efforts to gather relevant and robust data on snakes and their behaviour and clarify key factors contributing to snakebite risk, which will help in human-snake conflict management planning and resource mobilisation.

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Chapter 3: The spatial distribution of snakebite incidences in South Africa for nine venomous snake species

3.1 Abstract

As natural landscapes have been modified due to human development, human-wildlife conflict is on the rise. In particular, human-snake conflict has become more prominent as snakes increasingly find themselves in human-inhabited areas. To investigate the spatial distribution of snakebite incidences for nine medically important snake species in South Africa, I mapped snake occurrence records and snakebite incidences. I also mapped snakebite incidences in relation to human population density and protected areas. Snakebite incidences are largely distributed in the eastern parts with some hotspots in the southwestern region of South Africa. Hotspots are concentrated in densely populated areas including Johannesburg, Durban and Cape Town and the majority of these incidences were outside of protected areas. The results indicate that human-snake conflict is likely the main driving factor of snakebite incidences in South Africa and that increasing natural spaces could possibly reduce the likelihood of snakes moving into human-inhabited areas. However, targeted education measures in the snakebite hotspots will be most useful in preventing snakebite.

3.2 Introduction

In recent decades, rapid changes in environmental patterns have become a major threat to biodiversity and the functioning of ecosystems (Onoh et al. 2024). Influenced by anthropogenic factors, there has been a notable increase in average global temperatures which is expected to continue (Shukla et al. 2022). Climate change models predict that weather events are likely to become more extreme and more unpredictable (Bathiany et al. 2018; Engelbrecht et al. 2015), making it difficult to predict changes in the environment. Within these changing landscapes, some species have shown resilience to changing climates, but others have not fared so well (Manes et al. 2021). Additionally, as human populations continue to grow, the associated food, housing and transportation needs are creating new relationships between people and wildlife, resulting in conflict or coexistence (Dickman 2010; Mateo-Tomás et al. 2012).

Changing environmental conditions will continue to have significant effects on ecosystem dynamics including changes to the geographical distribution of many species (Freeman et al. 2018; Konvicka et al. 2003; Petford and Alexander 2021). Subsequently, this will have an impact on human-wildlife conflict challenges such as snakebite, which is also a public health issue that stems from human-snake conflict (Malhotra et al. 2021). Despite being a global issue, human-snake conflict has not received necessary attention (Feitosa et al. 2015; Gutiérrez et al. 2010) and since there is a large diversity of venomous snake species around the world coinciding with human populations, it is important to investigate the spatial relationship between the two factors (Martín et al. 2021). Although venomous snakes are found in most regions of the world, the highest venomous snake species richness is in the tropics (Chippaux 1998; White 2000; WHO 2017). Several studies show that tropical and subtropical countries with a high diversity of snakes and more vulnerable populations experience the highest impact of snakebite (Hansson et al. 2013; Kasturiratne et al. 2008), as these are the areas where farmers and rural community members are affected by the clearance of land for subsistence agriculture (Borges et al. 1999; Habib and Warrell 2013; Habib et al. 2015).

Different biotic (climate, topography, and vegetation) and abiotic factors (prey species and availability) can shape the geographical distribution of medically important snake species and affect their activity (Soberón and Nakamura 2009; Yañez-Arenas et al. 2014). These patterns change as the overlap in resource use between people and wildlife increases and in the case of snakebite incidences, become asymmetrically prevalent across rural agricultural communities in tropical climates (Samuel et al. 2020). Climate and land use change are further shown to have widespread impacts on medically important venomous snake species (Bohm et al. 2016) and global environmental change is likely to shift the geographic distribution of venomous snakes (Nori et al. 2014; Yañez-Arenas et al. 2016). Since mapping the distributions of species is an important tool for conserving and managing biodiversity, it can also be applied to snakebite management.

Several studies have focused on modelling the impact of climate change on the distribution of venomous snakes under current and future scenarios (Martinez et al. 2024; Yañez-Arenas et al. 2016; Zacarias and Loyola 2018). These studies have shown that snakebite risk areas will increase as venomous snakes shift their current ranges and human populations increase, causing increased areas of overlap. A recent study also used niche modelling to identify the distribution of snake species within Ancient Egypt to identify which species bit the Ancient Egyptian people in the past and the present (McBride et al. 2023). Since the high density of venomous snakes in a small area may not necessarily equate to an increased number of snakebite incidences (Luiselli et al. 2020), knowledge of spatial patterns of snakebite incidences and the factors that influence hotspots of potential contact between people and snakes can be useful (Hansson et al. 2013; Nori et al. 2014). While climate may be the main factor limiting the geographical ranges of snakes due to temperature and humidity (Chaves et al. 2015; Kearney et al. 2018), at a finer scale, factors such as reproduction and food availability are more likely to have the largest influence on spatial movements (Chaves et al. 2015; Sengupta et al. 1994). As natural spaces and protected areas are becoming sparse, snake spatial movements in human-inhabited areas are integral in investigating the relationship between people and snakes.

Previous studies have placed significant focus on the spatial epidemiological patterns of snakebite incidences, and changes in land use and habitat transformation have not been considered. Pintor et al. (2021) showcase several studies that have already used environmental niche models to estimate snake species distributions in different regions of the world. However, studies in Africa are limited with only one study focused on Mozambique (Zacarias and Loyola 2019) and one other on puff adder (*Bitis arietans*) (Barlow et al. 2013). Given that natural habitats are being transformed and that there is larger overlap in resource use by people and wildlife (Ma et al. 2024), spatial patterns of snakebite incidences need to include changes occurring in natural spaces, human population densities and access to healthcare in changing environments. Specifically, variation in the abundance of venomous snake species in communities across different habitats and regions has not yet been explored. Thus, spatiotemporal patterns can provide a baseline for snake ecology research.

In South Africa, the highest reptile species richness is found in the northeastern and southwestern parts of the country and this pattern is similar for snake species (Bates et al. 2014; Tolley et al. 2023a), possibly due to heterogeneous landscapes that allow for greater variation in habitats (Alexander et al. 2004). Category 1 medically important snake species have broad distributions and feature in biting incidences frequently, likely because their larger range increases the likelihood of interactions with humans (WHO 2017). Category 2 medically important snake species on the other hand, are not considered to be as widely distributed as Category 1 species but still bite people often (Chapter 2). Within venomous snake distributions, it is unknown whether snakebite incidences occur at random or whether there are trends which show an increase in the frequency of encounters with venomous snakes.

To effectively implement prevention measures for snakebite, the spatial distribution of incidences in relation to the abundance of snake species is required. In this study, I investigated the spatial patterns of snakebite incidence records for nine medically important venomous snake species in South Africa (Chapter 2) in relation to their distribution based on occurrence records across the country. I created heat maps to show the areas of high risk in relation to human population density and protected areas. I predicted that high incidence risk areas are likely to be in highly populated areas, outside of the current 9.2% of protected areas. These findings will improve our knowledge of the relationship that snakes have in transformed habitats and the impact of land use change on species occurrence and activity as well as improve snakebite management.

3.3 Methods

3.3.1 Species Occurrence Records

Presence-only records of *Atractaspis bibronii*, *Bitis arietans*, *Causus rhombeatus*, *Dendroaspis polylepis*, *Dispholidus typus*, *Hemachatus haemachatus*, *Naja annulifera*, *Naja mossambica* and *Naja nivea* were gathered from the Southern African Reptile Conservation Assessment (SARCA). SARCA includes data from ReptileMap (https:// vmus.adu.org.za), iNaturalist (https://www.inaturalist.org/) and various museums. The dataset included 550 locality records for *Atractaspis bibronii*, 3501 records for *Bitis arietans*, 1207 records for *Causus rhombeatus*, 667 records for *Dendroaspis polylepis*, 2131 records for *Dispholidus* *typus*, 824 records for *Hemachatus haemachatus*, 658 records for *Naja annulifera*, 1174 records for *Naja mossambica* and 1105 records for *Naja nivea* in southern Africa. Snake species that are not featured much in snakebite incidences: *Thelotornis capensis*; *Naja subfulva*, *Naja nigricincta* and *Bitis gabonica* were not included in the study. Although *C. rhombeatus* is not listed on the WHO list of medically significant snakes, it has been included in this study as it is commonly featured in snakebite incidences in South Africa (Chapter 2) and will be referred to as medically important in this chapter. As the dataset is large, there is a higher likelihood for sampling bias to occur. Thus, I cleaned and filtered the dataset using a 5 km radius in the *spThin* package in R.4.4.0 (R Core Team 2024). The final dataset of locality records was reduced to 235 (*Atractaspis bibronii*), 1030 (*Bitis arietans*), 373 (*Causus rhombeatus*), 228 (*Dendroaspis polylepis*), 592 (*Dispholidus typus*), 371 (*Hemachatus haemachatus*), 226 (*Naja annulifera*), 322 (*Naja mossambica*) and 413 (*Naja nivea*) and only records within South Africa were used.

3.3.2 Snakebite incidence records

Based on available location data on snakebite incidences in South Africa (Chapter 2), incidence records for bites from the nine medically important venomous snakes were collected from the National Snakebite Database (69) and the AfriTox Telelog database (399). Hospital records did not include names for snake species or incidence locations and were not included in this study. Spitting incidences from *N. mossambica* and *H. haemachatus* were not included.

3.3.3 Mapping

Snake occurrence records and incidence records were used in QGIS v.3.32 (QGIS Development Team 2024) to create heat maps to show hotspots of known snakebite incidences for each of the nine snake species in South Africa. Heat maps were created for all species using the Kernel density estimation (KDE) function which is a nonparametric neighbour-based smoothing function that can be used to identify hotspots (Nelson and Boots 2008).

Snakebite incidences were overlayed with data on population density to show where bites were clustered. Data on population density at a resolution of 1 km^2 grid cells was collected from the Humanitarian Data Exchange (https://data.humdata.org/). To assess the spatial patterns of snakebite incidences in relation to the current protected area network in South Africa, incidences were overlayed with the protected areas. Data on protected areas were downloaded from the South African National Biodiversity Institute (https://bgis.sanbi.org/). The South African National Environmental Management: Protected Areas Act (Act 57 of 2003) currently recognises protected area as Forest Nature Reserves, Forest Wilderness Areas, Island Nature Reserves, National Parks, Provincial Nature Reserves, Wilderness Areas, and World Heritage Sites (Tolley et al. 2019). Using the nearest neighbour function, the distance between snakebite incidences and the closest and farthest hospital were calculated and mapped to show accessibility to healthcare facilities. Hospital data was collected from the ArcGIS hub (https://hub.arcgis.com).

3.4 Results

Snakebite incidences in South Africa from nine medically important snake species are distributed widely across the country with larger clusters mostly in the eastern parts of South Africa with a few hotspots in the south-western parts (Fig. 1). Large clusters are found in suburbs around Johannesburg in Gauteng, Durban in KwaZulu Natal and Cape Town in Western Cape. The lowest number of incidences are shown in Northern Cape and Free State, which are also the least densely populated provinces (Statistics South Africa).

Hotspots for snakebite incidences from *A. bibronii* were predominantly in areas surrounding Johannesburg in Gauteng and Durban in KwaZulu Natal (Fig. 2a). Incidences from *Causus rhombeatus* were similar but some hotpots were also found in East London in Eastern Cape and Mbombela in Mpumalanga (Fig. 2c). Hotspots for snakebite incidences from *Bitis arietans* were predominantly in areas surrounding Johannesburg in Gauteng, Durban in KwaZulu Natal, Cape Town in Western Cape and East London in Eastern Cape (Fig. 2b) but generally widespread across the country. Incidences from *D. typus* were also distributed widely across the country but were mostly in the western parts of the country (Fig. 2e). Hotspots for incidences from *D. polylepis* were concentrated in Durban in KwaZulu Natal with some clusters in the north-eastern parts of the country (Fig. 2d). Gauteng was a major hotspot for snakebite incidences from *H. haemachatus* (Fig. 2f) as well as *N. annulifera* (Fig. 2g). Hotspots for snakebite incidences from *N. mossambica* were concentrated on the north-western edge of Gauteng bordering North West Province (Fig. 2h) and hotspots for *N. nivea* incidences were concentrated in Cape Town in Western Cape (Fig. 2i).

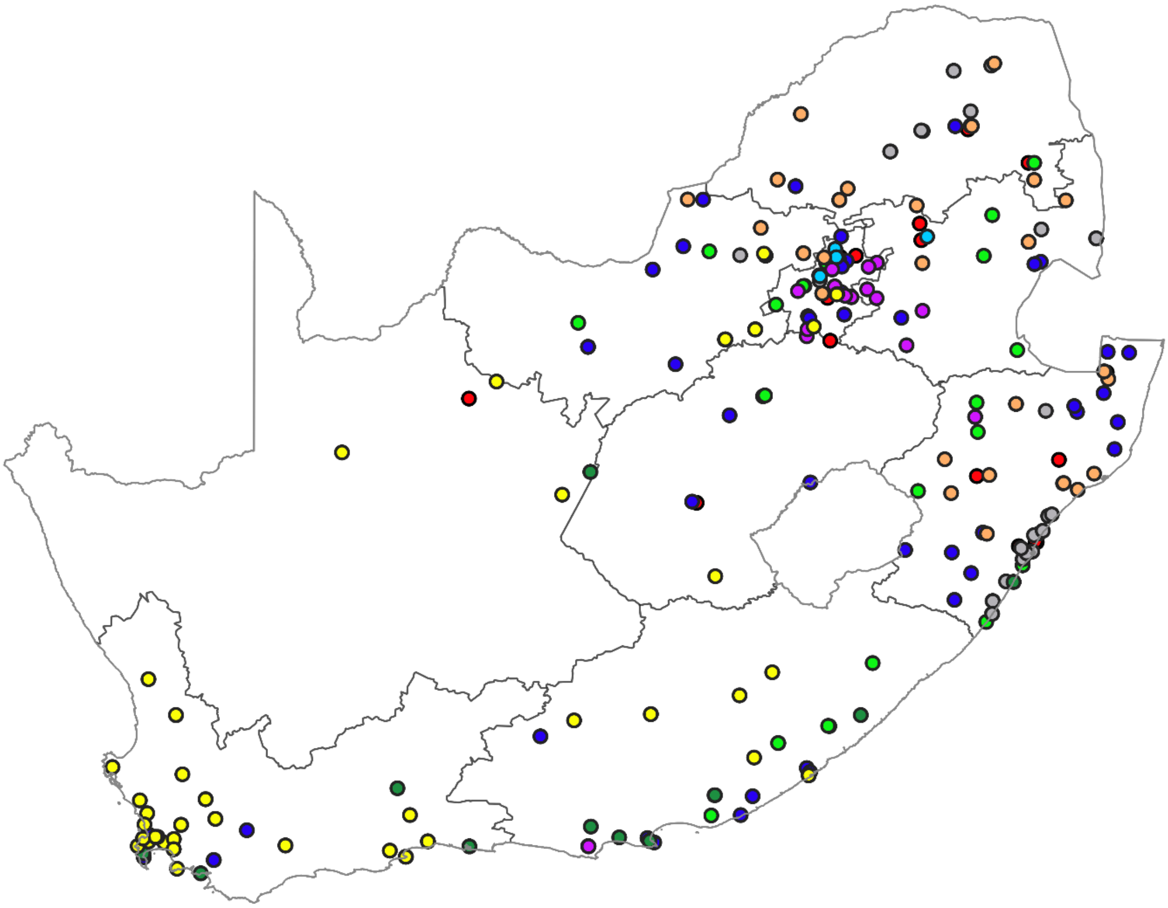

Figure 1. Snakebite incidence records in South Africa for nine medically important snake species from June 2015 to January 2024. *Atractaspis bibronii* (red), *Bitis arietans* (dark blue), *Causus rhombeatus* (light green), *Dendroaspis polylepis* (grey), *Dispholidus typus* (dark green), *Hemachatus haemachatus* (purple), *Naja annulifera* (light blue), *Naja mossambica* (orange), *Naja nivea* (yellow).

