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THE ECOLOGY OF OTTERS IN AN URBAN ENVIRONMENT

Damian Winter Ponsonby

0502362F

School of Animal, Plant and Environmental Sciences

Supervisor: Dr Ute Schwaibold

A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree of Doctor of Philosophy.

Johannesburg, 2018

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



Damian Winter Ponsonby

22nd day of January 2018

ABSTRACT

Urban development has rapidly become the largest contributing factor of biodiversity decline across the planet. Regardless, certain species can survive these novel environments due to their opportunistic nature or occurrence in niche specific areas within urban areas. The aim of my study was to establish whether two otter species, the African clawless otter (*Aonyx capensis*) and the spotted-necked otter (*Hydrictis maculicollis*), in an urban environment showed similar ecological patterns in distribution (spatial arrangement), diet, and habitat use compared to conspecific individuals in areas with moderate to low levels of human disturbance. Firstly, I investigated the distribution and frequency of each species based on occurrence of signs (e.g. spraints and footprints) in relation to urban and peri-urban areas of central Gauteng, South Africa. Both species were present in central Gauteng, with a greater number of *A. capensis* signs found. No difference was observed between the number of signs found in urban and peri-urban areas for both species. Secondly, habitat variables measured near otter signs showed several differences in variables between urban and peri-urban areas. Otter movement through urban areas appeared to be associated with tall grass species, reed beds, and trees, which provided means of concealment for the animals, as well as avoidance of buildings to reduce human encounters. Thirdly, diet analysis based on prey remains in otter faecal samples resulted in lower than expected levels of crab and fish, and revealed higher than expected numbers of less common prey (e.g. birds and mammals) being consumed. Finally, the genetic diversity of individuals was measured using allele frequency to determine the level of reproductive success (ability to survive and produce viable offspring; Fisher, 1915) of *A. capensis*. Results showed a high level of genetic recombination between individuals in the population suggesting no movement restrictions are being experienced by otters, but genetic diversity was low. Otters are able to utilise resources available in this novel environment, which does not appear to be affecting movement, habitat utilisation or diet. A high genetic flow suggests successful use of urban areas, although there is concern about future genetic health in Gauteng based on the lower level of genetic variance.

DEDICATION

To my

grandparents

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CHAPTER ONE

Introduction

Currently, 54% of the world's population reside in urban areas with this number predicted to increase to 66% by 2050 (United Nations, 2015). The ever-increasing human population attracted to cities has resulted in a need for urban development further into natural environments (Grimm *et al*., 2008). The replacement of natural environments with cities and suburbs has greatly transformed the landscape and the associated plant and animal species with an increase in homogenisation of these areas (McKinney, 2008). Cities also introduce land cover features (e.g. paving and roads) that lead to increased surface water runoff, increasing flow rates and sedimentation in surrounding rivers, as well as the introduction of pollutants to water sources. These disturbances to rivers ultimately affect the occurrence of aquatic plant and animal species. Disturbed and homogenous environments provide less diverse resources to surviving species able to remain in the novel modified environments (McKinney, 2008). Many cities also have open spaces such as parks and sports fields which break the sprawl of buildings and concrete as a means for people to escape the built environment. These areas provide a novel habitat for animals to occupy, but not all animal species are suited to living in cities (Maciusik, Lenda and Skórka, 2010; Gese, Morey and Gehrt, 2012).

1.1 Wildlife in urban areas

Humans alter their surroundings to make the environment more accommodating to their needs, such as building homes for shelter and roads to improve movement across an area. These activities result in physical changes to the environment and often the disturbance of established natural systems and the removal of resources for the creation of new commodities, resulting in the introduction of waste products into the environment (Grimm, *et al*., 2000). This results in significant

impacts to the chemistry of ecological systems and can lead to changes in the structure of a system (Coucerio *et al*., 2007). The microclimate in cities is usually warmer than in the surrounding less disturbed areas due to the high level of paved surfaces such as parking areas and roads, which can absorb solar heat, resulting in an increase in average ambient temperature. High-rise buildings act as wind barriers, resulting in a decreased removal of warm air (Begon, Townsend and Harper, 2005). In addition, buildings create new habitats for organisms, providing them with shelter and protection from predators. Suburban homes have gardens that receive water in periods that are generally dry, allowing organisms species constant access to water. Food is abundant in cities in various forms, from fruiting trees in gardens to refuse accumulated in landfills. This high level of food availability is ideal for animals as it reduces energy exerted during foraging (Maciusik *et al*., 2010).

