The behavioural ecology of African clawless otters, *Aonyx capensis*, in KwaZulu-Natal, South Africa

by

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Abstract

African clawless otters (*Aonyx capensis*) are the most widely distributed otter species in Africa, and they occur in a wide variety of habitats. Despite their extensive distribution there is a paucity in knowledge on their ecology and, especially their social behaviour. Latrines play important roles in intraspecific olfactory communication of many mammals. In this research project several aspects related to latrine sites and the role these sites play in the behavioural ecology of African clawless otters were assessed. Latrine site selection, population densities, activity time, and scent-marking behaviours were investigated and compared across two study areas (uMlalazi Nature Reserve and Zini Fish Farm) on the north coast of KwaZulu-Natal, South Africa. In addition, volatile organic compounds (VOCs) of 14 African clawless otter scats were described through gas chromatography mass spectrometry (GC-MS). Most of the latrine sites were located at the ecotone between two vegetation units or at the ecotone between a vegetation unit and a water source and were associated with little vegetation cover but lower wind exposure. It is hypothesised that this may increase their conspicuousness to conspecifics, while areas exposed to less wind likely aid in the retention of scent. Otters were strictly nocturnal around latrine sites and behaviours recorded were dominated by sprainting ("jiggle dances") and sniffing suggesting latrine sites to be important for intra-specific communication. Many of the identified VOCs are commonly associated with reproduction and sex pheromones in other animals. This, combined with substantial inter-scat variation in VOCs, lend further support to the hypothesis that latrine sites are mainly used for intra- (and not inter) communication purposes. Future research will benefit from individual-level identification of otters when investigating the olfactory landscape of latrine sites and the interpretation of their social function to African clawless otters.

Key terms:

African clawless otter, behavioural ecology, density, camera traps, random encounter model, latrine site, habitat selection, ethogram, faeces, spraint, scent-marking, olfactory communication, gas chromatography–mass spectrometry, volatile organic compounds

Declaration

Name: Stephanie Giselle Nicolaides

Student Number: 6061 8868

Degree: Master of Science in Life Sciences (Zoology Stream)

I, Stephanie Giselle Nicolaides, declare that this thesis, with the title:

The behavioural ecology of African clawless otters, *Aonyx capensis*, in KwaZulu-Natal, South Africa

which I hereby submit for the degree Master of Science in Life Sciences (Zoology Stream) in the Department of Life and Consumer Sciences, University of South Africa. It is my own original work, I further declare that I have not previously submitted this work, or part of it, for examination at Unisa for another qualification or at any other higher education institution.

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Stephanie Giselle Nicolaides

Research Outputs

Journal articles

Nicolaides, S.G., Mostert, T.H.C. & McIntyre, T. (2022). Latrine site selection by African clawless otters, *Aonyx capensis*, and their behaviour during latrine visitations. *Journal of Mammalogy*, Submitted.

Conference presentations

Poster presentations

15th IUCN/SSC OSG International Otter Congress (2022)

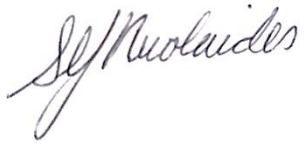
Title: Assessing habitat selection and features characterising the location of African clawless otter latrine sites in a marine habitat, KwaZulu-Natal, South Africa

11th Oppenheimer Research Conference

Title: Assessing habitat selection and features characterising the location of African clawless otter latrine sites in a marine habitat, KwaZulu-Natal, South Africa

Preface

The research contained in this dissertation was completed by the candidate while based in the Discipline of Zoology, School of Life Sciences, College of Agriculture and Environmental Science, University of South Africa, Pretoria, South Africa, under the supervision of Associate Professor Trevor McIntyre and under the co-supervision of Dr Theodorus H.C. Mostert. The contents of this work have not been submitted in any form to another university. The results reported are due to investigations by the candidate.



Stephanie Giselle Nicolaides

Associate Professor Trevor McIntyre

Dr. Theodorus Mostert

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I am very grateful for the warmth and support of my family. Their unwavering support in the field, reading countess drafts and their continual encouragement along this academic journey. I would not be who and where I am now without the love, confidence and support of my parents Angelo and Angelique and my little brother, Alexander to whom I dedicate this thesis.

I also thank my friends (too many to list here but you know who you are!) for providing support and friendship that I needed through this journey.

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LIST OF ABREVIATIONS

GLM	Generalized linear model
AGS	Anal gland secretions
REM	Random encounter model
VOC	Volatile Organic Compound
uMNR	uMlalazi Nature Reserve

CHAPTER I – INTRODUCTION

1.1 Title

The behavioural ecology of African clawless otters, *Aonyx capensis*, in KwaZulu-Natal, South Africa.

1.2 Background

Small and medium sized carnivores carry out important roles where they influence ecosystem structure and provide many ecosystem services (Marneweck et al., 2021). Sentinel species are defined as a specific form of indicator species that respond and adapt to ecosystem changeability in a manner that is measurable and interpretable, these species provide insights into the functioning and condition of ecosystem processing (Zacharias & Roff, 2001; Hazen et al., 2019). Small and medium sized carnivores can serve as global 'sentinels' of changes to the structure and functioning of ecosystems. Several examples of small carnivores used as sentinels including meerkats (*Suricata suricatta*) as sentinels of climate change (Van de Ven et al. 2020), ocelots (*Leopardus pardalis*) as sentinels of landscape connectivity and large infrastructure impacts (Perez 2019), Eurasian otters (*Lutra lutra*) as sentinels of bioaccumulation (Brand et al. 2020). Several small and medium sized carnivore mammal species are of particular concern in terms of their monitoring and conservation. This can be attributed to their elusive, shy, cryptic, secretive, crepuscular, nocturnal behaviour of these small mammals (Estes,1991). In addition, management and research are challenging and often limited for these species given the diminutive size of these creatures, that they range over large areas and occur at low densities (Kindberg et al., 2009; Streicher, 2020). There is a need for more research to be conducted given a paucity of ecological and behavioural research on several small mammal species, including the African clawless otter (*Anoyx capensis*), in Southern Africa and the continent as a whole. For example, over the past decade there have been a mere total of 23 research papers published that involved African clawless

otters in some way (McIntyre et al., 2022), illustrating a small body of knowledge that exists regarding the ecology and behaviour of African clawless otters.

The African clawless otter (*Aonyx capensis*) is the most widely distributed of the otter species in Africa (Estes, 1991). Despite their wide distribution, little is known about their ecology, and behavioural ecology in particular – such insights being potentially important to inform predictions about their adaptability to anthropogenically driven changes to their environment. Latrine sites play important roles in the intra- and interspecific communication of other carnivores and are particularly useful places to study the behavioural ecology of animals that use them (Rodgers et al., 2015; King et al., 2017; Allen et al., 2017).

1.3 Project outline

Improving information on habitat features and characteristics of latrine sites is essential, for the development of efficient management and conservation, based on the important role these sites play in otter ecology and behaviour. Latrine sites provide the opportunity to relate latrine site features and characteristics to activity and behavioural patterns of otters. Detailed descriptions of habitat variables and characteristics that influence the presence of latrine sites were investigated. Latrine sites were assessed to determine if they are selected based on environmental characteristics that provide environmental cover and vertical and horizontal security. Latrine sites were assessed to determine if they are optimally situated for olfactory cues to spread by air movements to aid intraspecific communication and if they are situated to offer security against predators. Habitat variables were quantified to assess the location of latrine sites, these were a combination of vegetation characteristics and shoreline topography.

Latrine use and behaviour at latrine sites has not been extensively studied in African clawless otters. Social behaviour and communication around latrine sites were analysed through camera trapping where behavioural information was recorded and described to

build on our knowledge of this species. Behavioural reactions were recorded through camera trapping. Given that the sexual dimorphism is not well pronounced in the African clawless otter, this study will also assess whether the sex of African clawless otters can be determined through the direction (orientation of urine stream in relation to the faecal stream) of their urine streams. Latrines are sites of intraspecific communication where anal-gland marking, faeces and urine convey information relating to sex, age and territory. Odour emission is a critical feature in ensuring other individuals are able to obtain this information (Vickers, 2000). Analysing the chemical characterisation of spraint (faeces deposited as scent marks (Kruuk, 1992)) and faeces of African clawless otters and how it changes over both short and long time periods was examined.

1.4 Dissertation structure

The dissertation is divided into five chapters. The first chapter includes an introduction and literature review on the African clawless otters and their distribution, ecology, population density, conservation status, latrine site use and scent marking behaviour. The aims and objectives of the dissertation are specified in this chapter. The second chapter addresses the comparative density, activity and behaviour of African clawless otters in a natural habitat and in an anthropogenically disturbed habitat (Aim 1 and 2). The third chapter addresses latrine site selection and its likely implications for the possible social function of latrine sites (Aim 3). Chapter four includes the preliminary characterisation of the volatile organic compounds (VOCs) identified in African clawless otter spraint and their potential behavioural roles (Aim 4). The final chapter highlights the major findings of the study and includes the recommendations for management strategies and factors that future research studies can assess.

1.5 Research problem

There is a significant gap in the knowledge on the behavioural ecology of African clawless otters around latrines sites (and the social function thereof) and on the predatory and foraging behaviour of these mammals in marine environments. Additionally, there is an

absence of published accounts of African clawless otter research based in the KwaZulu-Natal province of South Africa. In particular, this research study involved data collection from the uMlalazi Nature Reserve (uMNR) in Mtunzini, a small coastal town situated on the northern coast of KwaZulu-Natal.

1.6 African clawless otters

1.6.1 Taxonomy and phylogeny of *Aonyx capensis*

The African clawless otter belongs to the order Carnivora, family Mustelidae, and sub-family Lutrinae (Andarge et al., 2017). The classification of *Aonyx capensis* (Schinz, 1821) is as follows (Integrated Taxonomic Information System (ITIS), 2021):

Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Mammalia
Order:	Carnivora
Suborder:	Caniformia
Family:	Mustelidae
Subfamily:	Lutrinae
Genus:	*Aonyx*
Species:	*Aonyx capensis*

The genus *Aonyx* is monophyletic and phylogenetically linked to that of the New World otters (Van Zyll de Jong, 1987). The original scientific application was *Lutra capensis* (Schinz, 1821). The common names for *Aonyx capensis* are Cape clawless otter and African clawless otter (used here). The only two congeneric species to *Aonyx capensis* are the Congo otter (*Aonyx congica*) and the Asian small-clawed otter (*Aonyx cinereus*) (Koepfli & Wayne, 1998; Nel & Somers, 2013). The time of divergence between *Aonyx*

capensis and *Aonyx congica* is estimated to be around 2.6 MYA (Bininda-Emonds et al. 1999). *Aonyx* and *Hydrictis* are the two extant genera of otter that occur in Africa, there are three species recognised, namely, *Aonyx capensis*, *Aonyx congica*, and *Hydrictis maculicollis* (International Otter Survival Fund, n.d.).

1.6.2 Distribution and status of *Aonyx capensis*

The African clawless otter is endemic to Africa and is widespread in suitable habitats south of the Sahara, from Senegal to Ethiopia and southwards to South Africa (Kowalsky, 2013). In South Africa this species is widely distributed along the south and east coasts, with a sporadic distribution along the western coast, they are patchily distributed in the arid western interior of the country at sites where there are permanent bodies of water (Estes, 1991; Okes et al., 2016) (see Figure 1.1).

Currently there are little to no conservation measures in place for the protection of the African clawless otters and they are categorized by the IUCN Red List of Threatened Species as Near Threated (Jacques et al., 2021). One of the major threats to the natural environment and biodiversity is climate change and the impact of climate change in the South African context could decrease the availability of suitable habitats for otters (Jacques et al., 2015). Moreover, the continued threat of habitat destruction and human encroachment threatens the availability of scarce resources like land, water, food availability and denning sites (Okes et al., 2016). African clawless otters are faced with a variety of threats in their environment– the major threats being habitat degradation (through bush clearing and deforestation), marginal agricultural practices, pollution and degradation of freshwater sources (through invader species like that of water hyacinth (*Pontederia crassipes*) and the draining of wetlands) (Jacques et al., 2015). These threats are thought to have contributed to population declines of this species throughout most of their range and the estimated population of African clawless otters is believed to range from 21 500 to 30 276 individuals based on several density estimate studies (Verwoerd, 1987; Rowe-Rowe, 1992; Butler & du Toit 1994; Carugati & Perrin, 2006; Somers & Nel, 2013).

Despite the fact that subpopulations of this species are stable, the threats posed by climate change, and continued human encroachment and development along coastlines and riverbanks could result in substantial declines of this species. Even though the African clawless otter is not endangered in South Africa this species is rare throughout its range because of its specialised niche (Jacques et al., 2015). Further research and monitoring are required to increase our knowledge of behavioural aspects of this species.

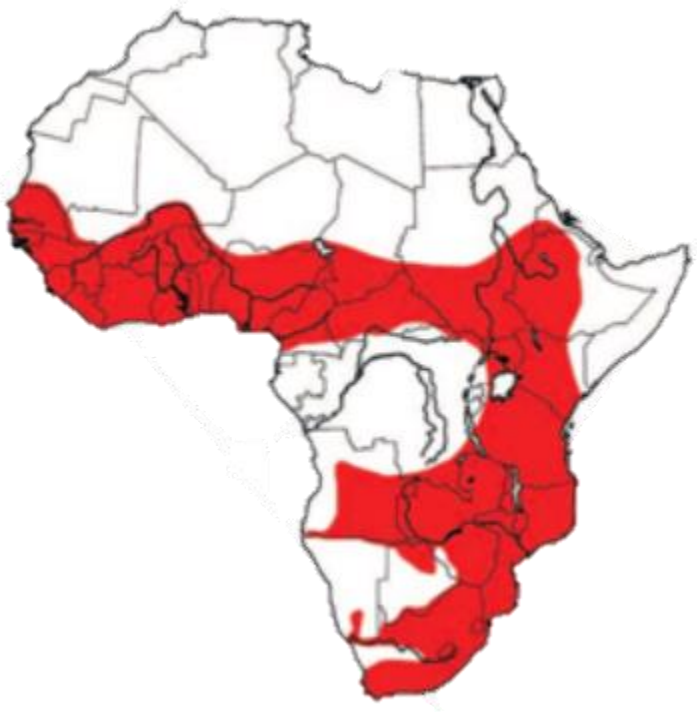

Figure 1.1 Distribution (shaded) of the African clawless otter (*Aonyx capensis*) (Somers & Nel, 2013)

Conflict between otters and fishermen is common, particularly in rural areas where fishing is primary source of income and otters are regarded as competitors and pests for food (Jacques et al., 2015). An example of this was seen in the Kairezi River Protected Area of Zimbabwe where fishery managers blamed trout declines in the area on otter predation. However, faecal analyses showed that only 1% of otter faeces in this region contained trout remains (Butler, 1994). Otters are often blamed and considered to be the primary cause of depredating netted fish and causing damage to fish nets (which they accidently get caught and drowned in) (Rowe-Rowe, 1990). A recent study by Jordaan et al. (2019)

found that in freshwater habitats not all African clawless otters had trout in their isotopic niche, despite this prey item being abundantly available in the study area. Their results suggested that African clawless otters are capable of substantial foraging plasticity, enabling them to make use of environments affected by humans but where sufficient quantities of prey are readily available (Jordaan et al., 2019).

At the moment there is a vague understanding of the social behaviours and olfactory communication of these semi-aquatic mammals. Studying animal behaviour is the connection between the molecular and physiological aspects of biology. By studying behaviour, we are able to find the link between species and their environment, in addition the behaviour of animals often provides the early signs of possible environmental degradation (Snowdon, n.d). According to Mench (1998), behaviour can be described as animals first 'line of defence' in response to environmental change. Thus, through careful observations behaviour can provide us with information regarding the animal's requirements, preferences, and internal states (Mench & Mason, 1997), which would undoubtedly improve our general understanding of this species the African clawless otter.

1.6.3 Morphology and ecology of *Aonyx capensis*

African clawless otters (see Figure 1.2) are characterised by their long-streamlined bodies, short legs, long muscular tails, fine dense hair and scent glands located at the base of their tails (Estes, 1991). The African clawless otter is the largest species of the Old-World otters and they are the third largest of all otter species (Kowalsky, 2013). The average length of male African clawless otter's ranges from 111 cm to 138 cm while females range in length from 114 cm to 160 cm (Estes, 1991). Their tail is rather muscular and tapered, measuring an average length of 51 cm in both males and females (Estes, 1991). These otters can weigh anything between 10 and 22 kilogram and there is minimal sexual dimorphism (Kowalsky, 2013). African clawless otter colouration ranges from tan to chocolate brown with distinctive white markings on their upper lips, cheeks, neck, throat and belly (Estes, 1991). Their thick coats are composed of two kinds of hair, an undercoat

composed of short white fine hair while the outer guard hair gives them a silky, luxurious appearance (Kowalsky, 2013).

Figure 1.2 African clawless otter (© Frans Vandewalle). Source: https://www.flickr.com/photos/snarfel/6956904931 .

African clawless otters are a predominantly aquatic species, with freshwater sources being an essential habitat requirement (Estes, 1991). Freshwater sources are not only essential for drinking and feeding requirements but is also for the rinsing of their dense fur of accumulated salt (for those inhabiting coastal areas) to restore thermo-insulation (Kruuk, 2006). This is vital as otters do not have a layer of subcutaneous fat (blubber) like other aquatic mammals to insulate heat and store energy in an aquatic environment (Harding, 2017). African clawless otters typically select habitats that contain overhanging vegetation and reed beds as these offer an abundance of favourable prey like crustaceans and fish (Harding, 2017). African clawless otters' shelter in burrows under rocks, tree roots and dense vegetation, these burrows are shared by several otters and used for resting, eating and nursing their young (burrows are typically composed of grass and vegetation) (Kowalsky, 2013). African clawless otters have been documented digging

burrows in sandy soil that reach up to 3 m deep, with entrances both above and below the water's surface (Arden-Clarke, 1986).

African clawless otters can also inhabit marine environments, provided there is access to freshwater sources, like estuaries and dams (Estes, 1991). In marine habitats rocky shores are particularly favoured by otters when foraging (Van Niekerk et al., 1998). The ability of African clawless otters to subsist on a variety of different prey items from fish, crabs, frogs and a variety of invertebrate groups allows these mammals to inhabit a variety of different habitat types (Estes, 1991). The habitat of African clawless otters is characterised by dense bush, long grass and overhanging vegetation (Somers & Nel, 2004a; Perrin & Carugati, 2006). However, the vegetation type does not affect the habitat selection of these otters. African clawless otters inhabit a diverse range of habitats from rain forest regions in Liberia that have 2000 mm of rainfall per annum to sub-desert regions that experience a mere 100 mm of rain per annum (Rosevear, 1974).

1.7 Literature review

1.7.1 Assessing population density

1.7.1.1 Available methods to measure otter population density

The elusive, secretive and nocturnal habits of African clawless otters make it challenging for these semi-aquatic mammals to be effectively monitored and research to be conducted. A unique challenge is posed to researchers in procuring accurate estimates of otter population parameters and habitat selection (Prigioni et al., 2006; Gallant, 2007a). Consequently, the field census methods developed to monitor otter populations have involved indirect field census techniques like counting tracks, faeces, latrines sites, feeding traces and active dens (Mason & Macdonald, 1987; Rowe-Rowe, 1992; Wilson & Delahay, 2001). Such techniques have drawn conflicting conclusions of population and density statuses and as a result there are doubts about the validity and reliability of conclusions derived from these techniques (Birks et al., 2005). Several authors have suggested caution when employing such monitoring techniques to assess populations (Jefferies, 1986; Kruuk et al., 1986; Mason & Macdonald, 1987).

The majority of global otter research has been built on standardised otter-specific field surveying methods developed in the 1970s concerning the need to monitor the Eurasian otter (Lenton et al., 1980; Ruiz-Olmo et al., 2001). This approach was based on the identification of indirect yet indubitable otter signs like tracks and spraint. This method became the standardised method to estimate key ecological characteristics like distribution, home range, density, relative abundance and habitat use of otter species (Van der Zee 1981; Chehebar, 1985; Arden-Clarke, 1986; Mason & Macdonald, 1987; Prakash et al., 2012).

While these methods were widely accepted, its accuracy and validity have been the subject of much debate in the ecological arena. For example, the precision and accuracy of the aforementioned method has been critiqued on the following findings and assessments: the number of spraints does not infer the number of otters inhabiting an area, and in this regard the absence of spoor does not imply the absence of otters (given the possibility of 'false negatives). Similarly, the number of signs does not necessarily correlate to intensity of use and is a poor method for measuring habitat selection (Kruuk et al., 1986, Ruiz-Olmo et al., 2001). Perhaps the biggest downfall of this approach is that it fails to consider that otter surveying protocols must be formulated to best suit the circumstances and habitat factors where the particular otter species occurs (O'Sullivan, 1993; Romanowski et al., 1996).

Density estimates of African clawless otters were initially measured by assessing characteristic signs, like holts, spoor and faeces deposited at latrine sites (Rowe-Rowe, 1992). Employing these methods Van der Zee (1981) and Arden-Clarke (1986), reported on the densities and distribution of African clawless otters along the Cape south and Southwest coasts, where densities were estimated to be one otter per 2 km of coastline. While African clawless otter densities in freshwater habitats in the KwaZulu-Natal Drakensberg were estimated to be one otter for every 1.25 (Perrin & Carugati, 2000) and on otter per 2.5 km of river (Perrin & Carugati, 2006).

The precision and accuracy of sign surveying techniques have variability and bias based on the experience of field workers, differences in habitat, the season in which data collection takes place, age and sex related differences in behaviour of the target species and the spatial and seasonal differences in the deposition and traceability of signs (Hutchings & White, 2000; Wilson & Delahay, 2001; Ruiz-Olmo et al., 2001). While this stands true for measuring and assessing otter populations, the surveying of otter spraint is a useful method in the broad scale identification of otter population statuses, provided that factors like the seasonality of sprainting activity and months of heavy rainfall have been taken into account (Mason & Macdonald, 1987).

In recent times, additional non-invasive techniques have been developed and improved upon to effectively estimate density of cryptic species: molecular identification (Hung et al. 2004; Beja-Pereira et al., 2009; Hájková et al. 2009; Martín et al., 2017) and camera trapping (Rowcliffe et al., 2008; Gil-Sánchez & Antorán-Pilar, 2020; Jayasekara et al., 2021). These two approaches are discussed in more detail below.

1.7.1.2 Molecular identification

A powerful and non-invasive molecular technique to estimate population density is through genetic analyses. Genetic analyses involve the use of species-specific sets of DNA primers which allows for the number of unique genotypes in a sample area to be identified (Deiner et al., 2017). DNA samples can be extracted through hair samples (obtained through hair snares) or through faecal sampling by extracting DNA held within the epithelial cells shed from the gut of the animal along with their faeces (Kohn et al., 1999; Prigioni et al., 2006). These techniques enable the genetic fingerprint of individual animals to be obtained in an area and then calculate population density and abundances (Deiner et al., 2017). Genotyping though non-invasive sampling has been effectively employed to validate the density and population parameters measured through ecological methods (Aristizábal Duque et al., 2018). The abundance and spatial organisation of the Eurasian otter were estimated by Hung et al. (2004), through DNA extraction of faecal

samples. Lanszki et al. (2008), employed sample genotyping and found a positive relationship between fresh stool density and population size.

A DNA barcoding technique developed by Madisha et al. (2015) allowed for African clawless otters to be differentiated from spotted-necked otter (*Hydrictis maculicollis*) through genetic analysis of faecal samples. In another study by Ponsonby et al. (2019) the genetic diversity and population structure (although not population density) of African clawless otters was effectively measured by using 10 microsatellite primers developed for the Eurasian otter. While genetic analyses may provide more accurate population density estimates, the techniques involved in this method are costly, time consuming and labour intensive (Hájková et al. 2009).

1.7.1.3 Camera trapping

Camera trapping is a quantitative technique that involves the use of fixed, remote cameras, triggered by infra-red sensory that capture images of passing animals (Rowcliffe et al., 2008). Advancements in camera trapping technology and capabilities have made this research method an increasingly reliable and mainstream tool for surveying wildlife (Gren et al., 2020; Meek et al., 2020). Camera trapping is a non-invasive technique, that has minimal environmental disturbance and enables researchers to investigate important information relating to a wide range of ecological aspects including: distribution (Wevers et al., 2021), density (Rowcliffe et al., 2008), abundance (Moeller et al., 2018; Tanwar et al., 2021), population structure (Silveira et al., 2003; Wegge et al., 2004), population monitoring (Rode et al., 2021), activity (Rowcliffe et al., 2014), and habitat use and behaviour of a species (Head et al., 2012; Caravaggi et al., 2017). In addition, camera traps are robust, weather and waterproof, and are capable of collecting data 24/7, allowing for important data to be captured on cryptic and elusive species (Nichols et al., 2011).

The established and conventional methods to assess population density through camera trapping usually necessitate additional information. Distance models require estimates on the distances of individuals from detection devices (Barlow & Taylor, 2005; Corlatti et al., 2020; Buckland et al., 2001), capture-recapture models require that each individual be uniquely marked and easily identifiable (Karanth, 1995; Karanth & Nichols, 1998; Trolle & Kery, 2003; Rich et al., 2014), while spatially explicit capture-recapture (SECR) density estimation (Efford & Fewster, 2013) further requires the incorporation of a precise spatial component to the detection history of each individual as well as a defined state-space (polygon encompassing the furthest traps of a particular array) from which the density is estimated (Borchers & Efford, 2008; Green et al., 2020). These methods are limited to species with individually unique markings (sufficiently variable for individual recognition) or to those for which a sample that can be individually marked before camera trapping is set up (Trolle & Kery, 2003).

Random Encounter Modelling (REM)

The development of the Random encounter model (REM) is formulated on the underlying process of contact between animals and camera traps, thereby eliminating the prerequisite of other models for the individual recognition of animals (Rowcliffe et al., 2008). This model is essentially an adaptation and modification of the ideal gas theory (Hutchinson & Waser, 2007), to describe rates of contact between moving animals (which does not require uniquely identifiable individuals) and static camera traps through which an estimate for animal density can be derived (Rowcliffe et al., 2008). The models require a species' encounter rate to be estimated, the detection zone of the camera should be specified by its radius and angle and the average estimated speed of movement of the target species should be known (Rowcliffe et al., 2008). The REM model assumes that cameras are placed randomly in regard to target species movement (Rowcliffe et al., 2013), that the target species population is closed and detections represent independent contacts between animals and the camera traps (Rowcliffe et al., 2008). Target species do not necessarily move in a random manner but by deploying camera traps in suitable habitat in a randomised array such that preferentially used areas like food or water

sources are avoided allows for random encounter opportunities (Rowcliffe et al., 2008; Cusack et al., 2015). The placement of camera traps can be used to approximate the random movement assumption of the model (Rowcliffe et al., 2013).

The REM model has been tried and tested on several occasions against the known densities of a population previously derived through other methods (Rovero & Marshall, 2009; Zero et al., 2013; Anile et al., 2014; Cusack et al., 2015a; Santini et al., 2022). It has been effectively employed to assess the population density of several carnivore species (e.g., wildcat (*Felis silvestris*), Anile et al., 2014; lion (*Panthera leo*), Cusack et al., 2015b; European pine marten (*Martes martes),* Balestrieri et al., 2016 and 12 meso-mammal carnivores in Sri-Lanka, Jayasekara et al., 2021). These studies concluded that the REM model is a promising method to record population density estimates.

1.7.2 Latrine site use and olfactory communication

1.7.2.1 Olfactory communication

Olfactory communication is defined by Eisenberg and Kleiman (1972) as:

"The process whereby a chemical signal is generated by a presumptive sender and transmitted (generally though the air) to a presumptive receiver who by means of adequate receptors can identify, integrate and respond (either behaviourally or physiologically) to the signal".

For instance, olfactory communication is commonly employed in discriminating and selecting potential mating partners. The greater sac-winged bat (*Saccopteryx bilineata*) relies on odorous cues for courtship rituals and social communication, the males of this species have a specialised pouch organ on their wing membranes which they fill with an odoriferous substance, which comprises of urine and salivary fluid (Voigt & Helversen, 1999). This is used by males to attract female mates; the males will hover in front of a female allowing them to fan their scent (Voigt, 2002).

There are several advantages to the use of olfactory communication, a major benefit being that chemical signals offer an 'honest' indication of characteristics and status of the depositor (Zala et al., 2004). This was observed in a study on mice (*Mus musculus*) where females were able to detect the health status of male mice based on their urinary odours, where they showed a greater preference for non-parasitized males (Kavaliers & Colwell, 1995). While another advantage is that olfactory cues can persist in the environment even once the sender has gone (Mitro et al., 2012). For example, the faeces of black rhinoceros (*Diceros bicornis*) were found to still stimulate an investigatory behaviour 32 days after they had been deposited, which suggests that were still able to emit an olfactory signal (Linklater et al., 2013). Moreover, olfactory communication can be employed to transmit spatial information and physiological information of the depositor (Marneweck, 2014). For example, chemical signals emitted by the pre-orbital gland of klipspringers (*Oreotragus oreotragus*) demarcate the territory and movements of an individual (Roberts & Lowen, 1997). An example of physiological information being transmitted through olfactory cues is in horses (*Equus cabalus*) where males are able to detect specific oestrus odours from female urine, such that stallions elicit sexual behaviour in response to oestrous urine (Ma & Klemm,1997).

Scent marking occurs through an animal depositing glandular secretions (faeces and urine can also act as the media through which glandular secretions are deposited) either on the ground or onto exposed objects and surfaces (prominent locations) within the animal's environment (Johnson, 1973). There are some disadvantages to chemical signalling and scent marking, as they cannot be directed in a specified direction or towards particular individuals (Eisenberg & Kleiman, 1972). The active space (diffusion resulting in a concentration gradient) of a scent are also affected by wind speed and direction, which may reduce its longevity when exposed to environmental conditions (Bossert & Wilson, 1963).

1.7.2.2 The function of scent-marking and latrine sites in animal behaviour

The functional success of social animal groups is dependent upon the communication system they predominantly utilise to convey information to their conspecifics and other species (Schaller, 1972). In most animal groups the social organisation of members is influenced by the information exchange between group members (Espirito-Santo et al., 2007). Communication occurs through visual and auditory signalling, with the more commonly used method being olfactory communication (Macdonald, 1995). Such olfactory chemical signals are derived from excretory products (faeces, urine and glandular secretions) (Eisenberg & Kleiman, 1972).

Scent marking describes the deposition of these products (chemical signals) on features in the immediate environment of the animal (Macdonald, 1980; Balakrishnan & Alexander, 1985), while the repeated use of specific locations for the deposition of urine and faeces results in the build-up of these excretory products in areas termed latrine sites. These sites serve an important role in olfactory communication (Wronski, et al., 2013). Latrine sites will either be established as territorial boundaries or they will be located within core areas of home ranges - the factors determining this including aspects such as group size and population density (Ziege et al., 2016). Scent marks can relay information pertaining to an individual's status and condition (Blaustein, 1981), territory (Roberts, 2012), competitive ability (Rich & Hurst, 1998), sex (Ferkin, & Johnston, 1995), reproductive state (Ziegler, 2013), as well as the age of an individual and individual identity (Gosling, 2010). Moreover, scent marking has also been found to convey information pertaining to the genetic 'quality' of an individual (Kean et al., 2017), for example female brown bears (*Ursus arctos*) display a greater preference for males that are genetically dissimilar (Mays & Hill, 2004).

It can be difficult to correctly determine and evaluate the role of faeces as scent marks and to discriminate between its role in communication and general excretion events (Espirito-Santo et al., 2007), as not all excretion events are necessarily linked to scent-marking. This becomes less difficult when faeces and scent marks are positioned in

conspicuous regularly visited latrine sites, which is a common behaviour in carnivorous mammals (Macdonald, 1995). Based on the diversity of information that can be coded within a signal, chemical cues are likely to serve multiple functions at the same time (Gosling, 1990). Lazaro-Perea et al. (1999), have proposed several hypotheses that are classified into five broad categories regarding the function of scent marking in mammals. The first hypothesis involves territorial demarcation, where scent marking is a form of territorial ownership (Lazaro-Perea et al., 1999). This is observed in jaguars (*Panthera onca*) and pumas (*Puma concolor*) that use a scrape marking behaviour, where scent secretions from glands between their toes are clawed onto trees to mark and maintain territorial boundaries (Harmsen et al., 2010). Brown hyaenas (*Hyaena brunnea*) of the southern Kalahari will deposit secretions in latrine sites throughout the entirety of their territory, while spotted hyaenas (*Crocuta crocuta*) in Eastern Africa will only place their scent marks in latrines along their territorial borders (Mills & Gorman, 2009).

The second hypothesis is termed the ownership hypothesis, such that an animal will scent mark to indicate 'ownership' of food sources within its home range (Lazaro-Perea et al., 1999). The third hypothesis, the 'mate attraction hypothesis' involves scent marking by females so as to advertise their reproductive status, as observed in female pumas where an increase in scent marking behaviour correlated with their oestrus period (Lazaro-Perea et al., 1999; Allen et al., 2015). The fourth hypothesis describes how scent marking can also be employed as a form of non-combative fighting, such that individuals will scent mark more during intergroup encounters, while the fifth and final hypothesis involves self-advertisement where an animal will scent mark on unmarked substrates in the environment to avoid any masking effects (that might occur with over-marking) (Lazaro-Perea et al., 1999; Bantihun, 2018). These five hypotheses do not always adequately explain scent marking. For example, Verreaux' sifaka (*Propithecus verreauxi verreauxi*) mostly scent mark along the perimeter of their home range, but do not preferentially mark food trees or increase marking behaviour during the mating season or during intergroup encounters (Lewis, 2006). In some cases, latrine sites may simply be the result of particular animal behaviours leading to an aggregation of faeces, for example with repeated perch use, sleeping sites, mating rituals and lek formation (Wenny, 2000).

Latrine sites play an important role in the behavioural and habitat use patterns of the Himalayan musk deer (*Moschus leucogaster*) (Kattel, 1992). Olfactory communication and in particular latrine site use in these ungulates has evolved to be highly developed as they occur in densely forested regions with minimal visual contact and no vocalisation between conspecifics (Lai & Sheng, 1993). An important feature of scent marking (particularly in frequently used latrines) is that it can remain effective for long periods of time (Espirito-Santo et al., 2007). Chemical communication through scent marking occurs through frequent marking onto substrates forming a latrine (Gorman & Trowbridge, 1989). The volume, location, spatial distribution and behaviours associated with latrine sites vary from species to species and according to the function they serve (Mitchell et al., 2004).

Latrine sites serve various purposes depending on the social status, sex and territorial status of individuals (Ben-David et al., 2005). In the only study to date related to the role of latrines sites in any African otter species Jordaan et al (2017) described the sprainting behaviour of African clawless otters and speculated on the possible role of latrines in inter-clan territorial behaviour. This has not been investigated further and there is no information regarding the potential social functions of behaviour associated with latrines and the selection of latrine sites. Latrines undoubtedly play a significant role in otter ecology and allow for otter activity patterns to be deciphered based on latrine site use and characteristics. According to Crowley et al (2012), both the selection and rate of latrine site use by otters is a trade-off between several selective pressures that affect and influence their behaviour at various spatial scales. Latrine site selection by otters is likely influenced by factors at both the coarse and fine scale, like particular habitat characteristics, vertical and horizontal security, prey availability and territorial factors (Crowley et al., 2012).

1.7.2.3 Latrine site use and scent marking behaviour in mammals

In most mammals, scent marking is known to occur via urine, faeces and glandular secretions (Ralls, 1971; Thiessen & Rice, 1976). Latrine site use, that is the preferential, frequent and repeated use of the same area for defaecation, urination and scent marking

is well researched in the Class Mammalia (Gorman & Trowbridge, 1989; Dröscher & Kappeler, 2014). Latrine sites are typically shared by several members of a family, social group or even by different neighbouring groups (Buesching & Jordan, 2019). The deposition of urine, faeces and anal gland secretions at latrine sites function in olfactory communication, such that the olfactory cues that result from scent marking behaviours and secretions convey information influencing the behaviour of conspecifics and other species (Lumkes et al., 2019). Latrine behaviour is defined as the non-random selection of a site for defaecation and scent marking such that faeces, urine and anal gland secretions accumulate in a specific location, that is, a latrine site (Irwin et al., 2004).

Latrine sites are established through the consistent deposition of faces, urine and anal gland secretions at discrete sites (Estes, 1991; Ben-David et al., 1998). This persistent and continual use of latrine sites results in them producing a distinct and persistent odour (Wagnon & Serfass, 2016). Latrine sites facilitate the information transfer and intraspecific communication, such that the faeces, urine and/or scent gland secretions deposited convey information relating to resource use (Stewart et al., 2001) and habitat quality (Ben-David et al., 2005). Several other functions have been proposed regarding the potential information conveyed through olfactory communication at latrine sites including: (1) information pertaining to the sex, diet, reproductive state, movements of an individual (Gorman & Trowbridge, 1989), (2) demarcate territorial boundaries (Roper et al., 1993), (3) to defend food resources (Piñeiro & Barja, 2015), (4) social recognition (Oldham & Black, 2009), (5) the social status of males (Rostain et al., 2004), (6) mate attraction and selection (Allen at al., 2015), along with other intra and interspecific communication functions.

1.7.2.4 Mustelid scent marking for intraspecific communication

The intensely malodorous nature of mustelid scent marks and their role in intraspecific communication has been the subject of chemical research for over 140 years (Burger, 2005). Mustelids are mostly solitary with ranges of up to 40 km (Erlinge, 1967; Kruuk, 2006), making encounters with conspecifics rare, where the use of visual or auditory

communication is limited making scent a key form of communication (Kean et al., 2011). The persistence of olfactory cues in the environment enables Mustelid species to have indirect communication with their conspecifics, such that the signaller does not need to interact directly with other individuals to convey information (Johnston, 2008).

Olfactory communication is a common feature across several taxa enabling communication and cohesion among group members (Buesching et al., 2003). Faeces and anal gland secretions thus facilitate intraspecific communication in the context of individual advertisement, mate attraction and territorial marking (Brown & Macdonald, 1985). While in more solitary species olfactory communication could provide a means for individuals to avoid potentially costly agnostic encounters with conspecifics (Erlinge et al., 1982). The discrimination of species, sex and social status has been identified in North American river otters (*Lontra canadensis*) through behavioural research (Rostain et al. 2004), while chemical evidence has identified sex signatures for age, sex and reproductive status in Eurasian otters *(*Kean et al. 2011). While the primary function of scent marking in mustelids is for intraspecific communication, it is hypothesized to also play a role in interspecific communication, given there are both quantitative and qualitative differences in the chemical composition of anal gland secretions (AGS) (Brinck et al., 1983).

1.7.2.5 Mustelid scent marking behaviour and its role in olfactory communication

Olfactory communication is regarded as the most important communication channel for mustelids (member of the family Mustelidae), most likely because these carnivorous mammals are territorial, and scent mark to indicate territorial ownership (Mumm & Knörnschild, 2018). Furthermore, it is likely that mustelid chemical signalling relays information pertaining to an individual's reproductive state, identity and food resources (Mumm & Knörnschild, 2018). Scent marking is achieved by mustelids through the deposition of faeces, urine, and through glandular secretions (Estes, 1991). The powerful olfactory secretions are produced by mustelids in the anal, ventral, foot and caudal glands (the degree of development, potency and quantity of secretion by these glands all vary

between mustelid species) and are marked on particular objects in the environment through defaecation, urination and the animal body rubbing, dragging or scratching (Estes, 1991; Mumm & Knörnschild, 2018). Several mustelid species including the likes of the striped polecat (*Ictonyx striatus*) and the African striped weasel (*Poecilogale albinucha*) are capable of producing chemical defensive sprays (Apps et al., 2015; Larivière, 2001), while American mink (*Neovison vison*) and the honey badger (*Mellivora capensis*) produce deterrent secretions when they are threatened (Brinck et al., 1978; Vanderhaar & Hwang, 2003). These chemical sprays are similar but not comparable to those produced by skunks (Mephitidae) (Mumm & Knörnschild, 2018). The anal glands of mustelids are not only responsible for producing and storing of defensive chemical sprays and secretions but are also responsible for the musky secretions employed by mustelids in scent-marking (Estes, 1991).

Otters belong to the family Mustelidae and all species in this family have well developed anal scent glands (Hutchings & White, 2000). Scent marking and the malodorous nature of secretions is an integral part of intraspecific communication in mustelids and as such they have been the focus of chemical and olfactory research (Burger, 2005). Otters possess two anal sac glands that are located on either side of the rectum with ducts that open close to the anus (Kean et al., 2017). According to Hutchings & White (2000), there are two primary functions of mustelid olfactory communication, the first one being the communication of reproductive status and the second the availability of resources. Analysis into the chemical characterisation of scent marks can provide a great deal of insight into the information being communicated about the signaller. As was found in a study on the European badger (*Meles meles*) scent marking communicated aspects like age, sex, reproductive status and body condition (Buesching et al., 2002). Group odours can be created by the secretional marking of conspecifics, such that cubs, mates and family members can be ‘labelled’ through body rubbing (Duplaix, 1980). European badgers use their subcaudal gland to create a group odour, this being achieved by individuals mutually marking and rubbing each other (Buesching et al., 2003). There are several anecdotal accounts of otters sniffing spraint and scat but these do not report the otters making direct contact with it (Trowbridge, 1983; Kruuk, 2006). Based on this it is

likely that otter olfactory communication maybe achieved through volatile organic compounds (VOCs).

The frequency of scent-marking and latrine site use in mustelids may vary with seasonal changes, peaking during the mating season (Mumm & Knörnschild, 2018). The honey badger (*Mellivora capensis*) displays distinctive marking differences between sexes, adult males predominantly using latrine sites while female favoured token urination, providing support to the 'scent-matching' hypothesis (Begg et al., 2003; Mumm & Knörnschild, 2018). Female honey badgers predominantly visit latrine sites when in oestrus, where they carry out intensive smelling and low-level scent marking behaviour suggesting a scent-matching function being carried out rather than reproductive advertisement (Begg et al., 2003). Latrine site use is also affected by the population density of a species. For example, European badgers form social groups in high density population areas, while in low density areas they occur pairs or remain solitary (Buesching et al., 2016; Mumm & Knörnschild, 2018). The larger groups have more latrine sites within their home range, but they typically invest in boundary latrines for territorial marking as space is limited in high-density populations (Buesching et al., 2016).