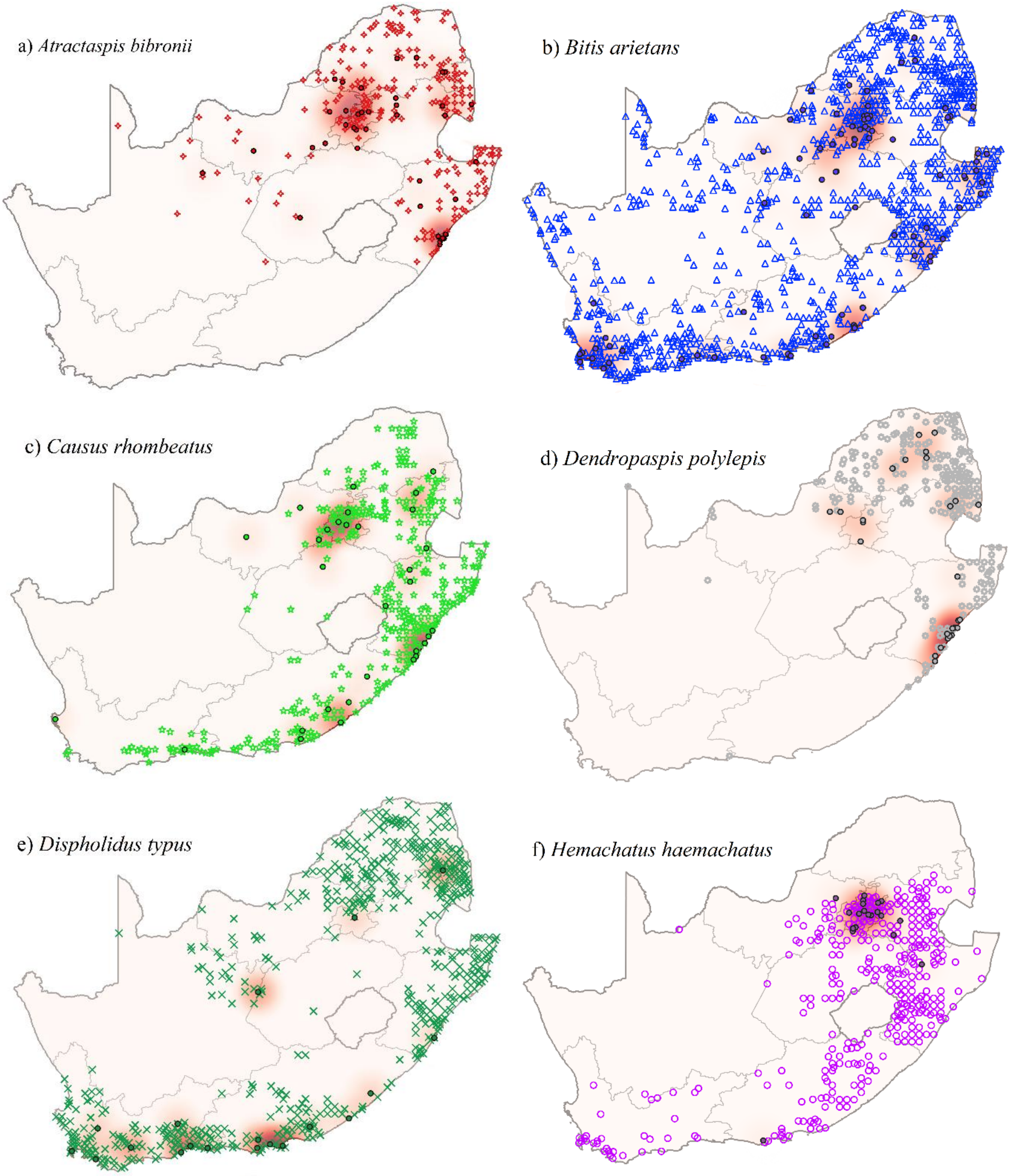

Figure 2. Hotspots for snakebite incidences (dots) for nine medically important snake species in South Africa showing areas where the incidence of snakebite was highest. In each map, open markers (shapes) show where the species has been recorded.

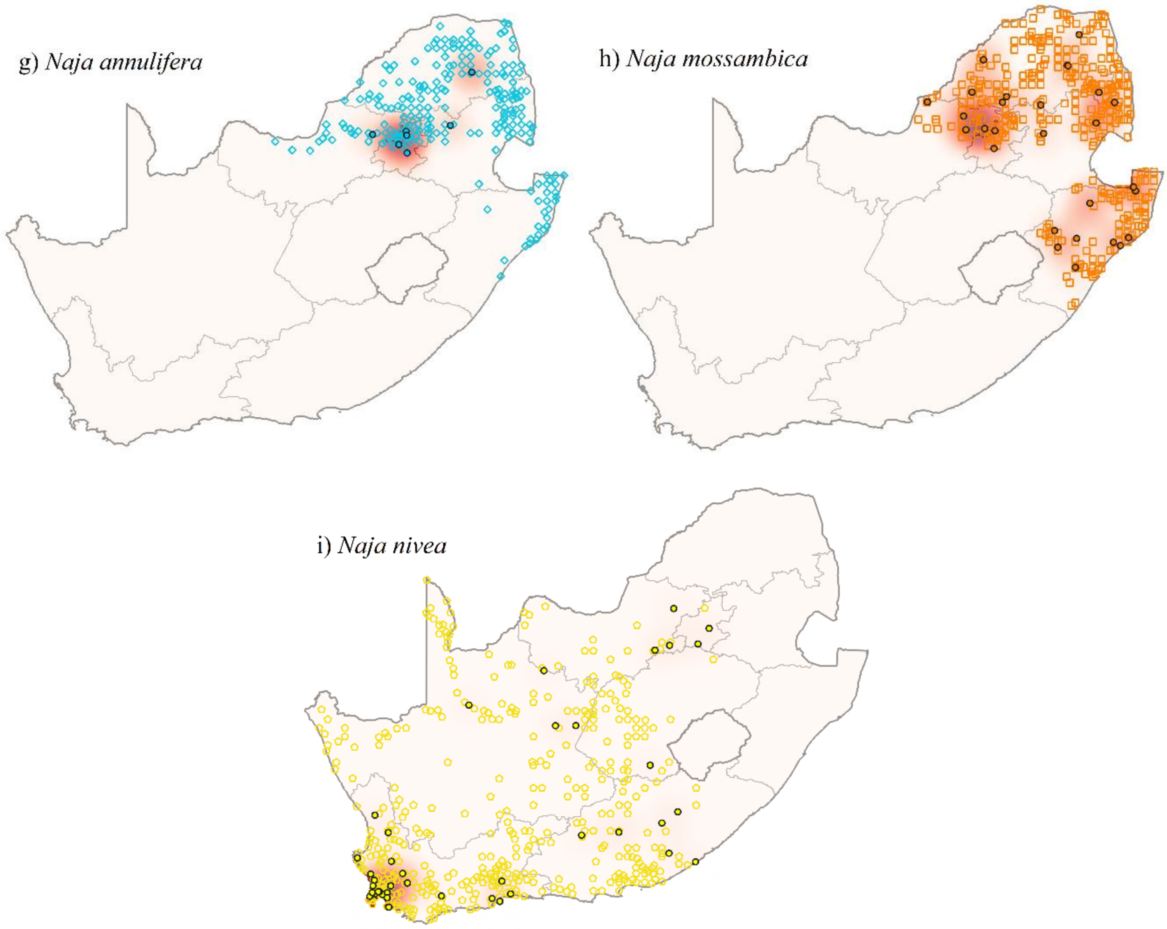

Figure 2 continued. Hotspots for snakebite incidences (dots) for nine medically important snake species in South Africa showing areas where the incidence of snakebite was highest. In each map, open markers (shapes) show where the species has been recorded.

Snakebite incidences mostly overlapped with areas of high human population density across South Africa with larger clusters recorded in areas where major cities are situated (Fig. 3). In general, snakebite incidences are widespread across the country and areas of low population density had much fewer bites. Approximately 98% (459/468) of snakebite incidences occurred outside of the 9.2% of protected areas (Fig. 3) but several incidences occurred in populated areas just outside the protected areas.

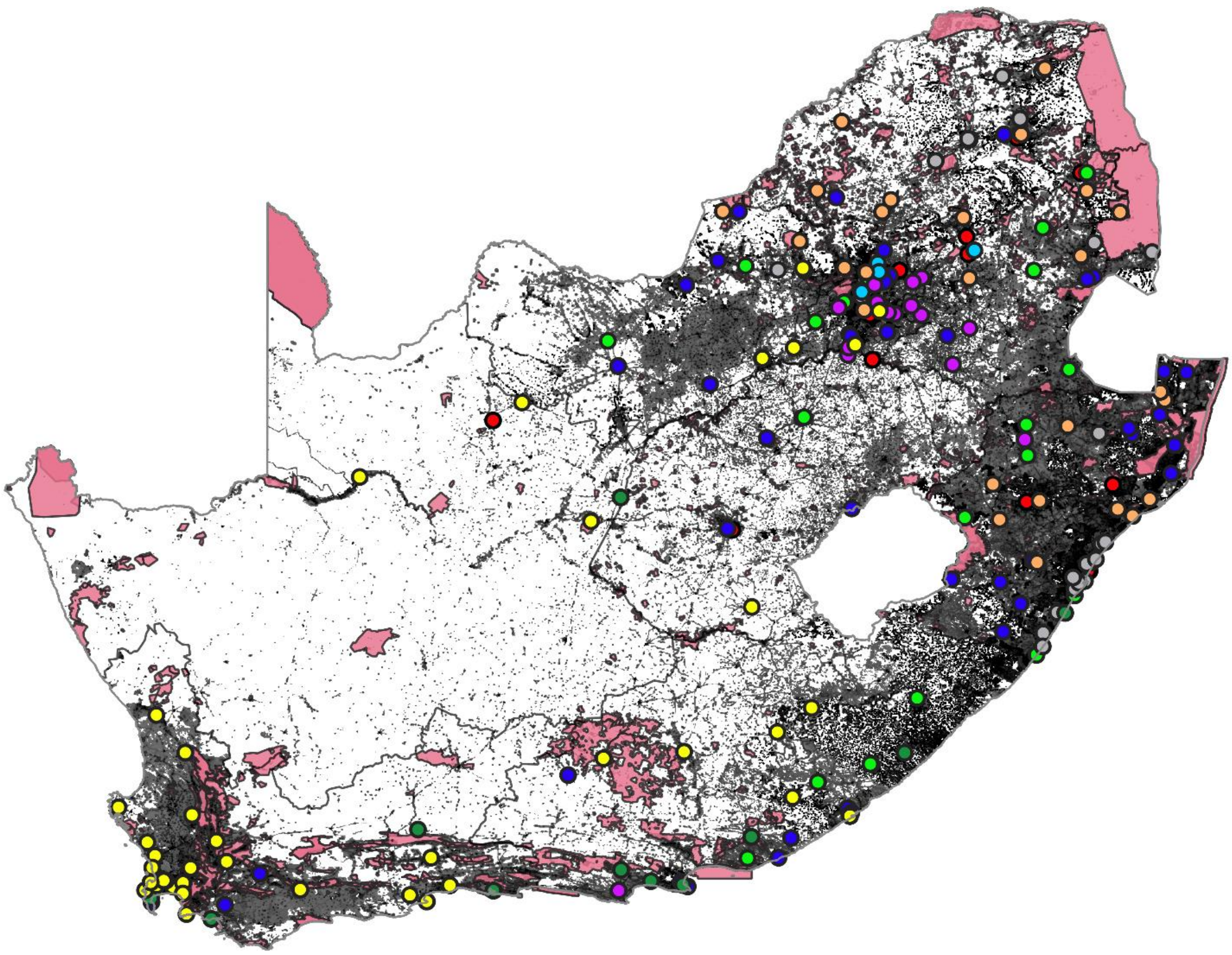

Figure 3. Snakebite incidence records in South Africa for nine medically important snake species shown in relation to human population density (black clustered areas indicate human population density) and protected areas (pink shaded areas). *Atractaspis bibronii* (red), *Bitis arietans* (dark blue), *Causus rhombeatus* (light green), *Dendroaspis polylepis* (grey), *Dispholidus typus* (dark green), *Hemachatus haemachatus* (purple), *Naja annulifera* (light blue), *Naja mossambica* (orange), *Naja nivea* (yellow).

Closest distances to the closest hospitals were less than 500 m for all nine species while the farthest distances ranged from 24 km (*Hemachatus haemachatus*) to 77 km (*Bitis arietans*) (Table 1). Since not all hospitals stock antivenom, the distances to the hospitals provide an indication of access to any medical facility not necessarily to a medical facility that is equipped to treat a snakebite.

Table 1. The minimum and maximum distance between a hospital and a snakebite incident from nine medically important snake species.

Snake species	Closest distance (km)	Location	Farthest distance (km)	Location
Atractaspis bibronii	0.14	Mbombela, Mpumalanga	33.06	Tonga A, Mpumalanga
Bitis arietans	0.14	Port Shepstone, KwaZulu-Natal	76.94	Molatedi, North-West
Causus rhombeatus	0.08	Vredenburg, Western Cape	38.46	Ganyesa, North-West
Dendroaspis polylepis	0.14	Port Shepstone, KwaZulu-Natal	33.06	Tonga A, Mpumalang
Dispholidus typus	0.07	Port Elizabeth, Eastern Cape	29.62	Patensie, Eastern Cape
Hemachatus haemachatus	0.16	Standerton, Mpumalanga	24.39	Madadeni, KwaZulu-Natal
Naja annulifera	0.29	Tzaneen, Limpopo	27.92	KwaMhlanga, Mpumalanga
Naja mossambica	0.14	Mbombela, Mpumalanga	68.93	Supingstad, North-West
Naja nivea	0.08	Vredenburg, Western Cape	48.63	Douglas, Northern Cape

3.5 Discussion

The results of this study revealed that hotspots for snakebite incidences in South Africa are concentrated in densely populated areas for all nine medically important snake species. Incidences generally followed the geographic distribution of the snake species responsible for the bite with most incidences in the biodiverse eastern region of the country and in the southwestern region. Overall, the largest clusters of incidences were shown to be in suburbs surrounding major cities such as Johannesburg, Durban and Cape Town which are also the most densely human populated areas in the country. Thus, medical facilities are generally in close proximity to snakebite incidences across the country and distances increase in remote location where longer travel times are required. However, since incidences are largely in densely populated areas, access to medical care is relatively available. The spatial patterns of snakebite incidences provide evidence that interactions between humans and snakes are occurring in areas of high human population density and these hotpots should be the target areas for prevention measures to be implemented.