However, animals do face many risks associated with living in an urban area. These threats include physical harm from vehicles and people, persecution of animals that pose a perceived threat to the safety of humans, pollution both in physical and chemical form and habitat degradation through city development (Gese *et al*., 2012). Continuous expansion of cities, increases the amount of natural land altered or destroyed to accommodate the required space. Development leads to the fragmentation of habitats, and the ability of animals to move freely between areas is greatly reduced if not prevented entirely (Sutherland, 1998; Thomas *et al*., 2001). A common example of this is the construction of roads which introduces a barrier through an undisturbed ecosystem causing habitat fragmentation and thereby limiting the dispersal of organisms, often leading to the isolation of organisms to a specific area (Sutherland, 1998; Didham, Kapos and Ewers, 2012). Barriers also reduce home ranges which can lead to increased conflict between conspecifics as home ranges begin to overlap and available resources are contested. In some cases, animals can be cut off completely from resources, resulting in no available food or water. This geographic isolation, or fragmentation, results in the decreased interaction between conspecifics, and less genetic mixing occurs through reproduction (Maciusik *et al*., 2010). Population fragmentation and genetic isolation lead to

reduced dispersal potential, possibly resulting in inbreeding. (Didham *et al*., 2012).

While some species are unable to survive in urban areas, others are attracted to them and can utilise the novel environment and resources presented by urban areas. Animals that display generalist tendencies are much more likely to thrive in the urban environment. Bonier, Martin and Wingfield (2007) used a worldwide dataset of bird species (217 urban species and 247 rural species) to show that birds adapted to a wider range of environmental conditions may be better suited to deal with disturbed habitats. Birds in an urban environment have a greater environmental tolerance than their rural counterparts, as was shown by their larger elevational and latitudinal distributions during breeding (Bonier *et al*., 2007). Species that have a widespread distribution tend to have broader environmental tolerance levels, allowing individuals to emigrate from a region if environmental conditions become unfavourable. Individuals of species with larger home ranges covering a broader range of niches are less likely to encounter closely related (e.g. offspring, siblings) conspecifics (Glazier, 1986). Widespread species, at an individual level, demonstrate relatively opportunistic behaviours which make them better equipped to use alternative resources and occupy disturbed habitats easily (Glazier, 1986). Gese, Morey and Gehrt (2012) found that coyotes (*Canis latrans*) can occupy urban areas by increasing their home range to increase the chances of encountering prey in the urban environment, which represents widely dispersed viable habitat patches. Coyotes were also found to prefer less developed areas with lower human activity levels, although some coyotes were forced to occupy developed areas with higher human activity due to a lack of adequate available space (Gese *et al*., 2012). Racoons (*Procyon lotor*) introduced into Japan as pets have adapted successfully across the majority of the country, displaying a wide variation in home ranges dependent on the surrounding environment. Smaller home ranges are reported in urban areas, possibly due to the omnivorous nature of the animal resulting in a greater availability of food (in the form of refuse), and availability of shelter which reduces the area needed to cover to locate resources (Ikeda, *et al*., 2004).

1.2 Behavioural flexibility

An individual's ability to change its behaviour in response to novel situations by employing new response methods to cope with novel stimuli is a demonstration of behavioural flexibility. Another definition of behavioural flexibility is the adjustment of existing responses to familiar stimuli when required to improve fitness (Shettleworth, 1998; Reader and Laland, 2002). Adaptation of behaviour in urban areas is important for the survival of animals as urban areas introduce novelties animals are unlikely to encounter in less disturbed areas. These novelties include reduction of preferred food and habitat or introduction of alternative food sources (e.g. refuse) (McKinney, 2006), increased physical threats such as cars, pets, and humans (Coffin, 2007).