Social Mustelid species like the European badger and giant otter (*Pteronura brasiliensis*) establish communal latrine sites, large areas for the deposition of excremental and secretional marking by all members of a group (Buesching & Macdonald, 2001; Carter & Rosas, 1997). Otters in particular tend to display rather elaborate marking behaviours which involve a scent-marking 'dance', for example giant otters, Spotted-necked otters and North American river otters have been recorded carrying out dance like stepping postures, body rubbing and intense sniffing at latrine sites (Mumm & Knörnschild, 2018; Groenendijk, 2019). Latrine use and behaviour at latrine sites has not been extensively studied in African clawless otters but significant findings were made by Jordaan et al. (2017), in the way secretions were deposited, before and during secretions a type of 'jiggle dance' (where hind legs were stomped and posteriors legs would move from side to side) was performed either by individuals or in groups.

1.7.2.6 Latrines and their role in the social behaviour of Mustelidae

Latrines are key sites of olfactory communication for North American river otters where various different behaviours have been observed, including the likes of stomping, sniffing, self-grooming, co-rubbing, wrestling, rolling and digging (Green et al., 2015). Latrine sites are also used as meeting sites, influencing and affecting the social structures of a population. The individual factors and behaviours that drive the complex social dynamic of otters have become the focus of several recent studies, to improve our understanding of the factors influencing their behaviour. Researchers have studied latrine sites to determine the density, distribution, occupancy and habitat selection of otters (Dubuc et al., 1990). Findings by Green et al. (2015), suggest that North American river otters were more likely to go to a latrine and engage in social behaviours, where they were commonly observed sniffing and standing, which supports the idea that latrine sites play a role in olfactory communication. The behaviours most frequently observed were standing and sniffing, suggesting that information is gathered about conspecifics by way of olfactory cues (Green et al., 2015). Dominant male North American river otters spent significantly more time investigating scats, possibly suggesting the role latrine sites play in communicating hierarchical roles and territories, furthermore olfactory communication at latrine sites could possibly facilitate mutual avoidance (Melquist & Hornocker, 1983; Ben-David et al., 2005).

In addition to anal gland secretions and faeces, otters are able to deposit scents at latrine sites through body rubbing and rolling (this form of scent marking is well-established in mustelids) (Estes, 1991). The study by Green et al. (2005), analysing North American river otter behaviour at latrine sites found body rubbing occurred more than defaecation. Where they make use of scent glands located in the pads of their feet and ventral region, North American river otters have been observed scraping sand into piles aiding scent marking with their digital scent glands (Kruuk, 2006). Novel behaviours in Neotropical otters (*Lontra longicaudis*) have been recorded along waterways in the eastern Brazilian Amazon. Individuals were recorded depositing urine on top of each other's, individual

digging and then scent marking as well as males rubbing their bellies and genitalia on sandy substrates (Michalski et al., 2021).

The social organisation and structure of animal groups have several implications for the foraging success (Aplin et al., 2012), information transfer (Sueur et al., 2011), disease transmission (Kappeler et al., 2015) and fitness of individuals (Silk, 2007). Otters frequently visit latrine sites, where the deposition of faeces and scent gland secretions play an important role in intraspecific communication between family members and social groups (Hutchings & White 2000). Studies conducted on captive otters indicated that scent-marking, played a powerful role in olfactory communication indicating an individual's 'identity', dominance and sex (Rostain et al., 2004). Body water type and the availability of food determine the number of scats that are accumulated at latrine sites of North American river otter (Crowley et al., 2012).

Resource availability and sex-specific differences could potentially create selective pressures on the fission-fusion dynamics within a population (Barocas et al., 2016). The social communication and information that is spread at latrine sites makes the system appropriate for analysing chemical and olfactory communication which drives their complex social organisation (Barocas et al., 2016). Findings on a population of coastal North American river otters found that latrine sites served various purposed depending on the social status, sex and territorial status of individuals (Ben-David et al., 2005). Latrine sites could play a role in the intragroup communication of otters, possibly aiding otter clan members in coming together for social foraging events (Ben-David et al., 2005). Latrine sites are suitable to investigate several aspects of African clawless otter biology including the likes of habitat use in a similar fashion to studies on North American river otters where fine-scale measurements of habitat indicate selection differed significantly between oiled and nonoiled study areas (following the Exxon Valdez oil spill in late March 1989) (Bowyer et al., 1995). Knowledge of habitat features and characteristics of latrine sites can hasten the process of finding their location, which could justify their

consideration during land management activities given the important role these sites play in the intraspecific olfactory communication for this species (Torgerson, 2014).

1.7.2.7 Faeces and anal gland secretions

The intestinal flora composition can reflect the social contact network of an individual (Tung et al., 2015), in this way faeces can code information pertaining to social and familial group membership (Carthey et al., 2018). For many mammals the excretory pathway for steroid hormones is through the liver/gut axis (Umapathy et al., 2013), such that faeces can code for information on the reproductive status (Martín et al., 2010), stress levels (Schatz & Palme, 2001), endocrine status, gender, social status, and age of an individual (Ferrero & Liberles, 2010). Given that faecal odour is affected by digestive processing and diet (Ferkin et al., 1997), faecal deposits alone are limited in their ability and suitability as individual specific advertisement signals (Noonan et al., 2019). In addition to this the information encoded in faecal odour is influenced and moderated by anal gland secretions (Macdonald, 1985). The anal glands allow for more complex and individual specific information to be transmitted as opposed to faeces alone (Buesching & Stankowich, 2017).

The anal gland secretions (AGS) in carnivores are produced in paired vesicular anal glands that are situated in the rectum on either side of the anus (Macdonald, 1985). The AGS when deposited with faeces is known as spraint (Kean et al., 2011). Although all mustelids have anal glands the degree of development, their potency, quantity of secretions and dispersal accuracy vary between species (Estes, 1991). All mustelids possess anal glands with the exceptions of the sea otter (*Enhydra lutris*), most likely attributed to the entirely aquatic nature of these otters (Albone, 1984; Kruuk, 2006).

Anal gland sections are excreted through mechanical stimulation together with faecal deposits during defaecation (McColl, 1967). The anal glands are compound, composed of several layers of secretory cells that encircle and empty into the ducts of the anal sacs (Estes, 1991). The chemical profiles of AGS have been determined to be individual

specific for several mustelid species including the Eurasian otter (Kean et al. 2011), steppe polecat, *Mustela eversmanii* (Zhang et al. 2002), Siberian weasel, *Mustela sibirica* (Zhang et al. 2002b), ferret, *Mustela furo* (Zhang et al. 2005) and European Badger (Noonan et al., 2019). Across most mustelid species the AGS are high in carboxylic acids and organosulfur compounds (Buesching & Stankowich, 2017).

1.7.2.8 Chemical properties of volatiles associated with latrines

The ability for conspecifics to recognise and differentiate individuals has evolved based on the associated fitness benefits for both the signaller and receiver (Tibbetts & Dale, 2007). Individual identity can be communicated through visual, vocal or scent cues, however scent is the most common modality in mammals (Brown & Mcdonald, 1985). Despite the various hypotheses and suggestions regarding the communicative functions of spraint, the exact chemical characteristics and messages conveyed through scent remain unknown (Kean et al., 2011). While anecdotal accounts of otters sniffing spraint exist (Trowbridge, 1983; Kruuk, 2006) there are no accounts of otters making direct contact with it. Based on this it seems likely that part of the olfactory communication is achieved through volatile organic compounds.

Olfaction is the primary mode of communication for several mammal species, the olfactory signals emitted from urine, faecal and scent mark deposits relaying information through VOCs (Marneweck, 2014). Volatile organic compounds are essentially a large group of carbon-containing molecules, which may have a biological or synthetic origin (Hough et al., 2018). The molecular weight of a compound determines its volatility, such that larger and heavier compounds are less volatile than those that are smaller and lighter (Stoddart, 1976). In order for a compound to be effective as a chemical signal it is believed that the compound should have a molecular weight ranging between 50 and 300 kDa (Wheeler, 1977). Indeed, the mean molecular weight of mammalian territorial and range marks is 208, while the mean molecular weight for mammalian sex attractants is 91 (Alberts, 1992). Volatile organic compounds are able to enter the gaseous phase at room temperature through a combination of their low molecular weight and high vapour

pressure and these contribute to the odours of faeces, urine, saliva and sweat (Hough et al., 2018). Compounds with a high volatility can be released as alarm signals and this rapid sudden discharge entails a rapid fade-out meaning the signal will not persist in the environment after the threat has disappeared (Bossert & Wilson, 1963). Volatile organic compounds can also be emitted continuously at a constant rate, allowing for a relatively constant depletion rate in the environment, allowing them to persist long enough in the environment that they can be received by conspecifics (Alberts, 1992).

Volatile organic compounds may vary in concentration based on individual characteristics like sex, as seen in the urine of lions (*Panthera leo*) (Andersen & Vulpius, 1999). Certain VOCs can also result in an immediate behavioural response termed the releaser effect; this is seen in aardwolves (*Proteles cristata*) which increase their scent marking rate when they encountered scent marks from same sex individuals within their territory (Bossert & Wilson, 1963; Sliwa & Richrdson, 1998). Another behavioural response that can arise in response to a VOC is termed the primer effect which has a physiological influence on the receiver (Bossert & Wilson, 1963). This was observed in mice, where the urine of males was capable of inducing oestrus in females (Jemiolo et al., 1986).

In terms of the particular VOC found in scent marking fluid, urine and faeces these differ from species to species. For example, the scent marking fluid and urine of the Himalayan Snow Leopard (*Panthera uncia*), contains several volatile compounds with low molecular weights belonging to different functional groups, namely, alcohols, aldehydes, ketones and sulphurous compounds (Das et al., 2019). Several saturated, monosaturated and polyunsaturated fatty acids were also reported in the urine of this felid, which play an important function in the durability and longevity of volatile compounds (Das et al., 2019). In another study by Marneweck (2014), which identified 326 volatile compounds from the dung odour profiles of white rhinoceros (*Ceratotherium simum*), nine compounds (predominantly alkanes and alkenes) were found to correlate with sex, while seven alcohol and alkane compounds were associated with the age of an individual. A higher number of acids and aldehydes were released by territorial males compared to their non-

territorial counterparts, while females in oestrous released lower proportions of acids, alcohols and alkanes than non-oestrus females (Marneweck, 2014). The findings of which indicate that middens are important information exchange centres for this species, allowing for information transfer between individuals through olfactory communication (Marneweck, 2014).

Information relating to signaller identity is communicated across taxa through scent (Kean et al., 2011). The sense of smell is one of the most important senses in the Mustelidae family to find prey and for complex social communication which is poorly understood (Estes, 1991; Ladds et al., 2017). Many otter species leave their faeces in exposed locations as well as in latrine sites or middens, which are typically in close proximity to dens (Estes, 1991). Scent marking is defined as the repeated deposition of small amount of anal gland secretion and faecal material at selected sites, while deposition that is purely for faecal elimination is characteristically more voluminous and do not have a particular pattern of distribution (Kleiman, 1966; Kean et al., 2011).

Little is known about what information is communicated or the social functions that are transmitted through spraint, anal gland secretions and/or faeces. The sniffing behaviour, including the like of head bobbing and nostril flaring, recorded of North American river otters at latrine sites indicate that they are capable of discriminating species, sex, and social familiarity on the basis of faeces (Rostain et al. 2004). Studies conducted on Eurasian otters and North American river otters have found that scent marking behaviour at latrine sites occurs year-round (Rostain et al., 2004), while some studies have suggested that scent marking is greater during mating seasons (Macdonald & Mason, 1987).

Early studies that attempted to investigate the scent marking of the Eurasian otter and the information conveyed through spraint identified a difference in the chemical composition between individuals, however the sample size of this study (n =3) was rather

small limiting the conclusions that could be drawn (Trowbridge, 1983). In a more recent study conducted by Kean et al. (2011), analysing otter scent gland physiology and chemical composition on 158 Eurasian otter carcasses allowed them to obtain additional data from individuals relating it to parameters like sex, age, reproductive status and size. This study used headspace solid-phase microextraction and gas chromatography–mass spectrometry to analyse volatile organic compounds and found that both univariate and multivariate differences were distinct between adult and juvenile otters, suggesting the importance of anal gland secretions in mate attraction of this species (Kean et al., 2011).

The compound *indole* was present in all juvenile samples with a significantly lower abundance of this compound found in adult samples, adult Eurasian otter gland sample collected were significantly 'sweeter' smelling (Kean et al., 2011). The distinct difference in the scent of juvenile and adults' spraints could be the result of an immature body function or point to differences in dietary preferences (Kean et al., 2011). Eurasian otter cub spraints were considerably larger than those of adults, (cubs could primarily be depositing spraint for faecal eliminations), while adult male spraints were also found to be smaller than those of females (Kruuk, 2006). This could be related to the possibility that males frequently deposit more spraint for communication purposes to demarcate the territory. The scent of spraint is significantly associated to the identity of individuals that occur in the same locations. For example, spraint samples collected from captive Eurasian otters, found a total of 162 VOCs across all samples collected (Kean et al., 2015). Spraint scent was significantly associated to the identity of individuals that occurred in the same geographic areas, possibly attributed to genetic similarity between individuals while hormonal fluctuations are believed to be responsible for the within-individual variation observed (Kean et al., 2015).

1.7.2.9 Selection of latrine sites

There are several factors believed to influence the location of latrine sites. These include scent dispersal or retention properties of scent marks, visual prominence of a site, cover that preserves scent mark integrity and a location that offers security (Ben-David et al.,

2005). The selective positioning of a latrine site is likely to increase the efficacy and transmission of chemical signals which ultimately prolongs their protection and longevity (Alberts, 1992). Several carnivorous species will establish latrine sites near their territorial borders allowing them to demarcate and defend their territory (Kruuk, 1972). In contrast, some species (particularly those with considerably larger home ranges) locate their latrines in core strategic locations within their home ranges (Gorman & Mills, 1984; Sillero-Zubiri & Macdonald, 1998).

Latrine site selection and patterns of latrine use could be associated with several factors relating to resource use and can furthermore have several implications in disease transmission and parasite avoidance (Hutchings et al., 2001; Riordan et al., 2011). For example, hog badgers (*Arctonyx collaris*) in China establish their latrines in relation to food resources, they have also been documented establishing latrines in areas categorised as having poorer food abundance (Zhou et al. 2015). The overall implications of this indicate that latrine sites could potentially be employed to demarcate the resources of a territory holder within a particular area. Latrine site use has also been found to vary with seasonal and habitat related fluctuations, and overall latrine site selection is skewed toward logged and selectively logged forest areas (Zhou et al., 2015). A similar behaviour has also been reported for coastal Eurasian otters, where scent marking and latrine sites are predominantly established to mark and signal the use of food patches and freshwater pools (Trowbridge, 1983).

Latrine sites in various locations may aid spatial memory to facilitate optimal foraging, while also allowing a species to mark and lay claim to scarce resources in an area (Garber, 1989; Espirito-Santo et al., 2007). For example, the distribution and latrine use by wild meerkats (*Suricata suricatta*) in the southern Kalahari enable the transfer of information to protentional intruders (Jordan et al., 2007). Groups of meerkats share latrines with neighbouring groups, likely facilitating intergroup communication and monitoring of surrounding areas, while remaining latrine sites of a group are concentrated in strategic territorial core regions of their home ranges (Jordan et al., 2007). The spatial

and temporal distribution of meerkat latrine sites suggest these sites play an important role in territory defence as well as mate defence (Jordan et al., 2007).

Latrine site selection is intrinsically linked to habitat selection given the importance these sites play in intra-specific communication. Habitat selection has been investigated in several otter species based on the presence of spraint, spoor, grooming sites and dens. There are a number of techniques that can be employed to assess habitat selection, one such method is assessing the density and features of latrine sites, dens and occupied shelters (Kruuk et al., 1989; Pardini & Trajano, 1999). Eurasian otters in Korea preferentially located their spraints in environments where weirs reduced the drift of water where a natural stream bank had formed, additionally shallow areas of streams and areas along the edges of water covered with trees and shrubs were favoured (Cho et al., 2009). North American river otters were found to favour latrine site selection where the following habitat features were present: large conifer trees, points of land, proximity to beaver bank dens, isthmuses and mouths of permanent streams (Newman & Griffin, 1994). Three sympatric otter species, the smooth-coated otter (*Lutrogale perspicillata*), the Eurasian otter and the Asian small-clawed otter were investigated in southern Western Ghats, India, their habitat preferences were found to be highly specific in terms of their diet and habitat, allowing them to coexist through resource partitioning (Raha & Hussain, 2016). The Asian small-clawed otter selected habitats at higher elevations, along narrow streams with dense vegetation and canopy cover, feeding predominantly on crabs. While the Eurasian otter and smooth-coated otter selected large rivers and dams at lower elevations, their diet is predominantly composed of fish, hence both species are well adapted to swimming and hunting in larger water bodies (Raha & Hussain, 2016).

Vegetation structure is commonly associated with latrine site selection in other mammals. For example, genet (*Genetta genetta*) latrines in southwestern Portugal are primarily located in areas with a diversity of landscape features, high understory height, close proximity to refuges as well as other latrine sites (Espirito-Santo et al., 2007). Latrine sites of the Himalayan musk deer are located in areas with specific habitat characteristics, for

example latrines typically occur in mixed and fir forests while blue pine forest and open areas are avoided, the latrine distribution is sparse at lower and higher altitudes but densely distributed between 3800 m and 4000 m above sea level, while the presence and distance from a water source is a critical feature in the establishment of a latrine site (Singha, 2018).

North American river otters select latrine sites based on multiple factors, such as security from predators, visual prominence, surrounding vegetation, scent dispersing properties and features that are likely to preserve scent marking (Newman & Griffin, 1994; Ben-David et al., 2005). A study conducted by Crowley et al. (2012), found that the selection of latrine sites by North American river otters were positively influenced by large diameter trees and horizontal visual obscurity. Furthermore, latrine sites that were consistently used were associated with conifer trees, a higher frequency of spruce trees and increased horizontal cover, indicating that horizontal cover (by way of large-diameter conifer trees with low hanging branches) plays an important role in latrine site selection (Crowley et al., 2012). Another study Torgerson (2014), into latrine site selection of a coastal river otter population found that several habitat variables distinguished the location of latrine sites, these included the presences of large fallen logs, greater shore heights, deeper water and canopy cover.

Given that latrines sites are ecologically important features for several species, knowledge on their use and the behaviours associated with them will improve knowledge systems and can be applied in ecological surveying and the implementation of conservation measures (Kattel, 1992).

1.7.2.10 Otter scent marking behaviour

There are minimal behavioural records of African clawless otter scent marking behaviour. A behavioural record by Rowe-Rowe (1978), suggested body rubbing, grooming and drying takes place at latrine sites. A more recent finding by Jordaan et al. (2017), found

African clawless otters performing elaborate scent marking behaviours at latrines, including body rubbing and the deposition of anal secretions through a ‘jiggle dance’.

North American River otter latrine sites are used for olfactory communication (Green et al., 2015). North American river otters have been documented performing several different behaviours at latrine sites including sniffing, body rubbing, self-grooming, stomping, digging, defaecating and wrestling (Green et al., 2015). Similar behaviours have been described in giant otters, where body rubbing, defaecation, urination and fore-paw rubbing against surrounding vegetation occurred at latrine sites (Leuchtenberger & Mourão, 2009). A recent study by Michalski et al. (2021) documented new scent marking behaviour in the Neotropical otter, where otters were recorded digging to scent mark with urine. In addition, couple scent marking behaviour in this species was recorded whereby males urinated on top (overmarked) of female’s fresh urine in newly dug shallow craters (Michalski et al., 2021).

1.7.2.11 Overmarking behaviour

Three types of over-marking have been described, 1) direct overmarking resulting in an overall blended group scent, 2) overmarking to mask and conceal other scent mark and 3) countermarking - marking adjacent to another scent thereby maintaining distinct individual scent signatures (Johnston et al., 1994; Kean, 2012). The exact benefit of using one strategy over another is not well understood. Overmarking, when one individual place their scent mark directly on top of the scent mark of another individual, is a common response in mammals when encountering scent marks (Johnston et al., 1994; Jordan et al., 2011; Brown & Macdonald and Rodgers et al., 2015).

Overmarking will typically occur within breeding pairs where males will scent mark over the scent of their mates (as described in the Neotropical otter above). Other examples of overmarking in breeding pairs include: meerkats, *Suricata suricatta* (Jordan, 2007); Kirk’s dik-dik, *Madoqua kirkii* (Brotherton, 1994); grey wolf, *Canis lupus* (Peters & Mech 1975)

and Wild Diademed Sifaka, *Propithecus diadema* (Miaretsoa et al., 2022). Overmarking also occurs where individuals of a species overmark the scents of same sex individuals. This form of scent marking is interpreted as a form of intrasexual competition, through the 'competing countermarks hypothesis' where only fit individuals are able to continually and effectively overmark the scent of their rivals (Rich & Hurst, 1999; Jordan et al., 2011). The social organisation of a species is believed to influence the type of overmarking used. Solitary species like the Golden hamster (*Mesocricetus auratus*) will employ countermarking where individual scent signatures remain distinct (Johnston, 1994). An analysis of scent communication in the Eurasian otter documented several instances of countermarking behaviour in response to scent presentation trials (Kean, 2012). These otters countermarked by depositing spraint or urine adjacent to test scents (Kean, 2012). The Neotropical otters are known to use scent marking as a form of olfactory communication between individuals (Rheingantz & Trinca, 2015; Michalski et al., 2021). Michalski et al. (2021) recorded novel behaviours whereby the otters would dig and deposit their urine in exposed craters, additionally individuals were recorded depositing their urine on top of each other's.

1.7.2.12 Otter social organisation

Mustelids are considered the least social carnivores (Gittleman, 1989) and otters are known to fluctuate intra-specifically from solitary to group living arrangements (Léliaso et al., 2021). Otters display considerable intraspecific variation as well in their social organisation, from solitary individuals (Kruuk, 2006), monogamous pairs (Ostfeld et al., 1989), male groups (Blundell et al., 2002), and extended family groups (Ribas et al., 2016; Schmelz et al., 2017). Changes in the social organisation of a group have implications on information transfer, foraging success and individual fitness (Barocas et al., 2016). The regular shift in the size and composition of a social group are described as fission-fusion dynamics (Barocas et al., 2016). Fluctuations in resource availability, sex-specific differences, competition, disease transmission and multiple contexts are all factors influencing group interaction and thereby create selective pressure on the fission fusion

dynamics of a population (Pépin & Gerard, 2008; Kashima, 2013 and Barocas et al., 2016).

The 13 species of otters show a great diversity of social systems and organisation, with some being fiercely territorial and solitary and others living in large complex social groups (Kruuk, 2006). The southern river otter (*Lontra provocax*) (Sepúlveda et al., 2007), marine otter (*Lontra felina*) (Medina-Vogel et al., 2007; Vianna et al., 2010), hairy-nosed otter (*Lutra sumatrana*) (Kanchanasaka, 2001; Nguyen et al., 2001), Congo clawless otter (*Aonyx congicus*) (Jacques et al., 2009), Eurasian otter (Mason & Macdonald, 1986) spotted-necked otter (Reed-Smith et al., 2014), and the neotropical otter (Kasper et al., 2008; Rheingantz et al., 2017) all exhibit a primarily solitary lifestyle, where individuals will only group together during reproductive and breeding periods. The smooth-coated otter (Nawab, 2009) displays both solitary and social habits, while the North American river otter will live in small social groups (DeLong et al., 2019). In contrast the Asian small-clawed otter (Foster-Turley, 1992; Hussain et al., 2011), giant otter (Leuchtenberger et al., 2014) and sea otter (Blundell et al., 2002) all have a high degree of sociality, living in groups with complex social interactions. Given that otter group associations may change seasonally, olfactory cues and scent marking through latrine sites could allow for individuals to re-establish identity with and among familiar individuals (Rostain et al., 2004). This intragroup communication between individuals could also involve the transmission of information relating to social hierarchy (Kruuk, 1972).

African clawless otters are largely considered to be solitary (Arden-Clarke, 1986; Ostfeld et al., 1989), however anecdotal accounts of groups of four to six individual travelling together have been recorded. Overall, there is presently a very vague picture of the social organisation of African clawless otters. The precise role of scent marking, communication and its role in the social make up of a population are not well understood.

1.7.2.13 Sexual communication

The role of odour in sexual communication among carnivores, in particular, is well supported (Dunbar, 1977; Wells & Bekoff, 1981; Gese et al., 1997; Molteno et al., 1998; Allen et al., 2016 and Janssenswillen et al., 2021). Scent marking at latrine sites is also believed to function in sexual communication and the advertisement of both male and female reproductive status (Kruuk, 1992). Eurasian otter and North American river otter scent marking at latrine sites occurs throughout the year, however some evidence exists that scent marking occurs at a higher frequency during the mating seasons (Humphrey & Zinn, 1982; Rostain, 2000). Eurasian otters vary their scent marking rate seasonally; this could be related to individuals signalling their reproductive status (Gorman & Trowbridge, 1989). North American river otters can discriminate the sex of conspecifics from the scent of spraint (Rostain et al., 2004). However, urine is believed to play a more significant role in the communication of an individual's reproductive status and oestrus condition (Gorman & Trowbridge, 1989). Volatile organic compound analysis of the anal scent gland secretions of Eurasian otters indicate sex differences were evident in adult otters but not younger individuals, suggesting the role of secretion and scent marking in mate attraction (Kean et al., 2011).

1.7.2.14 Territoriality and resource marking

Territorial marking allows an individual to gain an advantage over conspecifics by restricting or denying them access to resources like food or mates (Rostain et al., 2004). Territorial marking has been observed in several mustelids, including the European badger (Kruuk, 1984); stoat (*Mustela erminea*) *(*Erlinge, 1977*);* ferret (*Mustela furo*) (Clapperton, 1989) and the weasel (*Mustela nivalis*) (Erlinge, 1974). Scent marking is an important feature in maintaining social organisation and group dynamics of the territorial giant otters (Carter & Rosas, 1997). Giant otters maintain their territories for extended periods of time through re-establishing and constant scent marking of communal latrine sites in their territories (Leuchtenberger et al., 2015).

Mutual avoidance and territorial marking have been reported in otters in both freshwater and marine environments (Melquist & Hornocker, 1983; Gorman & Trowbridge, 1989; Kruuk, 1992; Shannon, 1993 and Mumm & Knörnschild, 2017). The ubiquitous distribution of latrine sites in the landscape suggests a different function of scent marking and territoriality in otters (Kruuk, 1992). The traditional interpretation of latrine site use and scent marking in mammals is to create a 'scent-fence' and 'advertisement of ownership' of one or several resources in an area (Hediger, 1949; Buesching & Jordan, 2022). This interpretation has been revised to account for the increasing variety of territorial intrusions by non-residents (Buesching & Jordan, 2019). Latrine sites are believed to establish a power symmetry between the territory holder and intruder (Maynard-Smith & Parker, 1976; Hammerstein, 1981). In addition to territoriality, scent marking may also mark the use and depletion of key resources and food patches and this could facilitate mutual avoidance on a small spatial–temporal scale (Kruuk, 1995; Remonti et al., 2011).

1.7.2.15 Active time

The differences in the seasonal activity patterns of otters are triggered by changes of the sunrise and sunset times. In northern latitudes otter activity patterns typically become unimodal as a result of shorter days and reduced foraging time, however in some localities otter may be primarily nocturnal (Mason & MacDonald, 1986; Kruuk & Moorhouse, 1990). Diel activity time of the Eurasian otter in Romania was found to change seasonally such that their peak activity times strongly correlated with darkness times and was likely linked to prey accessibility, in the summer months peak activity was between the hours of 20h00 and 08h00 while in the winter months it was between the hours of 16h30 and 07h30 (Bouros et al., 2016).

African clawless otters can be active at day or night; their activity peaks have been recorded during the early morning and late afternoon hours (Rowe-Rowe, 1978). Activity patterns of otters will likely vary depending on whether they inhabit areas that are undisturbed or disturbed (through human encroachment). Majelante et al. (2020), found significant differences in the group sizes of African clawless otters in transformed and

natural areas, where far more detections and activity time were recorded in transformed study areas. Illustrating how these otters display behavioural plasticity allowing them to take advantage of resource rich anthropogenic environments (Majelante et al., 2020).

1.8 Research aims and questions

This study sought to investigate previously unresearched aspects related to the ecology of African clawless otter. This project aimed to elucidate aspects of the behavioural ecology of African clawless otters in a coastal region of South Africa. The presence of otters in the general study area (uMlalazi Nature Reserve and Zini Fish Farm) was established through a pilot survey that determined the presence of African clawless otters though latrine sites and other signs.

The specific research aims and questions of this study included:

1.8.1 Aim 1: To model and determine African clawless otter population densities in the study area.

Research question:

- What is the population density of African clawless otters in the study area?

1.8.2 Aim 2: To assess social behaviour and communication of African clawless otters around latrine sites.

Research questions included:

- What behaviours do African clawless otters display at latrine sites?

1.8.3 Aim 3: To assess factors characterising the location of African clawless otter latrine sites.

Research questions included:

- Are African clawless otter latrines distributed randomly along water bodies?

- If not randomly distributed, which habitat characteristics correlate with the position of latrine sites in the landscape?

1.8.4 Aim 4: To describe the chemical properties of faeces and anal gland secretions of African clawless otters and assess the potential social roles of spraint marking.

Research questions included:

- What are the chemical properties of faeces and anal gland secretions of African clawless otters?

- Is there evidence for inter-sexual differences in the chemical properties of spraint and anal gland secretions of African clawless otters?

1.9 Significance of the study

This research on the African clawless otter populations in northern KwaZulu-Natal improves understanding of the behavioural ecology of a widespread, but understudied mammal. This research sought to specifically investigate and improve knowledge systems of African clawless otters in terms of their population density, social behaviours, latrine site selection, the chemical properties of faeces and anal gland secretions.

This research will aid in informing assessments of their likely behavioural plasticity and responses to changing environments. This study made use of several study approaches to assess the above-mentioned behaviours including camera trapping to assess behaviours at latrine sites, gas chromatography to determine olfactory composition and linear modelling to assess factors influencing latrine site selection.

1.10 Study area

Study sites were located in Northern Kwa-Zulu Natal, South Africa. Two study areas were located in the coastal town of Mtunzini (28°57'34.9"S, 31°45'00.4"E), while the third study area was located in Fairbreeze near the town of Gingindlovu (29°01'25.0"S 31°34'47.3"E). Population density analyses, behavioural data and faecal sample collection was conducted along the uMlalazi River (28°55'60" S; 31°48'0" E), located approximately 30 km south-west of Richards Bay in northern KwaZulu-Natal province, South Africa. The river drains into the Indian Ocean and is approximately 54 km long with a catchment area of 492 km^2 (South African Environmental Observation Network, 2021). The two study areas located in Mtunzini are the uMlalazi Nature Reserve (28°57'14.7"S, 31°45'59.3"E) and Zini Fish Farm (28°57'13.7", 31°45'57.2") (see Figure 1.3).

The uMlalazi Nature Reserve (uMNR) covers an area of 1 028 hectares in extent and forms part of both the Maputaland-Pondoland-Albany Biodiversity Hotspot and the Maputaland Centre of Floristic Endemism (Van Wyk & Smith, 2001; Zungu et al., 2018). The reserve is a natural area with relatively low anthropogenic disturbance. Zini Fish Farm is 45 hectares in extent, it comprises of 52 half-hectare earthen ponds. The primary product of the farm is saltwater tilapia (*Oreochromis mossambicus*). Zini Fish Farm is a transformed area with substantial anthropogenic disturbance. The uMNR and Zini Fish Farm are adjacent to one another, and although separated by a fence line, these two locations were be treated as a single study area, based on their close proximity and the permeability of the fence line, specifically to otters. The abundance of prey for African clawless otters and the absence of persecution by the farmers in the anthropogenically disturbed (augmented) Zini Fish Farm, makes this augmented environment attractant to otters.

In addition to this primary study area an additional study area was included, Cottonlands farm in Fairbreeze (29°02'16.5"S, 31°37'07.0"E), along the Nyezane River. Cottonlands farm is located 20 km south-west of the uMNR and Zini Fish Farm and is characterized by similar climatic conditions. At Cottonlands farm behavioural data and faecal samples

was solely collected for analysis, there was no population density or latrine site characteristic analysis assessed in this study area.

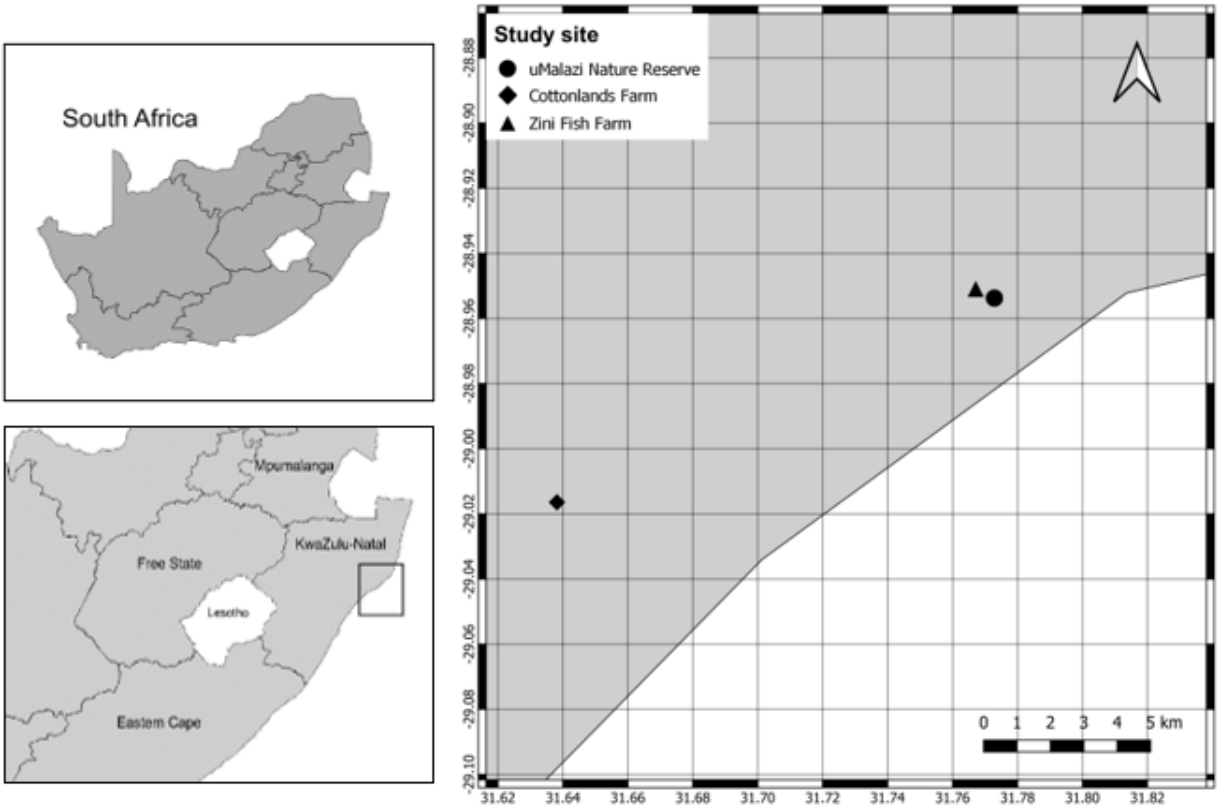

Figure 1.3. Study area in northern KwaZulu-Natal province, South Africa. Cottonlands Farm and Zini Fish Farm are both transformed areas, uMlalazi Nature Reserve is a natural area.

1.11 Climate and weather

The study area has a subtropical climate, with summers that are hot and humid and winters that are mild and frost free (Nevill & Nevill, 1995). The oceanic climate of the region is marked by precipitation in all months of the year without a clearly defined dry season. Temperatures range between a mean minimum of 11 °C in winter to a mean maximum of 33 °C in summer, with an average humidity of 75%. The temperature seldomly falls below 17 °C, with the sea water maintaining a temperature of 21 °C even throughout the winter months. As the temperatures rise toward the summer months so too does the precipitation, precipitation levels gradually increase from September

reaching a peak in January, as the temperature decreases towards the winter months do to does the amount of precipitation (Esterhuizen, 2019).

Mtunzini forms the southernmost tip of Maputaland of the Indian Ocean Coastal Belt (which stretches far north into Mozambique), this section of the KwaZulu-Natal coast receives a substantial portion of its annual rainfall in winter (Lubbe, 1996). There is a fixed pattern in the annual rainfall in this region, with high rainfall from October to March, and decreased but substantial rainfall from April to August, during the winter months (Esterhuizen, 2019). Approximately 60% of which fall in the mid-summer months and the remaining 40% falling in the early winter months (Tyson & Preston-Whyte, 2000; Mucina & Rutherford, 2006; Pretorius et al., 2014). The average annual rainfall in this region fluctuates between 819 mm and 1 272 mm (Mucina & Rutherford, 2006).

1.12 Ethical note

The study was performed under the approval of the University of South Africa (Research Ethics Committee reference: 2021/CAES_AREC/134). Research permits and permission letters were approved by Ezemvelo KZN Wildlife (OP 2373/2021), Zini Fish Farm and Cottonlands Farm.

CHAPTER II – COMPARATIVE DENSITY, ACTIVITY AND BEHAVIOUR OF AFRICAN CLAWLESS OTTER

2.1 Introduction

Reliable and unbiased estimates of population density are fundamental in ecological, conservation and wildlife management decisions as it facilitates our understanding into population dynamics, distribution, probability of survival, density dependent population growth, likelihood of local extinction, understanding of the autecology of an organism and sensitivity to stochastic processes (Wright & Hubbell, 1983; Maritz & Alexander, 2012; Myrvold & Kennedy, 2015 and Sittenthaler et al., 2020). Having knowledge on the number of individuals inhabiting a particular area can in turn provide valuable information relating to effects of ecological and anthropogenic factors (Challender et al., 2020).

Population abundance and density estimates form the basis for a wide range of studies in ecology. Population density is the measure of the absolute abundance of a species' population size per area unit, while relative abundance is essentially the relative representation of a species in an ecosystem (ENETWILD consortium et al., 2020). Accurate and reliable information of animal population densities enables researchers to elucidate and monitor trends of wild animal populations (Thompson et al., 1998). Obtaining such information is fundamental to ecological studies and is essential for the effective and efficient implementation of monitoring and conservation practices for vulnerable and threatened species (Nichols & Williams, 2006). Thus, the development of effective monitoring and conservation management strategies hinge upon reliable knowledge of a populations size and change over time.

African clawless otter density estimates have traditionally been based on their characteristic spoor and faeces at latrines (Estes 1991; Rowe-Rowe, 1992). This method faces certain drawbacks given that it is limited by bias due to fieldworker experience and it is also relatively labour intensive (Wilson & Delahay, 2001). A further downside to this

method is that it can either underestimate or overestimate the number of individuals in an area. Thus, measuring density through camera trapping has become a convenient tool in ecological research given it is cost effective and far less time consuming (Wearn & Glover-Kapfer, 2019; Santini et al., 2022;). Several camera trapping methods exist to estimate the population densities of unmarked individuals (Palencia et al., 2021). One such method is the random encounter model (REM), this model is well validated and has proven to perform better than traditional methods (sign surveying techniques such as identification of characteristic otter signs through holts, spoor and faeces) (Pettigrew et al., 2021; Jensen et al., 2022). In addition to population density estimates, remote photography through camera trapping can provide data on the activity time of the focal species (Rowcliffe et al. 2014). Activity time is an important (but often neglected and under researched) aspect of an animal's behaviour and ecology. Activity time, that is, the quantification of how a species distribute their time is an important aspect of animal behaviour (Frey et al., 2017). The manner in which a species use time as a resource provides valuable information on their ecological niche and behavioural ecology (Schoener, 1974). Obtaining reliable activity time patterns is a valuable feature in improving species knowledge, moreover, such information is also valuable from a research and management perspective (Gómez et al., 2005).

The aim of this study was to estimate the population density of African clawless otter population in two neighbouring study areas, one being anthropogenically disturbed and the other being a natural area. Given that African clawless otters are not individually identifiable from images, the population densities were estimated by applying the REM approach. African clawless otter home ranges can be large, with river lengths of up to 9.8 km being recorded as part of individuals' core ranges (Somers & Nel, 2004). Given that the two study areas here are located within close proximity to one another, it was considered likely that individual otters' home ranges would overlap between the two study areas – such areas therefore not supporting separate populations. Nonetheless, the estimate of otter density in Zini Fish Farm was predicted to be greater than that of the natural area (uMNR), given the rich patches of food available. Scent marking and latrine site use is employed by most mustelids, including otters (Ben-David et al., 2005;

Buesching & Jordan, 2019). Otter scent marking can occur in different ways, anal gland secretions (AGS) can be added to faeces prior to deposition or AGS can be voided without faeces (Kruuk, 2006). Latrine site use and behaviour have been documented and studied across several otter species, however research into the behaviour of African clawless otters (*Aonyx capensis*) specifically related to latrine sites has not been well documented. The other primary aim for this aspect of the research was to record and describe the behaviours of African clawless otters at latrine sites. Data collection was conducted in an undisturbed natural area and in an anthropogenically disturbed area. Given there is more human presence in the anthropogenically disturbed area of the fish farm, it is hypothesized that otters' activity time will more likely be strictly nocturnal. Moreover, the density of otters in the Fish Farm is also expected to be greater given the greater abundance and availability of prey.

2.2 Materials and methods

2.2.1 Study areas

The study area for the population density analysis and activity time comprised of the natural areas of uMNR and the anthropogenically disturbed area of Zini Fish Farm. The data for the analysis of the behavioural videos were collected from the uMNR, Zini Fish Farm and Cottonlands Farm.

2.2.2 Camera traps for density analysis

To assess African clawless otter population densities camera traps were set up at uMNR (n = 21) between 31 August and 25 November 2021 for a total of 815 camera days. Camera traps at Zini Fish Farm (n=16) were set up between 8 December 2021 and 2 February 2022 for a total of 381 camera days (see Figure 2.1). A population density analysis was not conducted at Cottonlands farm due to security and time constraints issues. The map generated of the study area to visually display the camera trap array was generated in the QGIS software programme (QGIS Development Team, 2022).