The eastern distribution of snakebite incidences follows the larger diversity and distribution of snake species in the eastern parts of South Africa. Perhaps most surprising is the increased number of incidences in the eastern parts of North West Province particularly from several different snake species including *D. polylepis*, *N. mossambica* and *N. nivea* which are closer to the edge of their distributions. This area is also the intersect between the eastern and western part of the country which is restricted in the south due to the Drakensburg Mountain range. Thus, Gauteng and the bordering region of North West Province is the major hotspot for snakebite incidences. Species such as *N. nivea* which are largely found in the western parts of the country are moving east (Tolley et al. 2023a) and range expansions to the west have been noted for *D. polylepis* (Tolley et al. 2023b). Possible explanations could be that the geographic range of some species may be changing due to climate change (Tolley et al. 2023b) or, as human populations are on the rise and natural spaces are encroached, the detection of species at the edge of their ranges is likely to occur North West Department of Rural, Environment and Agricultural Development (READ) 2015. The convergence in the distribution of snake species from the tropical east and arid west in conjunction with human population growth, could explain why this region is a hotspot for snakebite incidences. These data provide valuable information regarding the distribution and movement patterns of snake species in South Africa.

The large number of incidences outside protected areas indicate that random encounters with snakes in the wild are unlikely to lead to an envenomation but rather incidences are higher where there are more people. Thus, locations where high probabilities of encounters occur between people and snakes, are likely to be the hotspots for incidences. This is evident by the high number of incidences in densely populated areas for all snake species in the study. The increased negative interactions between people and snakes have been shown to contribute towards snakebite incidences (Martín et al. 2022) and this study provides support for such interactions in the South African context. Rodents which are common prey for snakes are also likely moving into human-inhabited areas, leading to increased snake activity in such areas. Since reptile richness has been shown to be higher inside protected areas in South Africa (Tolley et al. 2019), the encounter rate between people and snakes outside protected areas needs to be investigated. If we consider that the fear of snakes may be higher amongst people living in areas of high human activity (Pandey et al. 2016), where exposure to wildlife and nature may be more limited than in protected areas (Zsido et al. 2022), the probability of higher snakebite incidences in densely populated areas is apparent.

For all snakebite incidences, the shortest distance to a hospital was less than 500 m indicating that medical care is accessible in the event of many bites. However, while some urban hospitals may have antivenom, rural hospitals have few to none (Neumann et al. 2023). The farthest distances to travel are usually in more remote areas and in the case of a bite incidence from *B. arietans*, it could be up to 77 km away. Since it is likely that not all hospitals were included in the available dataset, the current patterns provide an approximation of distances required to travel to healthcare facilities. However, the data shows that reaching the nearest hospital allows access to care that may help the patient to live longer until they have access to antivenom. Due to the lack of information regarding access to antivenom, accurate predictions cannot be made regarding the outcome of a bite. However, based on the very low death rate due to snakebite in South Africa (Chapter 2), it is evident that access to any medical facility that can provide first aid and slow down the effect of venom, is highly useful.

Snakebite incidences were shown to occur in areas of high human population density. This is in contrast to other studies that have attempted to comprehensively characterise the geography of snakebite incidences in relation to human population density and found a reduction in incidences in areas of high human population density in Sri Lanka, Ghana and Nigeria (Ediriweera et al. 2016; Molesworth et al. 2003). This might be the result of a reduction of suitable habitats for snakes and increased human activities that significantly reduce the diversity of snake species (Ediriweera et al. 2016; Parkin et al. 2021). In other parts of the world, snakebite risk is also associated with agricultural workers who live in tropical areas (Ediriweera et al. 2016; Mise et al. 2019), and the factors that affect the susceptibility of agricultural workers than those in other occupations tends to differ in different regions. In South Africa, since the majority of incidences are in densely human populated areas, it is unlikely that agricultural workers are the main victims. An explanation for the patterns in South Africa could be that due to existing conservation areas and green spaces in close proximity to human populated areas, there are more refugia for snakes (Tolley et al. 2019). Expansion of natural spaces could allow snakes to move away from human habitats back into natural spaces.

There is also a bias in the reported data on incidences as it is likely that incidences from rural healthcare facilities have a lower probability of being reported. Other studies also report on the challenges in the lack of data from rural areas (Farooq et al. 2022; Fox et al. 2006). Thus, the current spatial patterns of snakebite incidences in South Africa only provide a partial view of the actual patterns. Patterns in snakebite incidences are further influenced by environmental factors that impact snake activity, which is likely to change from year to year. However, improved information on interactions between snakes and humans such as data from snake rescues and housing types, could offer more insight into the relationship between humans and snakes. Land use change is also thought to influence snakebite epidemiology and the probability of encountering local snake species, with factors such as climate change driving broad-scale changes in ensembles of venomous snakes (Martín et al. 2021). Therefore, it is evident that further investigation is needed on the relationship between humans and snakes in rural environments.

Overall, the spatial patterns of snakebite incidences correlate with the larger number of incidences reported in Chapter 2. This is especially evident for North West Province where the prevalence of snakebite is high for the 12.5 years of data assessed (8.41 per 100 000 individuals in the population), corresponding with the results of a previous study that also showed high prevalence in North West Province (Lermer et al. 2023). Although snakebite incidences in this study are an underestimate of the total number of incidences, these data provide a spatial overview of incidences and some of the factors that contribute towards them. It is likely that there will be range shifts for medically important venomous snake species in South Africa and investigating these shifts in relation to human inhabited areas could be beneficial to mitigate human-snake conflict. Padidar et al. 2023 provide a valuable breakdown of various population density factors influence risk areas and future studies could include this for South Africa.

Education and awareness campaigns need to be implemented in hotspots for snakebite incidences and since these are larger cities in South Africa, education efforts can be scaled up to be included within the current education system at schools and universities. The increased education efforts will create positive perceptions towards snakes and with more willingness to provide support to snake conservation initiatives (Farooq and Geldmann 2024; Knight 2008). This is a viable strategy to promote harmonious living with snakes and such strategies will not only benefit biodiversity conservation but also play an essential part in implementing national snakebite prevention protocols.

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Chapter 4: Determinants of striking behaviour in four South African medically important snake species

4.1 Abstract

Snakes in Africa display a range of defensive behaviours, but there is limited quantification on the retaliatory behaviours of most of these species when they are presented with stimuli that elicit a threat and the factors that might influence defensive behaviours. I investigated defensive striking behaviour of four African snakes: puff adder (*Bitis arietans*), black mamba (*Dendroaspis polylepis*), snouted cobra (*Naja annulifera*) and Mozambique spitting cobra (*Naja mossambica*), including the impact of snake body size and sex on striking behaviours and strike performance. Individuals were presented with a fake arm model to simulate a human arm and were used in two trials consecutively. Of the 14 *D. polylepis* individuals, ten defensively struck at the hand and eight individuals bit the hand. Three of the seven *N. annulifera* struck at the hand but of these only two bit the hand and of the six *B. arietans* individuals, four struck the hand and three bit the hand. None of the 21 *N. mossambica* individuals struck or bit the hand. Body size and sex were not significant predictors of striking behaviour and there was no significant difference between trials. However, defensive behaviours overall were higher in the second trial. Strike performance results showed that *B. arietans* ranked as the fastest striking among the three snakes contributing to its status as the species responsible for most snakebite incidences in South Africa. These results show that that snakes will flee when provided the opportunity but if continuously harassed, they will strike and bite.

4.2 Introduction

Animals typically defend themselves against predators for survival and this can lead to effective or ineffective retaliation. Thus, an animal's ability to effectively retaliate in any situation will influence the probability of its survival (Hayes et al. 2002). The effectiveness of antipredator behaviours depends on the context of predator-prey interactions and natural selection has equipped animals to use these traits flexibly (Llewelyn et al. 2010). Snakes are a notable example of animals that have many elaborate antipredator displays, allowing them to effectively defend themselves when necessary. Striking is an effective antipredator defence exhibited by many species of snakes (Greene 1988; Herzog et al. 1992) but snakes have also evolved additional forms of defence such as signalling warning displays. Defensive behaviours can either be primary, where snakes can avoid detection through crypsis or secondary, where they display warning signals to intimidate their predator (Edmund 1974). As snakes have maximised their ability to strike and inject venom into their target, they benefit from predatory and defensive success with reduced ways of getting injured (Herbert 2007). As a result, they are able to defend themselves effectively.

There are many factors that influence defensive behaviours in snakes. The severity of an attack from a predator has been shown to increase defensive behaviour (Arnold and Bennett 1984), while the impact of environmental and body temperatures on behaviour varies for different snakes (Goode and Duvall 1989; Keogh and DeSerto 1994; Rowe and Owings 1990; Whitaker et al. 2000). When some species of snakes are closer to their mean body temperature, they can delay launching a defensive strike (Whitaker et al. 2000). Other factors that influence defensive behaviour include body size (Hailey and Davies 1986; Whitaker and Shine 1999), reproductive condition (Graves 1989), and sex (Scudder and Burghardt 1983). However, both non-venomous and venomous snakes infrequently bite during an interaction with a predator as it can be costly and increase the risk of injury (Bauder et al. 2015; Gibbons and Dorcas 2002).

In recent years, negative interactions between wildlife and people have increased due to an overlap in resource use and encroachment of natural areas, which has led to widespread issues such as human-snake conflict (Madden and McQuinn 2014). The fear of snakes creates further conflict, arising through their persecution. The number of snakes killed per year is unknown but greatly outweighs human fatalities from snakebite incidences (Whitaker and Shine 2000). Snakes have been persecuted for many generations regardless of whether they are venomous. A study based in rural Australia found that 38% of the respondents to a community-based survey reported that they would try to kill snakes wherever possible (Whitaker and Shine 2000). Thus, defensive behaviours in snakes are likely to be more common.

Defensive behaviour has also been studied in cottonmouths (*Agkistrodon piscivorus*) (Glaudas 2004; Gibbons and Dorcas 2002; Glaudas and Gibbons 2005; Glaudas and Winne 2007; Glaudas et al. 2006; Roth and Johnson 2004), rat snakes (Penning et al. 2020), and prairie rattlesnakes (Goode and Duvall 1989), each providing insight into behavioural displays that snakes exhibit to warn off predators. Studies have shown that striking ability does not differ significantly between non-venomous and venomous snakes (Penning et al. 2020) and higher body temperatures can cause more intensive defensive striking behaviour (Whitford et al. 2020) but does not impact predatory striking (Whitford et al. 2019). In contrast, other studies show that body temperature does not affect the defensive behaviour in snakes (Stepp-Bolling 2012; Whitaker et al. 2000). The common theme across these studies is that snakes display complex defensive behaviours and are capable of adapting these for different ecosystems.

Several studies have investigated the defensive striking behaviour of snakes (Glaudas 2004; Glaudas et al. 2005; Herzog et al. 1989; Shine et al. 2000; Shine et al. 2002; Whitaker et al. 2000) and how this may relate to humans as potential 'predators' to snakes (Gibbons and Dorcas 2002). Whitaker and Shine (1999) showed that Australian brown snakes (*Pseudonaja textilis*) avoid encounters with humans by slithering away, reducing their risk of being encountered. Their findings also suggested that when observers wore darker shades of clothing, which more strongly contrasted the sky and surrounding environments, encounters with snakes were also reduced as the snake had a higher probability of moving away. Glaudas (2004), Glaudas et al. (2005) and Whitaker et al. (2000) also showed that testing the effectiveness of predatory strikes in snakes allows a better assessment of the relationship between people and snakes. Such studies are lacking in Africa despite the high diversity of venomous snakes.

Snakes in Africa display a large range of defensive strategies to defend themselves. Viperids, such as *Bitis* spp, are often solitary, ambush predators, highly cryptic and have reduced locomotor capacity due to their thick heavy bodies (Greene 1997). However, vipers also display some of the fastest strikes (Araujo and Martins 2006; Young 2010). Elapids such as mambas use their elongated bodies to move swiftly through their arboreal environments and in the case of black mamba (*Dendroaspis polylepis*), they use several warning signals to defend themselves (Pitman 1965). *Naja* spp exhibit morphological and behavioural adaptations in the form of hooding, hood markings and spitting venom (Jones 2017). Cobras have been shown to perform false strikes, spread a hood and hiss during a confrontation with predators (Nasoori et al. 2016; Rasmussen et al. 1995). These strategies may be used individually or in combination and aim to deter predators from a distance (Jones 2017). These behavioural displays offer insight into the ecology of snakes which is currently not known for many African snake species.

There is limited information on the retaliatory behaviours of African snakes when they are presented with stimuli that elicit a threat and the factors that might influence defensive behaviours. Here I describe and compare the defensive behaviours of four medically important snake species, puff adder (*Bitis arietans*), black mamba (*D. polylepis*), snouted cobra (*Naja annulifera*) and Mozambique spitting cobra (*Naja mossambica*). These snake species are ideal for this study because they display various different defensive behaviours and feature most in snakebite incidences in South Africa. Given that snakes and humans find themselves in close proximity, and snakes perceive humans to be a threat, I investigated the relationship of snake body size and sex on whether or not a strike is launched, and if so, the velocity and distance of the strike. I predicted that increased striking displays would be correlated with larger body sizes and striking would be elicited when the threat was < 0.5 m for individuals of all species except *D. polylepis* which has a larger body size.

4.3 Methods

Four venomous snake species were selected for striking behavioural experiments. These included the diurnal and semi-arboreal *D. polylepis* (Branch 2016), the primarily nocturnal and terrestrial *B. arietans* (Glaudas and Alexander 2016), the nocturnal and primarily terrestrial *N. mossambica* (Shine et al. 2007) and the diurnal and terrestrial *N. annulifera* (Shine et al. 2007). The number of individuals for each species varied based on appropriately non-habituated snakes available for data collection.

4.3.1 Experimental design and protocol

Test snakes were recently wild caught near the town of Hoedspruit, Limpopo Province, South Africa, as many of the medically important venomous snakes are found in the area and are often encountered by the local population. Snakes are active in the area from August to May, providing increased opportunities to encounter snakes. Snakes were collected through snake call outs from all local snake rescuers that had followed a standardised procedure to capture snakes in Hoedspruit and surrounding areas within a radius of 50 km. Snakes were also captured by actively searching for them by road cruising. All snakes that were collected were transported back to Hoedspruit Reptile Centre and individually housed in the laboratory for one week before trials began. Snakes were kept for no more than two weeks. Individuals of *N. mossambica* were kept for one month as they were used in chemical cue trials after the behavioural trials. During this period, snakes were housed in glass-fronted wooden enclosures lined with newspaper and fitted with a hide and water dish. Water was provided *ad libitum*. Snakes were not fed prior or during the experimental trials to eliminate the effect of recent feeding on defensive behaviour (Herzog and Baily 1987). The snout-vent length (SVL), tail length, mass and sex of each snake were recorded before and after the trials to get accurate measurements on snakes before and after the trials. Experiments ran from December 2022 to March 2024.