Adaptability demonstrated by individuals in urban areas will help them acquire food and find adequate shelter in the novel environment, improving the likelihood of the individuals reproducing (Sih, Ferrari and Harris, 2011). The ability to adjust behavioural patterns depending on surrounding environments is essential for the survival of all species, regardless of the level of human disturbance. This is necessary to tolerate seasonal changes, fluctuations in food availability and increased predator numbers (Atwell, *et al*., 2012; Miranda *et al*., 2013). Animals in urban areas can occur across a broader habitat range which reduces restriction to one specific habitat type, allowing them to move to another area if conditions become unfavourable. This has been observed in European stone martens (*Martens foina*) in urban areas that relocate denning sites from uninhabited buildings to inhabited buildings during winter, possibly due to inhabited buildings being insulated and heated (Herr *et al*., 2010). Red foxes (*Vulpes vulpes*) in Israel were found to increase their home ranges when easily accessible food sources (refuse from poultry farms) were removed (Bino *et al*., 2010).

A certain level of flexibility in behaviour, physiology and ecology may aid individuals present in urban areas in tolerating a broad array of environmental conditions, including habitat disturbances (Bonier *et al*., 2007). Animals may be able to change their behaviour in response to changing conditions due to this

flexibility, to decrease the chances of negative physiological effects of breeding in urban habitat, or to be able to utilise resources provided by the urban habitat, such as food or nest sites (Bonier *et al*., 2007). An example of this flexibility was found in the great tit (*Parus major*) exposed to high noise levels in urban environments. Slabbekoorn and Peet (2003) found that *P. major* changed the frequency of its call to compensate for high noise pollution in its urban habitat. *Parus major* utilises a higher frequency call to be heard above the low-frequency noises of its surrounding, resulting in other birds able to hear its call, thereby improving its reproductive success (individuals able to survive and reproduce viable offspring; Fisher, 1915) (Slabbekoorn and Peet, 2003). Generalist species tend to be opportunistic in nature, having a broader diet and are not restricted to one specific food source. Some opportunistic feeders make use of anthropogenic food sources such as refuse, or food set out to attract animals (e.g. placing fruits and seeds on a feeder for birds) (Maciusik *et al*., 2010). Raccoons show a tendency to utilise urban areas far more than other carnivores (coyotes and red foxes) in similar areas, demonstrating flexibility in habitat use by shifting to forage in urban areas that possible provide available food sources (in the form of refuge), which are not a part of their natural trophic niche (Randa and Yunger, 2006). Habitat use is possibly the most important aspect in the survival of an animal, and therefore requires some degree of flexibility to withstand environmental change. Habitat dictates the type of food present, the amount of cover to protect against predation, as well as predators present, and finally the abundance of viable mates (Rettie and Messier, 2000). To assess the ability of an animal to survive in an urban environment, the basic aspects of the animal's ecology and biology must be examined to identify innovative behaviours not expressed by individuals in less impacted areas, that improve overall fitness (Lowry *et al*., 2013). All these factors greatly impact on the overall fitness of an animal, and poor habitat selection can greatly impact the animal's chances of survival and reproduction.

Habitats are dynamic systems, constantly changing and dependent on external factors such as seasonality and rainfall, thus animal inhabitants must demonstrate certain levels of variability to withstand habitat change (Valdovinos *et al*., 2010). In colder months, available prey species numbers decrease, resulting

in a broadening of diet diversity expressed by, for example, honey badgers (*Mellivora capensis*) to maintain their body mass (Begg *et al*., 2003). A decrease in prey encounter rate by predators due to a reduction in prey abundance can result in a more intensive foraging pattern, but this increases active metabolism and energy loss, resulting in a greater requirement of food intake (Munk, 1995). Achieving an efficient balance between energy expenditure and intake is essential for the survival of predators, while at the same time avoiding inter- and intra-specific competition over prey (Miranda *et al*., 2013).