Camera traps (PRIMOS ProofCam 3) in the uMNR were positioned on the stretch of the Mlalazi River habitat bordering the uMNR (in locations where riverbank slope was accessible to otters) and along the drainage lines and water channels. The camera traps in Zini Fish Farm were set up along weirs and drainage lines. Camera traps in both study areas were deployed between 180 m and 300 m apart, placed on trees or wooden stakes and set to a height ranging between 20 cm to 100 cm above the ground. Camera traps positioned at heights between 70 cm and 100 cm were angled downwards to ensure they would capture as much of the demarcated area as possible. The demarcated area being the area in front of the camera sensor where movement and change in some activity in its vicinity triggers the camera (Maffei et al., 2004).

The camera traps were programmed to record for 24 hours a day (a camera- trap day is defined as the 24 h period for which a camera trap is functional). Camera traps were not baited (Rowcliffe et al. 2008). The camera traps were programmed to record a burst of four images when triggered with a 10 s delay period between trigger events. Distance labels, at 1 m intervals, were calibrated and marked for each camera trap (each camera trap placement along the river, drainage lines, weirs and water channels represent a camera station) to determine the distances within the field of view for each camera. All camera traps were checked on a weekly to bi-weekly basis to collect and replace picture storage cards, evaluate the equipment, and check for otter sign (Green et al., 2015).

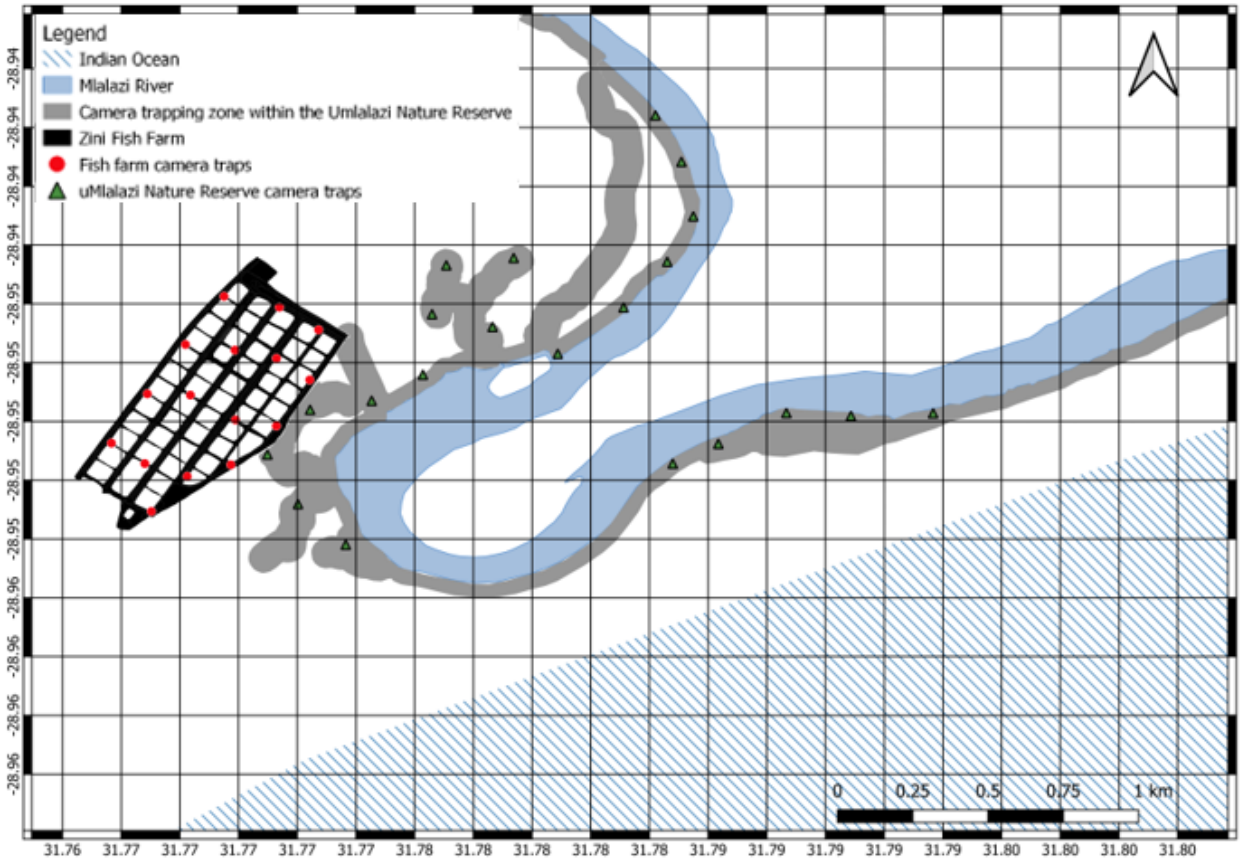

Figure 2.1 The distribution of camera trap arrays in the two study areas for African clawless otter population density analysis

2.2.3 Field work and camera trapping for behavioural analysis

Camera trapping was employed to document and record the visitation patterns and behaviour of African clawless otters at latrine sites. Accordingly, trail cameras (Bushnell Trophy Cam HD Essential) were placed at latrine sites between September 2021 and September 2022. The first study location was Zini Fish Farm (28°57'13.7", 31°45'57.2") where cameras were positioned around two latrine sites, which are referred to as Latrine site A and Latrine site B. While the second study location was at Cottonlands farm in Fairbreeze (29°1'0.83"S", 31°38'17.30"E), where the camera was positioned close to a weir along the Nyezane River (labelled as Latrine site C). The cameras were visited on a weekly basis to evaluate equipment, collect and replace video storage cards, change batteries and check for otter spoor. The activity time was determined based on all the otter data recorded from both study sites.

2.2.4 Study sites for behavioural analysis

Both latrine site A and B were located between two ponds on Zini Fish Farm. Both of these latrines were located in relatively open areas with sparse covering and short to medium height grass and reed in the surrounding area. Both of these latrines were approximately 5 m away from the ponds. There was a straight-line distance of approximately 80 m between latrine site A and B. Latrine site C was located approximately 10 m away from the Nyezane River. This latrine was positioned on and around the manmade artificial concrete substrate of the weir, which is surrounded by thick, dense shrubby vegetation. African clawless otter activity was confirmed at these sites through the presence of spoor and otter faeces. The camera traps were strategically positioned to capture the entirety of each latrine site. The camera traps were programmed to record a 60 second video when triggered followed by a 10 second delay before the next tigger event. Camera traps at all three latrine sites were placed on wooden stakes and positioned at heights between 60 cm and 100 cm above the ground. They were angled slightly downwards to ensure they would capture as much of the demarcated area as possible.

2.2.5 Activity time

All camera trap detections for the density analyses aspect of this dissertation and behavioural video data collected in the uMNR and Fish fam were utilised to determine African clawless otter activity time. A positive camera trap detection was defined as at least one image (in the burst of four images that were captured when a camera was triggered) where more than a third of the otter's body length was in frame (McIntyre et al., 2020). In order to assess African clawless otter activity time definitions provided by Gómez et al (2005) were used. Accordingly, to classify otters as being diurnal, <10% of records would have needed to be at night; mostly diurnal, 10–29% of records needed to be at night; nocturnal, ≥90% of records needed to be at night; mostly nocturnal, 70–89% of records needed to be at night; and cathemeral, 30–69% of records needed to be at night.

2.3 Data analyses

2.3.1 Density

All of the camera trap images were processed manually. When an African clawless otter was positively identified in an image, the following aspects were recorded: the study site, camera station, date, time and group size. Images of the same species at a particular site were treated as independent if separated by a period greater than 30 minutes, while the number of individuals recorded simultaneously in an image analysis were treated as separate detections (Wagnon & Serfass, 2016).

African clawless otter density (D km^{-2}) was calculated using a random encounter model (REM) that considers density as a function of trapping rate, animal speed and the dimensions of the camera detection zone (Rowcliffe et al. 2008)

$$\widehat{D} = \frac{y}{t} \times \frac{\pi}{vr(2+\theta)}$$

Where:

$y =$ the number of independent photographs of African clawless otters

$t =$ the survey effort (the total number of camera days - total amount of time the cameras were functional)

$v =$ average speed of African clawless otter movement (distance in km travelled per day)

$r =$ the detection distance of the camera trap

$\theta =$ angle of the camera detection zone (detection arc)

Animal speed of movement (v) was inferred to be 8.278 km/day from previous research on movement data (Somers & Nel, 2004b) and telemetry data (Majelantle et al., 2021) of African clawless otters. The detection distance of the camera trap was the estimated maximum distance that African clawless otters were detected from the camera traps in relation to the marked distances in the camera traps field of view. Analyses were

undertaken using R 4.0.2 software, through the R Studio interface (R Core Team, 2019). The REM density estimates and standard deviation were calculated in R using the remBoot package (Caravaggi, 2017). A bootstrapping approach (1000 iterations with replacement) was used to estimate standard deviations of density estimates.

Random encounter models assume that the populations being studied are closed. Given the relatively short survey period, the African clawless otter populations being assessed in this study area were considered to experience no migration and emigration. According to Estes (1991) it is unclear whether the breeding cycle is seasonal or perennial. There is believed to be a peak during the summer months. However, it must be noted that the breeding behaviour and sexual behaviour of African clawless otters is largely undescribed and requires further verification (Verwoed, 1987).

2.3.2 Behaviour assessment

All camera trap video footage was viewed and those with African clawless otters present were identified. The recorded behaviours were classified along with the duration of the behaviour. The videos captured were used to construct a detailed ethogram describing the observed behaviour. Focal sampling was employed to record the behaviour of each individual otters (Altmann, 1974). Focal sampling was selected as this method provides more detailed behavioural sampling compared to other sampling methods, in addition, little information is lost when an individual animal is observed though this method (Bosholn & Anciães, 2018). Camera trap video footage was re-evaluated ad libitum to improve the description and detail of events occurring around the behaviour of interest (Green et al., 2015). The total number of observations and the duration of each particular behaviour were calculated to determine the most common behaviour performed at latrines. The percentage of a behaviour was calculated as the total durations of a specific type of behaviour divided by the total duration of all behavioural events (Green et al., 2015). The otters lacked unique marking that would allow for individual recognition, each otter observed was considered a unique individual (Green et al., 2015). Thus, the

behavioural analysis provided here could be based on a minimum of two (n=2) African clawless otters.

2.3.2 Activity time assessment

To determine African clawless otter activity time all camera trap footage and video data collected in the uMNR and Fish Farm that record otters were included. Otter daily activity patterns were determined though a non-parametric circular kernel-density function (Ridout & Linkie, 2009; Rowcliffe et al., 2014). All of the statistical analyses were conducted using R, through the R Studio interface (R Core Team 2016).

2.4 Results

2.4.1 Density

Camera trap arrays, consisting of between 21 and 16 cameras, were placed in both sites, recording otter presence for a total of 19 560 camera hours in the uMNR and 9 144 camera hours in Zini Fish Farm. There were 10 and 14 African clawless otter detections from uMNR and Zini Fish Farm respectively. The detection rate in uMNR was 1.23 detections per 100 trap days while in the Fish Farm it was 3.67 detections per 100 trap days. African clawless otter density in the natural area of uMNR was 1 otter / 1.8 km, while in the anthropogenically disturbed area of Zini Fish Farm it was recorded as 1 otter / 2.3 km (see Table 2.1). The total number of camera trap images recorded on the Fish Farm was 46 810 and in the uMNR was 20 868. In addition to African clawless otter detections eleven other naturally occurring mammal species were recorded by camera traps during the study period in both study areas.

Table 2.1 Random Encounter Model estimation of African clawless otter densities at each study area, SD = Standard Deviation

Study area	Density (km^{-2})	SD
uMlalazi Nature Reserve	3.26	0.001
Zini Fish Farm	5.47	0.476

2.4.2 Behaviour video analysis

In total, the camera traps recorded 16 videos of African clawless otters visiting latrine sites. Across all 16 videos recorded no group scent-marking of otter groups were recorded and all individuals visiting the latrine sites were alone. The time otters were present in the 60 s videos ranged from 6 to 37 s. A total of 8 videos were recorded at latrine site A and 4 videos each were recorded at latrine site B and C. At latrine site A, 7 of the 8 videos were recorded on the 2nd of November 2021, and these videos were recorded consecutively, and were separated by time intervals between 20 to 30 minutes. The final video at latrine site A was captured on the 8th of November 2021. At latrine site B, the first two videos were captured on consecutive days, the 13th and 14th of October 2021, the other two videos were recorded on the 24th of October and the 2nd of November 2021. While at latrine site C, two videos were recorded on the 7th of November, and the other two videos were recorded on the 4th and 11th of November (see Table 2.2).

Table 2.2 The date and timestamp of the video footage of African clawless otters recorded at latrine sites in Zini Fish Farm and Cottonlands Farm.

Latrine site	Date	Time stamp
A	13/10/2021	03:11
A	2/11/2021	20:33
A	2/11/2021	20:36
A	2/11/2021	21:47
A	2/11/2021	22:08
A	2/11/2021	22:38
A	2/11/2021	00:07
A	8/11/2021	21:26
B	13/10/2021	19:40
B	14/10/2021	09:24
B	24/10/2021	22:13
B	2/11/2021	20:31
C	4/11/2021	04:23
C	7/11/2021	03:32
C	7/11/2021	01:22
C	11/11/2021	04:31

The otters were recorded displaying various scent marking and related behaviours. During these visits the otters were recorded displaying scent marking, sniffing, standing, stomping, body rubbing, defaecation, urination and the 'jiggle dance'.

Table 2.3 Latrine site ethogram for descriptions of the behaviours of African clawless otters (*Aonyx capensis*).

Behaviour	Definition
Standing	Stationary, no walking or running movement
Walking head down	Walking with head down, pointed at ground
Walking head up	Walking with head up, parallel to or not pointed at ground
Head raise up and down	Stationary or moving with head moving intermittently up and down
Running head down	Running with head down, pointed at ground
Running head up	Running with head up, parallel to or not pointed at ground
Sniffing	Nose to ground, head movement back and forth, either while the animal is stationary or walking
Jiggle dance	Anterior stomped and posterior legs would move from side to side
Urination	Urine is voided
Defaecate/sprainting	Elimination of faecal matter and/or anal gland secretions

The first two videos recorded of otters at latrine site A (13th of October and 2nd of November), recorded both otters running with their heads elevated. The first video recorded at latrine site A (13th of October) captured a short snippet of an otter running

through the latrine site with its head and tail elevated. In addition to this the first known recording of otters overmarking at a latrine site were captured at latrine site A. All of the otter videos captured at latrine sites A and B in the Fish Farm, or at latrine site C located at the farm in Fairbreeze recorded otters travelling and scent marking alone. Use of the ethogram to describe behaviours (Table 1.2) revealed that the most common behaviours recorded at latrine sites were sniffing (29%) and the jiggle dance (42%) facilitating the theory that latrine sites are primarily used for olfactory communication. Sprainting and defecating accounted for 7% of the total time African clawless otters were present at latrine sites. While the gaits where otters would elevate their heads at the run (6%) or walk (8%) accounted for a higher percentage than the gaits where their head would be positioned down at the walk (3%) or run (1%).

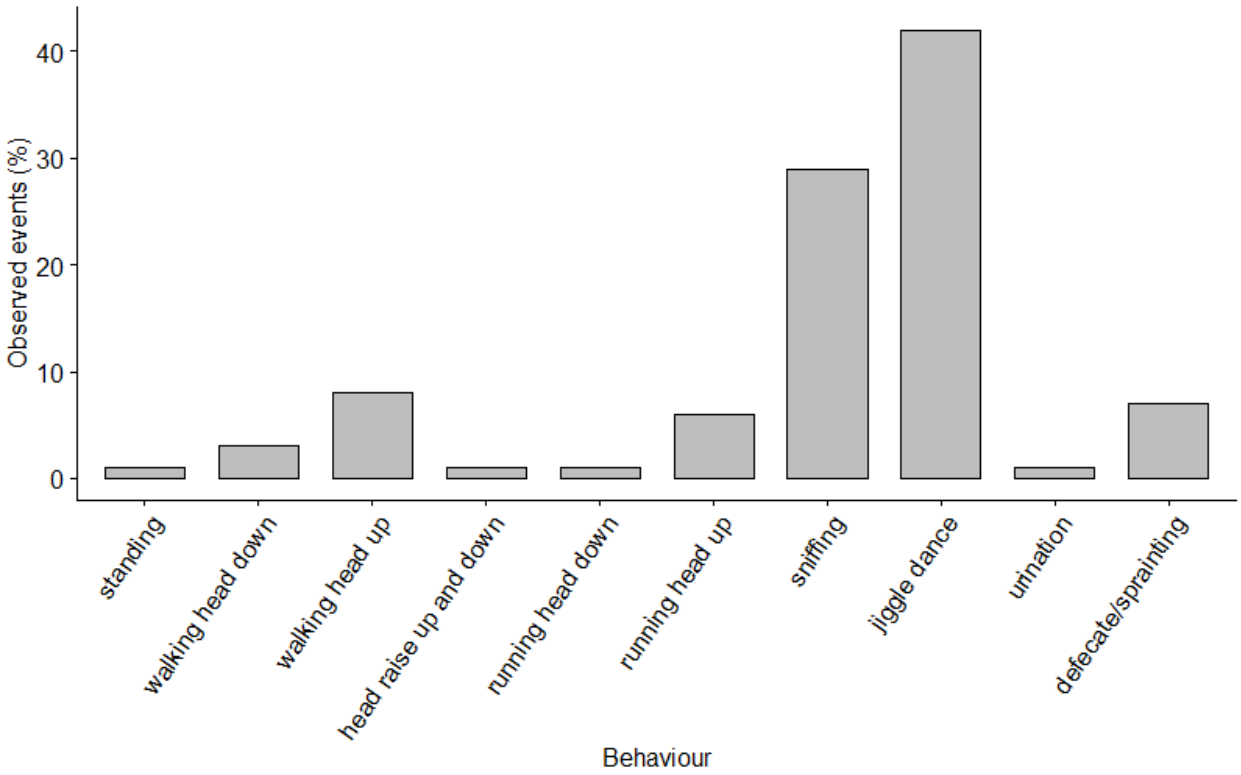

Figure 2.2 Percentages of the observed duration of the various behaviours of African clawless otter recorded at three latrine sites within Zini Fish Farm and along the Nyezane River in Northern KwaZulu-Natal from September 2021 to September 2022. Instances when the otters were out of view were excluded.

The first two videos recorded of otters at latrine site A (13th of October and 2nd of November), recorded both otters running with their heads elevated. The first video recorded at latrine site A (13th of October) captured a short snippet of an otter running through the latrine site with its head and tail elevated. The last video captured at latrine site A (8th of November) recorded an otter intensely sniffing the area for a period of 18s. All of the other videos captured at latrine site A were captured on the same day (2nd of November) from the hours of 20h00 to 00h00. In each of these videos the otters were recorded performing the 'jiggle dance' and intensely scent-marking through anal gland secretions on the low-level ground-cover vegetation of the latrine site. When performing the 'jiggle dance' the otters would pivot their forelimbs from side to side, while their hindlegs would be thrust from one side of their body to the other. The otter would scent-mark in this manner while rotating their body 180° to complete a semicircle. In some cases, the otter would complete the 'jiggle dance' pivoting their forelimbs while stomping and moving their hindlegs from side to side. It is unclear whether or not these were separate individuals or the same individual scent-marking the latrine site over the course of the night on the 2nd of November. Whether or not this was the same individual or separate individuals, this is the first known recording of otters overmarking at a latrine site.

At latrine site B only one of the four recordings at this site was of an otter's defecating. This recording was also the only recording of an otter during the daylight hours (09h24 on the 14th of October). The otter was first recorded urinating for 4 s while the defaecation took approximately 8 s, during this process the otter rotated completed a 360° rotation dispersed the urine and faeces on the ground cover vegetation. This otter was wet in the video, indicting it had likely just come out of the pond after a hunting expedition. All the recordings at latrine site C recorded otters' scent-marking through the 'jiggle dance', these recordings were all captured in the early hours of the morning between the hours of 03h00 and 05h00 (between the 2nd to the 11th of November). In three out of the four recordings taken in this locality the otters were observed intensely sniffing (for about 6 to 13 seconds) before they commenced the 'jiggle' dance. Once the otters had completed the 'jiggle dance', they would immediately leave the latrine site from the same area they had entered

the site from. The otters in this locality were also recorded as typically sprainting on the cement blocks and concrete weir along the Nyezane River.

2.4.3 Time of activity

Ninety-three percent of the otter data collected on Zini Fish Farm was obtained at night, between the hours of 18h00 and 05h00, thus this population are classified as “nocturnal” (see Figure 2.3). In the Umlalazi Nature Reserve eighty percent of the otter data was collected between the hours of 18h00 and 04h00 (accounting for the earlier sunrise during the summer months of October and November) classifying this population as ‘mostly nocturnal’ (see Figure 2.4).

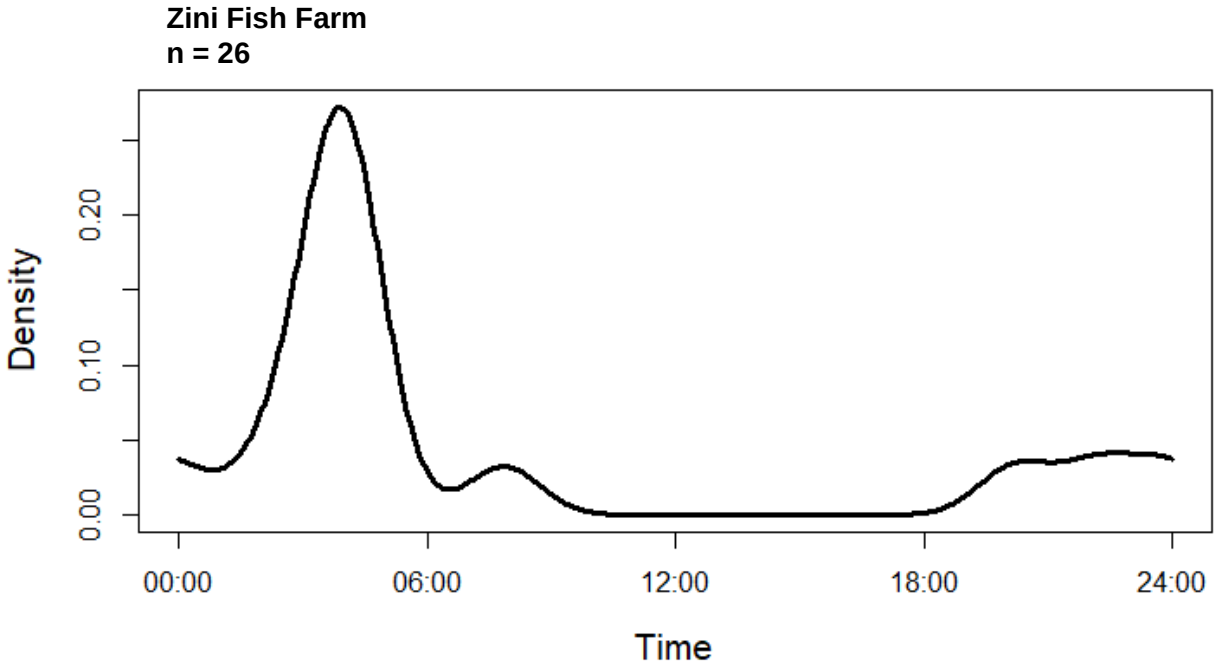

Figure 2.3 Fitted kernel density curve for African clawless otter activity time recorded by camera traps in Zini Fish Farm. n = number of records

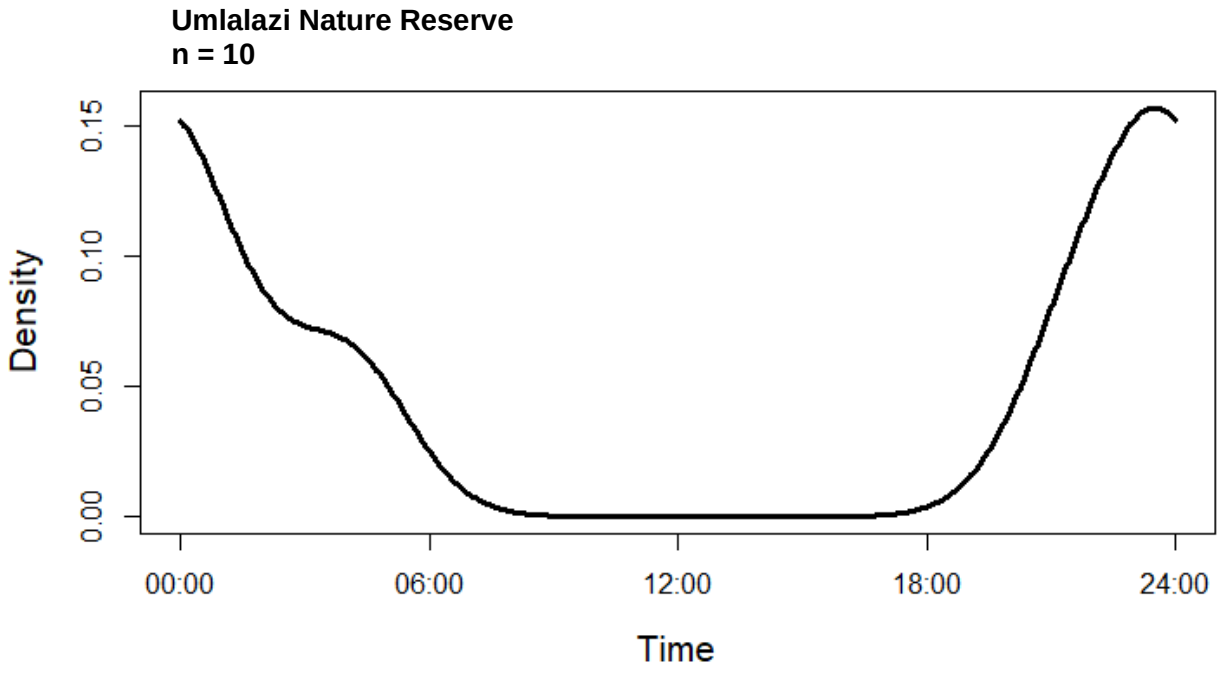

Figure 2.4 Fitted kernel density curve for African clawless otter activity time recorded by camera traps in the uMNR. n = number of records.

When combining the activity time data sets across both study sites it was determined that ninety-five percent of the otter data was obtained at night, between the hours of 18h00 and 05h00, and thus the overall population in this area are classified as “nocturnal” (see Figure 2.5).

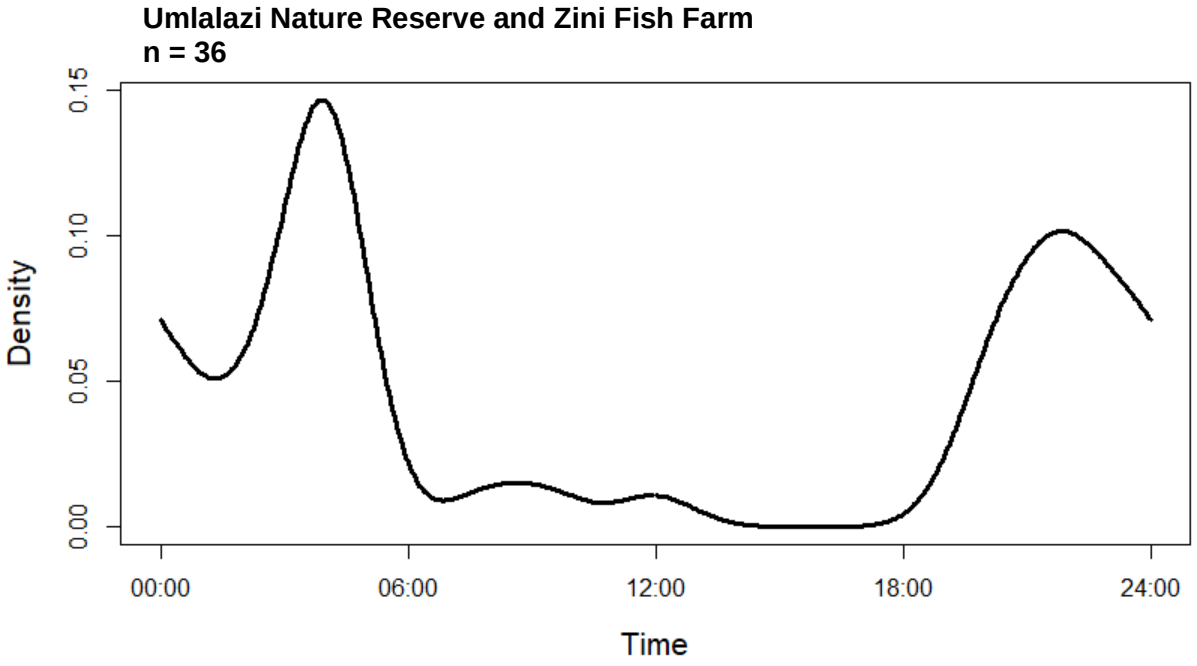

Figure 2.5 Fitted kernel density curve for African clawless otter activity time recorded by camera traps in both the uMNR and Zini Fish Farm. n = number of records

2.5 Discussion

2.5.1 Density

Density estimates of African clawless otters are reported from random encounter models applied to camera trapping surveys conducted. The results of the density analysis reveal that the otters occurred in greater densities in the Fish Farm (an augmented site with higher anthropogenic disturbance) compared to the natural area of the uMNR. The proximity of the two study areas to one another likely resulted in substantial overlap in terms of individual otters detected at either of the two study areas. It is therefore important to consider that the results reported here are unlikely to reflect independent population densities, but rather localised densities reflective of habitat use patterns by otters in the general vicinity. The overall population density of otters in the area are therefore likely to be somewhere between the reported values here of 3.26 and 5.47 otters/km^2. Previous

estimates of African clawless otter population density in marine habitats such as the southern coast of South Africa have been estimated at 1 otter / 1.9 km of coastline (Arden-Clarke, 1986) and 1 otter / 2 km of coastline (Van der Zee, 1982).

The density of otters in the transformed area of Zini Fish Farm, was expected to be higher than that of the uMNR, given the greater prey abundance. Such findings have been documented in both the Eurasian otter, smooth coated otter (*Lutra perspicillata*) and African clawless otter where higher population densities and visitation rates have been recorded around fish farms (areas with rich patches of artificial resources and high anthropogenic disturbance) (Anoop & Hussain 2004; Freitas et al. 2007; Majelantle et al. 2021). Findings by Ponsonby (2018), Jordaan et al. (2019) and Majelantle et al. (2021), have indicated that African clawless otters possess significant dietary linked behavioural plasticity, such that they can exploit anthropogenically disturbed areas where greater densities of prey are accessible.

The density estimates obtained in this study indicate that African clawless otter densities differed between the natural undisturbed area and the anthropogenically disturbed area. When converting the data to the same metric as that used by Perrin & Carugati (2006), the density of African clawless otters in the anthropogenically disturbed Fish Farm was 5.9 otters / 2.5 km, and in the natural area of uMNR it was recorded as 4.5 otters / 2.5 km. Overall, the density of African clawless otters in this study were comparatively similar to the density estimates obtained from previous research studies in South Africa (see Appendix A). In particular the more recent study by Majelantle et al (2021), obtained otter densities through random encounter modelling in two natural study areas of 3.6 otters / 2.5 km, and 2.1 otters / 2.5 km, while in an anthropogenically disturbed fish farm the otter density was comparatively higher recorded as 7.17 otters / 2.5 km.

Both this study and the study by Majelantle et al. (2021) assessed and compared African clawless otter densities in natural and anthropogenically disturbed areas. The disturbed

sites in both these studies included artificially stocked waterbodies with rich food patches for otters to exploit. The study by Majelantle et al. (2021) identified that African clawless otters exhibit behavioural plasticity, where they occurred in greater densities, formed larger groups, and concentrated their activity times to exploit a resource-rich anthropogenically augmented environment. The density of otters in the fish farm were expected to be greater than that of the natural area, given the rich patches of food. The greater African clawless otter density on the Fish Farm can potentially be attributed to the availability of resource rich food sources in the Fish Farm. The anthropogenic augmentation associated with the Fish Farm did not appear to influence otter density or visitation rates. The results reported here indicate that African clawless otter occurrence and density in both disturbed areas and undisturbed natural areas are not necessarily dependent on human activity alone but is likely influenced by a combination of factors like prey and resource availability.

North American river otter occupancy was found to not be determined by land use type or area development but rather by the availability of vegetative cover and freshwater sources (Hanrahan et al., 2019). Similarly African clawless otter presence in the Cape Peninsula region of the Western Cape was not found to be influenced by proximity to urban areas at the course (landscape) scale but rather that the otters displayed a preference for section of the river that were non-canalised and had low pollution levels (Okes & O'Riain, 2017). African clawless otter presence in urban areas of the Gauteng Province were associated with both urban and peri urban areas, however spraint site and burrow density estimate in these areas were lower (compared to what previous studies identified), suggesting that otters do not establishing core home ranges within heavily urbanised regions (Ponsonby & Schwaibold, 2019). Moreover, African clawless otter presence in urbanised areas were typically associated with tall grass and tree cover while the presence of buildings located near rivers reduced otter presence (Ponsonby, 2018).

To improve confidence in the population density of African clawless otters in this region longer-term monitoring could be conducted and/or radio tracking of individuals combined

with other field (direct observations of tagged and untagged individuals) and molecular data (relatedness estimates are determined through genotyping individuals) (Quaglietta et al., 2015). The use of multiple data sources to estimate otter population densities reduces bias and the limitations associated with single techniques and sampling methods (Arrendal et al., 2007; Hájková et al. 2009; Bonesi et al., 2013).

2.5.2 Scent marking behaviour

The elusive and secretive nature of African clawless otter combined with the difficulty of directly observing this species in the field (Estes, 1991) have resulted in many of the behavioural and ecological aspects of this species remaining largely unknown. Sprainting anal gland secretions have been well documented in otters (Ben-David et al., 2005; Ruiz-Olmo & Gosálbez, 1997; Rostain et al., 2004; Leuchtenberger & Mourão, 2009; Green et al., 2015; Jo & Won, 2020), however the manner in which the African clawless otter scent mark is not well documented. The African clawless otter scent marking behaviour previously identified as the ‘jiggle dance’ by Jordaan et al. (2017) was recorded at all three of the latrine sites identified in this study.

Stomping behaviour has been described in *Lontra canadensis* (Green et al., 2015; Rifenberg, 2020; Barocas et al., 2021), however this behaviour has only been associated with defaecation and urination. The stomping behaviour which forms part of the ‘jiggle dance’ has been documented and described in African clawless otters (Somers, 1997), and has recently been associated with both anal gland secretions and defaecation (Jordaan et al., 2017). The observations recorded in this study confirm the findings by Jordaan et al. (2017), where the foot stomping behaviour and jiggle dance’ are associated with anal gland secretions and defaecation. A common behaviour recorded in otters at latrine sites is body rubbing. It has been documented in the giant otter (Leuchtenberger & Mourão, 2009), the Neotropical otter (Michalski et al., 2021), the North American river otter (Green et al., 2015), the spotted-necked otter (Reed-Smith et al., 2014) and the African clawless otter (Estes, 1991; Jordaan et al., 2017).

The scent marking and overmarking behaviours reported here could potentially play a variety of roles from encoding information relating to resource availability, mate attraction, territorial marking and maintenance (Gosling & McKay, 1990; Rostain et al., 2004; Buesching & Jordan, 2019). The Eurasian otter are believed to scent mark in relation to the marking of key food resources (Remonti et al., 2011), while Kruuk (1992) speculates that Eurasian otters scent mark to signal resource use, enabling foraging efficiency to be increased. The observations reported here where the African clawless otter's visited latrine sites and scent marked individually, and where overmarking was observed possibly indicates that scent marking could function in intra-clan communication or territorial marking. Intra-clan communication also serves an important function in encoding and conveying social dominance, health and reproductive status (Arakawa et al., 2008; Hutchings & White, 2000). The scent marking behaviours reported here are likely associated with the scent marking and defence of territories. The observations reported here of otter scent marking do not preclude the alternative functions of scent marking where information is encoded relating to an individual's health, reproductive status (Arakawa et al., 2008; Buesching & Jordan, 2022), sex, age, dominance (Vaglio et al., 2016), social status and resource availability (Rostain et al., 2004).

2.5.3 Activity time

The peak activity time of African clawless otters between the hours of 00h00 and 06h00 may indicate that they have adapted to being most active when there is minimal human activity and disturbance. African clawless otters have been defined as crepuscular (Somers & Nel, 2004) and nocturnal (Njoroge et al., 2014; Majelantle et al. 2021). There was no significant difference in the otter activity time between the uMNR and Zini Fish Farm. Majelantle et al. (2021), found a significant difference in otter activity time between the natural area and anthropogenically disturbed area this could potentially be linked to otters avoiding direct encounters with humans or may be linked to differences in prey activity and accessibility.

The results obtained in this study indicate that the otters in uMNR and Zini Fish Farm were ‘mostly nocturnal’ and ‘nocturnal’, respectively, where the majority of the detections occurred between 00h00 and 06h00. The nocturnal activity patterns of the otters could be linked to prey accessibility and/or avoiding direct encounters with humans. Similar patterns were observed in American black bears (*Ursus americanus*), where their activity time would shift in urban areas from crepuscular to nocturnal such that they were active for shorter durations such that were still able to take advantage of anthropogenic food sources but still minimise direct contact with humans (Beckmann & Berger, 2003).

Human disturbance and infringement and external factors in an area can result in shifts in the activity patterns of a species (Melquist & Hornocker, 1983; Bluett & Hubert, 1995). North American river otters that inhabit a wetland complex in Ohio state, United States of America were found to be more active during the hours of 04h00–10h00 (68% active), and 16h00–22h00 (45% active). Diel activity in spotted-necked otters was found to be diurnal, suggesting these species hunt by sight which was inferred by their higher nocturnal activity during moonlight (Rowe-Rowe, 1977; Perrin & Carranza, 2000).

2.6 Conclusion

The findings of this chapter highlight that African clawless otters are capable of exploiting both natural and anthropogenically disturbed areas. This ability for otters to exploit such environments is potentially indicative of the opportunistic tendencies of this species. The scent-marking and overmarking behaviour displayed by African clawless otters could potentially be linked to territorial maintenance, resource marking, mate attraction or defence and information sharing (Trowbridge, 1983; Buesching & Jordan, 2022). The findings by Jordaan et al. (2017) suggest that the terrestrial movements of African clawless otters take place in groups suggesting a likely role in the marking and maintenance of clan territories. Given that none of the African clawless otters were recorded traveling or scent marking in a clan or group), it is possible that the function of the scent-marking behaviours reported here are associated with possible intra- or inter-clan communication related to resource availability (Prenda & Granadolorencio, 1996;

Rostain et al., 2004) and potentially reproductive status (Barocas et al., 2021; Buesching & Jordan, 2022;). However, given the limited time period the data was collected in, further research is required to investigate and solidify research findings linked to these aspects of African clawless otter behavioural ecology.

Furthermore, in addition to the above, more research is required to differentiate between opportunistic tendencies and the behavioural plasticity of this species. Given the ever-increasing anthropogenic development along coastlines it is imperative that research studies assess and monitor how African clawless otters adjust their population densities and activity time to exploit or avoid anthropogenically augmented landscapes (Okes et al. 2016; Majelantle et al., 2021).

CHAPTER III. LATRINE SITE SELECTION AND IMPLICATIONS FOR LIKELY SOCIAL FUNCTION OF LATRINES

3.1 Introduction

Latrine sites are the accumulation of faeces through the repeated use of a site by one or several individuals. Latrine sites are believed to play an important role in intraspecific communication (Vitale et al., 2020). Many carnivores deposit their faeces in specific dedicated latrine sites that are shared by several animals from a social group or by animals from neighbouring territories (Buesching & Jordan, 2022). There are several species of social mammals that deposit scent containing excretion as a means of intraspecific olfactory communication (Torgerson, 2014).

The spatial distribution of latrine sites can reflect on their likely adaptive significance (Vitale et al., 2020). For instance, latrine sites placed peripherally within an animal's home range are intuitively linked to have a territorial function (Vitale et al., 2020). The optimal spacing and distribution of latrines likely depends on the economic costs of maintaining one or several sites and the probability of intercepting territorial intruders (Gosling & Roberts, 2001). The establishment of latrine sites along territorial boundaries act as both a visual and olfactory fence, indicating the occupancy and competitive ability of the territory owner (Ziege et al., 2016). Core marking where latrine sites are established centrally within a home range such that an individual is able to 'monopolise' and mark key resources (Roper et al., 1993; Dröscher & Kappeler, 2014). Latrine site located in core areas of home ranges facilitate the information exchange, enhancing social bonds between members of a social group and maintaining dominance hierarchies (Mykytowycz & Gambale, 1969; Roper et al., 1993). A further factor to consider is temporal variability in scent marking and latrine site use, such changes may indicate short term and seasonal changes in breeding behaviour, environmental conditions, and possible long-term changes in a group's population size and demography (Roper et al.,1993; Rosell, 2001).

Habitat features like vegetation cover, ground elevation, water depth and average wind speed are all features that might influence site selection. The habitat characteristics of a site that are selected for a latrine can be used as potential indicators and clues to their role. For instance, habitat characteristics can influence a number of factors including scent dispersal and persistence, prey availability, protection from predators and visual prominence to conspecifics (Swimley et al., 1998; Depue & Ben-David, 2010; Crowley et al. 2012; Raha & Hussain, 2016).

Based on this information, there are two hypotheses that can be inferred. If latrine sites are important features that advertise territorial boundaries and inter clan communication, they are expected to be placed in areas with maximal exposure and prominent location within the environment such that other individuals or groups of otters are unlikely to miss them. African clawless otters show flexibility in their social organisation and they have been described as typically foraging alone but have also been documented travelling and scent marking in groups (Somers & Nel, 2004b; Kruuk, 2006; Jordaan et al., 2017). The second hypothesis is that given that otters typically travel in clans and groups the placement of latrine sites for the purpose of intra-clan communication will be random. The ultimate objectives of this section of the research study are to identify and assess the factors that influence the selection of latrine sites by African clawless otters at two ecological spatial scales (microscale and macroscale) and how these are likely to influence their behaviour at latrines. The two spatial scales that were employed to assess latrine site selection will be (1) microscale (fine-scale) and (2) macroscale (coarse scale) analysis.