4.3.2 Behavioural trials

To assess the response of snakes to visuals cues and thus assess behavioural responses, snakes were evaluated in visual cue trials. Trials were conducted at Hoedspruit Reptile Centre in a semi-captive setting in an open arena (Fig. 1). Snakes were provided with an identification number (i.e., DP #1) to identify individuals. Snakes were used in trials between 0 – 7 days after arrival at the Centre unless they were shedding in which case, the snakes were used up to 14 days after arrival at the Centre. A 1 m x 1 m wooden scale grid (10 cm x 10 cm increments) was placed on the ground in the arena for distance measurements and a barrier was placed in the top left corner (lateral view camera) to prevent snakes from immediately fleeing. A GoPro Hero 9 camera (GoPro Inc., San Mateo, CA) was placed parallel to the grid and the snake to record all behavioural observations (Fig. 1). The grid was laterally visible in the field of view of the GoPro. Each snake was placed under a hide between the grid and the barrier in the open arena (Fig. 1).

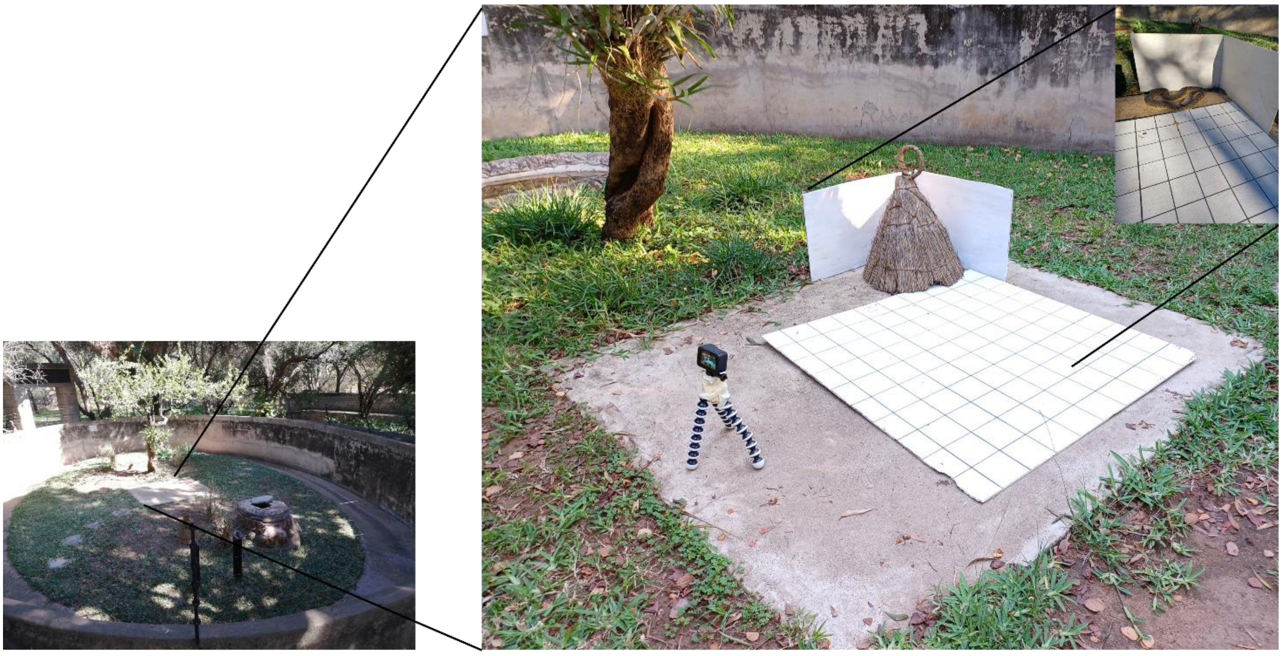

Figure 1. Semi-captive arena to conduct behavioural trials to test defensive striking in snakes.

Snakes were placed under a hide and allowed to rest for 60 seconds before the trial began. Snakes were then presented with a fake hand and forearm model to simulate a human arm (Fig. 2). The hand was fitted onto a pole and a distance of 1 m was maintained. Following the methodology of Burns and Penning (2021) the hand was moved closer to the snake and tapped around and on the snake to simulate a threat. Each trial began with the hand moving from the greatest distance possible (1 m) and moving closer towards the snakes at 10 cm intervals in a systematic manner. All strikes at the hand were interpreted as defensive behaviours because no snakes stayed attached to the hand after a strike was launched. Each individual was used in two trials consecutively. As soon as the snake fled after Trial 1, it was placed back in the hide for one minute before beginning Trial 2.

a)
Dendroaspis polylepis striking at fake arm

b)
Naja annulifera striking at fake arm

c)
Bitis arietans striking at fake arm

Figure 2. Various venomous snakes displaying biting behaviour after being presented with a fake hand.

Striking behaviour was interpreted as defensive when snakes made contact with the hand and then retracted back to a normal posture. Since no reliable methods could be used to assess whether venom was injected into the hand, no distinction was made between a dry bite and an actual bite in this study. Environmental temperature (°C) was also recorded using a Kestrel-3000 Pocket Weather Meter (Kestrelmeters.com) (NK, Boothwyn, PA, USA).

4.3.3 Video analysis

GoPro Player was used to analyse videos frame-by-frame and to score all behaviours. Behavioural responses were categorised as follows: striking, biting, spitting, attempting to escape, hooding, tongue flicking (number of times the tongue goes in and out the mouth) and gaping. Strike distance, velocity ($\frac{\Delta distance\ (m)}{\Delta\ time\ (seconds)}$) and acceleration ($\frac{\Delta Velocity}{\Delta\ time\ (seconds)}$) were also calculated for individuals that successfully executed a bite. Additional notes were also taken. A strike was defined as any movement forward with an opened or partially opened mouth and the snake extended its neck towards the hand. When the snake successfully made contact with the hand, a bite was considered successful.

4.3.4 Statistical analysis

A generalized linear mixed model (GLMM) was used to fit the model by maximum likelihood to examine predictors of defensive strikes in snakes. All analyses were conducted in R 4.4.0 (R Core Team 2024), and GLMM assumptions were tested in the R package DHARMa (Hartig 2018). I used a binomial dependent variable (i.e., strike (yes/no), while controlling for the effects of repeated observations on the same snakes. I did this by entering subjects (ID) as a random factor for all analyses in the R package lme4 (Bates et al. 2015). I tested for temporal variation in striking behaviour in snakes using temperature as a fixed factor, and I included two additional fixed factors, snake body size (SVL) and sex (M/F). The Akaike information criterion adjusted for small sample size (AICc) was used to compare the quality of the different models after adjusting for sample size (Burnham and Anderson 2002) and model comparisons were conducted by comparing model scores. I also used a GLMM to compare differences in striking between Trial 1 and Trial 2 for all snake species. I used an ANOVA to compare the strike acceleration, velocity, distance and duration between each species. No random effect was included.

4.4 Results

4.4.1 Behavioural trials

Forty-eight snakes were captured and used in defensive striking trials at Hoedspruit Reptile Centre. Fourteen *D. polylepis*, 21 *N. mossambica*, seven *N. annulifera* and six *B. arietans* were used in the trials. The GLMM showed that body size and sex were not a significant predictor of striking in *D. polylepis*, *N. annulifera*, or *B. arietans* (Table 1). Model 2 was the best model showing that there is variation in striking but this is not influenced by body size, but sex could be a small factor. Model weights were normalised so that the sum of weights across all models was 1, thus a value closer to 1, in this case 0.512, is the best model. An ANOVA comparing the full model to a null model with no predictors showed a non-significant chi-square test ($\chi^2 = 4.88$, df = 2, $P = 0.087$). *Naja mossambica* did not display any striking behaviour.

Table 1. AICc-ranked generalized linear mixed models predicting the impact of body size and sex on striking behaviour in venomous snakes with subject (ID) set as a random factor (n = 48, ID = 96). Each row shows the metrics of a different model, and the factors included in a particular model are depicted by the + sign. ΔAICc = difference between a model AICc score and the most parsimonious model AICc score (Model 2); *ωi* = Model weight (Highest = plausible; 0.512); SVL = Snake snout vent length; Sex = Snake sex.

Model	(Intrc)	Sex	SVL	df	AICc	ΔAICc	*ωi*
2	-1.312	+		3	102.2	0.00	0.512
1	-2.423			2	104.4	2.22	0.169
4	-1.555	+	0.0014	4	103.8	1.65	0.225
3	-2.854		0.0023	3	105.6	3.39	0.094

Of the 14 *D. polylepis* individuals tested, ten (71.4%) defensively struck at the hand when provoked. Eight of the individuals that struck the hand also bit the hand (57.1%, Fig. 2). Among other defensive behaviours, hooding was displayed in 11 individuals (78.6%), gaping was displayed in 13 individuals (92.9%) and six elevated their body (42.9%). Three of the seven *N. annulifera* individuals struck at the hand (42.9%) but of these only two bit the hand (28.6%). All individuals displayed hooding while 57.1% gaped. Of the six *B. arietans* individuals, four struck (66.7%) and three bit (50%), while 66.7% attempted to escape. Of the 21 *N. mossambica* individuals tested, none displayed striking or biting behaviours when presented with a threat. However, 76.2% spat at the hand, 90.5% displayed hooding and 33.3% gaped. All *D. polylepis, N. mossambica* and *N. annulifera* individuals attempted to escape after being presented with the threat stimulus. Four of the six *B. arietans* individuals attempted to escape at the end of the trial. For any individuals that attempted to flee, I increased the simulated aggression until a defensive strike was elicited or the snake fled. All individuals except two *B. arietans* attempted to escape immediately or 30 seconds after the threat was simulated.

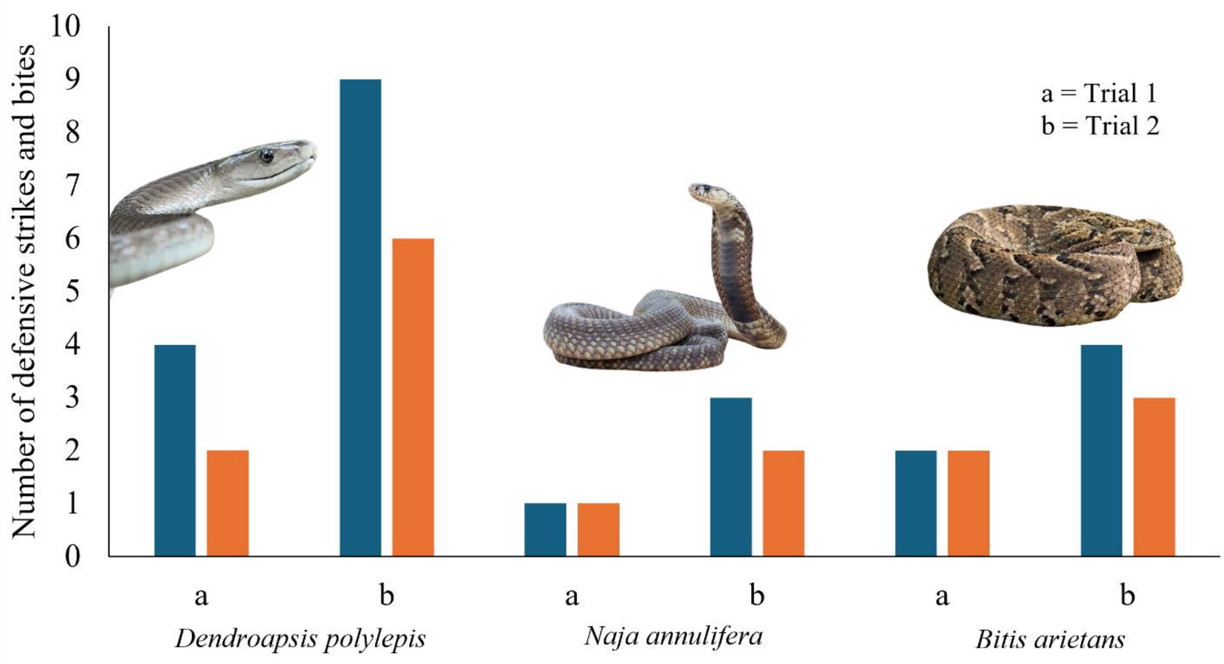

Figure 3. Striking and biting behaviours compared in Trial 1 (a) and Trial 2 (b) for *Dendroaspis polylepis*, *Naja annulifera* and *Bitis arietans*. Dark blue bars represent strikes and orange bars represent bites.

A GLMM showed that striking was significantly different between Trial 1 and Trial 2 (P = 0.036; Table 2), and snakes did show a tendency to bite and strike the fake hand more in the second trial than the first trial.

Table 2. AICc-ranked generalized linear mixed models showing the factors influencing the probability of a strike. The model that best fit the data included both Species and Trial as fixed effects, with a random intercept for individual snakes (ID). Each row shows the metrics of a different model, and the factors included in a particular model are depicted by the + sign. ΔAICc = difference between a model AICc score and the most parsimonious model AICc score (Model 4); *ωi* = Model weight (Highest = plausible; 0.883).

Model	(Intrc)	Species	Trial	df	AICc	ΔAICc	*ωi*
4	-9.860	+	+	6	78.8	0.00	0.883
2	4.404	+		5	83.3	4.48	0.094
3	-1.800		+	3	86.2	7.35	0.022
1	-2.423			2	104.4	25.58	0.000

4.4.2 Strike performance

Dendroaspis polylepis individuals did not strike at the hand if it was more than 0.5 m away (Fig. 4). In Trial 1, one individual struck at 20 cm, one at 15 cm, one at 10 cm and one at approximately 1 cm (very close to the snake). In Trial 2, four individuals struck at the hand when it was very close to the snake (~1 cm), two struck at 10 cm, two at 20 cm and one at 50 cm (Fig. 4). The average distance to strike was 4 cm. Of the three *N. annulifera* individuals that struck the hand, in Trial 1, one struck at a distance of 5 cm, one at 1 cm and in Trial 2, one struck at 1 cm, one at 5 cm and one at 10 cm (Fig. 5). The average distance to strike was 1.6 cm. Four of the six *B. arietans* individuals struck at the hand and only when it was 5 cm away or closer. In Trial 1, two individuals struck at 5 cm and in Trial 2, one individual struck from 5 cm away, one at 2 cm and two from 1 cm (Fig. 6). The average distance to strike was 1.6 cm. Spitting behaviour was displayed from distances > 1 m. During a single trial (30 – 60 seconds), *N. mossambica* individuals spat between 3-7 times before fleeing and individuals spat more in Trial 2 than in Trial 1(Fig. 7).