1.3 Eco-evolutionary factors

Eco-evolutionary dynamics investigates the feedback loop occurring between ecology and evolutionary factors influencing a species over a relatively short period of time. Environmental changes (habitat removal, climate change, introduction of invasive species) influence evolutionary change in species, which results in changes in ecological interactions (Pelletier, Garant and Hendry, 2009; Post and Palkovacs, 2009). In the case of urbanisation novel habitat and resources have been introduced that have had mixed (negative, positive or neutral) effects on animals (Clucas and Marzluff, 2011). Urban areas provide animals with novel food and shelter, which actually attracts some species to these urban areas and improves fitness of the species (Evans *et al*., 2009; Herr *et al*., 2010; Bateman and Fleming, 2012), but the viability of such behaviour may be questionable, and animals may occur in 'ecological traps' (Battin, 2004). Ecological traps are harmful habitats that species tend to prefer rather than avoid (Delibes, Gaona and Ferreras, 2001), often leading to localised extinction (Donovan and Thompson, 2001). Habitats affected by anthropogenic activities may offer unsuitable resources for native species (McKinney, 2006; 2008), as well as introducing other threats such as cars, pedestrians, companion animals, and pollution (Lowry *et al*., 2013). In some instances, individuals tend to become less cautious of humans in order to utilise these resources, which can be detrimental to both human and animal alike (Rauer, Kaczensky and Knauer, 2003). To fully appreciate the way a species can survive in a changing environment many aspects need to be

considered. It is not a simple process of identifying the vegetation cover a species seeks for shelter or the type of food consumed for nutrition, but how all these individual elements complement one another resulting in the optimal combination for the greater fitness of individuals in a species. Eco-evolutionary dynamics assesses the relationship between ecological factors and the evolutionary changes that result from these factors (Ezard, Côté and Pelletier, 2009; Pelletier *et al*., 2009). However, to fully understand the feedback system occurring, one needs extensive data relating to the various aspects influencing a species. These data are necessary to successfully determine how behavioural and physiological traits change in response to ecological stressors (Ezard *et al*., 2009; Post and Palkovacs, 2009). Once a firm understanding of the feedback system is acquired, it is possible to make predictions relating to the survivability of a species going forward into the future. This will further help determine how species will adapt to possible changes in the environment, which may result from impacts such as climate change or increased human disturbance in an area (Johnson, Vellend and Stinchcombe, 2009).

Due to the inherent ability to survive in a specific area, exposing species to an environment dramatically different from their original habitat may result in the local extinction of a species, because the physiology of individuals would not be able to tolerate a change in temperature or they would not be able to find food to which they are accustomed (Begon *et al*., 2005; Hofmann and Todgham, 2010). It is very unlikely that the habitat of a species will dramatically change over a short period of time, although populations do face a somewhat rapid habitat change in response to development (Sutherland, 1998; Grimm *et al*., 2000). The rapid rate of change presented by human development requires the study of animals affected by changing habitats to understand the risks to species' survival in the future.

A method of testing the survivability of a species is by assessing the reproductive success of the animal, measurable by the number of surviving offspring parented by two individuals that have a high level of genetic variance. This decreases the chance of homozygosity and the expression of recessive genes that may be detrimental to the health of the animal (Broom, 1991). The ability to find alternative food sources or habitat closely matching the animal's natural

environment is not all that is needed to succeed in the urban environment. Animals need to be able to interact and exchange genes for the species to be successful. This can be difficult in the city as barriers obstruct the movement of animals between suitable habitats, thus reducing the chance of interacting with conspecifics outside of family groups (Bascompte and Solé, 1996). A reduction in non-family interactions can result in an increased level of inbreeding, possibly causing a reduction in the overall health of the species (Amos *et al*., 2001). Therefore, to be successful, an animal is required to navigate through the environment with few barriers to movement and reproduce with unrelated conspecifics.

1.4 Southern African otters

Otters belong to the subfamily Lutrinae in the family Mustelidae, which is the largest family group in the order Carnivora. Lutrinae is divided further into seven genera: *Aonyx, Lutrogale, Lutra, Enhydra, Hydrictis, Lontra, and Pteronura* (Koepfli *et al*., 2008). Two otter species occur in South Africa, *Aonyx capensis* (Schinz, 1821) and *Hydrictis maculicollis* (Lichenstein, 1835). *Hydrictis maculicollis* was previously placed in *Lutra* based on work by Koepfli and Wayne (1998), but was later included in *Hydrictis* (Pocock, 1921) based on analyses by Koepfli *et al*. (2008).