3.2 Materials and methods

3.2.1 Field data collection

Analyses of latrine site selection took place at two spatial scales, a micro (fine scale analysis) and macroscale (course scale analysis), such that the environmental features of African clawless otter latrine sites (‘used’) were contrasted with characteristics at

control sites (‘available sites’) within both study areas. Based on the studies by Crowley et al. (2012) and Zaman et al. (2020), the microscale analysis assessed habitat features in a 1 x 1 m (1 m^2) grid, while the macroscale analysis assessed the habitat features in a 5 x 5 m (25 m^2) grid around the centre of each site. To assess the habitat characteristics, present at otter latrine sites, several habitat variables were measured at all latrine and control sites. For the micro-scale analysis ten candidate predictors were considered, as well as the interactions between these variables. While at the macroscale analysis three candidate predictors were considered.

The study area zone to be surveyed was determined by the maximum distance from a water source that an otter latrine was recorded within the study area. Based on this the study area was set to a 52 m buffer around all water bodies in the uMNR (the uMlalazi River, prominent water sources and drainage lines). While in the Fish Farm the study area, was limited to the fenced property surrounding the ponds. The distance from the Fish farm property fence line to the waters’ edge of the ponds ranged between 23 and 52 m in length.

Several intensive searches of the entire study area were conducted in uMNR and Zini Fish Farm, to ensure that the majority of latrine sites in the study areas were identified. Surveying for latrine sites in both study areas involved four people walking along the river in the uMNR and pond lines in the Fish Farm. The four people spaced themselves equidistantly over a distance of approximately 50 m to form a perpendicular transect line from the water’s edge and searched intensively along all accessible water edges. Surveys were conducted over a period of one month (1 – 30 August 2021) and the location of latrine sites in both study areas were mapped out in relation to the Umlalazi River and drainage lines within the reserve and in relation to the ponds on the Fish Farm.

3.2.2 Latrine site identification and selection

Latrine sites were identified through a series of surveys in the uMNR and Zini Fish Farm. Latrine sites were identified and included in the habitat selection analysis if it contained ≥1 scat (Barrett, 2014). African clawless otter faeces were identified based on its distinguished shape, size and characteristic sweet, pungent fishy like odour, as well as by the presence of crab carapace in the spraint (Rowe-Rowe, 1992; Stuart & Stuart, 2000). Latrine and control site measuring began in late August and ended in November. Global Positioning System (GPS) coordinates of the latrine sites located within the study area were recorded using a handheld Garmin GPS allowing it to a achieve an accuracy of approximately ≤5 m (Torgerson, 2014).

3.2.3 Control site selection

Control sites were selected through a systematic approach, independent of where latrine sites were located. This was done by dividing the study area into segments of homogeneous vegetation types and then allocating random sites within each segment where the habitat features for each control (non-latrine site) were recorded. The sampling area was stratified into homogeneous vegetation units based on the vegetation work done by Zungu et al (2018). The number of control sites allocated to each homogeneous vegetation type was calculated *pro rata* based on the percentage surface area covered by each unit within the study area. Similarly, the number of control sites to be sampled in the fish farm was determined in relation to the overall surface area (ha) of each vegetation type (see Table 3.1). A total of 100 control sites were randomly selected within each of the stratified vegetation units in the uMNR and 20 control sites in the Zini Fish Farm. In case of overlap with a latrine site, then the control site would be re-evaluated and another site picked nearby (the minimum distance that control sites were from any latrine sites was 15 m). Each of the control sampling plots at both the micro and macroscale were critically evaluated according to the Zurich-Montpellier sampling method such that the placement of the sampling plots fell within a representative homogeneous patch of the respective plant community (Werger, 1974).

Table 3.1 The surface area size and percentages of the vegetation types of the uMNR and Fish Farm and their respective number of control sites.

Vegetation types	Surface area (ha)	Surface area (%)	Number of control sites
uMlalazi Nature Reserve			
Grass and reed	8.49	9.93	10
Riverine woodland	15.17	17.75	18
Mudflat	4.71	5.51	6
Juncus beds	6.79	7.94	8
Mangrove forest	24.19	28.30	28
Dune forest	26.13	30.57	30
Total	**85.48**	**100**	**100**
Zini Fish Farm			
Grass and reed	12.84	63.06	12
Riverine woodland	2.52	12.38	3
Mudflat	1.96	9.63	2
Road	3.04	14.93	3
Total	**20.36**	**100**	**20**

The micro scale quantified the fine scale resource selection features of latrine sites. The landscape scale assessed habitat and broad ecosystem feature selection of latrines, while random sites were selected to model a course scale selection of latrine sites (Crowley et al., 2012). Plot sampling was employed to assess both microsite and landscape scale features. Plots at latrine and control sites consisted of the 1 x 1 m (1 m2) grid at the microscale, while plots at the macroscale analysis a 5 x 5 m (25 m2) grid around the centre of each site.

3.2.4 Microsite (fine-scale) selection

Sampling grids of 1 x 1 m, consisting of 10 x 10 cm cells were used to characterise the cover at each microsite. Features that were assessed at the microsite scale included total vegetation cover, dominant vegetative species, canopy cover, horizontal cover, bare ground (substrate) cover and the type of soil (clay, silt clay, sandy, gravel), slope, height/elevation above water, distance from water source, supratidal zone distance, and average wind speeds recorded on windy and calm days (see Table 3.2). Supratidal zone distance and distance from water were considered important features to measure given

the semiaquatic lifestyle and that the majority of their foraging occurs in water (Verwoerd, 1987; Estes, 1991; Somers, 2000; Somers & Nel, 2003). Dominant plant species were considered to be prominent/dominant in a specific plant community based on their high cover values or abundance relative to that of other species in the community (Avolio et al., 2019). The descriptions of these various habitat variables that were assessed at latrine and control sites are defined in further detail below:

3.2.4.1 Vegetation characteristics

Vegetation cover was divided into herbaceous (low growing plants, sedges, forbs, grasses and reeds) and woody (shrubs and trees) layers, where the species and its average height within the grid were recorded. The herbaceous layer cover includes both living herbaceous plants as well as decaying leaf-litter. This atypical approach of including both living and dead decaying vegetation was selected as otters are not likely to make distinction between living and dead vegetative material cover (Gallant et al., 2009, Crowley et al., 2012). This approach was also followed to enable standardisation in terms of structural cover between forest floors, covered by decaying plant material and other vegetation units, covered by living herbaceous plants. The vegetation features and bare substrate (bare patches) of each 1 x 1 m quadrat (1 m^2 sampling plots) and its corresponding 5 x 5 m quadrat (25 m^2 sampling plots) were recorded through visual estimation. Foliar (aerial) cover of woody species (which measures the vertical projection of exposed leaf area), within each quadrat was recorded at a height of 1 m. The percentage foliar cover was measured by sequentially adding the percentage cover value for each plant species, until a total was reached for each quadrat. Grass, forb, sedge, reed, shrub, and tree species were all identified to species level (where possible using practical field identification techniques) and recorded for each site. The bare patches (substrate type) were broadly divided into the following categories, namely, silt clay, sandy clay, sandy, gravel and paved surfaces (man-made). The following definitions and criteria were considered when visually determining the bare patch type, silt clay is defined as a mixture of clay and silt particles (with clay content more than 50%) generally brownish grey and rich in organic matter (Ye, 2017). Sandy clay has an organic-rich

subsurface horizon and contains a greater sand than clay content (> 35% clay and >45% sand) (Retallack, 2005). Sandy ground substrate is composed of finely divided mineral particles and gravel and paved surfaces are characterised as being composed of larger gravel stones and cement paved surfaces (Roy, 2013).

3.2.4.2 Horizontal and canopy cover

Horizontal cover is the visual vegetative obstruction of a site across a horizontal space, while canopy cover describes the vegetation obstruction of a site across a vertical space (Rutten et al., 2015). It is possible that horizontal and canopy cover could play a role in providing otters with cover and security from predators. Crocodiles, pythons and aerial raptor predators occur in both the study areas. This vegetative cover could also potentially aid otters in avoiding exposure of their latrine sites to other species that may also utilise them for marking, like the water mongoose (*Atilax paludinosus*). Additionally, the habitat variables of canopy cover and horizontal cover could guard latrine site (scent-marking areas) from environmental changes. Vegetative cover could potentially play a role in retaining and protecting scents from the elements prolonging its use for olfactory communication (Crowley et al., 2012).

In order to assess obstruction to olfactory cues around a latrine, the horizontal closure and canopy cover of the area were assessed based on the horizontal and canopy cover around a latrine site, following the procedures described by Joubert et al. (2014) and Toledo et al., (2008). This method has been successfully implemented in predator behavioural analysis studies (Potash et al., 2019). Given that olfactory communication and scent dispersal are difficult variables to measure, horizontal vegetation cover was implemented as an approximate proxy for olfactory obstruction. In this way horizontal vegetation obstruction is believed to play a role in limiting or restricting the dispersal of scent around latrine sites. Horizontal closure (visual vegetative obstruction) was measured using a 2 m pvc Robel pole (with alternating 10 cm bands of red and white, each band subdivided into four 2.5 cm regions) placed in the centre quadrant of the latrine (Joubert et al., 2014).

At each latrine site four observations were made of the pole from the four cardinal points, a 4-meter-long string was attached to the pole at a 1-meter height to provide the standard distance from the pole. Each recording noted the lowest visible segment of the Robel pole, that was completely obscured by vegetation. The total visual obstruction measurements obtained at each observation point were recorded and divided by the total number of readings for that particular site. This yielded the average horizontal obstruction (Potash et al., 2019). The canopy cover of a site was determined through visual estimation. Canopy cover classes, as described by Goloran et al., (2020) were used during visual estimations to determine the respective cover percentage of each site: open (10–39% of the sky is obstructed by tree canopies), moderately closed (40–69% of the sky is obstructed by tree canopies) and closed (70–100% of the sky is obstructed by tree canopy cover).

3.2.4.3 Elevation above water

Elevation above water was calculated by combining the clinometer estimate (i.e., the slope) with the diagonal distance (measured with a measuring tape) to the water edge.

3.2.4.4 Wind speed

To infer whether otter latrine sites are located in strategic locations to facilitate the wind dispersal of odour from latrine sites, the wind speed readings (m/s) were recorded at each latrine and control site in both study areas. Wind speed readings were measured at each site with a handheld anemometer (Benetech Wind Meter Anemometer). Sampling was conducted under a range of conditions to quantify variability. The relative exposure of wind at sites were defined as still and windy days were defined according to the Beaufort Wind Scale. Still days were defined as being between calm (0 m/s) and light air (0.5–1.5 m/s), while windy days were defined as having a light breeze (2–3 m/s), gentle breeze (3.5–5 m/s), moderate breeze (5.5–8 m/s) or fresh breeze (8.5–10.5 m/s).

A total of eight wind speed readings were conducted at each site over the course of four weeks from 13 November to the 16 of December 2021. Four readings were obtained at each of the sites on still days and four readings on windy days. Readings were taken in early mornings and in the late afternoons. Each individual reading recorded per site consisted of the average of three readings taken within a 5-minute window. Wind readings were recorded directly above each latrine site, approximately 5 cm above the ground surface. The categorical variables and species identified for each vegetation unit were used in the formulation of the vegetation descriptions for each of the seven vegetation units identified in both study areas. All of the continuous variables investigated at the microscale were used to model microscale selection of latrine sites (see Table 3.2).

Table 3.2 Variables used in the development of binomial count models for the selection of latrine sites by African clawless otters, based on microscale habitat characteristics.

Parameter code	Description	Variable type
Vegetation Cover	% Total vegetation cover (herbaceous and woody layer)	Continuous
Herbaceous cover	% Herbaceous cover	Continuous
Woody cover	% Woody cover	Continuous
Canopy cover	% Canopy cover	Continuous
Horizontal Cover	Horizontal cover (cm)	Continuous
Slope	% Bank slope	Continuous
Elevation above water	Elevation above water (cm)	Continuous
Distance from water	Distance from water (m)	Continuous
Supratidal zone distance	Distance from supratidal zone (m)	Continuous
Windy days average wind speed	Average wind speed recorded on windy days (m/s)	Continuous
Still days average wind speed	Average wind speed recorded on calm days (m/s)	Continuous

3.2.5 Macroscale (landscape) scale

The macroscale analysis assessed multiple grids, in a 5 x 5 m grid (25 m^2 sampling plots) at all latrine and control sites. The macroscale assessed each site by describing the ecosystem type, according to the herbaceous and woody layer cover percentages. The dominant tree and shrub species, dominant herbaceous species, their respective species

cover percentages and respective average heights were recorded for each site (see Table 3.3). In addition, sites at the landscape scale were also assigned to one of the following vegetation classes, adopted from the vegetation classification classes proposed by Edwards (1983). The Edwards (1983), framework for the description of vegetation structure was employed in this study as it is a priori system for the broad scale structural classification of vegetation across South Africa:

(0) mudflat, sparse herbaceous and woody cover

(1) low (< 200 mm tall) grassland/herbland, with sparse woody cover

(2) tall (> 200 mm tall) grassland/herbland, with sparse woody cover

(3) open savanna, with a clear herbaceous layer and a clear tree layer (stems of different trees more than 2 m apart, and/or less than 50% of the quadrat covered by woody vegetation

(4) dense to closed woodlands, thickets and forests (stems of different trees less than 2 m apart and/or more than 50% of quadrat covered by woody vegetation)

The categorical variables described above were employed to aid in the description and comparison of each of the vegetation units identified in the uMNR and Fish Farm, and the continuous variables of vegetation, canopy and substrate cover were used to model macroscale latrine site selection (see Table 3.3).

Table 3.3 Variables used in the development binomial count models for the selection of latrine sites by African clawless otters, based on macroscale habitat characteristics.

Parameter	Description	Variable type
Vegetation cover	% Total vegetation cover (herbaceous and woody layer)	Continuous
Canopy Cover	% Canopy cover	Continuous
Substrate Cover	% Substrate cover	Continuous

3.2.6 Vegetation types

The uMNR and its surrounding areas comprise of the following major vegetation types Northern Coastal Forest, Swamp Forest, Mangrove Forest, Subtropical Estuarine Salt

Marshes, Subtropical Dune Thicket, Subtropical Seashore Vegetation and Subtropical Freshwater Wetlands (Mucina & Rutherford 2006). The study areas within the uMNR and Zini Fish Farm were stratified into several homogenous vegetation units based on both texture and colour classes of aerial imagery (Google Earth Pro 7.3., 2021).

3.2.6.1 Mapping

For vegetation unit descriptions and vegetation mapping a phytosociological delineation of both the uMNR and Zini Fish Farm study areas was conducted. The mapping of plant communities and their associated vegetation maps are considered to be reliable surrogates for the demarcation of macro-ecosystems (Brown et al., 2013). Vegetation types were used as proxies for, and as indicators of the underlying ecosystems in which otters made latrine site choices (see Figure 3.1). The scale at which the vegetation types (ecosystems) within both study areas were assessed and described was finer than the scale chosen by Zungu et al. (2018). This resulted in a refinement of the vegetation mapping within the predetermined 52 m belt utilised by otters along all rivers, drainage lines, wetlands and dams within the study areas (see Figure 3.1 and Table 3.1). The map generated of the study area to visually display the vegetation units and location of latrine sites in the uMNR and Zini Fish Farm was generated in the QGIS software programme (QGIS Development Team, 2022). Vegetation mapping of both study areas was conducted such that each area was divided into its different plant communities at the association level and sub-association level. Mapping was done at a scale of 1:2500 (as seen from an altitude of approximately 500 m above the surface) (Google Earth Pro 7.3., 2021). The vegetation classifications and descriptions compiled by Zungu et al. (2018) were used as a guide for further refinement in this study.

The following vegetation types are identified and described within the uMNR:

Vegetation unit 1: Reed beds and hygrophilous grasslands

Vegetation unit 2: Riverine woodlands and floodplain bush clumps

Vegetation unit 3: Mudflats

Vegetation unit 4: Mangrove Forest

Vegetation unit 5: Juncus beds

Vegetation unit 6: Dune Forest

In the Fish Farm, the following vegetation types are identified and described:

Vegetation unit 1: Reed beds and hygrophilous grasslands

Vegetation unit 2: Riverine woodland

Vegetation unit 3: Mudflats

Vegetation unit 7: Roads and paved surfaces

3.2.7 Statistical analysis

Binomial generalised linear models (GLM) were implemented in the R programming environment (R Core Team, 2019) to explore the influences of predictor variables on latrine site selection (by comparing latrine sites to control sites). Ten variables were used to develop models for micro-scale selection (Johnson et al., 2006), of latrine sites, and three variables were used in the development of models for macroscale selection. For comparison the mean and standard deviations of each of the predictor variables at both the micro and macroscale are reported for each of the vegetation units. Covariation between predictor variables were assessed using pairs plots and co-variates removed prior to analyses. The predictor variables that were found to covary and subsequently removed were herbaceous and woody layer vegetation cover at the microscale. Majority of the predictor variables were included in the initial binomial GLM models. Three predictor variables were used to develop models for macroscale selection.

All possible combinations of fixed variables were then compared to select the most parsimonious models using the ‘dredge’ function in the MuMIn package (Barton, 2020). Akaike’s information criterion for small sample sizes (AICc) and Akaike weights (wi) were used to identify the most parsimonious explanatory models of latrine selection by African clawless otters (Burnham & Anderson, 2004). In addition, model outputs also assessed

interactions between predictor variables to investigate whether there are any significant links between habitat variables. Model selection to identify the most parsimonious models was determined based on maximum likelihood, second-order AIC (AICc) scores, corresponding AIC weights and delta AIC values (ΔAIC<2) (Burnham & Anderson, 2002). Z-statistics were used to assess the importance of individual predictors contained in the most parsimonious models. Statistical significance was set at $p \leq 0.05$.

The model output results for the micro and macroscale were depicted through predicted probability plots, where latrine site selection in relation to significant predictors was displayed. Moreover, interaction plots were created to visually represent the relationship between statistically significant predictor variables. All plots were generated in the R programming environment (R Core Team, 2019).

3.3 Results

A total of 38 latrine sites were located across both sites, 25 latrines in the uMNR and 13 latrines in the Fish Farm. The latrines were located between July and September 2021, and the latrine and control sites (n = 100 in uMNR and n = 20 on the Fish Farm) were assessed from August to November.

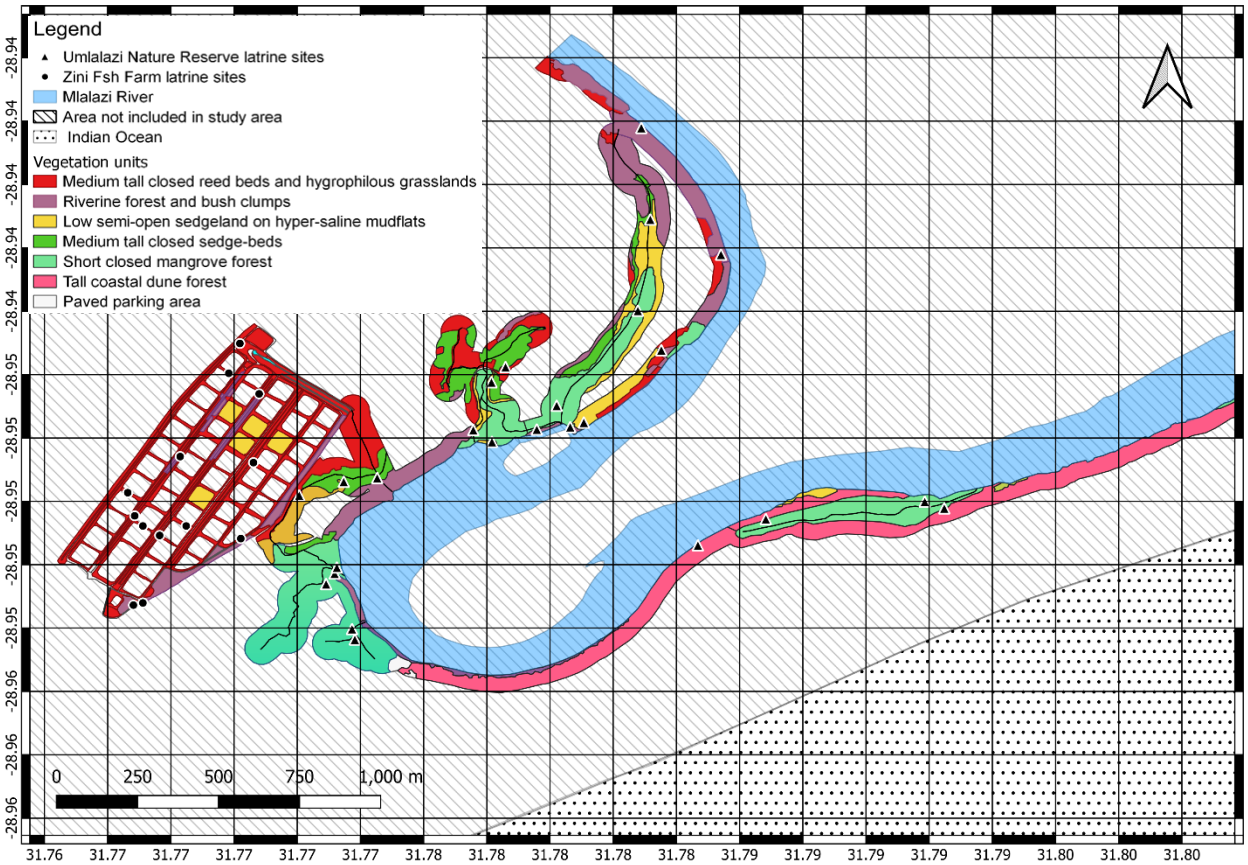

Figure 3.1 Vegetation units and latrine site location in the two study areas of the uMlalazi Nature Reserve and Zini Fish Farm

3.3.1 Vegetation unit descriptions

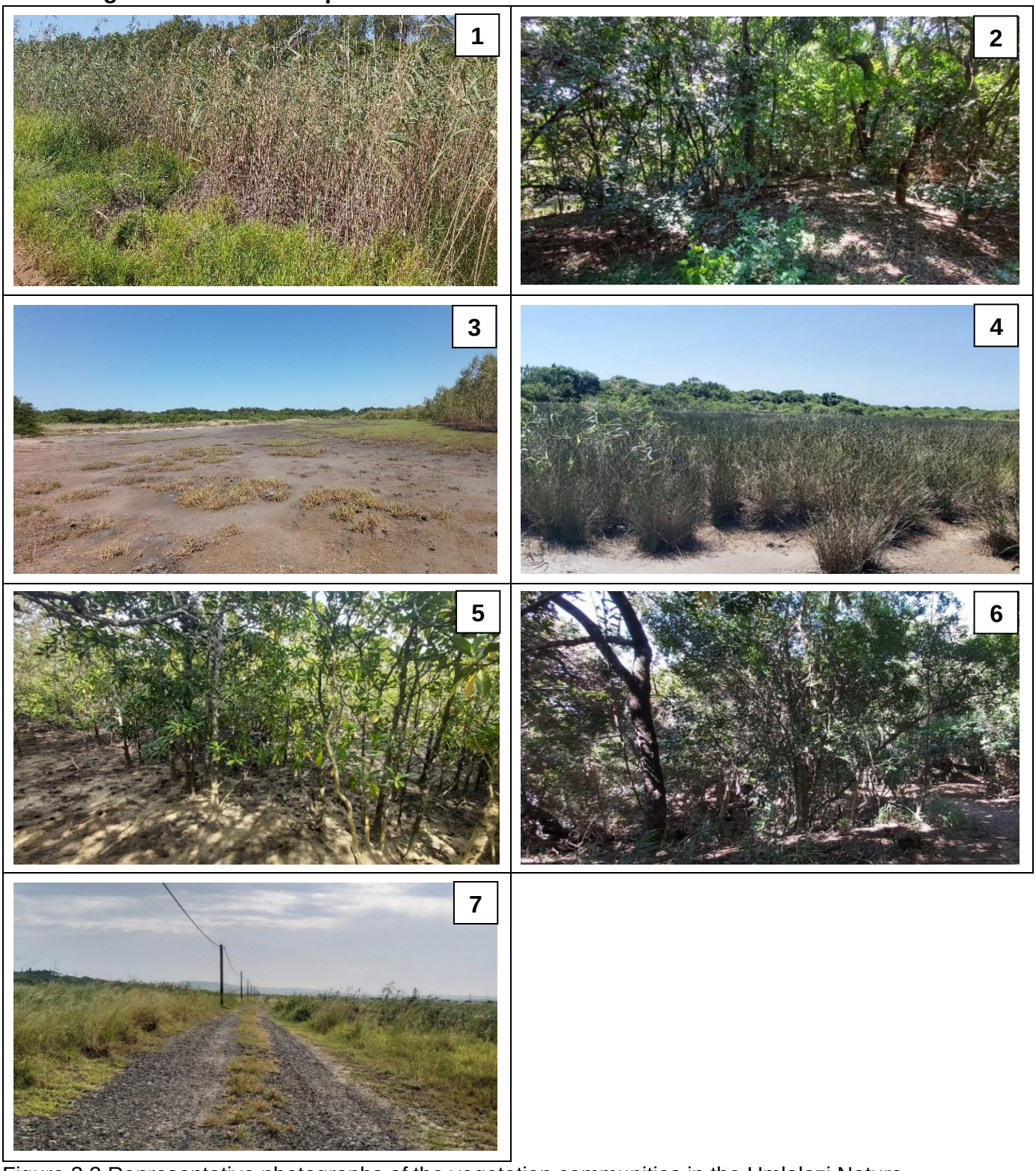

Figure 3.2 Representative photographs of the vegetation communities in the Umlalazi Nature Reserve and Zini Fish Farm at the macroscale: (1) *Phragmites australis–Stenotaphrum secundatum* medium tall closed reed beds and hygrophilous grasslands, (2) *Vachellia robusta–Euclea natalensis* riverine forest and bush clumps, (3) *Salicornia pachystachya* low semi-open

sedgeland on hyper-saline mudflats, (4) *Phragmites australis–Juncus kraussii* medium tall closed sedge-beds, (5) *Avicennia marina–Bruguiera gymnorrhiza* short-closed mangrove forest in tidal zone, (6) *Harpephyllum caffrum–Mimusops zeyheri* tall coastal dune forest, (7) Roads and paved surfaces.

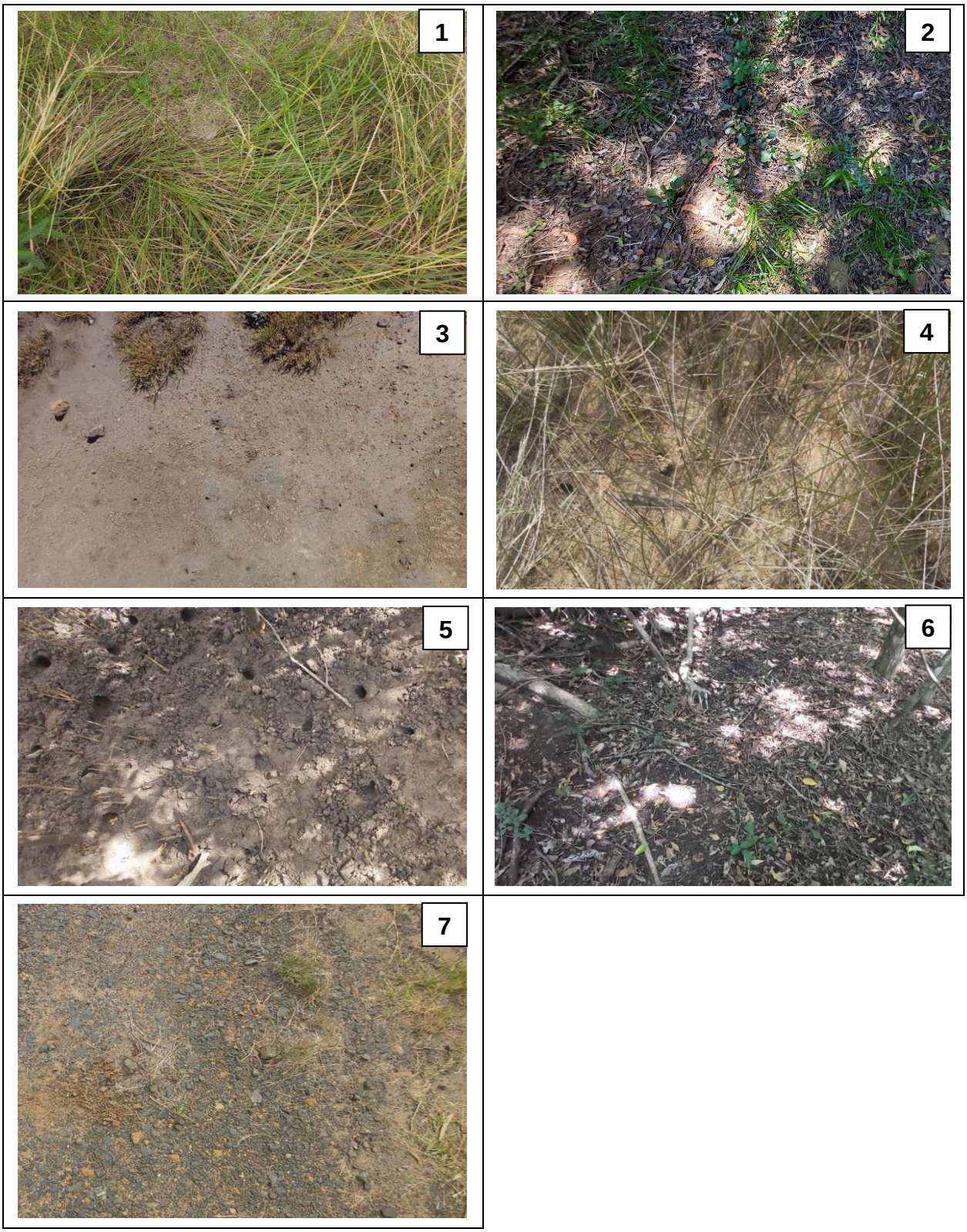

Figure 3.3 Representative photographs of the vegetation communities in the Umlalazi Nature Reserve and Zini Fish Farm at the microscale: (1) *Phragmites australis–Stenotaphrum secundatum* medium tall closed reed beds and hygrophilous grasslands, (2) *Vachellia robusta–Euclea natalensis* riverine forest and bush clumps, (3) *Salicornia pachystachya* low semi-open

sedgeland on hyper-saline mudflats, (4) *Phragmites australis–Juncus kraussii* medium tall closed sedge-beds, (5) *Avicennia marina–Bruguiera gymnorrhiza* short-closed mangrove forest in tidal zone, (6) *Harpephyllum caffrum–Mimusops zeyheri* tall coastal dune forest, (7) Roads and paved surfaces.

3.3.1.1 Vegetation unit 1: *Phragmites australis–Stenotaphrum secundatum* medium tall closed reed beds and hygrophilous grasslands

This vegetation unit forms part of the *Stenotaphrum secundatum–Phragmites australis* temporary wetlands plant community described by Zungu et al. (2018). It forms part of the southernmost outliers of the national vegetation type CB1 Maputaland Coastal Belt within the Indian Ocean Coastal Belt Biome (Bundy & Whitehead, n.d.; Mucina & Rutherford, 2006). According to Edwards' (1983) vegetation classification scheme, this vegetation unit can be described as a predominantly short-closed grassland. However, the presence of tall reeds within very wet sections of this unit, result in a more complex structure, with a patchy mosaic of vegetation height ranging from 100 mm to 3000 mm.

(i) Distribution within Study area 1 - uMlalazi Nature Reserve

This vegetation unit is associated with the floodplains beyond the uMlalazi River dyke wall. These floodplains are seasonally flooded with fresh water after high rainfall events within the river's catchment areas. The top soils are typically comprised of a large fraction of very fine alluvial silts and clays, with an increased amount of sand within the subsoils (Zungu et al. 2018). These grasslands are sporadically flooded by salt water from supratidal events, which often lead to dramatic die-off of plant species that are not adapted to saline conditions, and an increase in the dominance of the salt tolerant sedge *Juncus kraussii* (Colloty et al. 2002).

(ii) Distribution within Study area 2 - Zini Fish Farm

This is the dominant vegetation type on the Fish Farm. This vegetation unit is associated with all of the water channel drainage lines on the farm. Additionally, this vegetation type also surrounds each of the ponds on the farm. This border roughly forms an ± 8 m wide by ± 68 m long border line around each of the ponds on the farm. The artificial landscape

created within Zini Fish Farm, has created soil hydrology conditions similar to that seen along the floodplains beyond the uMlalazi River dyke wall. Similar to conditions along the floodplains, the interplay between the salt water pumped into the ponds and the fresh water diverted into artificial channels on the fish farm, seem to be a critical driver of the vegetation structure and composition of this vegetation unit.

(iii) Microscale (1 m^2 sampling plots)

The mean total herbaceous cover recorded was 94 ± 6.18% with mean herbaceous vegetation height at the time of surveys recorded as 248 ± 165 mm. A very sparse woody layer of scattered emergent shrubs occurred in this vegetation unit with cover averaging at 2.5 ± 0.7% and ranged between 0.5 and 1 m in height (see Figure 3.3). At the time of the surveys, the vegetation associated with these dynamic floodplains were dominated by the grass species *Stenotaphrum secundatum*, *Dactyloctenium australe*, *Cynodon dactylon*, *Digitaria eriantha*, *Hyparrhenia filipendula*, *Panicum maximum*, *Imperata cylindrica, Sporobolus africanus,* and the reed species *Phragmites australis*. Prominent forb species include *Pelargonium luridum, Ambrosia artemissiifolia, Rhynchosia caribaea, Hibiscus surattensis, Helichrysum ruderale*, *Wahlenbergia undulata, Desmodium incanum* and *Chromolaema odorata;* while prominent sedge species include *Kyllinga alata, Cyperus textillis* and *Juncus kraussii*. Small, prominent colonies of the following forb species were sporadically recorded *Centella asiatica*, *Sida rhombifolia*, *Conyza bonariensis*, *Aristea woodii*, *Sida rhombifolia*, and *Gomphrena celastroides*. The two fern species identified were the halophytic *Acrostichum aureum* and the hydrophilic *Microscorium scolopendrium*. The combination of such a salt-water affiliated fern and a fresh-water affiliated fern again highlights the complex interplay between salt and fresh water within the soil hydrology. Sparsely spread incidental emergent shrub and tree species include *Searsia nebulosa* and the forb *Tephrosia* sp.

The mean value of bare patches, devoid of vegetation cover, of the control sites was 6 ± 6%. The terrain associated with this vegetation unit is generally flat, with a slope of less than 1%, with some localised variability around debris mounds and berms, ranging from

one to seven degrees (mean = 4 ± 2°). Horizontal cover ranged between 110 to 500 mm (mean = 190 ± 140 mm), while canopy cover was minimal with a mean of 0.4 ± 2%. The terrain elevation above the nearest open water ranged from 210 mm to 3840 mm (2030 ± 2570 mm), while the distance from the nearest open water source ranged from 5 to 31 m (mean = 13.2 ± 10.58 m). The estimated distance from visual markings and debris indicating recent supratidal events ranged from 3 – 29 m (mean = 15 ± 10 m). Average wind reading for windy days was found to be 1.99 ± 0.45 m/s, while the average reading for calm days with no wind averaged at 0.08 ± 0.05 m/s.

(iv) Macroscale (25 m^2 sampling plots)

During relatively wet climatic cycles, this vegetation unit is dominated by the reed *Phragmites australis*, while during the drier cycles, the vegetation is dominated by the grasses *Stenotaphrum secundatum* and *Imperata cylindrica* (Zungu et al. 2018). At the macroscale the estimated total vegetation cover was estimated to be 92 ± 6.34% with average herb layer height being 313.42 ± 236.53 mm (see Figure 3.2). While the recorded woody canopy cover was 2 ± 3.61% for each 5 x 5 m sample plot, this can be attributed to the patchy and sporadic distribution of some tree species within this vegetation unit. The woody species identified at the macroscale include*, Vachellia robusta, Pavetta lanceolata, Lantana camara, Schinus terebinthifolius* and *Clerodendrum glabrum;* the semi-herbaceous woody shrubs include *Chrysanthemoides monilifera* and *Gomphocarpus physocarpus*. With mean woody species height measuring 0.65 ± 1.05 m.

3.3.1.2 Vegetation unit 2: *Vachellia robusta–Euclea natalensis* riverine forest and bush clumps

The riverine forest and floodplain bush clump vegetation unit comprises of a tall tree layer and a well-developed dense semi-herbaceous woody shrub layer. The fine textured sandy soils within this unit are exposed to seasonal flooding events. The soil does not remain waterlogged and drains freely. This vegetation unit forms part of the *Adenopodia*

spicata–Vachellia robusta riverine woodland community described by Zungu et al. (2018). According to Edwards' (1983) vegetation classification scheme, this vegetation unit can be structurally defined as tall forest.

(i) Distribution within Study area 1 - uMlalazi Nature Reserve

The *Vachellia robusta–Euclea natalensis* riverine forest and bush clumps vegetation unit occurs along topographically elevated sections of the uMlalazi River floodplain which drain relatively well. The flood plain is relatively heterogenous at both spatial and temporal levels. This can be attributed to the violent nature of flooding events that occur along this section of the uMlalazi River. Over time, successive flooding events deposit material along the more sheltered sections of the river. It is among these relatively sheltered refugia that forest and bush clumps develop and survive regular flooding events (Bundy & Whitehead, n.d.; von Maltitz et al., 2003; Mucina & Rutherford 2006). According to Zungu et al. (2018) this vegetation unit is an early successional stage of the Critically Endangered Lowveld Riverine Forest national vegetation type (Mucina & Rutherford 2006).

(ii) Distribution within Study area 2 - Zini Fish Farm

The riverine forest and bush clumps vegetation unit occurs along free-draining water rich patches on the farm, for instance, drainage lines. The most prominent stretch of riverine forest occurs along the boundary fence line with the uMNR, and forms part of a larger patch of forest within the reserve.

(iii) Microscale (1 m^2 sampling plots)

At the microscale the mean total vegetation cover recorded was 94 ± 7%. This vegetation unit was made up of both an herbaceous and woody layer at fine scale analysis. The mean herbaceous and woody layer cover values recorded were 91 ± 7% (average height 224 ± 165 mm) and 3 ± 3% (average height 1 ± 1 m) respectively (see Figure 3.3). The dominant species for this plant community include the grasses *Stenotaphrum*

secundatum, Cynodon dactylon, Oplismenus hirtillus, Imperata cylindrica, Digitaria eriantha; the forbs *Asystasia gangetica, Secamone filiformis, Rivina humulis, Oxalis latifolia, Ludwigia grandiflora*, *Euphorbia prostrata and* the woody climbing shrub *Senegalia kraussiana*. The diagnostic forb species identified include *Ipomoea cairica, Ludwigia grandiflora* and *Secamone filiformis*; along with the shrubs *Euclea natalensis, Apodytes dimidiata, Chromolaena odorata, Adenopodia spicata, Searsia nebulosa* and *Senegalia kraussiana*.

The high cover values recorded for the herbaceous layer can be attributed to the widespread coverage of leaf and plant litter. The canopy cover of this vegetation unit is rather dense (average canopy cover estimated to be 93 ± 9%). This can be attributed to the high cover values recorded for large trees with maximum heights ranging between 15 and 18 m. This dense tree layer results in minimal sunlight penetrating to the forest floor, leading to a patchily distributed shrub layer. Horizontal cover was measured to be 180 ±10 mm. Bare soil surface was minimal across control sites (5 ± 7.17%). The bare patches were predominantly made up of sandy soils with an organic rich orthic A-horizon. Terrain topography throughout this vegetation unit is predominantly flat, while some regions relatively undulating due to the deposition of sediment and debris during flooding events., with gentle slopes (mean = 5 ± 2°). The mean elevation above water for this vegetation unit is 1220 ± 71.9 mm. While the distance from open water ranged between 5 and 45 m (19 ± 12 m), the distance to the supratidal high tide line was recorded as 17 ± 12 m. Average wind speed recorded on windy days was 1.53 ± 0.04 m/s, while the average wind speed on calm days was measured to be 0.06 ± 0.05 m/s.

(iv) Macroscale (25 m^2 sampling plots)

At the macroscale this vegetation unit has a relatively dense herbaceous and woody canopy cover. The overall structure includes a tall (average tree height 7 ± 5 m), dense (average canopy cover of 91 ± 19%) tree layer, and a well-developed dense herbaceous layer (see Figure 3.2). The mean vegetation cover recorded was 65 ± 41% with a mean height of 219 ± 138 mm.

A relatively thick continuous layer of leaf litter was recorded within in these forests, with an average sub-canopy cover of 45 ± 21%. The dominant species at the herbaceous layer in this vegetation unit include the grasses *Cynodon dactylon*., *Imperata cylindrica*, *Digitaria eriantha* and *Stenotaphrum secundatum*; the forbs *Asystasia gangetica*, *Secamone filliformis*, *Rivina humilis*, *Achyranthes aspera;* the forb *Desmodium incanum;* and the shrubs *Euclea natalensis*, *Chromolaena odorata* and *Adenopodia spicata*. Bare patches made up an average of 35% of each plot. These forests are associated with fine-textured sandy soils which are the result of recent alluvial deposits subject to regular seasonal flooding (Zungu et al., 2018). Despite frequent flooding events these soils do not remain waterlogged for long periods of time due to the topography and elevation above open and ground water levels. The dominant woody species for this community include *Adenopodia spicata*, *Vachellia robusta*, *Sideroxylon inerme*, *Vachellia kosiensis*, *Bridelia micrantha, Euclea natalensis*, *Canthium inerme*, *Dovylais longispina*, *Barringtonia racemosa*, *Olea woodiana* and *Scutia myrtina*.