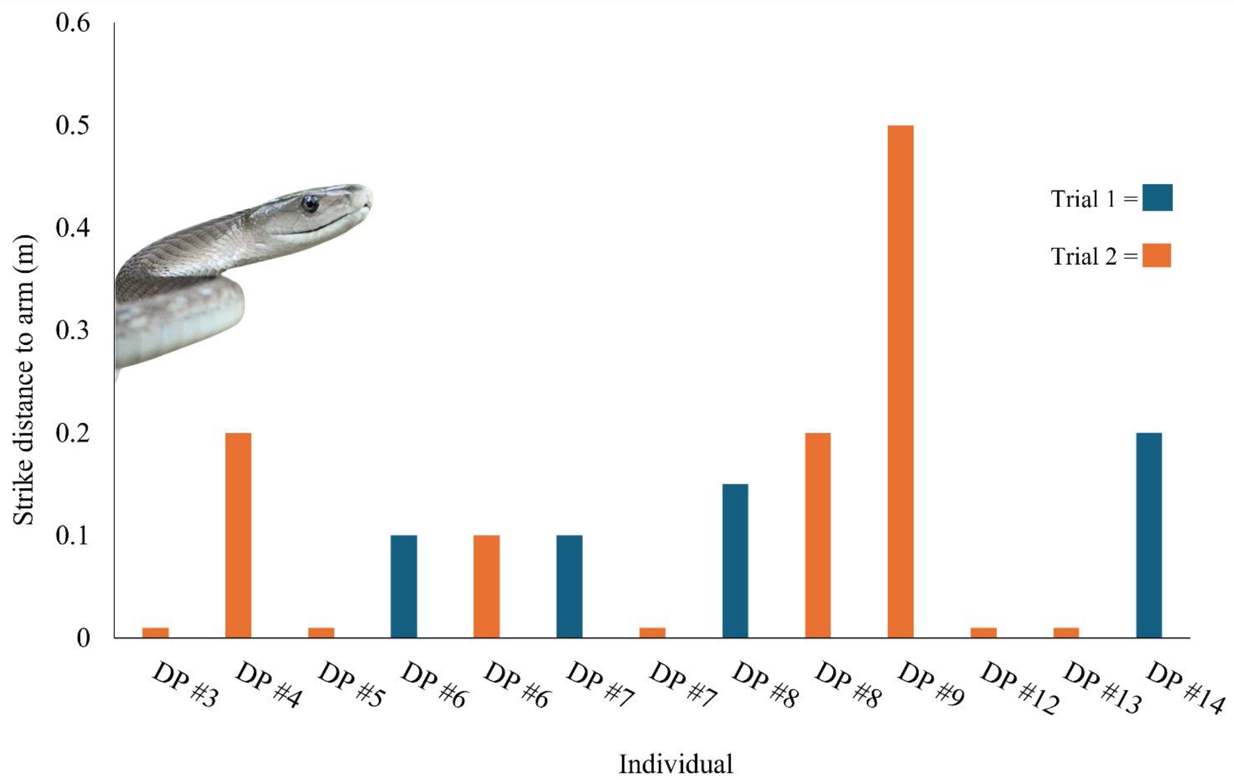

Figure 4. Distances at which *Dendroaspis polylepis* individuals struck the fake hand. Ten of the 14 individuals struck the arm and individuals not listed did not strike or bite the arm.

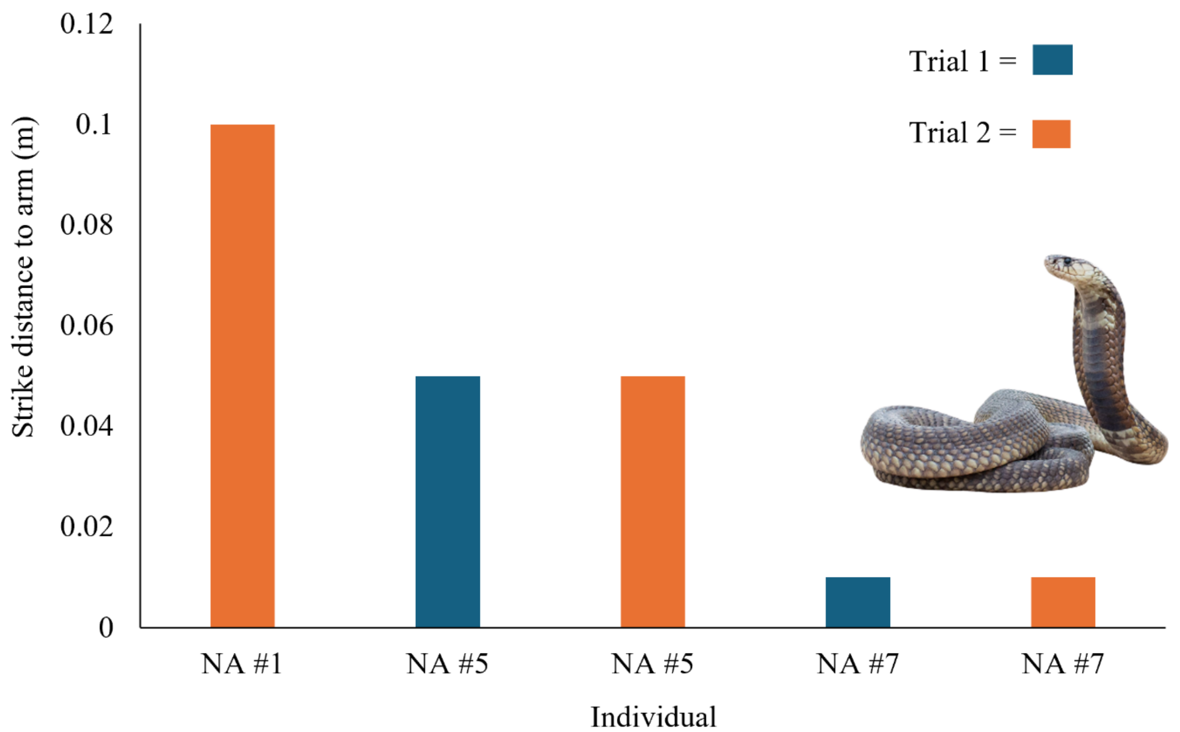

Figure 5. Distances at which *Naja annulifera* individuals struck the fake hand. Three of the seven struck the arm and individuals not listed did not strike or bite the arm.

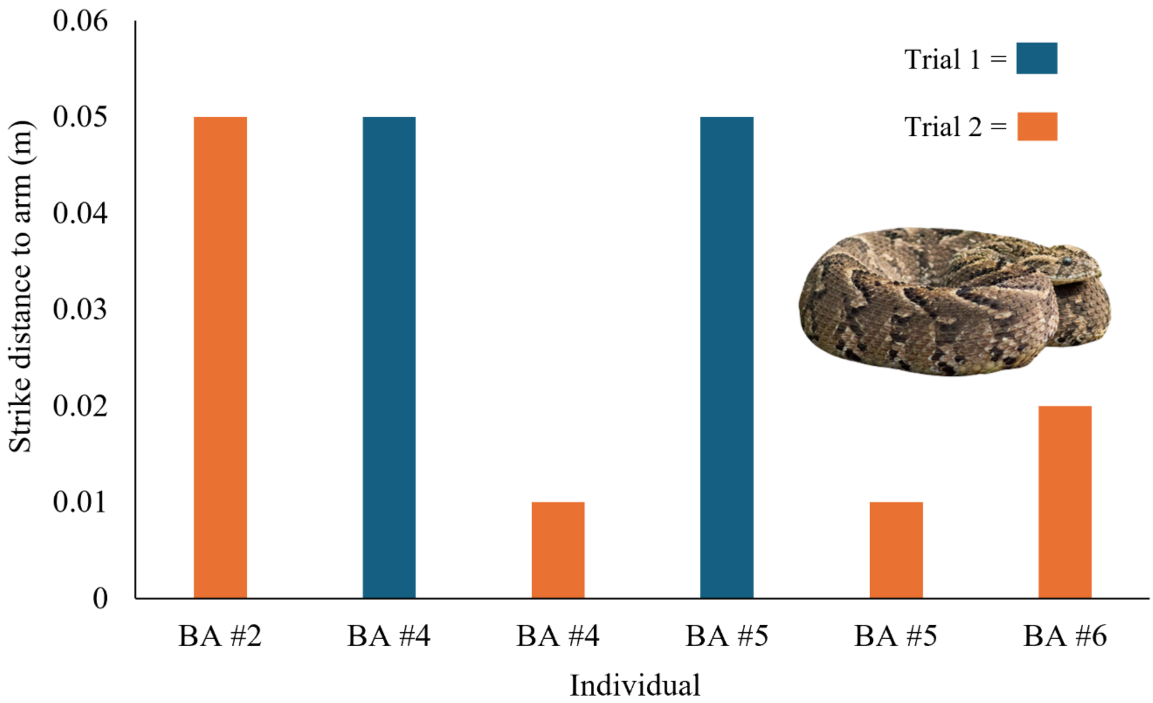

Figure 6. Distances at which *Bitis arietans* individuals struck the fake hand. Individuals not listed in the graph did not strike or bite.

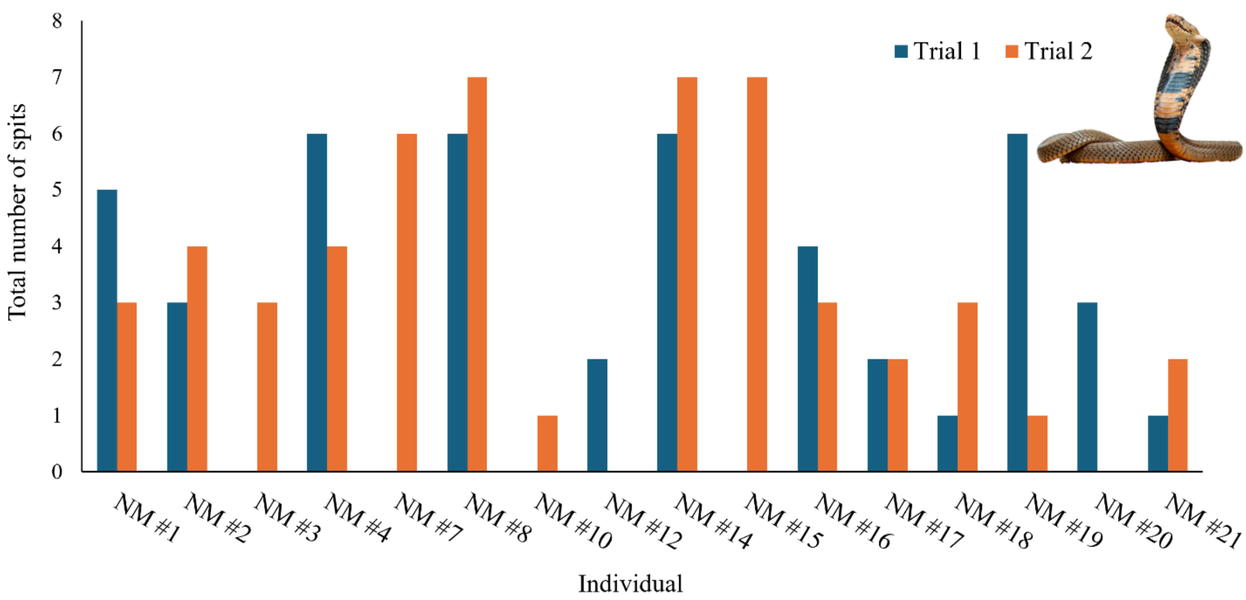

Figure 7. Total number of spits displayed by Naja mossambica individuals. Individuals not listed in the graph did not display any spitting.

All strikes were completed in less than 0.4 seconds. All snakes struck with high accelerations (range = 5.55 – 158.33 ms^{-2}) and velocities (0.03 – 1.56 ms^{-1}) over short distances (1 – 50 cm) and with short durations (0.06 – 0.38 s). There was a large variation between the strike accelerations and velocities for the different species (Table 3). The acceleration for the three species were statistically different from each other ($F_{2,21} = 3.89$, $P < 0.03$) but their velocities did not differ significantly ($F_{2,21} = 1.7$, $P > 0.20$). Strike distance was not significantly different between species ($F_{2,21} = 3.89$, $P > 0.20$) but strike duration was significantly different ($F_{2,21} = 3.89$, $P < 0.02$).

Table 3. Defensive strike performance by *Dendroaspis polylepis*, *Naja annulifera* and *Bitis arietans*. The range for each species is provided.

Species	Strike Acceleration (ms^{-2}) (mean ± SD)	Strike Acceleration (ms^{-2}) (range)	Strike Velocity (ms^{-1}) (mean ± SD)	Strike Velocity (ms^{-1}) (range)	Strike Distance (cm) (mean ± SD)	Strike Distance (cm) (range)	Strike Duration (s) (mean ± SD)	Strike Duration (s) (range)
Dendroaspis polylepis	43.69 ± 24.94	13.50 − 90.27	0.40 ± 0.30	0.03 − 1.56	0.14 ± 0.14	0.01 − 0.50	0.21 ± 0.08	0.12 − 0.38
Naja annulifera	10.74 ± 4.66	5.55 − 17.19	0.16 ± 0.08	0.05 − 0.29	0.06 ± 0.03	0.01 − 0.10	0.23 ± 0.09	0.16 − 0.38
Bitis arietans	60.86 ± 57.34	15.10 − 158.33	0.26 ± 0.25	0.10 − 0.83	0.06 ± 0.05	0.01 − 0.20	0.14 ± 0.08	0.06 − 0.24

4.5 Discussion

The behavioural trials in this study showed that of the four medically important venomous snake species, only *D. polylepis*, *N. annulifera* and *B. arietans* displayed striking and biting behaviour. In general, snakes delayed the initiation of a defensive strike until a simulated threat was in close proximity (< 50 cm), from the initial starting point (1 m). Individuals of *D. polylepis*, *N. annulifera* and *B. arietans* also displayed rapid strikes in less than 0.4 seconds duration. *Naja mossambica* did not strike but displayed spitting behaviour in response to the threat as spitting is its primary defence. When individuals of all species were presented with a fake hand, they reacted by either attempting to flee the strike arena or remaining in place and responding to the simulated threat by displaying several defensive behaviours. All snakes struck with high accelerations and velocities; however, body size and temperature did not influence striking behaviour. The behavioural experiments further show that snakes were less likely to bite if they had the option to flee but if aggravated consistently, they bite to defend themselves. This suggests that venomous snakes are typically hesitant to strike in encounters with humans, however, persistent provocation will elicit a strike and in the case of *D. polylepis, N. annulifera* and *B. arietans*, the likelihood of a bite is ~50%.

Striking distances were shorter than expected (less than 5 cm on average) and only one *D. polylepis* individual struck the farthest at 0.5 m, suggesting that a threat needs to be in close proximity before a snake will strike and bite. When bites occurred, they were extremely fast with high accelerations (range = 5.55 – 158.33 ms^{-2}), and this was observed in all three species with the highest acceleration observed in *B. arietans*. Although striking and biting were not statistically significant different between Trial 1 and 2, observations showed that defensive behaviours were higher in Trial 2, and the non-significance could be the result of a lower sample size. Since increased warning signals were displayed before a strike by all individuals, snakes are likely to provide ample warning to a predator when defending themselves.

The large variation in strikes, which were not influenced by body size or sex shows that striking behaviour is individual specific and striking in this context is not predictable based on selected variables. Since snakebite incidences mostly occur at random (Chapter 3), it is likely that venomous snakes such as those in this study strike and defend themselves as needed in a particular situation. The complexity of defensive behaviour in snakes has been researched in several other snake species (Stepp-Bolling 2012; Whitaker et al. 2000; Whitford et al. 2019; Whitford et al. 2020) and I provide evidence for the same complexity in *D. polylepis*, *N. annulifera* and *B. arietans*. As not all the individuals in the study displayed defensive strikes, it further indicates that provided that snakes have the option to flee, they are likely to do so.

Most individuals of *N. mossambica* and all individuals of *N. annulifera* displayed a hood as expected when presented with a threat. The two individuals of *N. mossambica* that did not display a hood did not show other defensive behaviours either and attempted to escape indicating that even when harassed, they prefer to flee rather than behave defensively. Since hooding is used as a defensive trait to appear larger to predators, it is no surprise that this was displayed before biting (*N. annulifera*) or spitting (*N. mossambica*). Instead of striking, *N. mossambica* only used spitting as a defence mechanism and spat long distances without attempting to strike. This supports the current literature on venom evolution as a defence in spitting snakes (Jones 2017) but does not explain the high number of snakebite incidences from this species in South Africa (Chapter 2). Further information is needed on the circumstances of bites to gain insight on why *N. mossambica* bite humans.