3.3.1.3 Vegetation unit 3: *Salicornia pachystachya* low semi-open sedgeland on hyper-saline mudflats

The *Salicornia pachystachya* mudflat community can be classified as low semi-open sedgeland on hyper-saline mudflats. This community has a fairly low vegetation cover and the vegetation structure being simple (Zungu et al., 2018). This vegetation unit is considered to form part of the major vegetation type AZe 3 Subtropical Estuarine Salt Marshes national vegetation type, as described by Mucina and Rutherford (2006). This vegetation unit has been described as being ‘semi-open’ given that colonies and clusters of the low herbaceous halophytic succulent *Salicornia pachystachya* are separated by large unvegetated gaps. This vegetation is structurally defined as a low sparse herbland according to the Edwards (1983) vegetation classification scheme. This vegetation unit dominates regions and floodplains that do not readily drain freely after seasonal flooding and heavy rainfall events and in this way act as natural evaporation pans.

(i) Distribution within Study area 1 - uMlalazi Nature Reserve

The hyper-saline mudflats are associated with very specific concave topographical features within estuarine regions of the uMlalazi River floodplains. The mudflats occur at the ecotone between the river and Mangrove forests, and at the ecotone between *Juncus kraussii* beds and the Mangrove forests.

(ii) Distribution within Study area 2 - Zini Fish Farm

The *Salicornia pachystachya* low semi-open sedgeland mudflats on the Fish Farm are associated with the remaining silt substrates left behind after five artificial ponds have been drained and retired from use as fish breeding facilities. These colonies of *Salicornia pachystachya* can be seen as the first seral stage in the primary succession of natural vegetation reclaiming these five abandoned ponds.

(iii) Microscale (1 m^2 sampling plots)

The saltwater exposure caused by tidal movements, sporadic floods and water evaporation from temporary pans leads to the hyper accumulation of salt levels in the soil, The resulting the alluvial soils typically contain large quantities of silt and clay, with little to no organic matter (Zungu et al., 2018) (see Figure 3.3). Bare soil surface was relatively high at these sites, mean cover recorded as 66 ± 35%. The high environmental stresses created by the hyper saline water-saturated soils likely contributes to the low plant species richness, with only a few specialised plant species being able to tolerate these extreme conditions within this vegetation unit.

The only dominant diagnostic species for this plant community is the salt tolerant (halophyte) succulent species *Salicornia pachystachya* (mean total herbaceous cover = 34 ± 35%, and average herbaceous layer height recorded as 29 ± 24 mm). There was no woody cover recorded in this vegetation unit. The horizontal vegetation cover was recorded as 29 ± 25 mm. The vegetation unit has a very low canopy cover, (2 ± 2%). Topographically, the mudflat vegetation unit is flat to slightly concave, with little to no

ground undulations, and no slope exceeding 3 degrees (1.5 ± 1°). Elevation above open water source for this vegetation unit averaged at 490 mm (± 360). Distance from water ranged between 5 and 35 m (mean= 20 ± 12 m). The distance to the supratidal line was measured as 0 m given that this vegetation unit is frequently exposed to tidal flooding events. Wind speeds recorded on the mudflats were relatively high compared to the other vegetation unit, averaging at 2.19 ± 0.32 m/s, while on still days it was recorded as 0.12 ± 0.05 m/s.

(iv) Macroscale (25 m^2 sampling plots)

The bare soil surface at the macroscale was found to be 56 ± 35%. The dominant species of this vegetation unit is the succulent *Salicornia pachystachya* had an average height of 564 ± 303 mm. The average vegetation cover was recorded as 44 ± 64% (see Figure 3.2). Other grass, sedge and reed species are patchily distributed on the outer regions of these units, and include *Juncus kraussii*, *Digitaria eriantha* and *Phragmites australis*. The macroscale canopy cover of this vegetation unit was extremely sparse and recorded as 1.28 ± 1.7%.

3.3.1.4 Vegetation unit 4: *Phragmites australis–Juncus kraussii* medium tall closed sedge-beds

This vegetation unit is a mosaic of reed dominated patches and sedge dominated patches. The high heterogeneity recorded is the result of fluctuating salinity levels at both spatial and temporal scales within this estuarine floodplain (Zungu et al., 2018). This plant community belongs to the vegetation type AZe Subtropical Estuarine Salt Marshes national vegetation type (Mucina & Rutherford, 2006). The *Juncus kraussii* dominated reed bed community is widespread along estuaries of the east coast of South Africa (Colloty et al., 2002).

(i) Distribution within Study area 1 - uMlalazi Nature Reserve

This vegetation unit is associated with saline evaporation pans that are regularly flooded by fresh water from the uMlalazi River, as well as salt water from oceanic tidal action. The *Juncus kraussii–Phragmites australis* medium tall-closed sedge-beds are patchily distributed in the floodplain regions of the uMNR, the majority of the vegetation unit falls in regions where influxes of saline water occur during supratidal events. The *Phragmites australis–Juncus kraussii* medium tall-closed sedge-beds vegetation unit was not recorded within Zini Fish Farm. This distinction between sites falling within or out of the supratidal zone is important given that sites that fall within the supratidal zone and that are affected by tidal movements on a regular basis are likely to influence otter latrine site selection.

(ii) Microscale (1 m^2 sampling plots)

The vegetation structure of this community is typically closed, dominated by stands of medium length sedgelands (0.9 m) and tall reed stands (3.5 m), ranging in heights from 0.9 m to 3.5 m. Total herbaceous cover recorded at micro scale is 59 ± 10.54%. The mean herbaceous layer height was recorded to be 927 ± 47 mm. There was no woody vegetation cover or canopy cover recorded for this vegetation unit. This species composition is dominated by the sedge species *Juncus kraussii* and the reed species *Phragmites australis*, which can both be regarded as diagnostic for this vegetation unit within this landscape (see Figure 3.3). There were no woody species recorded within this vegetation unit at the micro scale. The horizontal vegetation cover recorded was relatively high, 370 ± 70 mm, compared to the other vegetation units described here.

The substrate of this vegetation unit is saline containing high level of slit, clay and organic matter. Mean bare soil surface recorded at the microscale was found to be 42 (± 11%). The water drainage throughout this vegetation unit is slow and stagnant in some areas, resulting in an accumulation of organic materials (Zungu et al., 2018). The topography of this vegetation unit is mostly flat (3.5 ± 2°) with localised areas having gentle sloping (5

to 6 degrees) at the micro scale. These reed bed communities were in relatively close proximity to the water channel and drainage lines in the uMNR such that the mean distance to open water was recorded to be 15 ± 8 m, while the average distance to the nearest signs of supratidal activity was 8 ± 7 m. The elevation above the nearest open water source ranged from 33 mm to 253 mm (90 ± 77 mm). The windy day average wind readings within the *Phragmites australis–Juncus kraussii* vegetation unit were the lowest across all vegetation units. The average windy day reading measured at 0.74 ± 0.24 m/s and readings taken on still calm days averaged at 0.08 ± 0.05 m/s.

(iii) Macroscale (25 m^2 sampling plots)

At the macro scale, this vegetation unit is made up of dense, tall herbaceous patches, dominated by stands of *Juncus kraussii* and stands of *Phragmites australis* with a mean total vegetation cover of 84 ± 6% (with a mean height of 927 ± 47mm) and a bare soil cover of 14 ± 89%. Bare soil surface patches are restricted to localised areas within the reed bed community, with a mean value at the macroscale recorded to be 16 (± 5.63%) of the total surface area.

3.3.1.5 Vegetation unit 5: *Avicennia marina–Bruguiera gymnorrhiza* short-closed mangrove forest in tidal zone

Mangrove forests are unique, productive forests that form the interface between marine and terrestrial environments in tropical and temperate regions (Naidoo, 2016). Based on the structure, composition and extreme environmental conditions it is subjected to twice a day, it is dominated by specialist plant species. This vegetation unit forms part of the national vegetation type FOa 3 Mangrove Forest national vegetation type as described by Mucina & Rutherford (2006). The mangrove forest of the uMNR is akin to those described by Rajkaran and Adams (2011) in Northern KwaZulu-Natal (Kosi Bay, St Lucia and two mangrove forests in Richards Bay) and similar to what Naidoo (2016) recorded for the rest of South Africa.

(i) Distribution within Study area 1 - uMlalazi Nature Reserve

This vegetation unit is restricted to narrow intertidal zone regions along the estuarine section of the uMlalazi River. This vegetation unit is flooded with salt water through oceanic tidal movements and sporadic flooding events. The *Avicennia marina–Bruguiera gymnorrhiza* short-closed mangrove forest vegetation unit was not recorded within Zini Fish Farm.

(ii) Microscale (1 m^2 sampling plots)

The mangrove forest community has a low species richness and is dominated by two tree species, namely, *Avicennia marina* and *Bruguiera gymnorrhiza*. This community varies from medium to tall with tree heights ranging between 4 and 8 m. The total herbaceous cover recorded at the microscale was relatively low (27 ± 12%), with a ground cover of 4 ± 10%, that can be attributed to the pneumatophores (‘pencil roots’) of *Avicennia marina*. Mean herbaceous layer height was found to be 36 ± 78 mm. The woody layer is dominated by the mangrove tree species *Avicennia marina* and *Bruguiera gymnorrhiza*, with an average cover of 77 (± 6%), and a mean height of 5 ± 1 m. This vegetation unit is classified as a short forest (Edwards, 1983), with dense canopy cover 88 ± 19%. Horizontal vegetation cover in the mangrove forests was minimal (9 ± 2 mm) given the absence of shrubs and herbaceous ground cover (see Figure 3.3).

Bare soil surface recorded at the microscale level was 73 ± 12%. The silt and clay rich soils of the Mangrove forests are generally waterlogged, the soil is poorly drained, saline and anoxic (Zungu et al., 2018). The average terrain slope was measured to be 3 ± 1°, the terrain topography throughout this vegetation type was found to be variable, ranging from undulating to flat in places. The variable nature of the ground level can be attributed to the presence numerous mangrove crab (*Neosarmatium meinerti*) burrows that occur throughout the mangrove forest. The average elevation above the nearest water source was 890 ± 570 mm. The constantly changing environmental conditions this intertidal community is exposed to result in majority of the ground cover prone to flooding of saline

conditions through daily tidal movements, hence why the estimated average supratidal zone distance was recorded to be a mere 4 ± 7 m (while average distance to nearest water sources during normal tidal events was recorded as 17 ± 7 m). Wind speed on windy days averaged at 1.6 ±0.29 m/s while on calm days it was measured to be 0.08 ± 0.05 m/s.

(iii) Macroscale (25 m^2 sampling plots)

The mangrove forest community is composed of only two tree species, namely, *Avicennia marina* and *Bruguiera gymnorrhiza*. The macroscale vegetation cover was recorded as 9.29 ± 8%, while the bare soil cover was recorded as 90 ± 82% (see Figure 3.2). The macroscale canopy cover of the woody layer in these forests is extremely dense with an average cover of 92 ± 8%, with a relatively short tree height, according to Edwards (1983), of 5 ± 1 m). The mangrove forests are restricted to tidal areas fringing the uMlalazi River and areas within the floodplain where supratidal events result in localised inundation with saline water.

3.3.1.6 Vegetation unit 6: *Harpephyllum caffrum–Mimusops zeyheri* tall coastal dune forest

The dune forest vegetation unit of the uMNR is structurally complex and species rich. This vegetation unit is classified as a tall coastal dune forest. This forest is considered part of the FOz 7 Northern Coastal Forest national vegetation type as described by Mucina and Rutherford (2006).

(i) Distribution within Study area 1 - uMlalazi Nature Reserve

The *Harpephyllum caffrum–Mimusops zeyheri* tall coastal dune forest community is located along the north eastern edge of the uMNR.

(ii) Microscale (1 m^2 sampling plots)

At the microscale the average total herbaceous cover of the forest floor was estimated to be 79 ± 21%, with mean vegetation height at microscale being 135 ± 121 mm. While the mean woody cover percentage was 2 ± 3%, the mean shrub and tree height at the microscale was 1 ± 1 m. The canopy cover of the dune forest community is relatively dense (90 ± 13%, average tree height of 5 ± 3 m). This can be attributed to the multi-layered structure of the forest vegetation, ranging from very old and large trees with large canopies, to relatively short shrubs within the understory of the forest. Overall, this results in minimal sunlight reaching the forest floor, leading to a poorly developed herbaceous and shrub layer with, such that the forest floor is predominantly covered with leaf litter (see Figure 3.3). The dominant species at the herbaceous layer include the forb *Desmodium incanum*; the grasses *Oplismenus hirtellus*, *Stenotaphrum secundatum*, *Setaria megaphylla* and *Panicum deustum*. Other dominant species at the microscale include the ferns *Microsorum punctatum*, *Microsorum scolopendrium*, the forb *Bidens pilosa*; the vines *Secamone filiformis, Rhoicissus rhomboidea*, *Rhoicissus sessilifolia*, *Cissampelos torulosa*, and *Smilax anceps* and an unidentified moss species. The dominant shrubs at the woody layer include *Euclea natalensis, Peddia africana, Vepris lanceolata*, *Scolopia zeyheri*, *Dovyalis rhamnoides* and *Carissa bispinosa.*

The dominant tree species include *Ekebergia capensis*, *Harpephyllum caffrum*, *Cordia caffra*, *Mimusops caffra*, *Sideroxylon inerme*, *Trichilia emetica*, *Apodytes dimidiata*, *Empogona coriacea*, *Brachylaena discolor*, *Albizia adianthifolia*, *Dovyalis longispina* and *Olea woodiana*. The dune forest has sandy soil with high organic content in the upper layers. The average bare soil recorded along the forest floor is 16 ± 16.01%, while the remainder is covered by leaf-litter and decaying organic material. Compared to the floodplains along the uMlalazi River, the dune cordon through which the river cuts before emptying into the ocean, the dune system is relatively complex, with an ever-changing slope between dune crests and inter-dune straits. The dune forest is associated with the fast-draining sandy soils, which are well elevated above the underlying water table. Slope ranges from gentle to moderate throughout the landscape with the average slope recorded as 7 ± 5°. The mean horizontal cover was recorded as 5 ± 3 mm. The mean elevation above water at the microscale was 279 ± 219 mm, with the average distance

from the river being 22 ± 10 m (with the average distance from the supratidal zone 17 ± 9 m). The wind recordings taken on windy days in the dune forest did not measure particularly high, average reading measured at 1.1 ± 0.35 m/s, this can be attributed to the high vegetation density minimising the wind speed. While wind readings on still calm days in the dune forest were minimal averaging at a mere 0.058 ± 0.04 m/s.

(iii) Macroscale (25 m^2 sampling plots)

The dune forest community is characterised by continuous canopy cover throughout its range, with the mean canopy cover recorded as 92 ± 9% (average tree height estimated at 8 ± 4 m) (see Figure 3.2). The dominant tree species were *Empogona coriacea*, *Ekebergia capensis*, *Olea woodiana*, *Sideroxylon inerme*, *Harpephyllum caffrum*, *Apodytes dimidiata, Albizia adianthifolia*, *Cussonia zuluensis*, *Mimusops caffra* and *Brachylaena discolor*. The dominant shrub species included *Chaetacme aristata, Gymnosporia arenicola*, *Dovyalis longispina*, *Carissa bispinosa, Peddiea africana*, while the dominant vines included *Rhoicissus sessilifolia* and *Rhoicissus rhomboidea*.

Similarly, the vegetation cover at macroscale was very high with the average cover found to be 83 ± 15.58% (mean vegetation height recorded to be 186.36 mm ± 163.26). The dominant shrubs at the herbaceous layer were *Euclea natalensis*, *Chaetacme aristata*, *Brachylaena discolor*, *Vepris lanceolata*, *Peddia africana*, S*ynaptolepis kirkii*, *Carissa bispinosa*, *Scolopia zeyheri*, *Schrebera alata* and *Scutia myrtina*; the dominant woody climbers identified were, *Rhoicissus sessilifolia*, *Rhoicissus rhomboidea*, *Smilax anceps*, dominant forbs *Bidens pilosa*, and *Desmodium incanum*. In addition, the ferns *Microsorum scolopendrium* and *Microsorum punctatum* and grasses *Panicum deustum, Oplismenus hirtellus*, *Stenotaphrum secundatum* and *Digitaria longiflora* were recorded. The bare ground cover at macroscale was recorded as 16 ± 26%.

3.3.1.7 Vegetation unit 7: Roads and paved surfaces

(i) Distribution within Study area 2 - Zini Fish Farm

The roads and paved surfaces on the Fish Farm are in a grid like pattern forming the boundary lines between the rows of ponds and essentially following the perimeter of the farm.

(ii) Microscale (1 m^2 sampling plots)

At the microscale the roads and paved surfaces on the Fish Farm are composed of a mixture of gravel, cement and stone, with some areas that are overgrown with grasses and forbs. At the microscale the herbaceous cover was 21 ± 15% (with average herb height at 47 mm ± 23) (see Figure 3.3). The herbaceous species identified were the grasses *Cynodon dactylon*, *Stenotaphrum secundatum*; and the forbs *Aizoon canariense* and *Euphorbia prostrata*. The mean bare soil surface of the road was estimated to be 79 ± 15%. There was no woody vegetation cover or canopy cover recorded for this vegetation unit. The mean horizontal cover was a mere 10 ± 20 mm, while the mean slope at the microscale was found to be 3 ± 5°. Supratidal zone distance was nor recorded for this vegetation unit given that the ponds are not influenced by tidal movement. The average elevation above water was 600 ± 480 mm, while the average distance to a water source was 10 ± 5 m. Average wind speed recorded on windy days was 2.39 ± 0.46 m/s, while average wind readings on still calm days was 0.09 ± 0.02 m/s.

(iii) Macroscale (25 m^2 sampling plots)

The vegetation cover at the macroscale was a minimal 11 ± 7% with average herb height being 47 ± 23 mm. The herbaceous species identified at the microscale were the grasses *Cynodon dactylon*, *Stenotaphrum secundatum*; and the forbs *Aizoon canariense* and *Euphorbia prostrata*. The bare ground cover at the macroscale on the roads and paved services was recorded as 88 ± 32% (see Figure 3.2).

3.3.2 African clawless otter latrine sites: characteristics and features identified

(i) Distribution within Study area 1 - uMlalazi Nature Reserve

The majority of the latrine sites located in the uMNR were located at the ecotone between two vegetation units or at the ecotone between a vegetation unit and a water source. From the 38 latrine sites, 19 were located at the ecotone between two vegetation units or at the ecotone between a vegetation unit and a water source, 10 in the medium tall closed reed beds and hygrophilous grasslands, six on hyper-saline mudflats, two in mangrove forest, and one in the medium tall closed *Juncus krausii* reed beds. The ecotones between the mangrove forests and mudflats, between the mangrove forests and riverine forest bush clumps and the ecotone between the mudflats and *Phragmites australis–Juncus kraussii* medium tall closed sedge-beds were the most common latrine site locations within the uMNR (see Figure 3.1).

(ii) Distribution within Study area 2 – Zini Fish Farm

Thirteen latrine sites were located in the Fish Farm. Here, ten sites were located in the grass and reed vegetation type, while the remaining three were located at the ecotone between the road and grass and reed vegetation type (see Figure 3.1).

(iii) Microscale (1 m^2 sampling plots)

The average herbaceous cover recorded at latrine sites across both study areas was 25 ± 25%. The dominant herbaceous species recorded were the grasses *Stenotaphrum secundatum*, *Cynodon dactylon*, *Digitaria eriantha*, *Imperata cylindrica*; the forb *Euphorbia prostata*; the vine *Rhynchosia caribaea* and an unidentified moss species. The mean herbaceous cover at control sites varied between vegetation types ranging between 0 and 100%. At the microscale there were no woody species recorded at latrine sites. The common substrate types identified at latrine sites included sandy-silt clay, sandy soil and gravel sands.

Canopy cover at the microscale was minimal, 6 ± 14%, with the vast majority of the latrine sites located in open areas devoid of woody canopy cover. Canopy cover at control sites ranging between 0 and 100%. The horizontal cover recorded at latrine sites was minimal with the average height recorded being 4.6 ± 5.7 cm; with the control site horizontal cover ranging from 0 to 50.63 cm (41.4 ± 64.3 mm). Overall, most of the latrine sites were located in areas where the ground had a gentle slope, the average slope recorded was 5 ± 3° (at control sites slope ranged between 1° and 12°). The average distance latrine sites were located from a water source was 13 ± 11 m (control sites ranged between 3 and 49 m). The average distance from the supratidal zone was recorded as 3.36 ± 6.09 m. The average elevation recorded above water recorded as 900 ± 80 mm (control sites ranged between 27 and 909 mm. The average recording taken on windy days at latrine sites was 1.82 ± 0.43 m/s, while on still days it was 0.1 ± 0.05 m/s. The mean wind readings taken at control sites on windy days was 1.65 ± 0.37 m/s 0.43 and on still days was 0.08 ± 0.04 m/s.

(iv) Macroscale (25 m^2 sampling plots)

The vegetation cover at the macroscale averaged at 40 ± 28% (and ranged between 0 and 100% at control sites), with the average herb layer height measuring at 271 ± 323 mm. The dominant species at the herbaceous level include the grasses *Stenotaphrum secundatum*, *Cynodon dactylon*, *Digitaria eriantha* and *Sporobalus africanus*; the reeds *Phragmites australis* and the sedge *Juncus kraussii*; the herbs *Indigofera spicata*, *Euphorbia prostata*, *Conyza albida*, *Rhynchosia caribaea*; the succulent plant *Salicornia pachystachya* and an unidentified moss species. The bare patch substrate cover at the macroscale was relatively extensive at latrine sites with the average ground cover estimated at 60 ± 17% (ranged between 0 and 100% at control sites). The latrine sites on the Fish Farm did not contain any woody species at the macroscale, thus the average canopy cover is averaged from the latrine sites in the uMNR. The average canopy cover recorded at the macroscale was 17 ± 23%, with mean tree height recorded as 2.78 m ± 2.49. The canopy cover at control sites ranged between 0 and 100%.

3.3.3 Vegetation unit description results tables

The findings of the vegetation unit descriptions detailed above are summarised for both the microscale and macroscale below (see Table 3.4 and 3.5).

Table 3.4 The summarised findings of the predictor variables (mean and standard deviations are reported) at the microscale of the seven vegetation units and African clawless otter's latrine sites.

Predictor variables	Vegetation unit							
	1	2	3	4	5	6	7	African clawless otter latrine sites
	Reed beds and hygrophilous grasslands	Riverine woodlands and floodplain bush clumps	Mudflats	Mangrove Forest	Juncus beds	Dune Forest	Roads and paved surfaces	
Herbaceous cover (%)	94 ± 6.18	91 ± 7	34 ± 35	27 ± 12	59 ± 10.54	79 ± 21	21 ± 15	25 ± 25
Woody cover (%)	2.5 ± 0.7	3 ± 3	-	77 ± 6	-	2 ± 3	-	-
Canopy cover (%)	0.4 ± 2	93 ± 9	2 ± 2	88 ± 19	-	90 ± 13	-	6 ± 14
Horizontal Cover (mm)	190 ± 140	180 ± 10	29 ± 24	9 ± 2	370 ± 70	5 ± 3	10 ± 20	41.4 ± 64.3
Slope (degrees)	4 ± 2	5 ± 2	1.5 ± 1	3 ± 1	3.5 ± 2	7 ± 5	3 ± 5	5 ± 3
Elevation above water (mm)	2030 ± 2570	1220 ± 71.9	490 ± 360	890 ± 570	90 ± 77	279 ± 219	600 ± 480	900 ± 80
Distance from water (m)	13.2 ± 10.58	19 ± 12	20 ± 12	17 ± 7	15 ± 8	22 ± 10	10 ± 5	13 ± 11
Supratidal zone distance (m)	15 ± 10	17 ± 12	0 ± 0	4 ± 7	8 ± 7	17 ± 9	-	3 ± 6
Windy days average wind speed (m/s)	1.99 ± 0.45	1.53 ± 0.04	2.19 ± 0.32	1.6 ± 0.29	0.74 ± 0.24	1.1 ± 0.35	2.39 ± 0.46	1.82 ± 0.43
Still days average wind speed (m/s)	0.08 ± 0.05	0.06 ± 0.05	0.12 ± 0.05	0.08 ± 0.05	0.08 ± 0.05	0.06 ± 0.04	0.09 ± 0.02	0.1 ±0.05

Table 3.5 The summarised findings of the three predictor variables (mean and standard deviations are reported) at the macroscale of the seven vegetation units and African clawless otter's latrine sites.

Predictor variables	Vegetation unit							
	1	2	3	4	5	6	7	African clawless otter latrine sites
	Reed beds and hygrophilous grasslands	Riverine woodlands and floodplain bush clumps	Mudflats	Mangrove Forest	Juncus beds	Dune Forest	Roads and paved surfaces	
Vegetation cover (%)	92 ± 6	65 ± 41	44 ± 64	9 ± 9	84 ± 6	83 ± 16	11 ± 7	40 ± 28
Canopy cover (%)	2 ± 4	91 ± 19	1± 1.7	93 ± 8	-	92 ± 9	-	17 ± 23
Substrate cover (%)	8 ± 20	35 ± 13	56 ± 35	90 ± 17	16 ± 6	16 ± 26	88 ± 32	60 ± 17

3.3.4 Model outputs

3.3.41 Microscale

The four top-ranked models at the micro scale all retained canopy cover, horizontal cover, slope, vegetation cover, and moderate and minimal wind exposure (see Table 3.6). The top-ranked models at the microscale indicated that latrine sites were characterised as occurring in open areas with less canopy and horizontal cover on elevated areas that had lower wind speeds (Figure 3.4). The interaction of wind exposure and bank slope proved to be an important feature in latrine site selection. Accordingly, sites located on steeper slopes were more likely to be used as latrines when wind exposure was also higher – this association being the opposite in flatter areas (see Figure 3.4).

Latrines were characterised as occurring on sloping terrain in areas that were either exposed to wind or more sheltered from the wind. The structure of the four most parsimonious models at the microscale are presented in Table 3.6 and the respective binomial GLM model's estimated coefficients for Akaike's information criterion for the microscale habitat predicators are presented in Table 3.7. The model output of the remaining top models (models 2 to 4) is supplied in Appendix B.

Table 3.6 Summary of Akaike's information criterion for small sample sizes (AICc) model selection statistics for candidate models (binary GLM) predicting latrine site based on microscale habitat data collected in the uMNR and Fish Farm in Mtunzini, South Africa. wi = Akaike weight.

Model	Rank	AICc	ΔAICc	wi
Canopy Cover + Horizontal Cover + Slope + Still Average + Vegetation Cover + Windy Average + Slope*Still Average + Slope*Windy Average	1	71.2	0.00	0.151
Canopy Cover + Horizontal Cover + Slope + Still Average + Vegetation Cover + Windy Average + Slope * Windy Average	2	72.1	0.99	0.092
Canopy Cover + HAW + Horizontal Cover + Slope + Still Average + Vegetation Cover + Windy Average + Slope*Still Average + Slope*Windy Average	3	72.8	1.65	0.066
Canopy Cover + Horizontal Cover + Slope + Still Average + Vegetation Cover + Windy Average + Slope*Still Average	4	73.0	1.82	0.061

Table 3.7 The top ranked model's estimated coefficients summary of top-ranked model outputs predicting the selection of latrine sites based on microscale habitat data collected. ChiSq = Chi-squared; test df = degrees of freedom. p value significance * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001

Parameters	Estimate	Std. Error	ChiSq	df and residual df	p-value
Intercept	8.776	3.355	-	-	0.008 **
Canopy Cover	-0.081	0.023	40.870	1,160	<0.001 ***
Horizontal Cover	-0.127	0.058	5.698	1,159	0.017 *
Slope	-1.119	0.773	13.784	1,158	<0.001 ***
Still Average	-0.349	15.533	7.595	1,157	0.005 **
Vegetation Cover	-0.051	0.014	20.735	1,156	<0.001 ***
Windy Average	-3.799	1.340	6.257	1,155	0.012 *
Slope*Windy Average	0.571	0.362	4.060	1,154	0.072 *
Slope*Still Average	5.473	3.448	3.235	1,153	0.043 ·

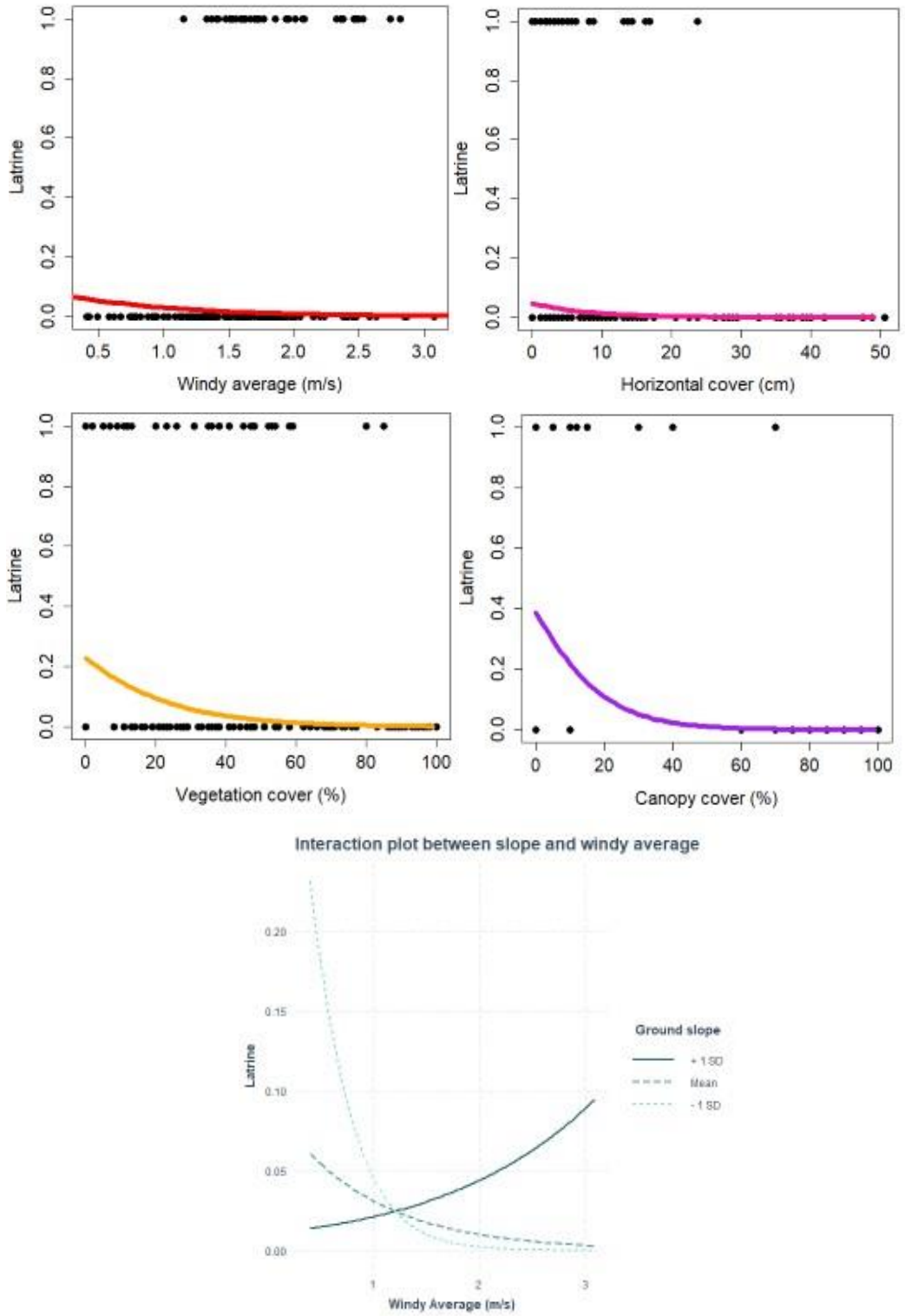

Figure 3.4 Predicted probability of latrine site selection in relation to the four predictors retained in all the top four models at the microscale that retained the same effect direction across all models. The interaction plot depicting the relationship between the predictor of slope and average wind speed on windy days is also depicted.

3.3.4.2 Macroscale

Latrine site selection at the macro scale was associated with areas containing little vegetative cover and minimal canopy cover. The top-ranked model at the macroscale retained the variables canopy cover and substrate cover (see Table 3.8). Latrine site selection at the macroscale indicated that substrate cover was positively related to the presence of latrine sites, while canopy cover had a negative association with latrine site selection by otters (see Figure 3.5). The structure of the three most parsimonious models at the macroscale are presented in Table 3.8 and the respective binomial GLM model's estimated coefficients for Akaike's information criterion of the three macroscale habitat predicators are presented in Table 3.9.

Table 3.8 Summary of Akaike's information criterion for small sample sizes (AICc) model selection statistics for candidate models predicting latrine site selection by African clawless otters based on macroscale data collected in the uMNR and Fish Farm in Mtunzini, South Africa. wi = Akaike weight.

Model	Rank	AICc	ΔAICc	wi
Canopy Cover + Substrate Cover	1	130.1	0.00	0.421
Herbacous Cover + Canopy Cover	2	130.3	0.20	0.381
Canopy Cover + Herbacous Cover + Substrate Cover	3	131.7	1.51	0.198

Table 3.9 The top ranked model's estimated coefficients summary of top-ranked model outputs predicting the selection of latrine sites based on macroscale habitat data collected. ChiSq = Chi-squared; test df = degrees of freedom. p value significance *** ≤ 0.001

Parameters	Estimate	Std. Error	ChiSq	df and residual df	p-value
Canopy Cover	-0.040	0.008	42.203	1,160	<0.001 ***
Substrate cover	0.033	0.008	0.789	1,159	0.375

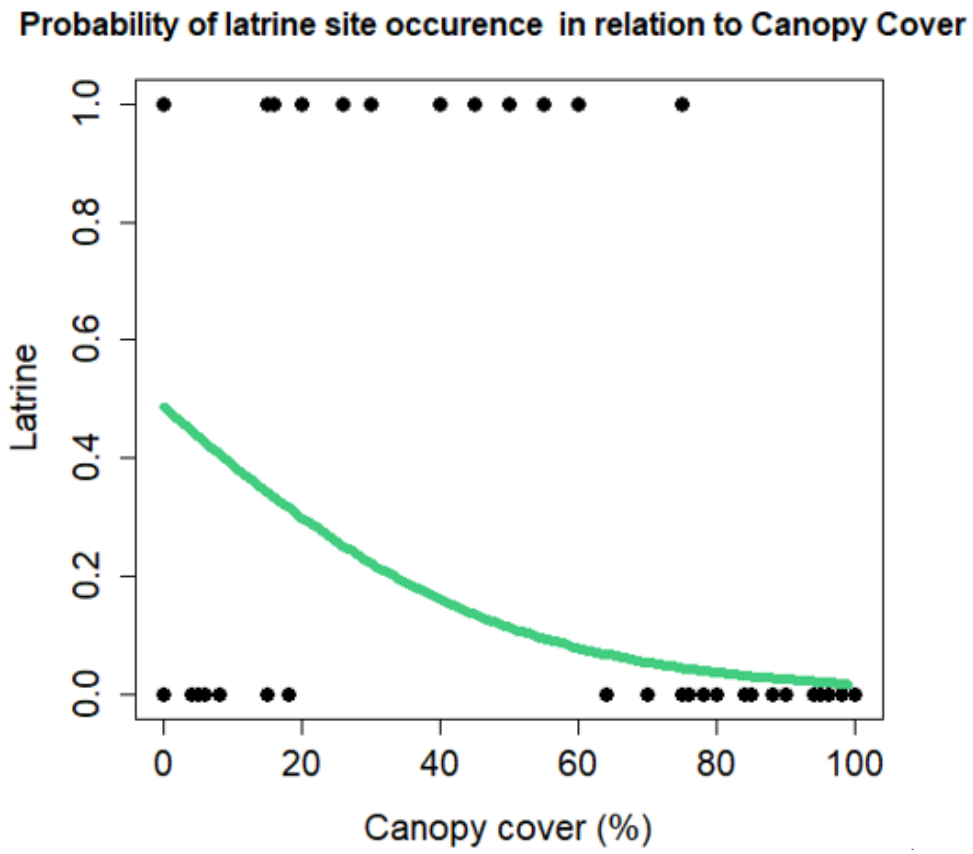

Figure 3.5 Predicted probability of latrine site selection in relation to canopy cover at macroscale

3.4 Discussion

This study was designed to ascertain the factors that influence the selection of latrine sites by African clawless otters at two ecological spatial scales in a coastal habitat of South Africa. The multiscale approach employed here provides a more detailed understanding of the habitat features linked to African clawless otter latrine site selection and the likely role these sites play in their behavioural ecology. Latrine site selection was expected to be driven by one of three factors: to facilitate the dispersal of scent, to facilitate the retention of scent or to avoid predation.

Otter latrine sites were well distributed throughout the study area and the majority of the latrine sites located in the uMNR were located at the ecotone between two vegetation units or at the ecotone between a vegetation unit and a water source. Ecotones are defined as multi-dimensional environmentally complex interaction zones between two or more ecological systems or communities (Hufkens et al., 2009). The selection of ecotones

as latrine site by otters may indicate the likely role of latrine sites in securing territorial boundaries. Many animal species use ecotones, breaks in natural vegetation and road-verge ecotones as the boundary of their territory (Geist & Walther, 1974; Underhill, 2003). The selection for latrine sites in areas with minimal vegetative, canopy and horizontal cover and areas with moderate wind exposure could potentially aid in making the latrine sites more conspicuous to conspecifics and even other species in the area. Whereas the combined selection for latrine sites with bank slope and minimal wind exposure could facilitate the retention of scent in an area (see Figure 3.4). The close proximity of latrine sites to water sources (an average distance of 13 ± 11 m) may provide otters with quick access to the parts of the river, water channels and ponds to limit their detectability.

Latrine sites at the microscale were associated with minimal herbaceous vegetation where the dominant herbaceous plants identified were grass, sedge, vine and moss species. Latrine site selection was positively associated with bare ground cover such that spraint was typically deposited on sandy-silt clay, sandy soil and gravel sand substrates. Sandy substrates were identified as an important feature for Neotropical otter olfactory communication (Michalski et al., 2021). The presence of latrine sites was negatively influenced by canopy and horizontal cover. These habitat features were hypothesized to play an important role, offering a form of protection from predation and the fact that these did not influence latrine site selection could possibly indicate that the otters in this area do not face major predation threats that would require their latrines to be concealed. The proximity of latrine sites to water sources and the supratidal zone was thought to be a potentially important feature given the proximity to potentially important food resources, such as crab burrows. Crowley et al. (2012) investigated otter activity and distance to stream mouth which was associated with increased fish density during spawning seasons, however given the fluctuation in availability of the food source the distance to stream mouth was not a significant variable. In the hog badger (*Arctonyx collaris*) latrine site selection was negatively correlated to high density food resources (Zhou et al., 2015).

Insights into latrine site selection facilitate our understanding of and provide insights into the mechanisms that drive the distribution and activity of otters (Crowley et al., 2012). The importance of lakeshore topography on the distribution patterns of latrine sites have been documented in North American river otters (Newman & Griffin, 1994; Swimley et al., 1998). As seen in the study by Albeke et al (2010), shoreline topography and terrestrial convexity were important habitat features predicting the presence of North American river otter latrine sites at a coarse scale. Detailed assessments of shoreline and overall study area topography were beyond the scope of this study and could be investigated in future research.

It is only through knowledge on habitat selection and scale-specific processes that, use-of-habitat assessments, effective conservation and management strategies can be implemented for otter populations (Mason & Macdonald, 1986; Douglas, 2003). Environmental variables were confirmed to have an influence on the selection and distribution of latrine sites by African clawless otters at both the micro and macroscale. This multiscale approach allowed for a more detailed description and understanding of African clawless otter latrine site selection. The widespread distribution of latrines sites across the uMNR and Zini Fish Farm may function in otter sociality. The otters could also potentially be travelling extensively along the water channels and river shoreline such that multiple latrine sites can be maintained (Crowley et al., 2012). If latrine site selection is connected to territorial marking, the substrate and microclimate of the latrine should be selected so as to maximise detection, scent dispersal and signal longevity (Buesching & Jordan, 2019). This can be achieved by selecting areas where air flow and elevation facilitate scent dispersal. There is consistency among the top four models at the microscale, where the predictors canopy cover, horizontal cover, vegetation cover and moderate wind speed that all had a negative influence on the probability of latrine site selection. The findings at the microscale are supported by the macro scale selection where latrine sites were positively associated with bare ground cover and negatively associated with canopy cover. The three top-ranked models all described this trend at the macroscale.

The findings of this research study may be relevant to African clawless otter populations inhabiting areas where predators, prey and habitat types are somewhat similar to the environmental and ecological features of this study area. While the specific micro and macroscale habitat features may vary between different localities, the importance of bare ground cover, minimal horizontal and canopy cover at both spatial scales have likely arisen as a result of selective pressures common to many coastal African clawless otter populations. According to Crowley et al. (2012), the selection and degree of latrine site use by otters is believed to be a trade-off between various selective pressures that influence their behaviour at several spatial scales. The study area of Mtunzini falls in a subtropical climate that is relatively warm, with very little daily temperature fluctuations. This lack of extreme daily and seasonal fluctuations, together with rainfall in both the summer and winter seasons, result in vegetation types and vegetation structures remaining relatively stable throughout the year. In this regard the stable vegetation and relatively constant temperature through the course of the year could favour latrine site selection in regions where there is greater scent dispersal so as to maximise scent detection by conspecifics.

Otters are elusive creatures and as a result extremely difficult to observe directly in their natural habitat, for this reason the distribution of spraints and latrine sites are used to assess activity, habitat preference and selection as well as population status (Mason & Macdonlad, 1986). The validity of the indirect sampling method employed here requires verification and appropriate correlation, evaluation and reliability can be achieved by comparing results with known otter populations (Kruuk & Conroy, 1987).

A limitation of this research is the correlation observed between habitat variables and latrine sile selection is influenced by the size of the sampling area, moreover it should be considered that the distribution of latrine sites alone does not necessarily indicate the relative importance of particular vegetation types over others (Kruuk & Conroy, 1987). Analysis of spraints and latrine sites for assessing and surveying otter populations alone should be used with caution and further research is required to test methodology and

confirm findings across a variety of habitats and densities (Kruuk & Conroy, 1987; Gallant et al., 2007).

3.5 Conclusion

The findings of this chapter provide valuable insights into the spatial scale of latrine site selection by African clawless otters. The selection of latrine sites with little vegetative cover and that are exposed to wind speed likely aids in the dispersion of scent. Whereas the combined selection for latrine sites with bank slope and minimal wind exposure could facilitate the retention of scent in an area. I suggest that latrine sites are selected in 'open' areas being devoid of vegetation as this increases the likelihood of conspecifics and/or other species coming across the site. Otters are seemingly not reliant on a substantial amount of scent dispersal by wind, while factors that would increase scent retention of a site also proved to not be a major factor in site selection either. The findings of this research study along with previous research studies have identified particular habitat variables and features that are associated with otter latrine site selection.

The scale of analysis assessed in this research should be employed to future conservation and management actions that assess the habitat requirements of African clawless otters. While the findings of this research are likely to be area specific the identification of particular habitat features that influence latrine site selection can be applied to other sites. Such findings may require additional consideration when prioritising management actions, habitat protection and reducing the activities that might negatively affect otters. The overall findings of this research provide valuable insight into otter ecology and provide an important first analysis of latrine site selection features in this species. In time such research findings could also facilitate and aid future research, monitoring, management and conservation strategies.

CHAPTER IV – PRELIMINARY CHARACTERIZATION OF VOLATILE ORGANIC COMPOUNDS IN AFRICAN CLAWLESS OTTER SPRAINT

4.1 Introduction

One of the primary questions in animal chemical communication research is how information is coded. This is believed to occur through a combination of behavioural and chemical means (Sun & Müller-Schwarze, 1998). Olfactory signals persist in the environment for prolonged periods of time (compared to visual and auditory signals) such that communication can occur over longer time frames where senders and receivers of signals do not necessarily need to be in close proximity (Vitale et al., 2020). Olfactory communication through scent marking is a common feature in mammals (Bradbury & Vehrencamp, 1998) employed for a variety of reasons including individual recognition (Brennan & Kendrick, 2006; Kulachi et al., 2014), group identity (Vaglio et al., 2016), territorial marking (Black-Decima & Santana, 2011; Marneweck, 2013), and reproduction (Scordato & Drea, 2007; Melo & González-Mariscal, 2010).

Mammals have complex chemical signals with multiple intricate components. Consequently, the desirable initial method in deciphering these signals is to begin with the chemical analysis approach (Sun & Müller-Schwarze, 1998). The anal gland volatiles of the following Mustelid species have been chemically analysed: the American mink, *Mustela vison* (Brinck et al., 1978); the stoat (*Mustela erminea*); the ferret, *Mustela putorius furo* (Crump & Moors, 1985); the European polecat, *Mustela* putorius (Brinck et al., 1983); the steppe polecat, *Mustela eversmanni* (Zhang et al., 2002a); the Siberian weasel, *Mustela sibirica* (Zhang et al., 2002b); European badgers (Noonan et al., 2019); and the Eurasian otter (Kean et al., 2011). The chemical information of these species has made them valuable model systems in the broad category of mammal chemical communication research (Zhang et al., 2002). Otters represent 13 of the 58 extant species in the family Mustelidae, yet to date there has been little research to ascertain the composition of scent marking and olfactory communication (Kean et al., 2011).

4.2 Materials and Methods

4.2.1 Sample collection

Otter faecal and anal gland secretions were identified based on their shape, size and characteristic odour (Stuart & Stuart, 2019). African clawless otter faeces are typically sausage shaped (one end pointed) and anal gland secretions typically accompany faeces as dark jelly like deposits (Stuart & Stuart, 2019). The faeces typically range from 25 to 35 mm and are full of fish scales, fish bones and shell fragments. African clawless otters have a characteristic odour that is descried as being very musky, fishy combined with a sweet taint (Estes, 1991; Stuart & Stuart, 2019).

Upon discovery of otter spraint, coordinates were recorded using a hand-held GPS device. Fresh and unbroken spraint samples were collected, the samples were placed into sterile plastic sealable vials and stored at -20°C. Unbroken and fresh spraint samples were targeted since compound deterioration was likely to be minimal. The freshness of otter spraint was assessed by heat and colour (Marneweck et al. 2018).

4.2.2 Chemical analysis

Faecal sample analysis involved gas chromatography mass spectrometry (GCMS). Gas chromatography (GC) is a technique that separates compounds by exploiting the partitioning of analytes between a flowing ‘mobile phase’ and a ‘stationary phase’ (Hough et al., 2018). Mass spectrometry (MS) is involved in the detection of analytes as they elute from the chromatographic system (Hough et al., 2018).

Volatile organic compounds (VOCs) emitted by the faecal and anal gland secretion samples were sampled and analysed using solid-phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS). Approximately 0.2 g of frozen samples were transferred to 1.5 ml analytical glass vials (Machery Nagel, Separations, South Africa). Sample vials were then placed in a heating block at 40°C to ensure a consistent temperature during sampling. A 65µm polydimethylsiloxane/divinylbenzene SPME fibre

(Supelco, Merck South Africa) was exposed to the headspace above each sample for 15 min. Preliminary testing of exposure times of up to 30 min indicated that 15 min was sufficient to reach equilibrium. Fibres were conditioned according to manufacturer's recommendations and reconditioned for 6 min in the GC injection port at 300°C if the fibre had not been used for several hours. An analysis of the fibre not exposed to a sample was conducted to detect non-sample compounds and any contamination or deterioration of the fibre.

Following exposure to headspace volatiles, the fibre was immediately manually injected into the GC-MS (Agilent 7890B gas chromatograph coupled to a 7977MSD quadrupole mass spectrometer). Samples were analysed on a 30 m, 0.25 mm inner diameter, 0.25µm film thickness, HP5 column (J&W, Agilent, South Africa), with helium as the carrier gas at constant flow rate of 1.2 L/min. Separation was achieved with a 2-min hold at 50°C, followed by a linear temperature increase of 10°C/min to 300°C and held at 300°C for 2 min, resulting in a total programme time of 29 min. An external hydrocarbon standard (1 µl) was injected using an automatic liquid injector for calculation of retention indices, in turn allowing for the calculation and standardisation of retention times.

The GC was coupled to a mass spectrometer (MS) operated in electron impact ionization EI+ mode, scanning from ion mass fragments 50 to 300 m/z. The mass spectra were deconvoluted using MassHunter 1.2 (Agilent) in conjunction with the NIST mass spectral library and the R-based statistics suite, Metababoanalyst. Data analysis and peak integration were performed using the program MassHunter (version 1.2). Compounds were identified based on comparison of mass spectra and retention times to the National Institute of Standards mass spectral library (NIST 2017) and by calculating their retention indices. Peaks with a short retention time below 4 min were not included in the analysis because signals with retention times were not measurable with sufficient accuracy. The faecal matter samples from which the volatiles were extracted were air-dried to determine their dry weight.

The Retention Time, Kovat's index, mean relative abundance, standard deviations, match factor and occurrence of each sample were determined. The Retention Time (min) is the measure of time taken for a solute to pass through the gas chromatograph column, it is calculated as the time from injection to detection. The retention time of each peak on the GC chromatogram is compared to pre-installed values of all compounds in the NIST library (Zang et al., 2021) (see Table 4.1). The Kovat's Retention Index (KI) is a dimensionless quantity that describes the rate at which a compound passes through a gas chromatograph column (Qu et al., 2021). The KI is regarded as a quantity that is independent of many experimental variables and is regarded as a universal descriptor of a compounds retention time in the chromatography column. The Match factor is the percentage identity match of the mass spectrum of the analyte being assessed with the mass spectrum of analytes deposited in the NIST library. If the spectra are identical the match factor will be 100%.

The mean relative abundance value of a compound is a fraction of the peak area on the chromatograph. The relative abundance essentially quantifies the amount of an ion produced in relation to the base peak (amount of the most abundant ion). These fractions can then be compared between compounds to determine their relative abundance. The mean relative abundance is a relative value, such that one compound can be higher than another but there is no absolute high or low value. Analysis of VOCs identified in African clawless otter spraint will be expanded on through a search of available literature and records on these behavioural roles of these compounds in other Animalia.

4.3 Results

The VOCs of 14 anal gland secretion and faecal samples of African clawless otters were identified. The number of compounds per sample ranged from 24 to 51 (mean 32 ± 7.83). Across all samples a total of 88 compounds were found of which a total of 34 were provisionally identified using the NIST library. The compounds identified comprised of a complex mixture of organic acids, esters, alcohols, ketones, benzenes and aromatic compounds. The use of SPME made it possible to identify 34 compounds (see Table

4.1). The following chemical groups (and their respective percentages) were present in African clawless otter faeces and anal gland secretions: alcohols (40.54%), esters (10.81%), ketones (5.41%), benzenes (5.41%), phenols (5.41%), aldehyde (5.41%), aromatics (5.41%), alkanes (5.41%), diterpene (5.41%), monoterpenoids (2.7%) organic disulfide (2.7%), alkenes (2.7%) and long-chain fatty acid (2.7%). The compound provisionally identified through the NIST library that was common across all 14 samples was N1,N1,N4-Tris(tert-butyldimethyl)amide. The Retention Time, Kovat's index, mean relative abundance, standard deviations, match factor and occurrence of the compound in each sample are detailed in Table 3.1.

Table 41. Volatile organic compounds (VOCs) in the anal gland secretion and faeces (n=14) of African clawless otters, *Aonyx capensis*, with Retention Times (RT min), Kovat's Retention Index (KI), mean relative abundance, standard deviation (Std dev), match factor (probability %), sample and occurrence.

Compound Identified	RT (min)	KI	Mean Relative Abundance	Standard Deviation	Match Factor	Sample	Occurrence
Phenol derivative 1	5.182	978.02	6.21	0.007		7,8	2
Phenol derivative 2	5.194	978.89	6.86	1.27		6,10	2
1-Pyrrol[tert-butyl(dimethyl)silyl]oxy morphopropan-2-ol	5.205	979.96	3.95	0.76		1,10	2
Methanol	5.211	980.54	6.73	2.64		4,11,12	3
Phenol	5.223	981.71	14.01	2.04		9,13	2
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	6.366	1105.98	2.1	3.61		9,12,13	3
UNKNOWN	6.493	1121.46	26.68	5.06		10,11	2
UNKNOWN	6.499	1122.20	33.68	2.28		4,7,9	3
Ethyl n-butyl disulphide	6.476	1119.39	15.23	5.98		8,12	2
1-Nonanol	6.77	1155.24	0.0098	0.0048		2,3, 11	3
UNKNOWN	6.793	1158.05	8.22	0.43		8,9,12,14	4
UNKNOWN	7.313	1223.78	6.94	8.31		1,3,7,13	4
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-	7.319	1224.59	4.8	5.01		4,5,6	3
Benzyl isothiocyanate 1	8.001	1318.05	3.64	0.53		7,8,9	3
Benzyl isothiocyanate 2	8.006	1318.78	6.58	7.24		1,4,5,10,11,12,13	7
1-Undecanol 1	8.301	1361.72	1.52	0.77		1,11	2
1-Undecanol 2	8.307	1362.59	1.11	0.042		6,8	2

1-Tetradecene	8.486	1388.65	0.83	0.43	3,8	2
Dodecanal	8.491	1389.37	0.47	0.38	4,6,10,11	4
UNKNOWN	8.595	1404.79	0.7	0.29	4,5,6,7,9,12,13	7
UNKNOWN	8.971	1462.91	0.12	0.04	10,13	2
UNKNOWN	9.248	1506.06	3.06	1.74	1,4,5,6,8,9,11,12	8
Methyl salicilate	9.254	1507.03	1.69	1.54	2,7,10	3
1-Tridecanol 1	9.496	1546.57	4.45	2.69	5,7,8,11	4
1-Tridecanol 2	9.502	1547.55	7.29	6.8	1,4,6,10,13	5
2-Tridecenal, (E)-	9.641	1570.26	34.83	49.25	3,4	2
Zingiberenol	9.86	1606.41	0.44	0.35	3,5,6	3
UNKNOWN	9.8739	1608.82	0.006	0.001	6,8,9,12,13	5
UNKNOWN	10.322	1686.48	0.86	1.21	6,8,11,12,13	4
UNKNOWN	10.328	1687.52	1.07	0.34	1,4,7,11	3
2,6,10,14-tetramethyl-pentadecane	10.449	1708.93	1.14	0.76	2,7,10	2
UNKNOWN	10.576	1732.06	0.15	0.086	1,6,7,8,11,13	6
7-Methylheptadecane	10.646	1744.81	0.94	0.79	1,6,8,11,13	5
Benzyl benzoate	10.651	1745.72	0.54	0.32	5,7,9,10,11,12	5
Tetradecanoic acid	10.767	1766.85	0.44	0.22	6,7,10,11,13	5
Ethyl tetradecanoate	10.825	1777.41	0.26	0.16	12,14	2
(Z)-9-Tetradecenyl acetate	10.859	1783.61	0.19	0.035	4,7,13	3
UNKNOWN	10.963	1802.69	0.26	0.026	5,7,13	3
Phytane	11.021	1813.85	2.62	3.32	1,2,6	3
UNKNOWN	11.131	1835	0.69	0.34	4,6,8,9	4
UNKNOWN	11.217	1851.54	0.68	0.73	4,6,8,9	4
9-Heptadecanone	11.223	1852.69	2.48	2.76	4,7,10,11,12,13	6
UNKNOWN	11.35	1877.12	18.92	11.36	4,7,10,11,12,13	6
1-Hexadecanol 1	11.345	1876.15	5.05	2.04	1,3,9	3
1-Hexadecanol 2	11.344	1875.96	3.96	1.34	6,8,9	3
1-Hexadecanol 3	11.443	1895	13.2	9.43	2,14	2
UNKNOWN	11.495	1905.23	0.25	0.28	1,2,14	3
UNKNOWN	11.714	1949.30	1.39	0.83	1,3,4,5,6,7,8,9,10,11,12,13	12

Abietatriene	12.13	2034.24	4.07	4.33		3,4,6,7,10,11,13	7
UNKNOWN	11.939	1994.57	0.16	0.03		11,14	2
UNKNOWN	12.176	2043.84	0.47	0.66		1,14	2
UNKNOWN	12.222	2053.44	0.84	0.11		2,12	2
9,12-Octadecadien-1-ol, (Z,Z)-	12.205	2049.90	5.25	4.15		4,10,11	3
UNKNOWN	12.274	2064.30	1.35	1.34		3,5,7,10,11,13	6
UNKNOWN	12.199	2048.64	3.08	2.02		7,13	2
UNKNOWN	12.442	2099.37	1.9	1.02		1,5,6,7,8,9,10,11,12,13	10
UNKNOWN	12.211	2051.15	0.16	0.01		3,5	2
Pyrene	12.338	2077.66	0.39	0.32		1,3,5,11	4
Phytol	12.448	2100.66	0.29	0.01		2,3	2
UNKNOWN	12.569	2127.19	0.15	0.07		1,4,6,7,8,9,11,12,13	9
UNKNOWN	12.748	2166.45	0.34	0.13		6,7,8,9,10,11,12,13	8
UNKNOWN	12.938	2208.55	0.09	0.03		6,12	2
1-Octadecanol, TMS derivative	12.742	2165.13	0.31	0.01		4,14	2
UNKNOWN	13.406	2317.06	0.63	0.59		1,5,6	3
UNKNOWN	12.944	2209.93	0.13	0.09		8,9	2
UNKNOWN	13.62	2367.7	0.19	0.14		1,2,5,13,14	5
UNKNOWN	13.943	2446.29	0.8	0.43		4,7,8,9,10,11,12,13,14	9
UNKNOWN	13.891	2433.42	0.13	0.02		6,13	2
cis-13-docosenol, tBDMS	14.561	2603.47	1.46	0.85		2,3,4,5,6,7,8,9,10,11,12,13	12
UNKNOWN	13.949	2447.77	0.5	0.37		1,5,6	3
N1,N1,N4-Tris(tert-butyldimethyl)amide	14.752				66.8	1,2,3,4,5,6,7,8,9,10,11,12,13,14	14
1-(2-Hydroxyethyl)-2-imidazolidinone	15.705				57.5	9,13,14	3
n-Hexadecanoic acid	18.974				96.36	9,13	2

The odour profiles and published reports of the thirty-four compounds identified African clawless otters and the role and behaviour in several Animalia are detailed in Table 3.2. Several VOCs define the characteristic odour of African clawless otter's spraint with the characteristic odour descriptors of 'faintly sweet', 'fruity', 'waxy', and 'strong penetrating aroma' (see Table 4.2).

Table 4.2. The 34 volatile organic compounds identified in African clawless otter's spraint and the published report of their odour profiles and their biological role in other Animalia.

No	Compound name	Odour profile	Cited relevance to behaviour	
			Behaviour	Species
1	Phenol derivative	smoky, woody and dusty/musty		
2	1-Pyrrol[tert-butyl(dimethyl)silyl] oxymorphopropan-2-ol			
3	Methanol	faintly sweet pungent odour		
4	Phenol	sickeningly sweet and tarry	estrus, sexuality, differentiating female reproductive states, age differentiation	*Idea leuconoe* (Nishida et al., 1996); *Bos Taurus* (Sankar et al., 2007); *Mamestra brassicae* (Jacquin et al., 1991); *Bubalus bubalis* (Brahmachary & Poddar-Sarkar, 2015); *Meles meles* (Noonan et al., 2019); *Lutra lutra* (Kean et al., 2015); *Panthera leo* (Soso & Koziel, 2017); *Canis lupus signatus* (Martín et al., 2010)
5	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	maple-, caramel-, smoky-, coffee-like		
6	Ethyl n-butyl disulphide	sulfureous aroma		
7	1-Nonanol	rose, fruity	sex pheromone	*Achroia innotata* (Francke & Schulz, 1999); *Raphicerus campestris* (Burger et al., 1999)
8	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-			
9	Benzyl isothiocyanate	strong penetrating aroma		
10	1-Undecanol	mild odour		
11	1-Tetradecene	mild pleasant odour		
12	Dodecanal	strong floral odour	sexual attraction	*Lemur catta* (Shirasu et al., 2020) *Tachyglossus aculeatus setosus* (Harris et al., 2012)
13	Methyl salicilate	sweet, fruity odour		
14	1-Tridecanol	mild, pleasant odour		*Lutra lutra* (Kean et al., 2015)
15	2-Tridecenal, (E)-	oily, citrus aroma		
16	Zingiberenol	spicy type odour-ginger	sex pheromone	*Tibraca limbativentris* (Blassioli-Moraes et al., 2020)

17	2,6,10,14-tetramethyl-pentadecane	odourless		
18	7-Methylheptadecane		sex pheromone	*Lambdina athasaria* (Duff et al., 2001); *Lambdina pellucidaria* (Duff et al., 2001)
19	Benzyl benzoate	weak, sweet-balsamic odour		
20	Tetradecanoic acid	waxy, fatty or soapy odor	possible sex differentiation	*Caracal caracal* (Goitom, 2017); *Suricata suricatta* (Leclaire et al., 2017)
21	Ethyl tetradecanoate	waxy odour		
22	(Z)-9-Tetradecenyl acetate		sex pheromone	Lepidoptera (Byers, 2005); O*strinia* zealis (Huang et al., 1998); O*strinia* zaguliaevi (Ishikawa et al., 1999); *Agrotis segetum* (Löfstedt et al., 2014*)*; *Spodoptera frugiperda* (Malo et al., 2015)
23	Phytane	odourless	reproduction	*Vipera ammodytes* (Shafi et al., 2021)
24	9-Heptadecanone		trail following behavior	*Pachycondyla tarsata* (Janssen et al., 1999)
25	1-Hexadecanol	odourless	sex pheromone, oestrus, kin recognition, stimulating parental care	*Caracal caracal* (Goitom et al., 2009); Bovidae (Shafi et al., 2021); *Junco hyemalis carolinensis* (Whittaker et al., 2016; Mas & Kolliker, 2008), *Raphicerus campestris* (Burger et al., 1999), Suricata suricatta (Leclaire et al., 2017), *Mungos mungo* (Jordan et al., 2010)
26	Abietatriene	woody odour		
27	9,12-Octadecadien-1-ol, (Z,Z)-			
28	Pyrene			
29	Phytol	faint floral aroma		
30	1-Octadecanol, TMS derivative			
31	cis-13-docosenol, tBDMS			
32	N1,N1,N4-Tris(tert-butyldimethyl)amide			
33	1-(2-Hydroxyethyl)-2-imidazolidinone			
34	n-Hexadecanoic acid	waxy type odour		

4.4 Discussion

Olfactory communication plays an important role in the ecology of otters and their socio-spatial organisation (Johnson et al., 2000; Berzins & Helder, 2008; Kean et al., 2015 and Mumm & Knörnschild, 2018). The precise mechanistic knowledge of why scent communication at latrine sites is conveyed between individuals is hitherto an under-investigated topic. Many carnivores advertise their territory and resource ownership as a pre-emptive measure to avoid conflict and potentially costly agonistic encounters with conspecifics (Buesching & Stankowich, 2017). This is effectively achieved with low-maintenance long term signals that do not require the continued physical presence of the

owner, but that can be matched to the individual who marked the site through individually identifiable scent. Given that a scent remains in the environment for some time it allows for the owner of the scent to be identified by rivals, even in its absence, reducing the costs of physical conflicts (Gosling, 1982; Leuchtenberge, 2018).

The results of this study indicate that African clawless otter scent profiles are diverse and are believed to play a role in encoding individual specific information. The complexity of chemical profiles varied between the samples. The variation across the 14 scent profiles may be associated with variables like sex, age, reproductive status, dominance and health that were not identified in this study given it assessed wild otter populations. Future research could initially profile individuals and identify individual variables (e.g., sex, reproductive status and age) through DNA analysis techniques to aid in encoding specific VOCs. Moreover, the substrate type, time difference between deposition and collection and between the scats collection and analysis are all factors that contribute to the differences between the samples (Kean et al., 2015). The one compound common to all 14 samples was provisionally identified as the compound N1,N1,N4-Tris(tert-butyldimethyl)amide through the NIST library.

The only other otter species where faecal VOC analysis was conducted to date is the Eurasian otter. Two compounds identified in African clawless otters have also been identified in the Eurasian otter, these include Phenol and 1-Tridecanol (Kean et al., 2015). Some of the compounds identified in this research have been identified in the urine, faeces and anal gland secretions of other animals, where the compounds function in various behavioural roles (see Table 4.2).

Studies assessing odour and olfaction are oftentimes able to equate certain behaviours with scent (Soso & Koziel, 2017), such that the role of individual compounds can be identified and linked to specific behaviour. For example, the ability of elephants to detect the compound cyclohexanone has led researchers to suspect that some must signals

may be single compounds (Rasmussen et al., 1996). Further research on individual chemical compounds and its role in scent-marking and olfactory communication are required to gain an understanding of the influence of particular VOCs on elucidating behaviour (Soso & Koziel, 2017).

The biological roles for nine of the compounds identified in the otter spraint are associated with reproduction and as sex pheromones in other animals, potentially indicating that scent-marking and chemical signalling in African clawless otters signals sexual behaviour and reproduction. However, the function of specific VOCs may differ between different species. For example, the compound 1-Hexadecanol is a common compound in the excrement of several mammals. In the Steenbok (*Raphicerus campestris*) (Burger et al., 1999), meerkat (*Suricata suricatta*) (Leclaire et al., 2017) it functions as a sex pheromone and in the caracal (*Caracal caracal*) (Goitom et al., 2009) and Bovidae (Shafi et al., 2021) it plays a role in oestrus signalling. The compound 1-Hexadecanol has also been identified in the avian species the Dark-eyed Junco (*Junco hyemalis carolinensis*) where it functions in kin recognition (Célérier et al., 2011) and in stimulating parental care (Mas and Kolliker, 2008).

The compound Phenol has also been identified to play different functions across different species. In the large tree nymph butterfly (*Idea leuconoe*) (Nishida et al., 1996), cabbage moth (*Mamestra brassicae*) (Jacquin et al., 1991), and water buffalo (*Bubalus bubalis*) (Brahmachary & Poddar-Sarkar, 2015) phenol plays a role in signalling sexuality. Phenol plays different roles in other speices, in cattle (*Bos Taurus*) (Sankar et al., 2007) it indicates oestrus, in the European badger (Noonan et al., 2019) it differentiates female reproductive state and in the African lion (*Panthera leo*) (Soso & Koziel, 2017) and Iberian Wolf (*Canis lupus signatus*) (Martín et al., 2010) it communicates age differentiation. The ubiquitous presence of some of these VOCs across several different mammal species could potentially indicate that the role they play in olfaction are not necessarily species specific (Burger, 2005). The other compounds listed in Table 4.2 that have been identified as playing a specific role in the behaviour of species, like the sex pheromone (Z)-9-

Tetradecenyl acetate, 7-Methylheptadecane and Zingiberenol could potentially play a similar role in the behaviour of African clawless otters. However, the precise role of these VOCs would need to be verified through behavioural bioassays. Through olfactive behavioural bioassays the responses elicited by particular VOCs can be determined through the animal's response to a stimulus and its sensory power. Olfactive behavioural bioassays were employed in the study by Zou et al. (2015), where mice were presented with ascending concentration of an odorant to measure olfactory function and behaviour.

4.5 Conclusion

The results obtained here indicate significant differences in the volatile scent signatures of the 14 African clawless otter's spraint samples collected, indicating that these compounds likely play a key role in their olfactory communication. Further investigations are required to ascertain the precise sources of variation between individual and group scent profiles, the individual scent cues and their role in coding for factors like sex, age, health and reproductive status.

CHAPTER V. CONCLUSIONS

5.1 Conclusions

The broad aim of this MSc study was to improve understanding on several aspects of African clawless otter behavioural ecology. To achieve this, three investigations were carried out assessing several aspects of otter behavioural ecology:

(1) The first chapter of this dissertation gives a broad background into small mammal research, followed by the aims and objectives of this study. Chapter I provides a comprehensive literature review on the major themes of this dissertation and on previous and ongoing research related to density, behavioural analyses, latrine site selection and VOC analysis.

(2) In chapter II population densities and active time of otters were assessed in an undisturbed natural area and in anthropogenically disturbed area. Additionally, this chapter assessed the scent-marking behaviour of otters at three latrine sites through camera trapping. The findings of this chapter indicate that African clawless otters can exploit both natural and anthropogenically disturbed habitats given that the population densities between the two sites were found to be very similar. The greater density recorded on the fish farm could be linked to the otters being attracted to the greater prey abundances and minimal persecution they experience from the farmers on the fish farm. The peak active time of the otters in this area being between the hours of 00h00 and 06h00 may indicate that they have adapted to being most active when there is minimal human activity and disturbance.

(3) The primary focus of the third chapter was to investigate aspects related to latrine site selection, behaviour associated with latrines. An analysis of latrine sites and control sites was conducted in both study areas to assess the factors that influence latrine site

selection by African clawless otters. In order to achieve this latrine and control sites were measured and assessed at two ecological spatial scales (micro and macroscale), in order to determine how likely the habitat features are to influence otter behaviour at latrine sites.

Studies assessing habitat selection and latrine site selection in African clawless otters in particular are rare, Chapter 3 is therefore an original and novel contribution to the behavioural ecology of this species. The results from this chapter reveal that African clawless otter's latrine sites were associated with areas containing little vegetative substrate cover and minimal canopy cover. The top-ranked models at the micro scale indicated that latrine sites were characterised as occurring in open areas with less canopy and horizontal cover on flatter areas that are relatively wind still. It is hypothesised that the selection of latrine site's locations with little vegetative cover, that are exposed to little wind speed likely aids in the retention of scent in the area and to facilitate detection by conspecifics.

(4) Lastly, the VOC profiles of African clawless otters were investigated to determine the composition of odour profiles and infer on the potential role of particular compounds. Faecal and anal gland secretions were analysed through gas chromatography mass spectrometry (Chapter IV). Studies investigating olfactory communication and scent communication in wild animal populations are rare. Chapter IV of this study is an original and novel contribution to the chemical ecology of African clawless otters with regards to the VOCS present in their faeces and anal gland secretions.

5.2 Implications and future research

The overall results of this research study have provided insights on African clawless otters in the following aspects of the behavioural ecology:

- Provided population density estimates and activity times of African clawless otters in a previously unresearched study area. Acquiring knowledge on population densities and activity time is important given the paucity of knowledge we have on these aspects of this species' ecology. Moreover, knowledge on population

densities provides vital information on population status, the effects of ecological and anthropogenic factors and in turn aids conservation assessments (Morin et al., 2020).

- Characterised and quantified the habitat parameters associated with latrine site selection. The findings of this study provide some insights into the likely social function that latrine sites play in African clawless otter behavioural ecology. Latrine sites are an important feature in otter ecology and provide an opportunity to relate latrine characteristics and habitat features to patterns of otter activity. The habitat features linked to latrine site selection have important consideration for conservation practices. Effective and efficient conservation and management practices can only be implemented through knowledge of scale specific processes. Given the rapidity of urbanisation, this knowledge is particularly important for conservation practitioners and urban planners to prioritise and conserve high priority species (Stevens et al., 2023; Crowley et al., 2012).

- Characterised the VOCs associated with African clawless otter latrines. The results reported here provide a platform for future research studies to assess the theoretical and practical applications of VOCs in olfactory communication. This information in combination with the data collected on: the spatial distribution of latrines, selection of latrine sites that benefit scent dispersal, the presence of overmarking at latrines, and the chemical make-up of AGS could all be interpreted to suggest that the major function of latrines is related to (a) intraspecific communication, likely associated with (b) reproduction/reproductive status and (c) territorial maintenance.

Future research studies should analyse how odour changes over time, and in turn assess the olfactory landscape of latrine sites and which compounds are responsible for differentiating and distinguishing the age, sex, health, and reproductive status of individuals, in order to create a complete understanding of the behavioural implications

and role of scent. The exploration of individual compounds and odour profiles were beyond the scope of this study but an important feature for future research on particularly small carnivorous mammals where olfactory communication plays an important role in their behavioural ecology. Moreover, future research studies should assess the location of latrine sites in relation to home ranges (and whether there are overlaps with female home ranges), territorial boundaries as well as the frequency of latrine site use. It would also be useful to link behavioural patterns of individual animals to individual latrine site use, this could aid in investigating the role of latrine site use and olfactory signals in any focal species (Buesching & Jordan, 2019). In addition to this, the adaptability and plasticity of African clawless otters should be investigated given the rapid and continued human encroachment and development along coastlines and riverbanks. Moreover, human-otter conflict is another research area that should be given attention, particularly in rural areas where fishing is a primary source of income and otters are regarded as competitors and pests.

REFERENCES

Albeke, S.E., Nibbelink, N.P., Mu, L. & Ellsworth, D.J. (2010). Measuring boundary convexity at multiple spatial scales using a linear “moving window” analysis: an application to coastal river otter habitat selection. *Landscape Ecology*, *25*(10), 1575-1587.

Alberts, A.C. (1992). Constraints on the design of animal communication systems in terrestrial vertebrates. *American Naturalist*, 139, S62eS89.

Albone, E.S. (1984). Mammalian Semiochemistry: The Investigation of Chemical Signals between Mammals. New York: Wiley.

Allen, M., Yovovich, V. & Wilmers, C. (2016). Evaluating the responses of a territorial solitary carnivore to potential mates and competitors. *Scientific Reports*, 6, 27257.

Allen, M.L., Gunther, M.S. & Wilmers, C.C. (2017). The scent of your enemy is my friend? The acquisition of large carnivore scent by a smaller carnivore. *Journal of Ethology*, 35, 13–19.

Allen, M.L., Wittmer, H.U., Houghtaling, P., Smith, J., Elbroch, L.M. & Wilmers, C.C. (2015). The role of scent marking in mate selection by female pumas (*Puma concolor*). *Plos One*, 10(10), e0139087.

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49, 227–267.

Andarge, E., Wube, T. & Balakrishnan, M. (2017). Diet analysis of the African clawless otter (*Aonyx capensis*) in and around Lake Tana. *Journal of Ecology and the Natural Environment*, 9(4), 53–61.

Andersen, K.F. & Vulpius, T. (1999). Urinary volatile constituents of the lion, *Panthera leo*. *Chemical Senses*, 24, 179–189.

Anile, S., Ragni, B., Randi, E., Mattucci, F. & Rovero, F. (2014). Wildcat population density on the Etna volcano, Italy: a comparison of density estimation methods. *Journal of Zoology*, 293, 252–261.

Anoop, K.R. & Hussain, S.A. (2004). Factors affecting habitat selection by smooth-coated otters (*Lutra perspicillata*) in Kerala, India. *Journal of Zoology*, 263, 417–423.

Aplin, L.M., Farine, D.R., Morand-Ferron, J. & Sheldon, B. C. (2012). Social networks predict patch discovery in a wild population of songbirds. *Proceedings of the Royal Society B: Biological Sciences*, 279(1745), 4199e4205.

Apps, P.J., Viljoen, H.W., Pretorius, V. & Rohwer, E.R. (2015). Volatile components of the anal gland secretion of the striped polecat *Ictonyx striatus*. *South African Journal of Zoology*, 23(2), 136–137.

Arakawa, H., Blanchard, D.C., Arakawa, K., Dunlap, C. & Blanchard, R.J. (2008). Scent marking behavior as an odorant communication in mice. *Neuroscience & Biobehavioral Reviews*, 32(7), 1236-48.

Arden-Clarke, C.H.G. (1986). Population density, home range size and spatial organisation of the Cape clawless otter, *Aonyx capensis*, in a marine habitat. *Journal of Zoology London*, 209, 201–211.

Aristizábal Duque, S.L., Orozco-Jiménez, L.Y., Zapata-Escobar, C. & Palacio-Baena, J.A. (2018). Conservation genetics of otters: Review about the use of non-invasive samples. *Therya*, 9(1), 85–93.

Arrendal, J., Vila, C. & Björklund, M. (2007). Reliability of noninvasive genetic census of otters compared to field censuses. *Conservation Genetics*, 8(5), 1097–1107.

Avolio, M. L., Forrestel, E. J., Chang, C. C., La Pierre, K. J., Burghardt, K. T., & Smith, M. D. (2019). Demystifying dominant species. *New Phytologist*, 223(3), 1106–1126.

Balakrishnan, M. & Alexander, K.M. (1985). Sources of body odour and olfactory communication in some Indian mammals. *Indian Review Life Science*, 5, 277–313.

Balestrieri, A., Aritz Ruiz-González, A., Vergara, M., Capelli, E., Tirozzi, P., Alfino, S. et al. (2016) Pine marten density in lowland riparian woods: a test of the random encounter model based on genetic data. *Mammalian Biology*, 81, 439–446

Bantihun, G. (2018). Animal Scent Marking. Adama Science and Technology University, Ethiopia. MedCrave Group LLC. pp. 3.

Barlow, J. & Taylor, B.L. (2005). Estimates of sperm whale abundance in the northeastern temperate Pacific from a combined acoustic and visual survey. *Marine Mammal Science*, 21, 429–445.

Barocas, A., Golden, H.N., Dudenhoeffer, M. & Ben-David, M. (2021). Sociality and signaling activity modulate information flow in river otter communication networks. *Behavioral Ecology*, 32(1), 60–68.

Barocas, A., Golden, H.N., Harrington, M.W., McDonald, D.B. & Ben-David, M. (2016). Coastal latrine sites as social information hubs and drivers of river otter fission-fusion dynamics. *Animal Behaviour*, 120, 103–114.

Barocas, A., Golden, H.W., Dudenhoeffer, M. & Ben-David, M. (2021). Sociality and signaling activity modulate information flow in river otter communication networks. *Behavioral Ecology*, 32(1), 60–68.

Barrett, D.A. (2014). Status and population characteristics of the northern river otter (*lontra canadensis*) in central and eastern Oklahoma. MSc thesis, Southeastern Oklahoma State University.

Barton, K. (2020). MuMIn: Multi-model inference (R package). Available at https:// cran.r-project.org/package=MuMIn [verified 18 May 2022].

Beckmann, J.P. & Berger, J. (2003). Rapid ecological and behavioural changes in carnivores: the responses of black bears (*Ursus americanus*) to altered food. *Journal of Zoology*, 261, 207–212.

Begg, C.M., Begg, K., du Toit, J. & Mills, M. (2003). Scent-marking behaviour of the honey badger, *Mellivora capensis* (Mustelidae), in the southern Kalahari. *Animal Behaviour*, 66, 917–929.

Beja-Pereira, A., Oliveira, R., Alves, P.C., Schwartz, M.K. & Luikart, G. (2009). Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources*, 9(5), 1279–1301.

Ben-David, M., Blundell, G.M., Kern, J.W., Maier, J.A.K., Brown, E.D., Jewett, S.C. (2005). Communication in river otters: Creation of variable resource sheds for terrestrial communities. *Ecology*, 86(5), 1331–1345.

Ben-David, M., Bowyer, R.T., Duffy, L.K., Roby, D.D. & Schell, D.M. (1998). Social behavior and ecosystem processes: river otter latrines and nutrient dynamics of terrestrial vegetation. *Ecology*, 79, 2567-2571.

Berzins, R. & Helder, R. (2008). Olfactory communication and the importance of different odour sources in the ferret (*Mustela putorius f. furo*). *Mammalian Biology*, 73, 379–387.

Bininda-Emonds, O.R.P., Gittleman, J.L. & Purvis, A. (1999). Building large trees by combining phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia). *Biological Reviews*, 74, 143–175.

Birks, J., Messenger, J., Braithwaite, T., Davison, A., Brookes, R. & Strachan, C. (2005). Are scat surveys a reliable method for assessing distribution and population status of pine martens? *Martens and fishers (Martes) in human-altered environments: an international perspective*, 235-252.

Black-Decima, P. & Santana, M. (2011). Olfactory communication and counter-marking in brown brocket deer *Mazama gouazoubira*. *Acta Theriologica*, 56(2), 179–187.

Blassioli-Moraes, M.C., Khrimian, A., Michereff, M.F.F., Magalhães, D.M., Hickel, E., de Freitas, T.F.S., Barrigossi, J.A.F., Laumann, A., Silva, A.T., Guggilapu, S.D., Silva, C.C., Sant'Ana, J. & Borges, M. (2020). Male-produced sex pheromone of T*ibraca limbativentris* revisited:absolute configurations of zingiberenol stereoisomers and their influence on chemotaxis behavior of conspecific females. *Journal of Chemical Ecology*, 46, 1–9.

Blaustein, A.R. (1981). Sexual selection and mammalian olfaction. *The American Naturalist*, 117, 1006–1010.

Bluett, R, & Hubert, G.Jr. (1995). Background. In: Illinois River Otter Recovery Plan (Bluett, R., ed). Illinois Department of Natural Resources, Springfield, IL, USA. pp. 1–22.

Blundell, G.M., Ben-David, M. & Bowyer, R.T. (2002). Sociality in river otters, cooperative foraging or reproductive strategies? *Behavioral Ecology*, 13, 134–141.

Bonesi, L., Hale, M. & Macdonald, D.W. (2013). Lessons from the use of non-invasive genetic sampling as a way to estimate Eurasian otter population size and sex ratio. *Acta Theriologica*, *58*(2), 157–168.

Borchers, D.L. & Efford, M.G. (2008). Spatially explicit maximum likelihood methods for capture-recapture studies. *Biometrics*, 64, 377–385.

Bosholn, M., & Anciães, M. (2018). Focal animal sampling. *Encyclopedia of Animal Cognition and Behavior*, 1-3.

Bossert, W.H. & Wilson, E.O. (1963). The analysis of olfactory communication among animals. *Journal of Theoretical Biology*, 5, 443–469.

Bouros, G., Ionescu, D.T. & Hodor, C. (2016). Observation of Eurasian otter's diel activity using camera trapping in Central-eastern Romania. *Vestnik Zoologii*, 53(1), 47–56.

Bowyer, R.T. & Testa, J.W. & Faro, J.B. (1995). Habitat selection and home ranges of river otters in a marine environment: effects of the Exxon Valdez oil spill. *Journal of Mammalogy*, 76, 1–11.

Bradbury, J.W. & Vehrencamp, S.L. (1998). Principles of Animal Communication. 2nd edn. Sinauer Associates, Sunderland, Massachusetts, USA.

Brahmachary, R. & Poddar-Sarkar, M. (2015). Fifty years of tiger pheromone research. *Current Science*, 108, 2178–2185.

Brand, A-F., Hynes, J., Walker, L.A., Pereira, M.G., Lawlor, A.J., Williams, R.J., Shore, R.F. & Chadwick, E.A. (2020). Biological and anthropogenic predictors of metal

concentration in the Eurasian otter, a sentinel of freshwater ecosystems. *Environmental Pollution*, 266, 115280

Brennan, P.A. & Kendrick, K.M. (2006). Mammalian social odours: attraction and individual recognition. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361(1476), 2061–2078.

Brinck, C., Erlinge, S. & Sandell, M. (1983). Anal sac secretion in mustelids: a comparison. *Journal of Chemical Ecology*, 9, 727–745.

Brinck, C., Gerell, R. & Odham, G. (1978). Anal pouch secretion in mink, *Mustela vison*. *Oikos*, 38, 68–75.

Brotherton, P.N.M. (1994). The evolution of monogamy in the dik-dik. Ph.D. thesis, Cambridge University.

Brown, L. R., Bezuidenhout, H., Du Preez, P. J., Bredenkamp, G. J., & Mostert, T. H. (2013). Guidelines for phytosociological classifications and descriptions of vegetation in southern Africa: checklist. *Koedoe: African Protected Area Conservation and Science*, *55*(1), 1-10.

Brown, R.E. & Macdonald, D.W. (1985). *Social Odours in Mammals*. Oxford University Press, Oxford.

Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J.L., Borchers, D.L., & Thomas, L. (2001). Introduction to distance sampling: Estimating abundance of biological populations (1st ed.). Oxford University Press.

Buesching, C. & Stankowich, T. (2017). Biology and conservation of the musteloids (badgers, otters, skunks, raccoons and their kin). In Communication amongst the musteloids: signs, signals, and cues. Oxford University Press. pp. 149–166.

Buesching, C. D., & Jordan, N. R. (2022). The function of carnivore latrines: review, case studies, and a research framework for hypothesis testing. *Small Carnivores: Evolution, Ecology, Behaviour, and Conservation*, 131–171.

Buesching, C.D. & Jordan, N. (2019). The social function of latrines: a hypothesis-driven research approach. Springer. pp. 94–103.