Bitis arietans primarily relied on inflating and deflating its body while making a hissing sound to advertise a warning and also moved backwards with the head and neck in s-shaped defensive movements before a strike was elicited. When ready to strike, the jaw opened, and the fangs on the maxillary bone would rotate forward, becoming vertically aligned at the top of the mouth indicating that body curvature and head placement are important indicators for defensive strikes. As heavy bodied ambush foraging snakes (Young 2020), the distance between the snake and the target (in this case the fake arm) needs to be covered by the striking action, before the target can evade the strike. As the fastest striking snake in this study (< 0.2 seconds) and best overall strike performance, *B. arietans* show that despite only being able to strike at shorter distances, they can strike effectively. Their effective striking is also likely the reason why they are responsible for most snakebite incidences in South Africa (Chapter 2).

Overall, *D. polylepis* showed the greatest willingness to bite in comparison to the other species tested in my study but were still reluctant to strike and bite in response to harassment. There is ample evidence to suggest that snakes rely on displaying warning signs before striking and this has been shown in various other species (Glaudas and Twine 2007; Shine et al. 2002). Almost all *D. polylepis* individuals displayed gaping and exposed the inky black colour on the inside of their mouth, thus supporting the evolution of the black mouth colouration as a warning signal (Marques and Sazima 2022). In addition to gaping, *D. polylepis* individuals are also capable of hooding and both hooding and gaping behaviours were displayed when the simulated threat was > 0.5 m away. This further supports the effectiveness of warning signals in defence. The adaptive significance of displaying warning signs is to maximise the prey's survival and decrease the probability of an attack by a predator and this is done effectively by *D. polylepis*, despite their ability to strike and escape quickly when needed.

The accelerations and velocities of strikes for all three snake species in this study showed that snakes have the ability to reach their predator quickly. When bites occurred, the strikes were fast, and individuals released their jaws quickly from the hand after the bite, supporting the rapidity observed in defensive striking in snakes in other studies (Araujo and Martins 2006; Teshera and Clark 2021). Strikes often occur within a blink of an eye (Burns and Penning

2016) and snakes have a unique ability to use their body as anchors while thrusting their open mouths to strike towards a predator (Cundall 2009; Cundall and Greene 2000). Snakes in this study used the first third of their body to lunge forward during a strike while the rest of the body remained in the same position. This indicates that their striking ability needs to be accurate enough to deliver a bite if needed and still be able to flee. Glaudas (2021) found that proximity to venomous snakes and encounter frequency are not necessarily linked to snakebite incidences. However, encounter rates are increased when humans and snakes find themselves in the same space and in such circumstances, it is the behaviour of the snake that predicts the likelihood of a bite occurring. The fast strikes over short distances by *D. polylepis*, *N. annulifera* and *B. arietans* provide insight into the delivery of effective bites when humans and snakes are in close proximity.

Since most snakes attempted to flee in this study, there is no evidence of external factors influencing fleeing. Snakes show tolerance towards harassment and most seek to escape rather than retaliate. Contrary to popular opinion, even *D. polylepis* are reluctant to strike and bite in response to human harassment unless they are persistently provoked. The results of this study provide insights into the defensive behaviours of African snakes in relation to human threats which creates opportunities to consider prevention measures to human-snake conflict challenges such as snakebite. Future studies could consider venom expenditure from envenomation for defensive bites and assess differences between defensive behaviours between venomous and non-venomous snakes that are becoming more prominent in snakebite incidences. The fake arm could also be swabbed for presence of venom as one way to determine envenomation the arm could be modified to collect venom in a receptacle inside the arm in a similar manner to how snakes are milked.

Striking and envenomation are likely to increase when snakes are stressed and within disturbed, high stress environments where human encounters with snakes are increased. Thus, if environmental stressors are mitigated, the burden of snakebite is likely to be reduced (Herr et al. 2017). While we cannot accurately predict when venomous snakes will strike, their pre-strike behaviours and warning signs are indicative of an impending strike and potentially a bite. Strikes from venomous snakes are also fast and thus if humans interact with them in close proximity, there is an increased likelihood of envenomation. Further studies could replicate this design in other provinces to observe if snakes behave differently in different regions and increased sample sizes for different species will provide more accurate behavioural data. This study provides further insight into the growing knowledge on the behavioural ecology of venomous snakes which is becoming a crucial factor in mitigating conflict with humans.

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Chapter 5: Unveiling the allure: Chemical cue investigation in Mozambique spitting cobra (*Naja mossambica*)

5.1 Abstract

Snakebite incidences in sleeping humans present a significant public health concern in several regions of the world. In South Africa, the Mozambique spitting cobra (*Naja mossambica*) is predominantly responsible for such incidents. Given that many bites occur non-confrontationally, there is some speculation that snakes misidentify humans as prey, potentially attracted by human sweat. To test the mistaken identity hypothesis, I investigated the behaviour of *N. mossambica*, when individuals were presented with human sweat, prey scent, and a control stimulus. Body size was also used as a factor to assess differences in behaviour among individuals. The results indicate that *N. mossambica* investigated sweat and prey samples significantly more than the control and body size was positively correlated with investigative behaviour. *Naja mossambica* did not display defensive behaviours such as biting, striking or spitting which are commonly associated with a threat response. Most behaviours observed were associated with general exploration and active foraging. *Naja mossambica* like many other snakes that bite sleeping humans, are nocturnal active foragers and most likely come across sleeping humans and bite them accidentally mistaking them as prey. Thus, this study supports the mistaken identity hypothesis. This research underscores the importance of assessing snake behaviour in mitigating the risks associated with human-snake interactions, particularly during vulnerable periods such as sleep.

5.2 Introduction

Snakes are exposed to different types of predators in various habitats, causing them to display multiple defensive behaviours (Greene 1988). With increasing human pressure on the environment, encounters between humans and snakes have increased, resulting in more defensive behaviours observed in snakes (Araujo and Martins 2006; von Takach et al. 2023). These defensive encounters can sometimes lead to envenomation from venomous snakes, leading to a general increase in snakebite incidences. The majority of snakes that cause envenomation are from the snake families Viperidae and Elapidae (Gutiérrez et al. 2017). Viperids are generally ambush-foraging snakes that rely on camouflage to avoid detection, and elapids are predominantly actively foraging snakes and display more notable defensive behaviours (Greene 1997).

Among the elapids, the core cobra clade is profoundly interesting for their behavioural adaptations and ecological traits (Shine et al. 2007). All core cobra species have highly toxic venom which is comprised of cytotoxins, cardiotoxins and neurotoxins, all of which can cause neurological damage and local necrosis (Chu et al. 2010). Many commonly recognised defensive traits of snakes are observed in the *Naja* clade. Some of these species have evolved hooding and display a variety of hood colourations, and others have additionally evolved the ability to spit venom (Jones 2017). Hooding requires the snake to spread the ribs in the neck and reveal brightly coloured patterns to appear larger to predators (Berthé et al. 2009). Hooding is also present to a lesser extent in other elapids, such as mambas (*Dendroaspis*). Cobras have been shown to perform false strikes, spread a hood and hiss during a confrontation with predators (Nasoori et al. 2016; Rasmussen et al. 1995). Selection pressures from predators may have caused the evolution and use of a combination of these traits (Jones 2017).

Spitting has arisen several times independently in the elapids (Slowinski et al. 1997; Westhoff et al. 2005; Wüster and Thorpe 1992). The evolution of spitting in cobras has offered an ideal system for exploring the evolution of defensive toxins as the behaviour targets specific mammalian sensory tissues and among ~600 venomous snake species, it is the only long-distance, injurious defensive adaptation (Kazandjian et al. 2021). To successfully hit their target, spitting cobras have evolved specialised fangs that eject their venom at high pressure in a forward motion (Avella et al. 2021). Thus, spitting cobras are a striking example of snakes that have been shaped by similar selection pressures and shared adaptations which have driven convergence in molecular, morphological, functional and behavioural traits

(Kazandjian et al. 2021). These adaptations have allowed cobras to occupy diverse ecosystems where they can successfully evade and defend against predators.

Some snakes have been shown to perform antipredator behaviours more effectively at night (*Cryptophis nigrescens*; *Hoplocephalus bungaroides*) (Llewelyn et al. 2010), most likely because visual cues become impaired. Thus, in darkness these species must rely on olfactory cues to effectively navigate their surroundings, assess potential threats, and engage in predator detection and prey capture (Mason 1992). Not much is known about the ecology of nocturnal cobras, however, they are quite often reported to bite people at night (Broadley and Baylock 2013). Snakes are likely to get into homes as they are attracted to the smell of food or seeking shelter (Jayawardana et al. 2020) and many bites occur in newborn babies (Broadley and Blaylock 2013; Fritts et al. 1994; Suraweera et al. 2020). There have been several reported cases of sleeping humans bitten by venomous snakes from common kraits (*Bungarus caeruleus*) in Nepal (Chappuis et al. 2007) and India (Alirol et al. 2010; Chauhan et al. 2015; Sharma et al. 2016), and by hump nosed vipers (*Hypnale hypnale*) and *B. caeruleus* in Sri Lanka (Jayawardana et al. 2020). Several studies have also been conducted to assess why brown tree snakes (*Boiga irregularis*) bite people at night in Guam (Chiszar et al. 1988; Chiszar et al. 1993). While in several parts of Africa, reports show that people have been bitten when they sleep by zebra spitting cobra (*Naja nigricinta*) in Namibia (Saaiman and Buys 2019) and red spitting cobra (*Naja pallida*) in Kenya (Tianyi et al. 2024).

In South Africa, there have been several reported cases of bites in sleeping humans by Mozambique spitting cobra (*Naja mossambica*) (Blaylock 2005; Coetzer and Tilbury 1982; Tilbury 1982; Vermaark et al. 2010) and in Chapter 2, I have provided a summary of these incidences. Among these, Vermaark et al. (2010) report on bites from *N. mossambica* in three sleeping people, two of which were neonates who sustained multiple bites on the hand. Tilbury (1982) also reports on bites in sleeping neonates. As neonates are unlikely to annoy or cause harm to the snakes, the bites are likely predatory rather than defensive (Chiszar et al. 1993), perhaps meaning that snakes are attracted to the chemical cues from neonates (Chiszar et al. 1988). Considering that most bites in sleeping humans are non-confrontational, it is likely a case of misidentification of the victim as prey (Natusch et al. 2021). Perhaps, like some mosquito species, cobras could be attracted to human sweat.

Since defensive behaviour can often take the form of visual or acoustic displays, *N. mossambica* primarily relies on spitting to defend themselves. However, when visual and acoustic displays fail, snakes will rely on biting defensively (Westhoff et al. 2005). Given that *N. mossambica* is responsible for most bites in sleeping humans in South Africa, I investigated the behaviour of *N. mossambica* when presented with chemical cues in a testing arena to assess whether behaviours were predatory or defensive. I also investigated whether body size, sex and temperature impacted the chemosensory investigation. I hypothesised that

5.3 Methods

5.3.1 Study animal

Naja mossambica are slate grey to olive brown snakes with dark-edged scales with a black outline. The underbelly is salmon pink or yellowish and some individuals have black crossbars and blotches on the throat/neck area forming a band across the ventral scales (Marais 2022). They are mostly active during dusk and night but can be found basking during the day. They typically inhabit mesic savanna, and lowland forests close to a permanent source of water (Marais 2022). The venom is predominantly cytotoxic that causes significant damage to the tissue, causing necrosis but the venom also has additional neurotoxic effects (Hus et al. 2024). They feed on a variety of prey including amphibians, mammals, young birds, eggs and other snakes (Shine et al. 2007). *Naja mossambica* spreads a hood as a defence and spits its venom up to almost 3 m (Rasmussen et al. 1995).

5.3.2 Experimental design and protocol

The study was conducted in the town of Hoedspruit, Limpopo Province, South Africa and snakes were wild caught within a radius of 50 km. Snakes were collected through callouts from all local snake rescuers that followed standardised procedures. Individuals of the target species, *N. mossambica*, were collected from the field and transported back to Hoedspruit Reptile Centre. Snakes were kept for up to one month as they were used in behavioural trials (Chapter 4) and for chemical cue trials. During this period, snakes were housed in glass-fronted wooden enclosures lined with newspaper and fitted with a hide and water dish. Water was provided *ad libitum*. Snakes were not fed before or during the experimental trials to remove the effect of recent feeding on defensive behaviour (Herzog and Baily 1987). Snakes were fed purpose-bred laboratory rats before release back in the wild. The snout-vent length (SVL), tail length, mass and sex of each snake were recorded before and after the trials to get accurate measurements of the snakes before and after the trials. Experiments ran from December 2022 to March 2024.

5.3.3 Chemical cue trials

To test the ability of *N. mossambica* to discriminate the scent of human sweat against the scent of a prey item and a control, cotton applicators were used to collect scent samples. Sweat samples were collected from the project investigator (myself). Sweat was collected on the applicator from the forehead after physical exercise to stimulate sweat production (Braks et al. 2001). Scent from a prey item (amphibian) was collected from pond water which housed a resident toad (*Sclerophrys gutturalis*) at Hoedspruit Reptile Centre. The control was a non-scented applicator. Fresh applicators were used for each trial. To assess the response of snakes to chemical cues, the behaviours of individuals were assessed in chemical cue trials. A large, round, open aquaculture tub was used as the testing arena (diameter = 1.75 m, height = 0.63 m).

Since *N. mossambica* actively forage from early evening into the night and based on the hypothesis that antipredator responses have been shown to match activity schedules in some animals (Llewelyn et al. 2010), experimental trials took place between 16:30 – 19:00. Experimental trials took place one week after the behavioural experiments (Chapter 4), to avoid any impact from the behavioural trials to affect the chemical cue trials. The testing arena was disinfected using F10SC, a veterinary disinfectant comprised of quaternary ammonium and biguanide compounds generally used as a surface-acting biocidal (Health and Hygiene Pty). Applicators with the scent of prey, sweat and the control were placed in the arena immediately after collection of sweat and prey sample (Fig. 1). To avoid the scent of the observer having any impact on the snake, latex gloves were used to place applicators in the arena. A hide was used to allow snakes to habituate before trials began.

Trials lasted for 30 minutes including 10 minutes for habituation. The observer left the immediate vicinity of the testing arena as soon as the snake was released from the hide. Time, duration and environmental temperatures were recorded for each trial. Environmental temperature and humidity were recorded using a handheld Kestrel 1000 Weather Meter (NK, Boothwyn, PA, USA), which records temperature, humidity, barometric pressure, and wind speed. Each individual was used in a single trial and trials were recorded using a GoPro Hero 9 camera, positioned above the arena.

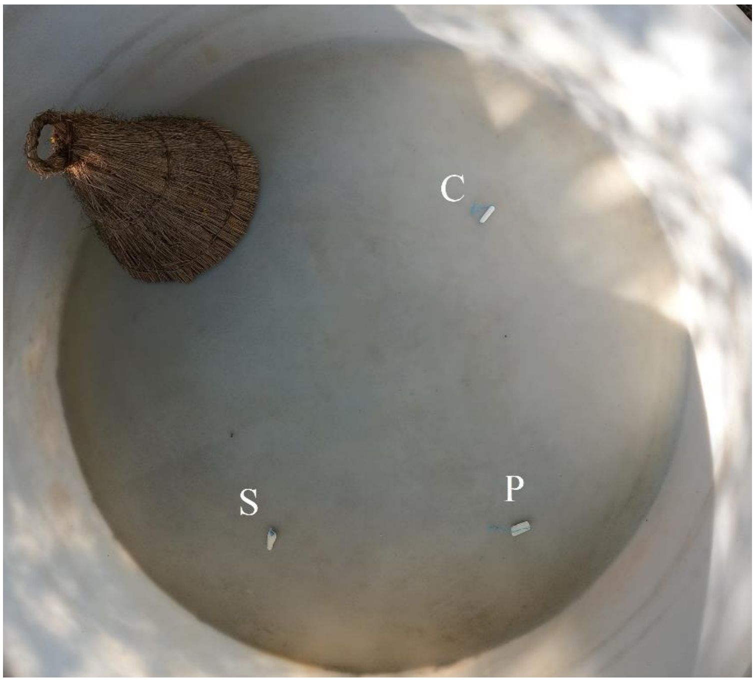

Figure 1. Testing arena for chemical cue trials with a sweat-scented applicator (S), a prey scented applicator (P) and a control (C). A hide was used to allow snakes to habituate before trials began.

GoPro Player was used to analyse videos frame-by-frame and to score all behaviours. Behavioural responses were categorised as follows: attempting to escape, hooding and tongue flicking (number of times the tongue goes in and out the mouth). Investigative behaviour was defined as an individual stopping at a sample and spending a few seconds or more tongue flicking and observing the sample. Tongue flicks were measured from the time the snake stopped at the sample until it moved away from the sample. The tongue flick rate was also measured and calculated by dividing the number of tongue flicks by time (seconds).

5.3.4 Statistical Analyses

Statistical analyses were carried out in R 4.4.0 (R Core Team 2024) and were considered significant at, $P < 0.05$. A Multiple Logistic Regression was used to assess the impact of body size (SVL), sex and temperature as predictor variables on the investigative behaviour (a binary outcome: 0 = no behaviour, 1 = behaviour) of the three samples. Total tongue flicks and were compared for the different samples using a Poisson regression with body size (SVL), sex and temperature used as predictor variables. A linear regression was used to assess the time (seconds) spent on each sample using body size (SVL), sex and temperature as predictor variables.

5.4 Results

Fourteen individuals of *N. mossambica* were captured and used in chemical cue trials. Both juveniles and adults were used to maximise the sample size in the study. None of the individuals in the study attempted to bite any of the applicators. Individuals that investigated samples, stopped at the sample, tongue flicked, smelled the sample and moved their snout around the sample before moving off. The control sample was investigated the fewest number of times (Fig. 2). Individuals displayed tongue flicks while exploring the arena and most attempted to escape the arena.

The Multiple Logistic Regression showed that body size had a significant impact on investigative behaviour ($\chi^2 = 6.8$, df = 2, $P < 0.05$) and individuals with larger body sizes were associated with increased investigative behaviour (Fig. 3). Temperature and sex had no significant impact on investigative behaviour.

The Poisson regression showed that body size was positively correlated with tongue flicks ($P < 0.05$). There was a significant difference between tongue flicks for each sample. The control sample had a significantly lower number of tongue flicks ($P < 0.001$) than the prey sample (by 63%) and the sweat sample had a slightly lower number of tongue flicks ($P < 0.04$) than the prey sample (by 26%). The linear regression showed that time spent on the control sample by *N. mossambica* was significantly lower ($F_{5,36} = 3.2$, $P = 0.003$) than time spent on the prey and sweat sample and time spent on the sweat sample was only marginally non-significant ($F_{5,36} = 2.3$, $P = 0.07$) than time spent on the prey sample.

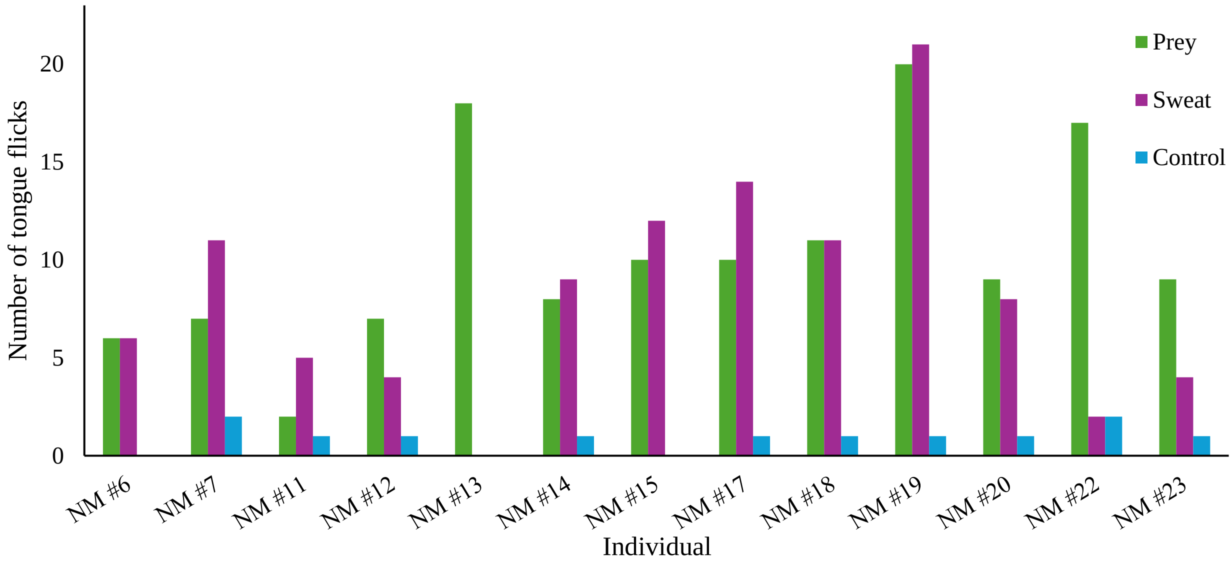

Figure 2. The number of tongue flicks for each of the three samples investigated (Prey, Sweat and Control). NM #21 did not investigate any of the samples and was not included in the graph.

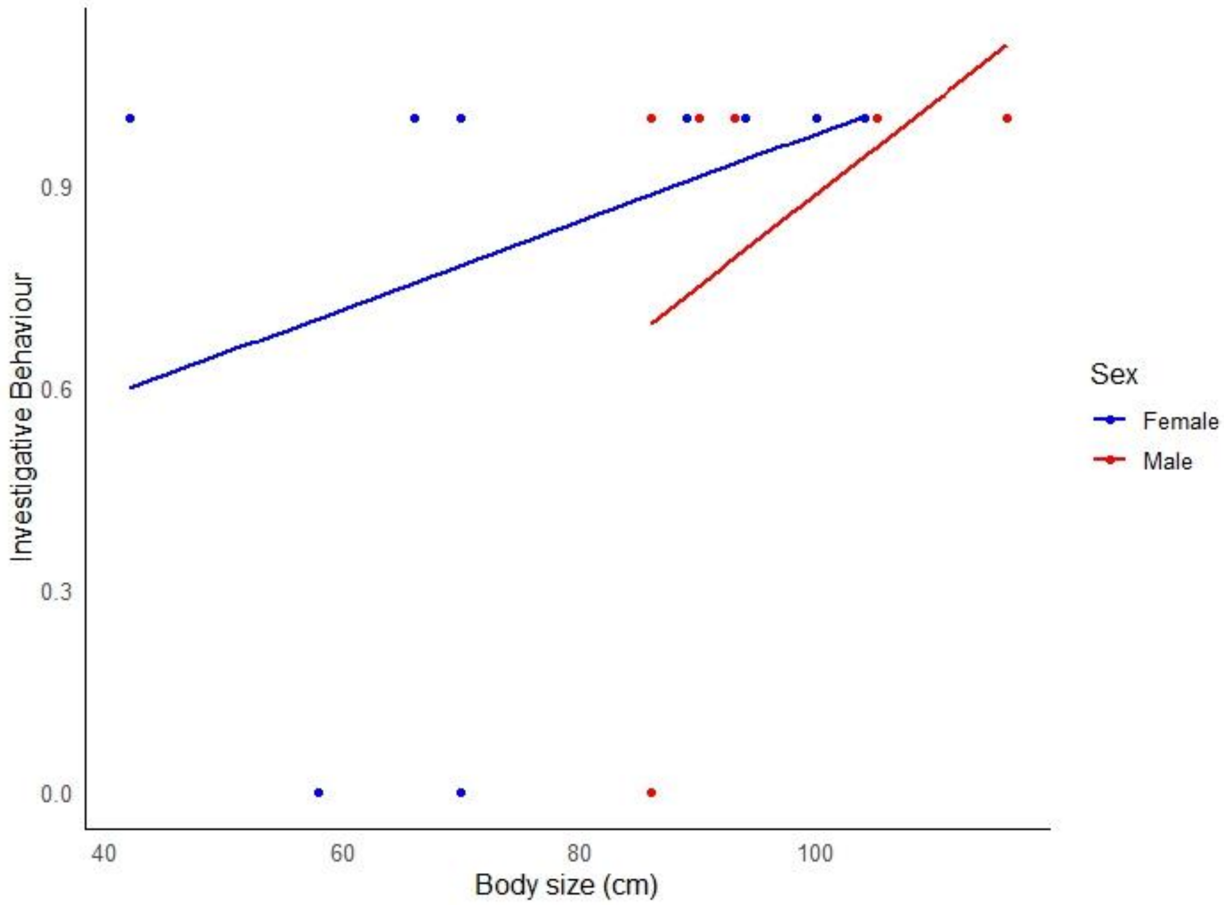

Figure 3. The positive correlation between body size and investigative behaviour in *Naja mossambica* comparing males and females. Larger bodied snakes investigated the arena more than smaller bodied snakes with no difference between males and females. The logistic regression model was fitted with body size as the predictor variable and the model intercept was -23.18 and the body size coefficient was 0.088. The curve illustrates the predicted probabilities, with probabilities increasing as body size increases. The model's residual deviance is 24.60, indicating the fit of the model to the data.

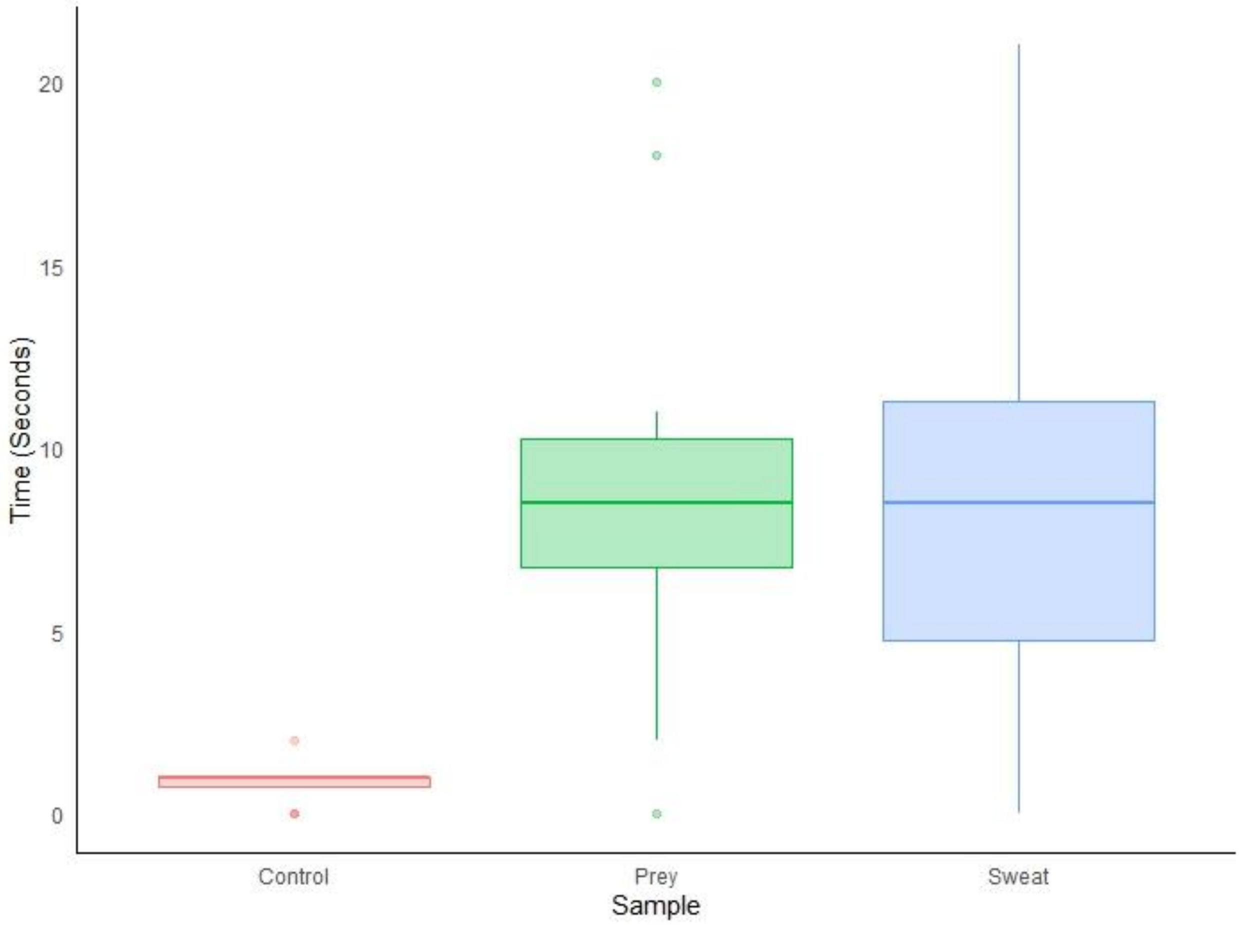

Figure 4. Total time spent investigating the control, prey and sweat samples in the arena.

Hooding was observed mostly when snakes elevated their body while attempting to flee. Three individuals displayed hooding when encountering sweat and prey samples (Fig. 5). Hooding only occurred when first encountering the prey sample by NM #17. NM #19 hooded at the sweat sample twice through the trial and NM #20 hooded at the prey sample when first encountered.

Figure 5. Hooding behaviour when encountering sweat samples (a and b) and prey samples (c and d).

5.5 Discussion

This study showed that *N. mossambica* failed to display biting or striking behaviour when presented with chemical cues and generally both prey and sweat samples were investigated more than the control. When investigating prey and sweat samples, *N. mossambica* sometimes displayed hooding, most likely as a response to being startled. Body size was found to influence investigative behaviour as larger individuals investigated samples more than smaller individuals, but most snakes explored the entire arena. The rate of tongue flicks did not differ between the samples, but time spent on samples was significantly different and the prey sample was investigated the most. Overall, these results show that *N. mossambica* largely displayed behaviours associated with general exploration and active foraging and fewer defensive behaviours. Thus, the study supports the hypothesis that foraging Mozambique spitting cobras are most likely biting humans accidentally because they are mistaking them for prey while actively foraging at night. To my knowledge, this is the first study to assess the ability of *N. mossambica* to discriminate between various chemical cues and test the hypothesis of mistaken identity in snakebite incidences.