Buesching, C.D. & Macdonald, D.W. (2001). Scent-marking behaviour of the European badger (*Meles meles*): resource defence or individual advertisement? In Chemical signals in vertebrates. Springer, Boston, MA. pp. 321-327.

Buesching, C.D., Newman, C., Service, K., Macdonald, D.W., Riordan, P. & Parmenter, R.R. (2016). Latrine marking patterns of badgers (*Meles meles*) with respect to population density and range size. *Ecosphere*, 7(5), e01328.

Buesching, C.D., Stopka, P. & MacDonald, D.W. (2003). The social function of allo-marking in the European badger (*Meles meles*). *Behaviour*, 140(8), 965–980.

Buesching, C.D., Waterhouse, J.S. & Macdonald, D.W. (2002). Gas-chromatographic analyses of the subcaudal gland secretion of the European badger (*Meles meles*) part I: chemical differences related to individual parameters. *Journal of Chemical Ecology*, 28, 41–56.

Bundy, S. & Whitehead, A. (n.d.). Indian ocean coastal belt biome. Strategic environmental assessment for the expansion of electricity grid infrastructure in South Africa. [Web:] https://gasnetwork.csir.co.za/wp-content/uploads/2019/04/3.4.3.-Annexure_IOCB_EGI_Final_150419.pdf, [Date of access: 10 June 2022]

Burger, B.V. (2005). Mammalian semiochemicals. *Topics in Current Chemistry*, 240, 231–278.

Burger, B.V., Greyling, J., & Spies, H.S.C. (1999). Mammalian exocrine secretions. XIV: Constituents of preorbital secretion of steenbok, *Raphicerus campestris*. *Journal of cCemical Ecology*, 25(9), 2099–2108.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference: A practical information-theoretic approach. 2nd ed., Springer. pp. 512.

Burnham, K.P. & Anderson, D.R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods and Research*, 33, 261–304.

Butler, J.R.A. & du Toit, J.T. (1994). Diet and conservation status of Cape clawless otters in eastern Zimbabwe. *South African Journal of Wildlife Research*, 24, 41.

Butler, J.R.A. & Marshall, B.E. (1996). Resource use within the crab-eating guild of the upper Kairezi River, Zimbabwe. *Journal of Tropical Ecology*, 12, 475–490.

Butler, J.R.A. (1994). Cape clawless otter conservation and a trout river in Zimbabwe: A case study. *Oryx*, 28, 276–282.

Byers, J.A. (2005). Chemical constraints on the evolution of olfactory communication channels of moths. *Journal of Theoretical Biology*, 235, 199–206.

Caravaggi, A. (2017). remBoot: an R package for random encounter modelling. *Journal of Open Source Software*, 2(10), 176.

Caravaggi, A., Banks, P.B., Burton, A.C., Finlay, C.M.V., Haswell, P.M., Hayward, M.W., Rowcliffe, M.J. & Wood, M.D. (2017). A review of camera trapping for conservation behaviour research. *Remote Sensing in Ecology and Conservation*, 3(3), 109–122.

Carter, S.K. & Rosas, F.C.W. (1997). Biology and conservation of the Giant *Otter Pteronura brasiliensis*. *Mammal Review*, 27(1), 1–26.

Carthey, A.J., Gillings, M.R. & Blumstein, D.T. (2018). The extended genotype: microbially mediated olfactory communication. *Trends in Ecology & Evolution*, 33(11), 885–894.

Carugati, C. & Perrin, M.R. (2006). Abundance estimates of the Cape clawless otter *Aonyx capensis* (Schinz 1821) and the spotted necked otter *Lutra maculicollis* (Lichtenstein 1835) in the KwaZulu-Natal Drakensberg, South Africa. *Tropical Zoology,* 19, 9–19.

Célérier, A., Bon, C., Malapert, A., Palmas, P. & Bonadonna, F. (2011). Chemical kin label in seabirds. *Biological Letters*, 7, 807–810.

Challender, D.W.S., Nash, H.C., & Waterman, C. (2020). Pangolins, Science, Society and Conservation. In Biodiversity of World: Conservation from Genes to Landscapes, Pangolins. Academic Press. pp. 615–630.

Chehebar, C. E. (1985). A survey of the southern river otter *Lutra provocax* Thomas in Nahuel Huapi National Park, Argentina. *Biological Conservation*, 32, 299–307.

Cho, H.S., Choi, K.H., Lee, S.D. & Park, Y. (2009). Characterizing habitat preference of Eurasianriver otter (*Lutra lutra*) in streams using a self-organizing map. *Limnology*, 10, 203–213.

Clapperton, B.K. (1989). Scent-marking behaviour of the ferret, *Mustela furo L*. *Animal Behaviour*, 38, 436–446.

Colloty, B.M., Adams, J.B. & Bate, G.C. (2002). 'Classification of estuaries in the Ciskei and Transkei regions based on physical and botanical characteristics. *South African Journal of Botany*, 68, 312–321.

Corlatti, L., Sivieri, S., Sudolska, B., Giacomelli, S. & Pedrotti, L. (2020). A field test of unconventional camera trap distance sampling to estimate abundance of marmot populations. *Wildlife Biology*, 2020(4), wlb.00652

Crowley, S., Johnson, C.J. & Hodder. D. (2012). Spatial and behavioral scales of habitat selection and activity by river otters at latrine sites. *Journal of Mammalogy*, 93(1), 170–182.

Crump, D.R. & Moors, P.J. (1985). Anal gland secretions of the stoat (*Mustela erminea*) and the ferret (*Mustela putorius furo*): some additional thietane components. *Journal of Chemical Ecology*, 8, 1037–1043.

Cusack, J.J., Dickman, A.J., Rowcliffe, J.M., Carbone, C., Macdonald, D.W. & Coulson, T. (2015a) Random versus game trail-based camera trap placement strategy for monitoring terrestrial mammal communities. *PLOS ONE*, 10, e0126373.

Cusack, J.J., Swanson, A., Coulson, T., Packer, C., Carbone, C., Dickman, A.J., Kosmala, M., Lintott, C. & Rowcliffe, M.J. et al. (2015b). Applying a random encounter model to estimate lion density from camera traps in Serengeti National Park, Tanzania: density estimation of Serengeti lions. *The Journal of Wildlife Management*, 79, 1014–1021.

Das, S., Manna, S., Ray, S., Das, P., Rai, U., Ghosh, B. & Sarkar, M.P. (2019). Do urinary volatiles carry communicative messages in Himalayan snow leopards [*Panthera uncia*, (Schreber, 1775)]? *Chemical Signals in Vertebrates*, 14, 27–37.

Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., Vere, N., Pfrender, M.E. & Bernatchez, L. (2017). Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology*, 26, 5872–5895.

DeLong, C.M., Wright, C., Fobe, I., Wilcox, K.T. & Morrison, E. (2019). North American river otters (*Lontra canadensis*) discriminate between 2D objects varying in shape and color. *Learning & Behavior*, 47, 91–104.

Depue, J.E. & Ben-David, M. (2010). River Otter Latrine Site Selection in Arid Habitats of Western Colorado, USA. *Journal of Wildlife Management*, 74(8), 1763–1767.

Dias, S.J., White, P.J.C., Borker, A.S. & Fernandes, N.V. (2022). Habitat selection of smooth coated otters (*Lutrogale perspicillata*) in the peri coastal, urbanised landscape of Goa, India. *Mammal Research*, 67, 299–309.

Douglas Leclaire, S., Jacob, S., Greene, L. K., Dubay, G. R., & Drea, C. M. (2017). Social odours covary with bacterial community in the anal secretions of wild meerkats. *Scientific Reports*, 7(1), 1–13

Douglas, M. (2003). How can we apply theories of habitat selection to wildlife conservation and management? *Wildlife Research*, 30(4), 303-319.

Dröscher, I. & Kappeler, P.M. (2014). Maintenance of familiarity and social bonding via communal latrine use in a solitary primate (*Lepilemur leucopus*). *Behavioural Ecology and Sociobiology*, 68, 2043–2058.

Dubuc, L.J., W. B. Krohn, & R.B. Owen, Jr. (1990). Predicting occurrence of river otters by habitat on Mount Desert Island, Maine. *Journal of Wildlife Management*, 54, 594–599.

Duff, C.M., Gries, G., Mori, K., Shirai, Y., Seki, M., Takikawa, H., Sheng, T., Slessor, K.N., Gries, R., Maier, C.T., & Ferguson, D.C. (2001). Does pheromone biology of *Lambdina athasaria* and *L. pellucidaria* contribute to their reproductive isolation? *Journal of Chemical Ecology*, 27(3), 431–442.

Dunbar, I. F. (1977). Olfactory preferences in dogs: the response of male and female beagles to conspecific odours. *Behavioral Biology*, 20, 471–481.

Duplaix, N. (1980). Observation on the ecology and behavior of the giant river otter *Pteronura brasiliensis* in Suriname. *La Terre et la Vie – Revue d'écologie*, 34(4), 496–620.

Edwards, E. (1983). A broad-scale structural classification of vegetation for practical purposes. *Bothalia*, *14*(3/4), 705–712.

Efford, M.G. & Fewster, R.M. (2013). Estimating population size by spatially explicit capture-recapture. *Oikos*, 122, 918–928.

Eisenberg, J.F. & Kleiman, D.G. (1972). Olfactory communication in mammals. *Annual Review of Ecology and Systematics*, 3, 1–32.

ENETWILD consortium, Grignolio, S., Apollonio, M., Brivio, F., Vicente, J., Acevedo, P., Petrovic, K. & Keuling, O. (2020). Guidance on estimation of abundance and density data of wild ruminant population: methods, challenges, possibilities. *EFSA Supporting Publications*, 17(6), 1876E.

Erlinge, S. (1967). Home range of the otter *Lutra lutra L.* in southern Sweden. *Oikos*, 18, 186–209.

Erlinge, S. (1974). Distribution, territoriality and numbers of the weasel *Mustela nivalis* in relation to prey abundance. *Oikos*, 25, 308–314.

Erlinge, S. (1977). Spacing strategy in stoat, *Mustela erminea*. *Oikos*, 28, 32–42.

Erlinge, S., Sandell, M. & Brinck, C. (1982). Scent-marking and its territorial significance in stoats, *Mustela erminea*. *Animal Behaviour*, 30, 811–818.

Espirito-Santo, C., Rosalino, L. & Santos-Reis, M. (2007). Factors affecting the placement of common genet latrine sites in a Mediterranean landscape in Portugal. *Journal of Mammalogy*, 88, 201–207.

Esterhuizen, J. (2019). Coastline dynamics at Thukela Mouth and the Amatigulu and uMlalazi river estuary areas, KwaZulu-Natal. MSc thesis, University of Pretoria.

Estes, R.D. (1991). The behavior guide to African mammals. University of California Press, California, USA. pp. 419–424; 441–445.

Ferkin, M.H. & Johnston, R.E. (1995). Meadow voles, *Microtus pennsylvanicus*, use multiple sources of scent for sex recognition. *Animal Behaviour*, 49, 37–44.

Ferkin, M.H., Sorokin, E.S., Johnston, R.E. & Lee, C.J. (1997). Attractiveness of scents varies with protein content of the diet in meadow voles. *Animal Behaviour*, 53(1), 133–141.

Ferrero, D.M. & Liberles, S.D. (2010). The secret codes of mammalian scents. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 2(1), 23–33.

Foster-Turley, P. (1992). Conservation ecology of sympatric Asian otters *Aonyx cinerea* and *Lutra perspicillata*. PhD Thesis, University of Florida.

Francke, W. & Schulz, S. (1999). Pheromones. In: Comprehensive Natural Products Chemistry. Barton, D., Nakanishi, K. & Meth-Cohn, O. (eds.). pp.197–261.

Freitas, D., Gomes, J., Sales-Luis, T., Madruga, L., Marques, C., Baptista, G., Rosalino, L.M., Antunes, P., Santos, R. & Santos-Reis, M. (2007). Otters and fish farms in the Sado estuary: ecological and socio-economic basis of a conflict. *Hydrobiologia*, 587, 51–62.

Frey, S., Fisher, J.T., Burton, A.C. & Volpe, J.P. (2017). Investigating animal activity patterns and temporal niche partitioning using camera-trap data: Challenges and opportunities. *Remote Sensing in Ecology and Conservation*, *3*(3), 123-132.

G.J. Retallack,G.J. (2005). Soils. In Encyclopedia of Geology. Selley, R.C., Cocks, L.R. M. & Plimer, I. R. (eds.). Elsevier Academic. pp. 194-202.

Gallant, D. (2007). Species-wise disparity in scientific knowledge about otters: An obstacle to optimal management and conservation actions? *IUCN Otter Specialist Group Bulletin*, 24, 5–13.

Gallant, D. Vasseur, L., Dumond, M., Tremblay, E. & Bérubé, C.H. (2009). Habitat selection by river otters (*Lontra canadensis*) under contrasting land-use regimes. *Canadian Journal of Zoology*, 87(5), 422–432.

Gallant, D., Vasseur, L. & Berube, C.H. (2007). Unveiling the limitations of scat surveys to monitor social species: a case study on river otters. *The Journal of Wildlife Management*, 71(1), 258-265.

Garber, P.A. (1989). Role of spatial memory in primate foraging patterns: *Saguinus mystax* and *Saguinus fuscicollis*. *American Journal of Primatology*, 19, 203–216.

Geist, V. & Walther, F. (1974). Behaviour of ungulates and its relation to management. In Symposium on the Behavior of Ungulates and Its Relation to Management (1971: Calgary, Alta.). International Union for Conservation of Nature and Natural Resources.

Gese, E.M. & Ruff, R.L. (1997). Scent-marking by coyotes, *Canis latrans*: the influence of social and ecological factors. *Animal Behaviour*, 54, 1155–1166.

Gil-Sánchez, J.M. & Antorán-Pilar, E. (2020). Camera-trapping for abundance estimation of otters in seasonal rivers: a field evaluation. *European Journal of Wildlife Research*, 66, 72.

Gittleman, J.L. (1989). Carnivore Behavior, Ecology and Evolution. Vol. II. New York: Cornell University Press, McNutt.

Goitom, A.H. (2017). Identification of the putative urinary intraspecific recognition pheromone of the caracal *Caracal caracal*. PhD Theisis, University of Stellenbosch, South Africa.

Goloran, A.B., Demetillo, M.T. & Betco, G.L. (2020). Mangroves Assessment and Diversity in Coastal Area of Barangay Cagdianao, Claver, Surigao Del Norte, Philippines. *International Journal of Environmental Sciences & Natural Resources*, 26(3), 82-90.

Gómez, H, Wallace, R.B., Ayala, G. & Tejada, R. (2005). Dry season activity periods of some Amazonian mammals. *Studies on Neotropical Fauna and Environment*, E 40, 91–95.

Google Earth Pro 7.3. (2021). uMlalazi Nature Reserve. 28°57' 29.53" S,31°46'09.75" E, elevation 100 m. [Web:] https://earth.google.com/web/@-28.95947999,31.76763576,1018.91157481a,0d,35y,0.0121h,38.7693t,-0.0084r?utm_source=earth7&utm_campaign=vine&hl=en, [Date of access: 22 September 2021]

Gorman, M.L. & Mills, M.G.L. (1984). Scent marking strategies in hyaenas (Mammalia). *Journal of Zoology*, 202, 535–547.

Gorman, M.L. & Trowbridge, B. (1989). The role of odor in the social lives of carnivores. In: Carnivore Behavior, Ecology and Evolution. (Gittleman, J.L., ed.). Ithaca, New York: Cornell University Press. pp. 57–88.

Gosling, L.M. & McKay, H.V. (1990). Competitor assessment by scent matching: an experimental test. *Behavioral Ecology and Sociobiology*, 26, 415–420.

Gosling, L.M. & Roberts, S.C. (2001). Testing ideas about the function of scent marks in territories from spatial patterns. *Animal Behaviour*, 62, F7–F10.

Gosling, L.M. (1982). A reassessment of the function of scent marking in territories. *Zeitschrift für Tierpsychologie*, 60, 89–118.

Gosling, L.M. (1990). Scent Marking by Resource Holders: Alternative Mechanism for Advertising the Costs of Competition. Oxford University Press, Oxford.

Gosling, L.M. (2010). A reassessment of the function of scent marking in territories. *Zeitschrift für Tierpsychologie*, 60, 89–118.

Green, A.M., Chynoweth, M.W. & Şekercioğlu, Ç. (2020). Spatially Explicit Capture-Recapture Through Camera Trapping: A Review of Benchmark Analyses for Wildlife Density Estimation. *Frontiers in Ecology and Evolution*, 8. [Web:] https://doi.org/10.3389/fevo.2020.563477, [Date of access: 8 March 2022]

Green, M.L., Monick K., Manjerovic M.B., Novakofski J. & Mateus-Pinilla N. (2015). Communication stations: cameras reveal river otter (*Lontra canadensis*) behaviour and activity patterns at latrines. *Journal of Ethology*, 33, 225–234.

Green, S.E., Rees, J.P., Stephens, P.A., Hill, R.A. & Giordano, A.J. (2020). Innovations in Camera Trapping Technology and Approaches: The Integration of Citizen Science and Artificial Intelligence. *Animals*, 10(1), 132. https://doi.org/10.3390/ani10010132

Groenendijk, J. (2019). The Giant Otter: Giants of the Amazon. Pen and Sword.

Hájková, P., Zemanová, B., Roche, K. & Hájek, B. (2009). An evaluation of field and noninvasive genetic methods for estimating Eurasian otter population size. *Conservation Genetics*, 10, 1667–1681.

Hammerstein, P. (1981). The role of asymmetries in animal contests. *Animal Behaviour*, 29, 193–205.

Hanrahan, A.T., Rutter, A.U., Nielsen, C.K. & Schauber, E.M. (2019). Spatial ecology of river otters in a human-modified landscape. *Journal of Mammalogy*, 100(4), 1327–1339.

Harding, J. (2017). Cape Clawless Otter. Ocean Research Conservation Africa – ORCA. [Web:] http://orcafoundation.com/2017/08/cape-clawless-otter/#:~:text=Cape%20clawless%20otters%20are%20predominantly,food%20supply%20in%20their%20environment.&text=During%20this%20time%20they%20will,but%20also%20fish%20and%20amphibians., [Date of access: 19 April 2021]

Harmsen, B.J., Foster, R.J., Gutierrez, S.M., Marin, S.Y. & Doncaster, C.P. Doncaster. (2010). Scrape-marking behaviour of jaguars (*Panthera onca*) and pumas (*Puma concolor*). *Journal of Mammalogy*, 91, 1225–1234.

Harris, R.L., Davies, N.W., & Nicol, S.C. (2012). Chemical composition of odorous secretions in the Tasmanian short-beaked echidna (*Tachyglossus aculeatus setosus*). *Chemical Senses*, 37(9), 819–836.

Hazen, E.L., Abrahms, B., Brodie, S., Carroll, G., Jacox, M.G., Savoca, M.S., Scales, K.L., Sydeman, W.J. & Bograd, S.J. (2019). Marine top predators as climate and ecosystem sentinels. *Frontiers in Ecology and the Environment*, 17(10), 565–574.

Head, J.S., Robbins, M.M., Mundry, R., Makaga, L. & Boesch, C. (2012). Remote video-camera traps measure habitat use and competitive exclusion among sympatric chimpanzee, gorilla and elephant in Loango National Park, Gabon. *Journal of Tropical Ecology*, 28(6), 571–583.

Hediger, H. (1949). Säugetier-Territorien und ihre Markierung. *Bijdragen tot de Dierkunde,* 28, 172–184.

Hough, R., Archer, D. & Probert, C.A. (2018). Comparison of sample preparation methods for extracting volatile organic compounds (VOCs) from equine faeces using HS-SPME. *Metabolomics*, 14, 19.

Huang, Y., Honda, H., Yoshiyasu, Y., Hoshizaki, S., Tatsuki, S. & Ishikawa, Y. (1998). Sex pheromone of the butterbur borer, *Ostrinia zaguliaevi*. *Entomologia Experimentalis et Applicata*, 89, 281–287.

Hufkens, K., Scheunders, P., & Ceulemans, R. (2009). Ecotones in vegetation ecology: methodologies and definitions revisited. *Ecological Research*, 24(5), 977-986.

Humphrey, S.R. & Zinn, T.L. (1982). Seasonal habitat use by river otters and everglades mink in Florida. *Journal of Wildlife Management*, 46, 375–381.

Hung, C.M., Li, S.H. & Lee, L.L. (2004). Faecal DNA typing to determine the abundance and spatial organisation of otters (*Lutra lutra*) along two stream systems in Kinmen. *Animal Conservation*, 7, 301–311.

Hussain, S.A., Gupta, S.K. & de Silva, P.K. (2011). Biology and ecology of Asian small-clawed Otter *Aonyx cinereus*. Illiger, 1815, a review. *IUCN Otter Specialist Group Bulletin*, 28, 63–75.

Hutchings, M.R. & White P.C.L. (2000). Mustelid scent-marking in managed ecosystems: implications for population management. *Mammal Review*, 30, 157–169.

Hutchings, M.R., Kyriazakis, I. & Gordon, I.J. (2001). Herbivore physiological state affects foraging trade-off decisions between nutrient intake and parasite avoidance. *Ecology*, 82, 1138–1150.

Hutchinson, J.M.C. & Waser, P.M. (2007). Use, misuse and extensions of 'ideal gas' models of animal encounter. *Biological Reviews of the Cambridge Philosophical Society*, 82, 335–359.

Integrated Taxonomic Information System (ITIS). (2021). *Aonyx capensis* (Schinz, 1821). [Web:] https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=621911#null, [Date of access: 18 April 2021]

International Otter Survival Fund (IOSF). (n.d.). African Otters. [Web:] https://www.otter.org/public/AboutOtters_AfricanOtters.aspx, [Date of access: 5 November 2021]

Irwin, M.T., Samonds, K.E., Raharison, J. & Wright, P.C. (2004). Lemur latrines: observations of latrine behavior in wild primates and possible ecological significance. *Journal of Mammalogy*, 85(3), 420–427.

Ishikawa, Y., Takanashi, T. & Huang, Y. (1999). Comparative studies on the sex pheromones of *Ostrinia* spp. in Japan: The burdock borer, *Ostrinia zealis*. *Chemoecology*, 9, 25–32.

Jacques, H., Reed-Smith, J. & Somers, M.J. (2015). *Aonyx capensis*. The IUCN Red List of Threatened Species, 2015: e.T1793A21938767

Jacques, H., Véron, G., Allary, F. & Aulagnier, S. (2009). The Congo clawless otter *Aonyx congicus* Mustelidae, Lutrinae, a review of its systematics, distribution and conservation status. *African Zoology*, 44, 159–170.

Jacquin, E., Nagnan, P. & Frerot, B. (1991). Identificaiton of hairpencil secretion from male *mamestra-brassicae* (L) (*Leppidoptera, nostuidae*) and electroantennogram studies. *Journal of Chemical Ecology*, 17, 239–246.

Janssen, E., Hölldobler, B., & Bestmann, H. J. (1999). A trail pheromone component of the African stink ant, *Pachycondyla* (*Paltothyreus*) *tarsata* Fabricius (Hymenoptera: Formicidae: Ponerinae). *Chemoecology*, 9(1), 9–11.

Janssenswillen, S., Roelants, K., Carpentier, S., de Rooster, H., ` Metzemaekers, M., Vanschoenwinkel, B., Proost, P. & Bossuyt, F. (2021). Odorant-binding proteins in canine anal sac glands indicate an evolutionarily conserved role in mammalian chemical communication. *BMC Ecology and Evolution*, 21, 182.

Jayasekara, D., Dharshani Mahaulpatha, D. & Miththapala, S. (2021). Population density estimation of meso-mammal carnivores using camera traps without the individual recognition in Maduru Oya National Park, Sri Lanka. *Hystrix, the Italian Journal of Mammalogy*, 32(2), 137–146.

Jefferies, D.J. (1986). The value of otter *Lutra lutra* surveying using spraints: an analysis of its successes and problems in Britain. *Otters: the Journal of the Otter Trust*, 1, 25–32.

Jemiolo, B., Harvey, S. & Novotny, M. (1986). Promotion of the Whitten effect in female mice by synthetic analogs of male urinary constituents. *Proceedings of the National Academy of Sciences*, 83, 4576-4579.

Jensen, P.O., Wirsing, A.J. & Thornton, D.H. (2022). Using camera traps to estimate density of snowshoe hare (*Lepus americanus*): a keystone boreal forest herbivore. *Journal of Mammalogy*, 103(3), 693–710.

Jo, Y. & Won, C. (2020). The sprainting behavior and habitat preference of the Eurasian otter *Lutra lutra* along a montane stream in South Korea. *Mammal Study*, 45. doi: 10.3106/ms2018-0071

Johnson, C.J., Nielsen, S.E., Merrill, E.H., McDonald, T.L. & Boyce, M.S. (2006). Resource selection functions based on use – availability data: theoretical motivation and evaluation methods. *Journal of Wildlife Management*, 70, 347–357.

Johnson, C.J., Parker, K.L., Heard, D.C. & Gillingham, M.P. (2002). Movement parameters of ungulates and scale-specific responses to the environment. *Journal of Animal Ecology*, 71, 225–235.

Johnson, D., Macdonald, D. & Dickman, A. (2000). An analysis and review of models of the sociobiology of the Mustelidae. *Mammal Review*, 30, 171–196.

Johnson, R.P. (1973). Scent marking in mammals. *Animal Behaviour*, 21, 521–535.

Johnston, R.E. (2008). Individual odors and social communication: individual recognition, kin recognition, and scent over-marking. *Advances in the Study of Behavior*, 38, 439–505.

Johnston, R.E., Chaing, G. & Tung, C. (1994). The information in scent over-marks of golden hamsters. *Animal Behaviour*, 48, 323e330.

Jordaan, R.K., Somers, M.J. & McIntyre, T. (2017). Dancing to the message: African clawless otter scent marking behaviour. *Hystrix, the Italian Journal of Mammalogy*. doi:10.4404/hystrix-28.2-12264

Jordaan, R.K., Somers, M.J., Hall, G. & McIntyre, T. (2019). Plasticity and specialisation in the isotopic niche of African clawless otters foraging in marine and freshwater habitats. *Mammalian Biology - Zeitschrift fur Saugetierkunde*. doi: 10.1016/j.mambio.2019.07.00

Jordan, N. R., Mwanguhya, F., Kyabulima, S., Rüedi, P., & Cant, M. A. (2010). Scent marking within and between groups of wild banded mongooses. *Journal of Zoology*, 280(1), 72–83.

Jordan, N.R. (2007). Scent-marking investment is determined by sex and breeding status in wild meerkats. *Animal Behaviour*, 74, 531e540.

Jordan, N.R., Cherry, M.I. & Manser, M.B. (2007). Latrine distribution and patterns of use by wild meerkats: implications for territory and mate defence. *Animal Behaviour*, 73, 613e622.

Jordan, N.R., Manser, M.B., Mwanguhya, F., Kyabulima, S., Rüedi, P. & Cant, M.A. (2011). Scent marking in wild banded mongooses: 1. Sex-specific scents and overmarking. *Animal Behaviour*, 81, 31–42.

Joubert, D., Powell, L.A. & Schacht, W.H. (2014). Visual obstruction as a method to quantify herbaceous biomass in southern African semi-arid savannas. *African Journal of Range & Forage Science*. doi: 10.2989/10220119.2014.919960

Kanchanasaka, B.K. (2001). Tracks and other signs of the hairy-nosed otter (*Lutra sumatrana*). *IUCN Otter Specialist Group Bulletin*, 18, 57–63.

Kappeler, P.M., Cremer, S. & Nunn, C. L. (2015). Sociality and health: impacts of sociality on disease susceptibility and transmission in animal and human societies. *Philosphical transactions of the Royal Society B: Biological Sciences*, 370, 20140116

Karanth, K.U. (1995). Estimating tiger *Panthera tigris* populations from camera-trap data using capture—recapture models. *Biological Conservation*, 71, 333–338.

Karanth, K.U. & Nichols, J.D. (1998) Estimation of tiger densities in India using photographic captures and recaptures. *Ecology*, 79, 2852–2862.

Kashima, K., Ohtsuki, H. & Satake, A. (2013). Fission-fusion bat behavior as a strategy for balancing the conflicting needs of maximizing information accuracy and minimizing infection risk. *Journal of Theoretical Biology*, 318, 101–109.

Kasper, C.B., Bastazini, V.A.G., Salvi, J. & Hamilton, C.Z.G. (2008). Trophic ecology and the use of shelters and latrines by the Neotropical otter *Lontra longicaudis* in the Taquari Valley, Southern Brazil Iheringia. *Zoologia*, 98, 469–474.

Kattel, B. (1992). Ecology of the Himalayan musk deer in Sagarmatha National Park, Nepal [PhD thesis]. Colorado: Colorado State University

Kavaliers, M. & Colwell, D.D. (1995). Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proceedings of the Royal Society B: Biological Sciences*, 261, 31–35.

Kean, E.F. (2012). Scent communication in the Eurasian otter (*Lutra lutra*) and potential applications for population monitoring. PhD Thesis, Cardiff University.

Kean, E.F., Bruford, M.W., Russo, I.M., Műller, C.T. & Chadwick, E.A. (2017). Odour dialects among wild mammals. *Scientific Reports*, 7, 13593.

Kean, E.F., Chadwick, E.A. & Müller, C.A. (2015). Scent signals individual identity and country of origin in otters. *Mammalian Biology*, 80, 99–105.

Kean, E.F., Müller, C.T. & Chadwick, E.A. (2011). Otter scent signals age, sex, and reproductive status. *Chemical Senses*, 36, 555–564.

Kindberg, J., Ericsson, G. & Swenson, J. E. (2009). Monitoring rare or elusive large mammals using effort-corrected voluntary observers. *Biological Conservation*, 142(1), 159–165.

King, T.W., Salom, R., Shipley, L., Quigley, H. & Thornton, D. (2017). Ocelot latrines: communication centers for Neotropical mammals. *Journal of Mammalogy*, 98, 106-113.

Kleiman, D. (1966). Scent marking in the Canidae. *Symposia of the Zoological Society of London*, 18, 167–177.

Kohn, M., York, E.C., Kamradt, D.A., Haught, G., Sauvajot, R.M. & Wayne, R.K. (1999). Estimating population size by genotyping feces. *Proceedings of the Royal Society of London, B. Biological Sciences*, 266, 657–663.

Kowalsky, D. (2013). *Aonyx capensis* African clawless otter. Animal Diversity Web. University of Michigan. [Web:] https://animaldiversity.org/accounts/Aonyx_capensis/., [Date of access: 19 April 2021]

Kruuk H. (1992). Scent marking by otters (*Lutra lutra*): signalling the use of resources. *Behavioural Ecology*, 3, 133–140.

Kruuk, H. & Conroy, J.W.H. (1987). Surveying otter *Lutra lutra* populations: a discussion of problems with spraints. *Biological Conservation*, 41(3), 179-183.

Kruuk, H. & Moorhouse, A. (1990). Seasonal and spatial differences in food selection by otters *Lutra Lutra* in Shetland. *Journal of Zoology*, 221, 621–637.

Kruuk, H. (1972). The Spotted Hyena: a Study of Predation and Social Behavior. Chicago: Chicago University Press. pp. 335.

Kruuk, H. (1992). Scent marking by otters (*Lutra lutra*): signaling the use of resources. *Behavioral Ecology*, 3, 133–140.

Kruuk, H. (1995). Wild Otters: Predation and Populations. London: Academic Press.

Kruuk, H. (2006). Otters, ecology, behaviour and conservation. Oxford University Press, Oxford, UK.

Kruuk, H. (2014). Sprainting into the wind. *IUCN Otter Specialist Group Bulletin*, 31(1), 12–14.

Kruuk, H. Conroy, J.W.H., Glimmerveen, U. & Ouwererk, E.J. (1986). The use of spraints to survey populations of otters *Lutra lutra*. *Biological Conservation*, 35, 187–194.

Kruuk, H., Gorman, M.L. & Leitch, A. (1984). Scent marking with the subcaudal gland by the European badger, *Meles meles L*. *Animal Behaviour*, 32, 899–907.

Kruuk, H., Moorhouse, A., Conroy, J., Durbin, L. & Frears, S. (1989). An estimate of numbers and habitat preferences of otters *Lutra lutra* in Shetland, UK. *Biological Conservation*, 49(4), 241–254.

Kulahci, I.G., Drea, C.M., Rubenstein, D.I. & Ghazanfar, A.A. (2014). Individual recognition through olfactory–auditory matching in lemurs. *Proceedings of the Royal Society B: Biological Sciences*, 281(1784), 20140071.

Ladds, Z., Hoppitt, W. & Boogert, N.J. (2017). Social learning in otters. *Royal Society Open Science*, 4(8), 170489.

Lai, J. & Sheng, H. (1993). A comparative study on scent marking. In: Ohtaishi, N. & Sheng, H.I. (eds.). Deer of China: biology and management. Elsevier Science Publishers, Amsterdam, Netherlands. pp. 204–208.

Lanszki, J., A., Hidas, K., Szentes, T., Révay, I., Lehoczky, and S., Weiss. 2008. Relative spraint density and genetic structure of otter (*Lutra lutra*) along the Drava River in Hungary. *Mammalian Biology*, 73, 40–47.

Larivière, S. (2001). Poecilogale albinucha. *Mammalian Species*, 681, 1–4.

Lazaro-Perea, C., Snowdon, C.T. & Arruda, M. (1999). Scent marking behaviour in wild groups of common marmosets (*Callithrix jacchus*). *Behavioral Ecology and Sociobiology*, 46(5), 313–324.

Léliaso, M., Lemasson, A. & Lodé, T. (2021). Social organization of otters in relation to their ecology. *Biological Journal of the Linnean Society*, 133(1), 1–27

Lenton, E.J., Chanin, P.R.F. & Jefferies, D. J. (1980). Otter survey of England 1977–1979. London: Nature Conservancy Council

Leuchtenberger, C, Magnusson, W.E. & Mourão, G. (2015). Territoriality of Giant Otter Groups in an Area with Seasonal Flooding. *PLOS ONE*, 10(5), e0126073.

Leuchtenberger, C. & Mourão, G. (2009). Scent-marking of giant otter in the southern Pantanal, Brazil. *Ethology*, 115, 210–216.

Leuchtenberger, C. (2018). Scent-Marking. Vonk, J. & Shackelford, T. (eds.). Encyclopedia of Animal Cognition and Behavior. Springer, Cham.

Leuchtenberger, C., Sousa-Lima, R., Duplaix, N., Magnusson, W. E., & Mourão, G. (2014). Vocal repertoire of the social giant otter. *The Journal of the Acoustical Society of America*, 136(5), 2861–2875.

Lewis, R.J. (2006). Scent marking in sifaka: no one function explains it all. *American Journal of Primatology*, 68(6), 622–636.

Linklater, W.L. Mayer, K. & Swaisgood, R.R. (2013). Chemical signals of age, sex and identity in black rhinoceros. *Animal Behaviour*, 85, 671–677.

Löfstedt, C., Van Der Pers, J.N.C., Lofqvist, J., Lanne, B.S., Monica Appelgren, M., Bergström, G. & Thelin, B. (1982). Sex pheromone components of the turnip moth, *Agrotis segetum*. *Journal of Chemical Ecology*, 8, 1305–1321.

Lubbe, R. A. (1996). Vegetation and flora of the Kosi Bay Coastal Forest Reserve in Maputaland, northern KwaZulu-Natal, South Africa. PhD dissertation, University of Pretoria.

Lumkes, R.A., Day, C.C. & Zollner, P.A. (2019). Behavioral response of the mammalian community to river otter latrine activity. *The American Midland Naturalist*, 182(1), 75–88.

Ma, W. & Klemm, W. R. (1997). Variations of equine urinary volatile compounds during the oestrous cycle. *Veterinary Research Communications*, 21, 437–446.

Macdonald, D. (1985). The carnivores: order Carnivora. In. Social odours in mammals, vol 2 (eds: Brown, R.E. & Macdonald, D.W.). Clarendon Press, pp. 619–722.

Macdonald, D.W. (1980). Patterns of scent marking with urine and faeces amongst carnivore communities. *Symposia of the Zoological Society of London*, 45, 107–139.

Macdonald, D.W. (1995). European mammals. Evolution and behaviour. Harper Collins Publisher, London, United Kingdom.

Macdonald, S.M. & Mason, C.F. (1987). Seasonal marking in an otter population. *Acta Theriologica*, 32, 449–462.

Madisha, M.T., Ponsonby, D., Schwaibold, U., Kotzé, A., Jansen, R., Brettschneider, H. & Dalton, D.L. (2015). Differentiation of two south African otter species (*Aonyx capensis* and *Lutra maculicollis*) from spraint based on partial CytB primer sets. *Global Ecology and Conservation*, 4, 8–13.

Maffei, L., Cuéllar, E., & Noss, A. (2004). One thousand jaguars (*Panthera onca*) in Bolivia's Chaco? Camera trapping in the Kaa-Iya National Park. *Journal of Zoology*, 262(3), 295-304.

Majelantle, T., McIntyre, T. & Ganswindt, A. (2020). Monitoring the effects of land transformation on African clawless otters (*Aonyx capensis*) using fecal glucocorticoid metabolite concentrations as a measure of stress. *Integrative Zoology*, 15(4), 293–306.

Majelantle, T.L., Ganswindt, A., Jordaan, R.K., Slip, D.J., Harcourt, R. & McIntyre, T. (2021). Increased population density and behavioural flexibility of African clawless otters (*Aonyx capensis*) in specific anthropogenic environments. *Urban Ecosystems*, 24, 691–699.

Malo, E.A., Castrejón-Gómez, V.R., Cruz-López, L. & Rojas, J.C. (2004). Antennal Sensilla and Electrophysiological Response of Male and Female *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Conspecific Sex Pheromone and Plant Odors. *Annals of the Entomological Society of America*, 97(6), 1273–1284.

Maritz, B. & Alexander, G.J. (2012). Population Density and Survival Estimates of the African Viperid, *Bitis schneideri*. *Herpetologica*, 68, 195–202.

Marneweck, C. (2013). Silent signals: identifying dung odour profiles that indicate age, sex and status in white rhino olfactory communication. MSc Thesis, University of KwaZulu-Natal).

Marneweck, C., Butler, A. R., Gigliotti, L. C., Harris, S. N., Jensen, A. J., Muthersbaugh, M., Newman, B.A., Saldo, E.A., Shute, K., Titus, K.L., Yu. S.W. & Jachowski, D. S. (2021). Shining the spotlight on small mammalian carnivores: global status and threats. *Biological Conservation*, 255, 109005.

Marneweck, C., Jürgens, A. & Shrader, A.M. (2018). The role of middens in white rhino olfactory communication. *Animal Behaviour*, 140, 7–18.

Martín, E.A., Heurich, M., Müller, J., Bufka, L., Bubliy, O. & Fickel, J. (2017). Genetic variability and size estimates of the Eurasian otter (*Lutra lutra*) population in the bohemian Forest ecosystem. *Mammalian Biology-Zeitschrift für Säugetierkunde*, 86, 42–47.

Martín, J., Barja, I. & López, P. (2010). Chemical scent constituents in feces of wild Iberian wolves (Canis lupus signatus). *Biochemical Systematics and Ecology*, 38(6), 1096–1102.

Mas, F. & Kolliker, M. (2008). Maternal care and offspring begging in social insects: chemical signalling, hormonal regulation and evolution. *Animal Behaviour*, 76, 1121–1131.

Mason, C.F. & Macdonald, S.M. (1986). Otters – ecology and conservation. Cambridge: Cambridge University Press.

Mason, C.F. & Macdonald, S.M. (1987). The use of spraints for surveying otter *Lutra lutra* population: an evaluation. *Biological Conservation*, 41, 167–177.

Maynard-Smith, J. & Parker, G.A. (1976). The logic of asymmetric contests. *Animal Behaviour,* 24,159–175.

Mayor, S.J., Schneider, D.C., Schaefer, J.A. & Mahoney, S.P. (2009). Habitat selection at multiple scales. *Ecoscience*, 16(2), 238–247.

Mays, H.L. & Hill, G.E. (2004). Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, 19, 554–559.

McColl, I. (1967). The comparative anatomy and pathology of anal glands. Arris and Gale lecture delivered at the Royal College of Surgeons of England on 25th February 1965. *Annals of The Royal College of Surgeons of England*, 40(1), 36.

McIntyre, T., Awa-Eiseb, S., Conradie, S., Devos, M., Jordaan, R.K., Josling, J., le Roux, B.A., Lewis, C., Majelantle, T., Nicolaides, S. & Somers, M. (2022). Otters in southern Africa: research outputs, ongoing research and knowledge gaps. *15th IUCN/SSC OSG International Otter Congress, Sospel, Alpes-Maritimes, France, 19-23 September 2022.*

McIntyre, T., Majelantle, T.L., Slip, D.J. & Harcourt, R.G. (2020). Quantifying imperfect camera-trap detection probabilities: implications for density modelling. *Wildlife Research*, 47(2), 177-185.

Medina-Vogel, G., Boher, F., Flores, G., Santibañez, A. & Soto-Azat, C. (2007). Spacing Behavior of Marine Otters (*Lontra felina*) in Relation to Land Refuges and Fishery Waste in Central Chile, *Journal of Mammalogy*, 88(2), 487–494.

Meek P.D., Ballard G., Falzon G., Williamson J., Milne H., Farrell R., Stover J., Mather-Zardain A.T., Bishop J.C., Cheung E.K.W. & Lawson C.K. (2020). Camera trapping technology and related advances: Into the new millennium. *Australian Journal of Zoology*, 40(3), 392–403.

Melo, A.I., & González-Mariscal, G. (2010). Communication by olfactory signals in rabbits: its role in reproduction. *Vitamins & Hormones*, 83, 351–371.

Melquist, W.E. & Hornocker, M.G. (1983). Ecology of river otters in west central Idaho. *Wildlife Monographs*, 83, 3–60.

Mench, J. (1998). Why it is important to understand animal behaviour. *Institute for Laboratory Animal Research Journal*, 39(1), 20–26.

Mench, J.A. & Mason, G.J. (1997). Behavior. Appleby, M.C. & Hughes, B.O. (eds.) Animal Welfare. pp. 127–142.

Miaretsoa, L., Cascella, A., Vadàla, L., Valente, D., De Gregorio, C., Torti, V., Norscia, I., Ratsimbazafy, J., Friard, O., Giacoma, C. & Gamba, M. (2022). Marking Versus Overmarking: Spatial and Behavioral Patterns of Scent Marking in Wild Diademed Sifaka (*Propithecus diadema*). *International Journal of Primatology.* doi.org/10.1007/s10764-022-00292-0

Michalski, F., Martins, C.B., Rheingantz, M.L. & Norris, D. (2021). New scent marking behavior of neotropical otter (*Lontra longicaudis*) in the Eastern Brazilian Amazon. *IUCN Otter Specialist Group Bulletin*, 38 (1), 27–35.

Mills, M. & Gorman, M. (2009). The scent-marking behaviour of the spotted hyaena *Crocuta crocuta* in the southern Kalahari. *Journal of Zoology*, 212, 483–497.

Mitchell, T. Irwin, K., Samonds, E., Raharison, J., Wright, P.C. (2004). Lemur latrines: observations of latrine behavior in wild primates and possible ecological significance. *Journal of Mammalogy*, 85(3), 420–427.

Mitro, S., Gordon, A.R., Olsson, M.J. & Lundstöm, J.N. (2012). The smell of age: perception and discrimination of body odors of different ages. *PLoS One*, 7, 1–7.

Moeller, A.K., Lukacs, P.M. & Horne, J.S. (2018). Three novel methods to estimate abundance of unmarked animals using remote cameras. *Ecosphere*, 9(8), e02331.

Molteno, A.J., Sliwa, A. & Richarson, P.R.K. (1998). The role of scent marking in a free-ranging, female black-footed cat (*Felis nigripes*). *Journal of Zoology*, 245, 35–41.

Morin, D.J., Challender, D.W., Ichu, I.G., Ingram, D.J., Nash, H.C., Panaino, W., Panjang, E., Sun, N.C.M. & Willcox, D. (2020). Developing robust ecological monitoring methodologies for pangolin conservation. In Pangolins, (pp. 545-558). Academic Press. pp. 545-558.

Mucina, L. & Rutherford, M.C. (2006). The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19, South African Biodiversity Institute, Pretoria.

Mumm, C., & Knörnschild, M. (2017). Territorial choruses of giant otter groups (*Pteronura brasiliensis*) encode information on group identity. *PloS one*, 12(10), e0185733.

Mumm, C.A.S. & Knörnschild, M. (2018). Mustelid communication. In: Vonk, J. & Shackelford, T.K. (eds.), Encyclopedia of Animal Cognition and Behavior. Springer International Publishing.

Mykytowycz, R. & Gambale, S. (1969). The distribution of dung-hills and the behavior of free living wild rabbits, *Oryctolagus cuniculus* (L.), on them. *Forma et function*, 1, 333–349.

Myrvold, K.M. & Kennedy, B.P. (2015). Density dependence and its impact on individual growth rates in an age-structured stream salmonid population. *Ecosphere*, 6(12), 281.

Naidoo, G. (2016). The mangroves of South Africa: An ecophysiological review. *South African Journal of Botany*, 107, 101-113.

Nawab, A. (2009). Aspects of the ecology of Smooth-coated Otter *Lutrogale perspicillata* Geoffroy St. Hilaire, 1826: a review. *Journal of Bombay Natural History Society*, 106, 5–10.

Nevill, H. & Nevill, E.M. (1995). A survey of the Culicoides (Diptera: Ceratopogonidae) of the Umlalazi Nature Reserve in Zululand, South Africa, with notes on two species biting man. *Onderstepoort Journal of Veterinary Research*, 62, 51–58.

Newman, D.G. & Griffin, C.R. (1994). Wetland use by river otters in Massachusetts. *Journal of Wildlife Management*, 58, 18–23.

Nguyen, X.D., Pham, T.A. & Le, H.T. (2001). New information about the hairy-nosed otter *Lutra sumatrana* in Vietnam. *IUCN Otter Specialist Group Bulletin*, 18, 64–75.

Nichols, J.D. & Williams, B.K. (2006). Monitoring for conservation. *Trends in Ecology & Evolution*, 21, 668–673.

Nichols, J.D., O'Connell, A.F. & Karanth K.U. (2011). Camera Traps in Animal Ecology and Conservation: What's Next? In: O'Connell A.F., Nichols J.D., Karanth K.U. (eds). Camera Traps in Animal Ecology. Springer, Tokyo. pp. 253–263.

Nishida, R., Schulz, S., Kim, C.S., Fukami, H., Kuwahara, Y., Honda, K. & Hayashi, N. (1996). Male sex pheromone of a giant danaine butterfly, *Idea leuconoe*. *Journal of Chemical Ecology*, 22, 949–972.

Njoroge, C.H., Ogada, M.O. & Gichuki, N.N (2014). Temporal detection patterns of the African clawless otter *Aonyx capensis* (Schinz, 1821) in the Laikipia plateau of central Kenya. *IUCN Otter Specialist Group Bulletin*, 31(1), 44–48.

Noonan, M.J., Tinnesand, H.V., Müller, C.T., Rosell, F. Macdonald, D.W. & Buesching, C.D. (2019). Knowing me, knowing you: anal gland secretion of european badgers (*meles meles*) codes for individuality, sex and social group membership. *Journal of Chemical Ecology*, 45, 823–837.

Okes, N., Ponsonby, D.W., Rowe-Rowe, D., Avenant, N.L. & Somers, M.J. (2016). A conservation assessment of *Aonyx capensis*. In Child, M.F., Roxburgh, L., Do-Linh-San,

E., Raimondo, D. & Davies-Mostert, H.T., (eds.). The Red List of Mammals of South Africa, Swaziland and Lesotho. South African National Biodiversity Institute and Endangered Wildlife Trust, South Africa.

Okes, N.C. & O'Riain, M.J. (2017). Otter occupancy in the Cape Peninsula: Estimating the probability of river habitat use by cape clawless otters, *Aonyx capensis*, across a gradient of human influence. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27, 706–716.

Oldham, A.R. & Black, J.M. (2009). Experimental tests of latrine use and communication by river otters. *Northwestern Naturalist*, 90, 207–211.

Ostfeld, R.S., Ebensperger, L., Klosterman, L. & Castilla, J. (1989). Foraging, activity budget, and social behaviour of the South American marine otter *Lutra feline*. *National Geographic Research*, 5, 422–438.

O'Sullivan, W.M. (1993). Efficiency and limitation of the standard otter (*Lutra lutra*) survey technique in Ireland. *Proceedings of the Royal Irish Academy*, 93b, 49–53.

Palencia, P., Rowcliffe, M., Vicente, J. & Acevedo, P. (2021). Assessing the camera trap methodologies used to estimate density of unmarked populations. *Journal of Applied Ecology*, 58, 10.1111/1365-2664.13913.

Pardini, R. & Trajano E. (1999). Use of shelters by the neotropical River Otter (*Lontra longicaudis*) in an Atlantic Forest Stream, Southeastern Brazil. *Journal of Mammalogy*, 80(2), 600–610.

Parker, D.M., Burchell, R.K., & Bernard, R.T. (2005). The diet of Cape clawless otters at two sites along the Bloukrans River, Eastern Cape Province, South Africa. *African Zoology*, *40*(2), 330-334.

Pépin, D. & Gerard, J.F. (2008). Group dynamics and local population density dependence of group size in the Pyrenean chamois, *Rupicapra pyrenaica*. *Animal Behavior*, 75, 361–369.

Perez, R.S. (2019). Using Medium and Large-Sized Mammals as Indicator Species to Measure Connectivity and Large Infrastructure Impacts in Costa Rica. PhD thesis, University of Idaho, Moscow, Idaho, USA.

Perrin, M.R. & Carrranza, D.I. (2000). Activity patterns of the spotted necked otter in the Natal Drakensberg, South Africa. *South African Journal of Wildlife Research*, 30(1), 1–7.

Perrin, M.R. & Carugati, C. (2000). Habitat use by the Cape clawless otter and the spotted-necked otter in the KwaZulu-Natal Drakensberg, South Africa. *South African Journal of Wildlife Research*, 30, 103–113.

Perrin, M.R. & Carugati, C. (2006). Abundance estimates of the Cape clawless otter *Aonyx capensis* (Schinz 1821) and the Spotted-necked otter *Lutra maculicollis* (Lichtenstein 1835) in the KwaZulu-Natal Drakensberg, South Africa. *Tropical Zoology*, 19(1), 9–19.

Peters, R.P. & Mech, L.D. (1975). Scent-marking in wolves. *American Scientist*, 63, 628e637.

Pettigrew, P., Sigouin, D. & St-Laurent, M. H. (2021). Testing the precision and sensitivity of density estimates obtained with a camera-trap method revealed limitations and opportunities. *Ecology and Evolution*, 11(12), 7879–7889.

Piñeiro, A. & Barja, I. (2015). Evaluating the function of wildcat faecal marks in relation to the defence of favourable hunting areas. *Ethology Ecology & Evolution*, 27(2), 161-172.

Ponsonby, D.W. & Schwaibold, U. (2018). Country otter, city otter: The distribution patterns of two otter species in an urbanized area of Gauteng, South Africa. *African Journal of Ecology*, 1–7.

Ponsonby, D.W. (2018). The ecology of otters in an urban environment. PhD thesis, University of the Witwatersrand.

Potash, A.D., Conner, L.M. & McCleery, R.A. (2019). Vertical and horizontal vegetation cover synergistically shape prey behaviour. *Animal behaviour*, 152, 39–44.

Prakash, N., Mudappa, D., Shankar Raman, T. & Kumar, A. (2012). Conservation of the Asian small-clawed otter (*Aonyx cinereus*) in human-modified landscapes, Western Ghats, India. *Tropical Conservation Science*, 5, 67–78.

Prenda, J. & Granadolorencio, C. (1996). The relative influence of riparian habitat structure and dish availability on otter *Lutra lutra* L. sprainting activity in a small Mediterranean catchment. *Conservation Biology*, 76, 9–15.

Pretorius, M.L., Brown, L.R., Bredenkamp, G.J. & Van Huyssteen, C.W. (2014). The ecology and species richness of the different plant communities within selected wetlands on the Maputaland Coastal Plain, South Africa. Biodiversity: the dynamic balance of the planet. Rijeka: InTech Publishers. pp. 275-296.

Prigioni, C., Remonti, L., Balestrieri, A., Sgrosso, S., Priore, G., Mucci, N. & Randi, E. (2006). Estimation of European Otter (*Lutra lutra*) Population Size by Fecal DNA Typing in Southern Italy. *Journal of Mammalogy*, 87(5), 855–858.

QGIS Development Team. (2022). QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available at http://qgis.osgeo.org [verified 9 April 2022]

Qu, C., Schneider, B.I., Kearsley, A.J., Keyrouz, W. & Allison, T.C. (2021). Predicting kovats retention indices using graph neural networks. *Journal of Chromatography A*, 1646, 462100.

Quaglietta, L., Hájková, P., Mira, A. & Boitani, L. (2015). Eurasian otter (*Lutra lutra*) density estimate based on radio tracking and other data sources. *Mammal Research*, *60*(2), 127–137.

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing 2015, Vienna, Austria.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at https://www.R-project.org/ [verified 18 May 2022]

Raha, A. & Hussain, S.A. (2016). Factors affecting habitat selection by three sympatric otter species in the southern Western Ghats, India. *Acta Ecologica Sinica*, 36(1), 45–49, 1872-2032.

Rajkaran, A. & Adams, J.B. (2011). Mangrove forests of Northern Kwa-Zulu Natal: Sediment conditions and population structure of the largest mangrove forests in South Africa. *Western Indian Ocean Journal of Marine Science*, 10, 25–38.

Ralls, K. (1971). Mammalian scent marking. *Science*, 171(3970), 443–449.

Rasmussen, L.E.L., Lee, T.D., Roelofs, W.L., Zhang, A. & Daves, G.D. (1996). Insect pheromone in elephants. *Nature*, 379, 684–684.

Reed-Smith, J., Thomas, S., Kihudu, T. & Mussa, M. (2014). Preliminary report on the behaviour of Spotted-necked otter (*Lutra maculicollis*, Lichtenstein, 1835) living in a lentic ecosystem. *Zoo Biology*, 33.

Remonti, L., Balestrieri, A., Smiroldo, G. & Prigioni, C. (2011). Scent marking of key food sources in the Eurasian otter. *Annales Zoologici Fennici*, 48(5), 287–294.

Rheingantz, M., Leuchtenberger, C., Zucco, C., & Fernandez, F. (2016). Differences in activity patterns of the Neotropical otter Lontra longicaudis between rivers of two Brazilian ecoregions. *Journal of Tropical Ecology*, 32(2), 170-174.

Rheingantz, M.L. & Trinca, C.S. (2015). *Lontra longicaudis*. *The IUCN Red List of Threatened Species 2015*, e.T12304A21937379.

Rheingantz, M.L., Santiago-Plata, V. & Trinca, C. (2017). The Neotropical otter Lontra longicaudis: a comprehensive update on the current knowledge and conservation status of this semi-aquatic carnivore. *Mammal Review*, 47, 291–305.

Ribas, C., Cunha, H., Damasceno, G., Magnusson, W., Solé-Cava, A. & Mourão, G. (2016). More than meets the eye: kinship and social organisation in giant otters (*Pteronura brasiliensis*). *Behavioral Ecology and Sociobiology*, 70, 61–72.

Rich, L.N., Kelly, M.J., Sollmann, R., Noss, A.J., Maffei, L., Arispe, R.L., Paviolo, A., De Angelo, C.D., Di Blanco, Y.E. & Di Bitetti, M.S. (2014). Comparing capture-recapture, mark-resight, and spatial mark-resight models for estimating puma densities via camera traps. *Journal of Mammalogy*, 95(2), 382–391.

Rich, T.J. & Hurst, J.L. (1998). Scent marks as reliable signals of the competitive ability of mates. *Animal Behaviour*, 56(3), 727–735.

Rich, T.J. & Hurst, J.L. (1999). The competing countermarks hypothesis: reliable assessment of competitive ability by potential mates. *Animal Behaviour*, 58, 1027–1037.

Ridout, M.S. & Linkie, M. (2009). Estimating overlap of daily activity patterns from camera trap data. *The Journal of Agricultural, Biological and Environmental Statistics*, 14, 322–337.

Rifenberg, J. (2020). Observational study of two ex Situ North American River Otters (Lontra canadensis). *The Pegasus Review: UCF Undergraduate Research Journal*, 12(2), 4.

Riordan, P. Delahay, R.J., Cheeseman, C., Johnson, P.J. & Macdonald, D.W. (2011). Culling-induced changes in badger (*Meles meles*) behaviour, social organisation and the epidemiology of bovine tuberculosis. *PLoS ONE*, 6, e28904.

Roberts, S.C. & Lowen, C. (1997). Optimal patterns of scent mark in klipspringer (*Oreotragus oreotragus*) territories. *Journal of Zoology*, 243, 565–578.

Roberts, S.C. (2012). On the relationship between scent-marking and territoriality in Callitrichid primates. *International Journal of Primatology*, 33, 749–761.

Rode, J., Lambert, C., Marescot, L., Chaix, B., Beesau, J., Bastian, S., Kyrbashev, J. & Anne-Lise Cabanat, A. (2021). Population monitoring of snow leopards using camera trapping in Naryn State Nature Reserve, Kyrgyzstan, between 2016 and 2019. *Global Ecology and Conservation*, 31, e01850.

Rodgers, T.W., Giacalone, J., Heske, E.J., Pawlikowski, N.C. & Schooley, R.L. (2015). Communal latrines act as potentially important communication centers in ocelots *Leopardus pardalis. Mammalian Biology*, 80(5), 380–384.

Romanowski, J., Brzezinski, M. & Cygan, J.P. (1996). Notes on the technique of the otter field survey. *Acta Theriologica*, 41, 199–204.

Roper, T.J., Conradt, L., Butler, J., Christian, S.E., Ostler, J. & Schmid, T.K. (1993). Territorial marking with faeces in badgers (*Meles meles*): a comparison of boundary and hinterland latrine use. *Behaviour,* 127, 289–307.

Rosell, F. (2001). The function of scent marking in beaver (*Castor fiber*) territorial defence. PhD Dissertation, Norwegian University of Science and Technology

Rosevear, D.R. (1974). The Carnivores of West Africa. British Museum of Natural History, London, UK.

Rostain, R.R. (2000). Social behavior and olfactory communication in the North American river otter, *Lontra canadensis*. MSc. thesis, San Francisco State University.

Rostain, R.R., Ben-David, M., Groves, P. & Randall, J.A. (2004). Why do river otters scent-mark? An experimental test of several hypotheses. *Animal Behaviour*, 68, 703–711.

Rovero, F. & Marshall, A.R. (2009). Camera trapping photographic rate as an index of density in forest ungulates. *Journal of Applied Ecology*, 46, 1011–1017.

Rowcliffe, J.M., Field, J., Turvey, S.T. & Carbone, C. (2008). Estimating animal density using camera traps without the need for individual recognition. *Journal of Applied Ecology*, 45(4),1228–1236.

Rowcliffe, J.M., Kays, R., Carbone, C. & Jansen, P.A. (2013). Clarifying assumptions behind the estimation of animal density from camera trap rates. *Journal of Wildlife Management*, 77, 876–876.

Rowcliffe, J.M., Kays, R., Kranstauber, B., Carbone, C. & Jansen, P.A. (2014). Quantifying levels of animal activity using camera trap data. *Methods in Ecology and Evolution*, 5(11), 1170–1179.

Rowe-Rowe, D.T. (1977). Prey capture and feeding behaviour of South African otters. *Lammergeyer*, 23, 13–21.

Rowe-Rowe, D.T. (1978). Biology of the two otter species in South Africa. In Duplaix, N. (ed.), Otters. Proceedings of the First Working Meeting of the Otters Specialist Group. International Union for Conservation of Nature (IUCN). Gland, Switzerland. pp. 129–134.

Rowe-Rowe, D.T. (1990). Action plan for African otters. In: Foster-Turley, P., MacDonald, S. & Mason, C. (eds.). Otters: an Action Plan for their Conservation. IUCN, Gland, Switzerland pp. 41–51.

Rowe-Rowe, D.T. (1992). Survey of South African otters in a freshwater habitat, using sign. *South African Journal of Wildlife Research*, 22, 49–55.

Roy, T.K. (2013). Influence of sand on strength characteristics of cohesive soil for using as subgrade of road. *Procedia-Social and Behavioral Sciences*, 104, 218-224.

Ruiz-Olmo, J. & Gosálbez, J. (1997). Observations on the sprainting behaviour of the otter *Luta lutra* in NE Spain. *Acta Theriologica*, 42(3), 259–270.

Ruiz-Olmo, J., Saavedra, D. & Jiménez, J. (2001). Testing the surveys and visual and track censuses of Eurasian otters (*Lutra lutra*). *Journal of Zoology*, 253, 359–369.

Rutten, G., Ensslin, A., Hemp, A. & Fischer, M. (2015). Vertical and horizontal vegetation structure across natural and modified habitat types at Mount Kilimanjaro. *PloS one*, 10(9), e0138822.

Sankar, R., Archunan, G. & Habara, Y. (2007). Detection of oestrous-related odour in bovine (*Bos taurus*) saliva: bioassay of identified compounds. *Animals*, 1, 1321–1327.

Santini, G., Abolaffio, M., Ossi, F., Franzetti, B., Cagnacci, F., & Focardi, S. (2022). Population assessment without individual identification using camera-traps: A comparison of four methods. *Basic and Applied Ecology*, 61, 68–81.

Schatz, S. & Palme, R. (2001). Measurement of faecal cortisol metabolites in cats and dogs: a non-invasive method for evaluating adrenocortical function. *Veterinary Research Communications*, 25(4), 271–287.

Schmelz, M., Duguid, S., Bohn, M. & Volter, C. (2017). Cooperative problem solving in giant otters (*Pteronura brasiliensis*) and Asian small-clawed otters (*Aonyx cinerea*). *Animal Cognition*, 20, 1107.

Schoener, T.W. (1974). Resource partitioning in ecological communities. *Science*, 185, 27–39.

Scordato, E.S. & Drea, C.M. (2007). Scents and sensibility: information content of olfactory signals in the ringtailed lemur, Lemur catta. *Animal Behaviour*, 73(2), 301–314.

Sepúlveda, M.A., Bartheld, J.L., Monsalve, R., Gómez, V. & Medina-Vogel, G. (2007). Habitat use and spatial behaviour of the endangered Southern river otter *Lontra provocax* in riparian habitats of Chile, conservation implications. *Biological Conservation*, 140, 329–338.

Shafi, T.A., Siddiqui, M. F. & Wani, A.A. (2021). Chemical signaling in bovines: understanding the behavior and way of communication. In: Bovine Science - Challenges and Advances. IntechOpen. doi.org/10.5772/intechopen.99834

Shannon, J.S. (1993). Behaviour of Otters in a Coastal Marine Habitat: Abstract of Work in Progress. *IUCN Otter Specialist Group Bulletin,* 8, 23–27.

Shirasu, M., Ito, S., Itoigawa, A., Hayakawa, T., Kinoshita, K., Munechika, I., Imai, H. & Touhara, K. (2020). Key Male Glandular Odorants Attracting Female Ring-Tailed Lemurs. *Current Biology*, 30, 2131–2138.

Silk, J.B. (2007). The adaptive value of sociality in mammalian groups. *Philosphical transactions of the Royal Society B: Biological Sciences,* 362**,** 539–559.

Sillero-Zubiri, C. & Macdonald, D.W. (1998). Scent-marking and territorial behaviour of Ethiopian wolves *Canis simensis*. *Journal of Zoology*, 245, 351–361.

Silveira, L., Jacomo, A.T.A. & Diniz-Filho, J.A.F. (2003). Camera trap, line transect census and track surveys: a comparative evaluation. *Biological Conservation*, 114, 351–355.

Singha, P.B., Shresthac, B.B., Thapaa, A., Saudd, P. & Jianga, Z. (2018). Selection of latrine sites by Himalayan musk deer (*Moschus leucogaster*) in Neshyang Valley, Annapurna Conservation Area, Nepal. *Journal of Applied Animal Research*, 46(1), 920–926.

Sittenthaler, M., Schöll, E.M., Leeb, C., Haring, E., Parz-Gollner, R., & Hackländer, K. (2020). Marking behaviour and census of Eurasian otters (*Lutra lutra*) in riverine habitats: what can scat abundances and non-invasive genetic sampling tell us about otter numbers? *Mammal Research*, 65, 191–202.

Sliwa, A. & Richrdson, P.R.K. (1998). Responses of aardwolves, *Proteles cristatus*, Sparrman 1783, to translocated scent marks. *Animal Behaviour*, 56, 137–146.

Smith, C., Whitworth, A., Brunner, E. & Pomilia, M. (2020). Habitat selection and diet of the Neotropical otter (*Lontra longicaudis*) on the Osa Peninsula, Costa Rica, and range-wide monitoring recommendations. *Neotropical Biodiversity*, 6:1, 62–74.

Snowdon, C.T. (n.d). Significance of Animal Behavior Research. California State University, Northridge. [Web:] http://www.csun.edu/~vcpsy00h/valueofa.htm, [Date of access: 18 April 2021]

Somers, M. J., & Nel, J. A. (2003). Diet in relation to prey of Cape clawless otters in two rivers in the Western Cape Province, South Africa. *African Zoology*, *38*(2), 317-326.

Somers, M.J. & Nel, J.A.J. (2004a). Habitat selection by the Cape clawless otter (*Aonyx capensis*) in rivers in the Western Cape Province, South Africa. *African Journal of Ecology*, 42(4), 298–305.

Somers, M.J. & Nel, J.A.J. (2004b). Movement patterns and home range size of Cape clawless otters (*Aonyx capensis*), affected by high food density patches. *Journal of Zoology*, 262, 91–98.

Somers, M.J. & Nel, J.A.J. (2013). *Aonyx capensis* African Clawless Otter. In Kingdon, J. & Hoffmann, M. (eds.). Mammals of Africa. Volume V: Carnivores, Pangolins, Equids and Rhinoceroses. Bloomsbury, London, UK. pp. 104–108.

Somers, M.J. & Purves, M.G. (1996). Trophic overlap between three syntopic semi-aquatic carnivores: Cape clawless otter, spotted-necked otter and water mongoose. *African Journal of Ecology*, 34(2), 158-166.

Somers, M.J. (1997). Cape clawless otter Aonyx capensis. In: Mills, M.G.L. & Hess,L. (eds.). The Complete Book of Southern African Mammals. Struik Publishers. pp. 200–201.

Soso, S. B., & Koziel, J. A. (2017). Characterizing the scent and chemical composition of Panthera leo marking fluid using solid-phase microextraction and multidimensional gas chromatography–mass spectrometry-olfactometry. *Scientific Reports*, *7*(1), 1-15.

South African Environmental Observation Network (SAEON). (2021). South African Estuary Information System – Mlalazi. [Web:] https://saeis.saeon.ac.za/Info/8, [Date of access: 28 October 2021]

Stevens, T.K., Hale, A.M. & Williams, D.A. (2023). Environmental and anthropogenic variables influence the distribution of a habitat specialist (*Sylvilagus aquaticus*) in a large urban forest. *Conservation Science and Practice*, 5(2), e12882.

Stewart, P.D., Macdonald, D.W., Newman, C. & Cheeseman, C. (2001). Boundary faeces and matched advertisement in the European badger (*Meles meles*): a potential role in range exclusion. *Journal of Zoology*, 255, 191–198.

Stoddart, D.M. (1976). Mammalian odours and pheromones, London, UK, Edward Arnold Ltd.

Streicher, J.P. (2020). Aspects of the ecology of three mongoose species along a rural–urban landscape gradient of KwaZulu-Natal, South Africa. PhD Thesis, University of KwaZulu-Natal.

Stuart, C. & Stuart, T. (2019). A field guide to the tracks and signs of southern, Central and East African wildlife. Cape Town: Struik Nature.

Stuart, C., & Stuart, T. (2000). A field guide to the tracks and signs of southern and east African wildlife. Cape Town.

Sueur, C., King, A.J., Conradt, L., Kerth, G., Lusseau, D., Mettke-hofmann, C. Schaffner, C.M., Williams, L., Zinner, D. & Aureli, F. (2011). Collective decision-making and fission–fusion dynamics: a conceptual framework. *Oikos*, 120, 1608–1617.

Sun, L. & Müller-Schwarze, D. (1998) Anal gland secretion codes for relatedness in the beaver, *Castor canadensis*. *Ethology*, 104, 917–927.

Swimley, T.J., Serfass, T.L., Brooks, R.P. & Tzilkowski, W.M. (1998). Predicting river otter latrine sites in Pennsylvania. *Wildlife Society Bulletin*, 26, 836–845.

Tanwar, K.S., Sadhu, A. & Jhala, Y.V. (2021). Camera trap placement for evaluating species richness, abundance, and activity. *Scientific Reports*, 11, 23050.

Thiessen, D. & Rice, M. (1976). Mammalian scent gland marking and social behavior. *Psychological Bulletin*, 83(4), 505–539.

Thompson, W.L., White, G.C., & Gowan, C. (1998). Monitoring vertebrate populations. Academic Press, San Diego, California, USA.

Tibbetts, E.A. & Dale, J. (2007). Individual recognition: it is good to be different. *Trends in Ecology & Evolution*, 22, 529–537.

Toledo, D., Abbott, L.B. & Herrick, J.E. (2008). Cover Pole Design for Easy Transport, Assembly, and Field Use. *Journal of Wildlife Management*, 72(2), 564–567.

Torgerson, T.J. (2014). Latrine site selection and seasonal habitat use of a coastal river otter population. MSc thesis, Humboldt State University.

Trolle, M. & Kery, M. (2003). Estimation of ocelot density in the pantanal using capture-recapture analysis of camera-trapping data. *Journal of Mammalogy*, 84(2), 607–614.

Trowbridge, B.J. (1983). Olfactory communication in the European otter (*Lutra l. lutra*). University of Aberdeen (United Kingdom).

Trusso, S. (1997). Lake shore latrine site selection by river otters (*Lutra canadensis*). MSc Thesis, State University of New York.

Tung, J., Barreiro, L.B., Burns, M.B., Grenier, J.C., Lynch, J., Grieneisen, L.E., Altmann, J., Alberts, S.C., Blekhman, R. & Archie, E.A. (2015). Social networks predict gut microbiome composition in wild baboons. *eLife*, 4, e05224

Tyson, P.D. & Preston-Whyte, R.A. (2000). The weather and climate of southern Africa. 2nd edn. Oxford University Press, Cape Town.

Umapathy, G., Kumar, V., Kabra, M. & Shivaji, S. (2013). Detection of pregnancy and fertility status in big cats using an enzyme immunoassay based on 5α-pregnan-3α-ol-20-one. *General and Comparative Endocrinology*, 180, 33–38.

Underhill, J.E. (2003). Roads and wildlife: a study of the effects of roads on mammals in roadside habitats. PhD Thesis, University of Birmingham

Vaglio, S., Minicozzi, P., Romoli, R., Boscaro, F., Pieraccini, G., Moneti, G. & Moggi-Cecchi, J. (2016). Sternal gland scent-marking signals sex, age, rank, and group identity in captive mandrills. *Chemical Senses*, 41(2), 177–186.

Van de Ven, T.M., Fuller, A. & Clutton-Brock, T.H. (2020). Effects of climate change on pup growth and survival in a cooperative mammal, the meerkat. *Functional Ecology*, 34, 194–202.

Van der Zee, D. (1981). Density of Cape clawless otters *Aonyx capensis* (Schinz, 1821) in the Tsitsikarna Coastal National Park. *South African Journal of Wildlife Research*, 12(1), 8–13.

Van Niekerk, C.H., Somers, M.J. & Nel, J.A.J. (1998). Freshwater availability and distribution of Cape clawless otter spraints and resting places along the south-west coast of South. *South African Journal of Wildlife Research*, 28(3), 68–72.

Van Wyk, A.E. & Smith, G.F. (2001). Regions of floristic endemism in Southern Africa, Umdaus Press, Pretoria.

Van Zyll de Jong, C.G. (1987). A phylogenetic study of the Lutrinae (Carnivora; Mustelidae) using morphological data. *Canadian Journal of Zoology*, 65. 2536–2544.

Vanderhaar, J. M. & Hwang, Y. T. (2003). *Mellivora capensis*. *Mammalian Species*, 721, 1–8.

Verwoerd, D.J. (1987). Observations on the food and status of the Cape clawless otter *Aonyx capensis* at Betty's Bay, *South Africa. South African Journal of Zoology*, 22, 33–39.

Vianna, J.A., Ayerdi, P., Medina-Vogel, G., Mangel, J.C., Zeballos, H., Apaza, M. & Faugeron, S. (2010). Phylogeography of the Marine Otter (*Lontra felina*): Historical and Contemporary Factors Determining Its Distribution, *Journal of Heredity*, 101(6), 676–689.

Vickers, N.J. (2000). Mechanisms of animal navigation in odor plumes. *The Biological Bulletin*, 198(2), 203-212.

Vitale, J. D., Jordan, N. R., Gilfillan, G. D., McNutt, J. W., & Reader, T. (2020). Spatial and seasonal patterns of communal latrine use by spotted hyenas (Crocuta crocuta) reflect a seasonal resource defense strategy. *Behavioral Ecology and Sociobiology*, 74, 1-14.

Vitale, J.D., Jordan, N.R., Gilfillan, G.D., McNutt, J.W. & Reader, T. (2020). Spatial and seasonal patterns of communal latrine use by spotted hyenas (*Crocuta crocuta*) reflect a seasonal resource defense strategy. *Behavioral Ecology and Sociobiology*, 74(10), 1–14.

Voigt, C.C. & Helversen, O. (1999). Storage and display of odour by male *Saccopteryx bilineata* (Chiroptera, Emballonuridae). *Behavioral Ecology and Sociobiology*, 47, 29–40.

Voigt, C.C. (2002). Individual variation in perfume blending in male greater sac-winged bats. *Animal Behaviour*, 63, 907–913.

von Maltitz, G., Mucina, L., Geldenhuys, C., Lawes, M., Eeley, H., Adie, H., Vink, D., Fleming, G. & Bailey, C. (2003). Classification system for South African Indigenous forests. Environmentek report, CSIR Pretoria.

Wagnon, C.J. & Serfass, T.L. (2016). Camera traps at northern river otter latrines enhance carnivore detectability along riparian areas in eastern North America. *Global Ecology and Conservation*, 8, 138–143.

Wearn, O.R. & Glover-Kapfer, P. (2019). Snap happy: camera traps are an effective sampling tool when compared with alternative methods. *Royal Society Open Science*, 6(3), 181748.

Wegge, P., Pokheral, C.P. & Jnawali, S.R. (2004). Effects of trapping effort and trap shyness on estimates of tiger abundance from camera trap studies. *Animal Conservation*, 7, 251–256.

Wells, M.C. & Bekoff, M. (1981). An observational study of scent marking in coyotes, *Canis latrans*. *Animal Behaviour*, 29, 332–350.

Wenny, D.G. (2000). Seed dispersal of a high-quality fruit by specialized frugivores: High quality dispersal? *Biotropica*, 32, 327–337.

Werger, M.J.A. (1974). The Place of the Zürich-Montpellier Method in Vegetation Science. *Folia Geobotanica & Phytotaxonomica*, *9*(1), 99–109.

Wevers, J., Beenaerts, N., Casaer, J., Zimmermann, F., Artois, T. & Fattebert, J. (2021). Modelling species distribution from camera trap by-catch using a scale-optimized occupancy approach. *Remote Sensing in Ecology and Conservation*, 7, 534–549.

Wheeler, J.W. (1977). Properties of compounds used as chemical signals. In: Muller-Schwarze, D. & Mozell, M.M. (eds.). Chemical Signals in Vertebrates. Berlin, Germany: Springer US.

Whittaker, D.J., Gerlach, N.M., Slowinski, S.P., Corcoran, K.P., Winters, A.D., 6, Soini, H.A., Novotny, M.V., Ketterson, E.D. & Theis, K.R. (2016). Social Environment Has a Primary Influence on the Microbial and Odor Profiles of a Chemically Signaling Songbird. *Frontiers in Ecology and Evolution*.

Wiens, J. A. (1989). Spatial scaling in ecology. *Functional Ecology*, 3, 385–397

Wilson, G.J. & Delahay, R.J. (2001). A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation. *Wildlife Research*, 28,151–164

Wright, S.J. & Hubbell, S.P. (1983). Stochastic extinction and reserve size: a focal species approach. *Oikos*, 1983, 466–476.

Wronski, T., Apio, A., Plath, M. & Ziege, M. (2013). Sex difference in the communicatory significance of localized defecation sites in Arabian gazelles (*Gazella arabica*). *Journal of Ethology*, 31, 129–140.

Ye, Y.C. (2017). Marine geo-hazards in China. Elsevier.

Zacharias, M.A. & Roff, J.C. 2001. Use of focal species in marine conservation and management: a review and critique. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 11, 59–76.

Zala, S.M., Potts, W.K. & Penn, D.J. (2004). Scent-marking displays provide honest signals of health and infection. *Behavioral Ecology*, 15, 338-344.

Zaman, M., Tolhurst, B. A., Zhu, M., & Jiang, G. (2020). Den-site selection at multiple scales by the red fox (*Vulpes vulpes subsp. montana*) in a patchy human-dominated landscape. *Global Ecology and Conservation*, 23, e01136.

Zang, W., Sharma, R., Li, M. W. H., & Fan, X. (2021). Retention time trajectory matching for target compound peak identification in chromatographic analysis. arXiv preprint arXiv:2107.07658.

Zero, V.H., Sundaresan, S.R., O'Brien, T.G. & Kinnaird, M.F. (2013) Monitoring an Endangered savannah ungulate, Grevy's zebra *Equus grevyi*: choosing a method for estimating population densities. *Oryx*, 47, 410–419.

Zhang, J., Soini, H., Bruce, K., Wiesler, D., Woodley, S., Baum, M. & Novotny, M. (2005). Putative chemosignals of the ferret (*Mustela furo*) associated with individual and gender recognition. *Chemical Senses*, 30(9), 727–737.

Zhang, J., Sun, L., Zhang, Z., Wang, Z., Chen, Y. and Wang, R. (2002b) Volatile compounds in the anal gland of Siberian weasels (*Mustela sibirica*) and steppe polecats (*M. eversmanni*). *Journal of Chemical Ecology*, 28, 1287–1297.

Zhang, J., Zhang, Z., Wang, R., Chen, Y. & Wang, Z. (2002a) A headspace sampling method and its application in analysis of volatiles of anal glands of the American mink (*Mustela vison*). *Acta Zoologica Sinica*, 48, 137–141.

Zhou, Y., Chen, W., Buesching, C.D., Newman, C., Kaneko, Y., Xiang, M., Nie, C., Macdonald, D.W. & Xie, Z. (2015). Hog badger (*Arctonyx collaris*) latrine use in relation to food abundance: evidence of the scarce factor paradox. *Ecosphere*, 6(1), 19.

Ziege, M., Bierbach, D., Bischoff, S., Brandt, A.L., Brix, M., Greshake, B., Merker, S., Wenninger, S., Wronski, T. & Plath, M. (2016). Importance of latrine communication in European rabbits shifts along a rural-to-urban gradient. *BMC Ecology*, 16, 29.

Ziegler, T.E. (2013). Social effects via olfactory sensory stimuli on reproductive function and dysfunction in cooperative breeding marmosets and tamarins. *American Journal of Primatology*, 75, 202–211.

Zou, J., Wang, W., Pan, Y. W., Lu, S., & Xia, Z. (2015). Methods to measure olfactory behavior in mice. *Current protocols in toxicology*, 63, 11.18.1–11.18.21.

Zungu, N.S., Mostert, T.H.C. & Mostert, R.E. (2018). Plant communities of the uMlalazi Nature Reserve and their contribution to conservation in KwaZulu-Natal. *Koedoe*, 60(1), a1449.

Appendix A

Table A.1 Density estimates of African clawless otters (*Aonyx capensis*) obtained from previous research studies in South Africa

Research paper	Study areas	Natural/transformed	Density (km)
Van der Zee, 1982	Tsitsikamma Coastal National Park	natural	1 otter / 2 km
Arden-Clarke, 1986	Southern coast of South Africa	natural	1 otter / 2 km
Carugati, 1995	Mooi River (Kamberg Nature Reserves) - Stillerust section	natural	1 otter / 0.7 km
Carugati, 1995	Mooi River (Kamberg Nature Reserves) - "Hatchery" section	transformed	1 otter / 1.7 km
Kubheka et al., 2013	Kamberg Nature Reserves - Mooi River ("Hatchery" section)	natural	1/1.7 otter / 1 km
Carugati, 1995	Cobam Nature Reserves -Polela River	natural	½.5 otter / 1.7 km
Carugati, 1995	Loteni Nature Reserves - Loteni River	natural	1/2.5 otter / 1.25 km
Perrin & Carugati, 2006	KwaZulu-Natal Drakensberg park - Cobham section	natural	1 otter / 2.5 km
	KwaZulu-Natal Drakensberg park - Loteni section	natural	1 otter / 1.25 km
	KwaZulu-Natal Drakensberg park - Stillerust section	natural	1 otter / 1.25 km
	KwaZulu-Natal Drakensberg park – "Hatchery" section	transformed	1 otter / 2.5 km
	KwaZulu-Natal Drakensberg park - Farmland	transformed	1 otter / 2.5 km
Majelantle et al., 2021	Verloren Vallei Nature Reserve and Cobham Nature Reserve	natural	3.6 otters / 2.5 km
	Millstream Farm fish farm	transformed	7.17 otters / 2.5 km

Appendix B

Table B.1 The top ranked model's estimated coefficients summary of the 2nd ranked model output predicting the selection of latrine sites based on microscale habitat data collected. df = degrees of freedom. p value significance * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001

Parameters	Estimate	Std. Error	ChiSq	df and residual df	p-value
Intercept	5.902	2.665	-	-	0.027 *
Canopy Cover	-0.078	0.020	40.582	1,159	<0.001 ***
Horizontal Cover	-0.133	0.058	6.506	1,158	0.022 *
Slope	-0.317	0.450	13.784	1,157	0.482
Still Average	21.610	8.836	7.595	1,155	0.014 *
Vegetation Cover	-0.046	0.012	19.715	1,160	<0.001 ***
Windy Average	-3.442	1.284	5.688	1,156	0.007 **
Slope: Windy Average	0.420	0.282	3.273	1,154	0.137

Table B.2 The top ranked model's estimated coefficients summary of the 3rd ranked model output predicting the selection of latrine sites based on microscale habitat data collected. df = degrees of freedom. p value significance * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001

Parameters	Estimate	Std. Error	ChiSq	df and residual df	p-value
Intercept	8.759	3.420	-	-	0.010 *
Canopy Cover	-0.080	0.023	37.733	1,159	<0.001 ***
Elevation above water	-0.005	0.007	0.619	1,157	0.439
Horizontal Cover	-0.124	0.058	5.392	1,158	0.034 *
Slope	-0.944	0.836	14.395	1,156	0.259
Still Average	-0.518	15.770	7.299	1,154	0.974
Vegetation Cover	-0.055	0.016	21.299	1,160	<0.001 ***
Windy Average	-3.674	1.363	6.752	1,155	0.007 **
Slope: Still Average	5.584	3.580	3.283	1,152	0.119
Slope: Windy Average	0.514	0.381	2.668	1.153	0.177

Table B.3 The top ranked model's estimated coefficients summary of the 4th ranked model output predicting the selection of latrine sites based on microscale habitat data collected. df = degrees of freedom. p value significance * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001

Parameters	Estimate	Std. Error	ChiSq	df and residual df	p-value
Intercept	5.210	2.526	-	-	0.039 *
Canopy Cover	-0.104	0.029	48.297	1,159	<0.001 ***
Horizontal Cover	-0.128	0.055	6.625	1,158	0.021 *
Slope	0.030	0.266	13.784	1,157	0.910
Still Average	8.199	13.127	11.127	1,155	0.532
Vegetation Cover	-0.047	0.013	20.451	1,160	<0.001 ***
Windy Average	-2.183	0.948	6.257	1,156	0.021 *
Slope: Still Average	3.733	2.673	2.448	1,154	0.162