There was no evidence that *N. mossambica* behaved defensively towards any chemical cues and hooding was displayed when individuals elevated themselves to escape from the arena.

However, in three individuals that displayed hooding when encountering the sweat and prey scent sample, it was likely that the snake was startled when approaching an unknown object and reacted defensively. Hooding has only been reported to be a defensive behaviour (Jones 2016; Young and Kardong 2010) and it is likely that when individuals first encountered the sample, the response was to spread a hood even in low light conditions. Because cobras often spread a hood when confronting prey, this is not an unusual observation. Jones (2017) reports that hood patterns can be used to prevent an attack on cobras when they are fleeing. Similarly, in low light conditions at night, *N. mossambica* may rely on hooding when attempting to flee as a defence against predators.

When *N. mossambica* investigated prey and sweat samples, tongue flicking increased as the individuals approached the sample, but tongue flicking decreased when individuals moved closer and stopped to assess the sample for several seconds. Since tongue flick rate did not differ between samples but differed between individuals, and body size was a predictor for investigative behaviour, the results suggest that morphological rather than environmental conditions may influence investigative behaviour. Larger snakes investigated samples more than small snakes and it is likely that because smaller snakes have a higher perception of predation risk (Roth and Johnson 2004), they mostly attempted to escape. In this study, one small individual did not display any behaviours when placed in the arena and after the hide was removed, it remained in the same position with the first third of its body elevated and in a hooded position. This indicates that there are likely to be behavioural differences between individuals.

Individuals in the study spent longer durations investigating the prey sample than sweat and control samples. *Naja mossambica* is generally found actively foraging at night (Marais 2022) and it is the only nocturnal elapid that’s a generalist feeder in South Africa. Evidence from several case reports has shown that when bites occur in sleeping humans at night, multiple bites can occur on the same body part or the only exposed area of the body (e.g., face; Tilbury 1982; Vermaark et al. 2010). Given that significant time was spent on the prey sample and some snakes strike prey multiple times (Chiszar et al. 1982; Kardong 1986), there is evidence that bites are predatory rather than defensive. Visual cues are removed when snakes encounter immobile sleeping people, and they are unable to recognise the objects they encounter. Within this situation, snakes will rely on their sense of smell and if a snake is hungry, the scent of a human may be mistaken for prey (Natusch et al. 2021). Additional data from Chapter 2 showed that snake species other than *N. mossambica* have also been reported to bite people while they are sleeping. These include Bibron's stiletto snake (*Atractaspis bibronii*), black mamba (*Dendroaspis polylepis*), rinkhals (*Hemachatus haemachatus*) and cape cobra (*Naja nivea*). The common trait among these snake species is actively foraging thus, snakebite incidences are probably occurring when humans are accidentally mistaken for food. This provides further evidence to support the hypothesis of mistaken identity and is most likely applicable to bites occurring in sleeping humans in other parts of the world.

As actively foraging nocturnal snakes, *N. mossambica* are mostly likely searching for prey in different landscapes and due to their generalist diet, a variety of prey is ingested. Although venomous bites are associated with defence, bites in sleeping humans occurring as a feeding response is plausible as some snake species have been reported to ingest humans. Species such as reticulated python (*Malayopython reticulatus*) and green anaconda (*Eunectes murinus*), can weigh over 60 kg and exceed 5 m in body length and could conceivably subdue and swallow a small or medium-sized person (Headland and Greene 2011; Rivas 1998). Natusch et al. (2021) also suggest that snakes may misinterpret the situation (multiple sleeping humans) and detect the presence of multiple prey, such as a mother and her dependent young or a nest of juvenile mammals which presents a successful feeding opportunity. Chiszar et al. (1993) also report that snakes attempting to swallow appendages may not be uncommon since in their study on *B. irregularis*, an individual ate a blood-soaked applicator. Thus, seizing a small object may be a successful strategy, even if the snake realises it has grabbed a small part of a larger animal that it cannot consume because releasing the hold may cause the prey to escape (Natusch et al. 2021). According to optimal foraging theory, the possible benefit of a large meal can outweigh the risk of misjudging the prey's size, leading to strenuous efforts to ingest prey even if it proves impossible (Arnold 1993). Snake species reported to bite people in snakebite incidences such as *N. mossambica* usually weigh < 3 kg, and it is not possible that they would be able to ingest a small human (Fritts et al. 1994).

None of the trial individuals spat during the chemical cue trials, indicating that spitting likely does not play a role when bites occur while people are sleeping. Chapter 4 showed that *N. mossambica* will predominantly spit in defensive encounters and the lack of spitting in the chemical cue trials was somewhat expected. However, considering that bites from *N. mossambica* also occur when humans are not sleeping and in pets (Leisewitz et al. 2004; Tilbury 1982), it is likely that during confrontations, bites occur due to an aggressive interaction causing *N. mossambica* to bite defensively. In a different case where mistaken identity and aggressive interactions were combined, sea snakes (*Aipysurus laeviswere*) were reported to attack humans (Lynch et al. 2021) and the 'attacks' reportedly occurred by male sea snakes after failed courting endeavours. As humans and snakes are finding themselves in closer proximity, such encounters are not unusual. While defensive biting was not observed in *N. mossambica* in this study, the possibility of such behaviour occurring cannot be excluded and perhaps the sample size in this study was a limiting factor.

The majority of snake species reported to bite sleeping humans are elapids (*N. mossambica*, *N. nigricinta*, *N. pallida*, *B. caeruleus*) and since all of them are generalist feeders, gaining insight into the nocturnal ecology of these snakes is instrumental in assessing snakebite incidences. Current data on the prey consumed by *N. mossambica* indicates that mammals and amphibians make up the majority of their diet (Maritz et al. 2019; Shine et al. 2007). Future studies could assess if there are prey preferences by using their scent as an attractant to *N. mossambica* to further support the hypothesis of mistaken identity. As snake venom is highly potent and linked to prey, defensive traits are likely an extension of their foraging ecology. However, there are limited data on the link between defensive behaviours and foraging ecology.

Although there is much information to gather on the behavioural ecology of *N. mossambica*, this study is the first to provide some insight into snakebite incidences in sleeping humans. While some predictions can be made regarding how bites may occur from *N. mossambica*, the simplest explanation is that it is the only actively foraging nocturnal elapid species in South Africa with a generalist diet. Factors that influence the foraging ecology of *N. mossambica* could provide further insight into incidences. Thus, it is important to consider prevention measures such as the use of mosquito nets and elevated beds to prevent bites when sleeping (Chappuis et al. 2007). As snakes find themselves moving into human habitats, it is important to consider reducing conflict situations and reducing incidences leading to bites from venomous snakes.

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Chapter 6: Summary, Recommendations and Conclusion

6.1 Summary

The concept of 'coexistence' has gained prominence in conservation science, becoming a crucial framework for understanding human-wildlife interactions (IUCN 2023). However, some conflicts are complex such as human-snake conflict and require a multifaceted approach to manage. Recent literature has showcased that the public health challenge of snakebite would benefit from the collection of data on the different factors that drive the activity and behaviour of venomous snakes (Malhotra et al. 2021; Martín et al. 2021). In this thesis, I aimed to assess the current state of snakebite in South Africa and investigate the behaviour of several South African venomous snake species to identify the spatiotemporal patterns that provide perspective on the relationship between these snakes and the occurrence of snakebite incidences. These findings provide valuable insights into the relationship between people and snakes within the context of snakebite.

There are several factors to consider when assessing snakebite in South Africa and my thesis focused on means to define and overcome the associated challenges. In Chapter 2, I assessed the current incidences of snakebite in South Africa which presented several challenges in the data collection process due to access to data and the Protection of Personal Information Act, 2013. As snakebite is not a notifiable disease in South Africa, at the national level, there is limited involvement from the government to address challenges associated with snakebite. However, I addressed some of these challenges and provided insight into the current statistics on snakebite incidences in South Africa from the available data sources and shed light on the underestimation of the current numbers due to lack of reporting. Most epidemiological studies on snakebite also lack sufficient information regarding the circumstances of incidences and snake species involved. By gaining insight into information on location (KwaZulu Natal), demographics (Males under the age of 30) and activity (walking) provided in Chapter 2, the relevant measures that need to be taken to avoid conflict between humans and snakes can be identified.

The impact of climate change cannot be understated as the consequences have been experienced in different ecological systems globally (Grimm et al. 2013). While climate modelling has proven to be useful in snakebite studies (Yañez-Arenas et al. 2016; Zacarias and Loyola 2019), the broad-scale approach neglects to address the current ecological patterns at a small scale such as land use change (Martín et al. 2022). Although climatic modelling was considered for medically important venomous snake species in South Africa, the goal of Chapter 3 was to provide insight into local drivers and hotspots for snakebite incidences which were considered more necessary for creating prevention measures at present. A recent study also found that future human-wildlife overlap is associated with spatial and temporal variation and insight into the local dynamics is vital, especially for targeted area-based land-use and systematic conservation planning (Ma et al. 2024). The spatial patterns of incidences in relation to the distribution of snake species have provided valuable insight into the impact of human population density on wildlife and the value of protected areas in safeguarding natural spaces. In conjunction with climate modelling studies such as Zacarias and Loyola (2019) and others, the results from Chapter 3 show support that densely human populated areas are targets for snakebite incidences at present and the number of incidences in such areas is likely to increase.

While snakebite has largely been assessed from an epidemiological and medical perspective, the ecological perspectives have been neglected, particularly the behaviour of snakes. Snake defensive behaviour and particularly striking behaviour of snakes are crucial in investigating scenarios of snakebite incidences. The change in human population density, and the resulting consequences are already having an impact on human-wildlife conflict (Schell et al. 2021) and insight into the behaviour of venomous snakes can be used to create protocols for interventions between people and snakes. In Chapter 4, I assessed the defensive behaviour of puff adder (*Bitis arietans*), black mamba (*Dendroaspis polylepis*), snouted cobra (*Naja annulifera*) and Mozambique spitting cobra (*Naja mossambica*) when presented with a fake arm to simulate a human threat. Distance measurements showed that snakes on average would only strike if the arm was less than 50 cm away in very close proximity and the likelihood of biting increased with increased harassment. These results provide evidence that snakes would prefer to flee from conflict situations and will only behave defensively when required.

Insight into snake behaviour can also show how species navigate through their landscapes while influenced by different factors. In Chapter 5, I investigated the attraction of *N. mossambica* to various chemical cues such as prey and human sweat. There is no surprise that *N. mossambica* showed the highest attraction to the prey sample which suggests that bites in sleeping humans are most likely because *N. mossambica* is the only actively foraging nocturnal elapid in South Africa that is a generalist feeder. Bites from other snakes are most likely also related to their behaviours and there is much value in conducting behavioural studies as a means to mitigate human-snake conflict.

6.2 Recommendations

Several authors have proposed that human-snake conflict mitigation needs to be considered as the primary prevention method to mitigate snakebite incidences (Malhotra et al. 2021; Michael et al. 2019). While there is considerable interest in developing antivenom and the treatment of snakebite, this thesis highlights the need for further investigation into snake behaviours and how these behaviours affect the current dynamics between people and snakes. As detailed in Chapter 2, snakebite incidences are underreported in South Africa with a prevalence of 5.63 per 100 000 individuals for the 12.5-year period, and to overcome this challenge snakebite needs to be a notifiable disease in South Africa. Eswatini is a great example where significant progress has been made since the country declared snakebite as a notifiable disease, with every health facility required to report snakebite cases (Padidar et al. 2023). This ensures that snakebite is adequately addressed, and support can be provided by the government to implement prevention measures and treat snakebite. In South Africa, the National Snakebite Advisory Group could act as the agency to implement relevant measures in addressing snakebite issues with the assistance of the national government.

The current categories of medically important venomous snake species by the World Health Organisation should be updated to include species that are commonly featured in snakebite incidences, and not only based on the distribution of the species. Chapters 2 and 3 provide detailed information on snakebite incidences from rhombic night adders (*Causus rhombeatus*). Although not currently on the list of medically important snakes, this species features more commonly in snakebite incidences than several other Category 2 snake species (*Thelotornis capensis*, *Naja melanoleuca*, *Naja nigricincta* and *Bitis gabonica*). While the venom is moderately cytotoxic (Marais 2022), it can cause severe pain and swelling. Thus, I propose that *C. rhombeatus* should be included in Category 2 on the list of medically important snake species for South Africa.

While the name sub-Saharan Africa is widely accepted for regional identification, the clumping of the countries in the region provides problems when assessing challenges such as snakebite. The current incidence of snakebite is significantly different in central Africa than in southern Africa with a higher burden in central Africa. Not only are the populations living in these regions different, but the ecology of the snake species in these regions is also different. Additionally, South Africa has manufactured antivenom since 1928 (Chapter 1) and the antivenom is exported to neighbouring countries (Chippaux et al. 2019). Thus, countries in southern Africa have been better placed to treat snakebite incidences than countries in central, west and east Africa. Therefore, when addressing snakebite in sub-Saharan Africa, the differences in the subregions should be clearly stated in guidelines and protocols.

Effectively managing the risks of snakebite in response to environmental changes requires collaboration from both public health authorities and nature conservation agencies to ensure that positive results are achieved (Martín et al. 2021). Target 4 of the IUCN Convention on Biological Diversity focuses on mitigating human-wildlife conflict and this thesis forms an important contribution towards that target. There are currently no guidelines on addressing human-snake conflict anywhere in the world and in the latest IUCN Human Wildlife Conflict Guidelines (2023), snakes are only briefly mentioned. This thesis provides insight into the importance of human-snake conflict mitigation guidelines which can be implemented through various agencies (IUCN Snake Specialist Group, NGO's such as Save The Snakes etc.; Snakebite Community Engagement Network) in collaboration with the larger scientific and conservation community.

As animal behaviour is not within the control of people, the solution is to reduce risk of incidences by creating less attractive circumstances for snakes to come into homes. First, safe housing can hinder indoor access to snakes, and the use of elevated beds and mosquito nets can prevent snakebite incidences while people are asleep (Bawaskar and Bawaskar 2019; Kularatne 2002). Additionally, cleaner spaces are less likely to attract common prey items for snakes. It may also be beneficial to reduce items around houses that potentially attract snake prey (human food waste, piles of wood, litter, stocks of grain or other crops and sources of water). The use of torches and shoes that cover feet and ankles when walking is also recommended. Education and awareness programs need to be implemented in affected areas in conjunction with community engagement and knowledge of snakes and snakebite should be included in school curricula. Finally, experts from the natural sciences, health sciences and social science need to work in congruity to successfully tackle the multi-disciplinary challenge that is snakebite

6.3 Conclusion

Overall, increasing our knowledge of how snakes behave and interact with people and utilise resources in different ecosystems will enable us to test more complex hypotheses that focus on human-wildlife adaptations. This thesis not only contributes toward addressing knowledge gaps on the driving factors of snakebite incidences but also sheds light on the focus areas where prevention measures can be placed. By providing a broad to specific perspective on snakebite in South Africa, I have highlighted the key focus areas to mitigate human-snake conflict that can also be applied in other countries. The results of this study will be implemented in various capacities over the next few years, and I hope it contributes towards snakebite management.

6.4 References

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