Aonyx capensis (common names: African clawless or Cape clawless otter) is the larger of two otter species present in southern Africa, with males larger than females (♂♂ head and body length (HB) = 1130–1380 mm, ♂♂ weight (WT) = 10.0–18.0 kg; ♀♀ HB = 1140–1330 mm, mean ♀♀ WT = 10.0–13.8 kg; Somers and Nel, 2013). The dorsal side of the body is covered in dense dark brown fur consisting of guard hairs, ranging from 10 mm on the head and tail to 25 mm on the mid-back (Skinner and Chimimba, 2005). The chin, throat, chest and abdomen are lighter (white to off-white) than the dorsal region of the body and shorter white or off-white underfur occurs beneath the guard hairs and is often obscured by the top coat (Skinner and Chimimba, 2005). The tail is long and dorsoventrally flattened (♂♂ tail length (T) = 445–570 mm; ♀♀ T = 450–495 mm; Somers and

Nel, 2013). As the common name implies, the otter does not possess the usual claws on the hind and forefeet, but simple nails are present on the digits of the hind feet. Five digits are present on both fore and hind feet, with webbing only on half the length of the hind feet and barely visible on the fore feet (Skinner and Chimimba, 2005). The skull is robust with a large thick lower jaw and broad molars suited for breaking open the carapace of crustaceans. Upper canines are quite large and sharp (Skinner and Chimimba, 2005). Spraints (faeces) of adult *A. capensis* are 22–29 mm in diameter and are found within 10 m (usually 2–4 m) of water (Rowe-Rowe, 1992). Spraints predominantly contains fragments of crabs and have a fishy odour. Fresh spraints are dark brown in colour, fading to a creamy white over time. *A. capensis* spraints are often found at spraint sites (latrines) (Rowe-Rowe, 1992).

The distribution of *A. capensis* extends widely across Africa in most tropical and sub-tropical areas of sub-Saharan Africa from Senegal to Ethiopia and down to the Western Cape of South Africa (Somers and Nel, 2013; Figure 1.1). The species is always found near freshwater sources and occurs in most aquatic environments, ranging from freshwater lakes to marine environments (Nel and Somers, 2007). Home range length extend from 4.9 to 54.1 km with core home range (area of highest activity e.g. denning sites) length from 0.2 to 9.8 km (Somers and Nel, 2004a). It requires the cover of dense vegetation, fresh water (for drinking, foraging, cleaning its fur, and movement) and the presence of holes or rocks as a means of refuge (van Niekerk, Somers and Nel, 1998; Somers and Nel, 2004a). It shelters in holts (dens) in dense vegetation and under rocks or in burrows it has dug (Rowe-Rowe, 1992). Rowe-Rowe (1992) found holts covered by specific vegetation, namely *Merxmuellera* spp. and *Leucosidea sericea*. It has also been known to use hollow trees as refugia (Walker, 1996). Holts are dug in the riverbank, sometimes with a second hole which emerges in the vegetation on the bank. Holt entrance holes are oval-shaped with a mean width of 361 ± 85 mm and mean height of 246 ± 32 mm as recorded by Rowe-Rowe (1992). *Aonyx capensis* is more likely to use areas with dense reed beds and rocky substrate in riparian habitat (Nel and Somers, 2007). The presence of sprainting sites occurs in the grass above river banks, near deep water (0.5–1 m deep) and near holts

(Rowe-Rowe, 1992). Main activity patterns occur during the late afternoon and early evening, and occasionally at dawn or early morning (Somers and Nel, 2004a). *Aonyx capensis* are adept swimmers and will spend more time in the water than on land. After swimming, they dry themselves by rubbing against vegetation, the ground or rocks, this activity can lead to the flattening of vegetation, known as rolling sites (Skinner and Chimimba, 2005).

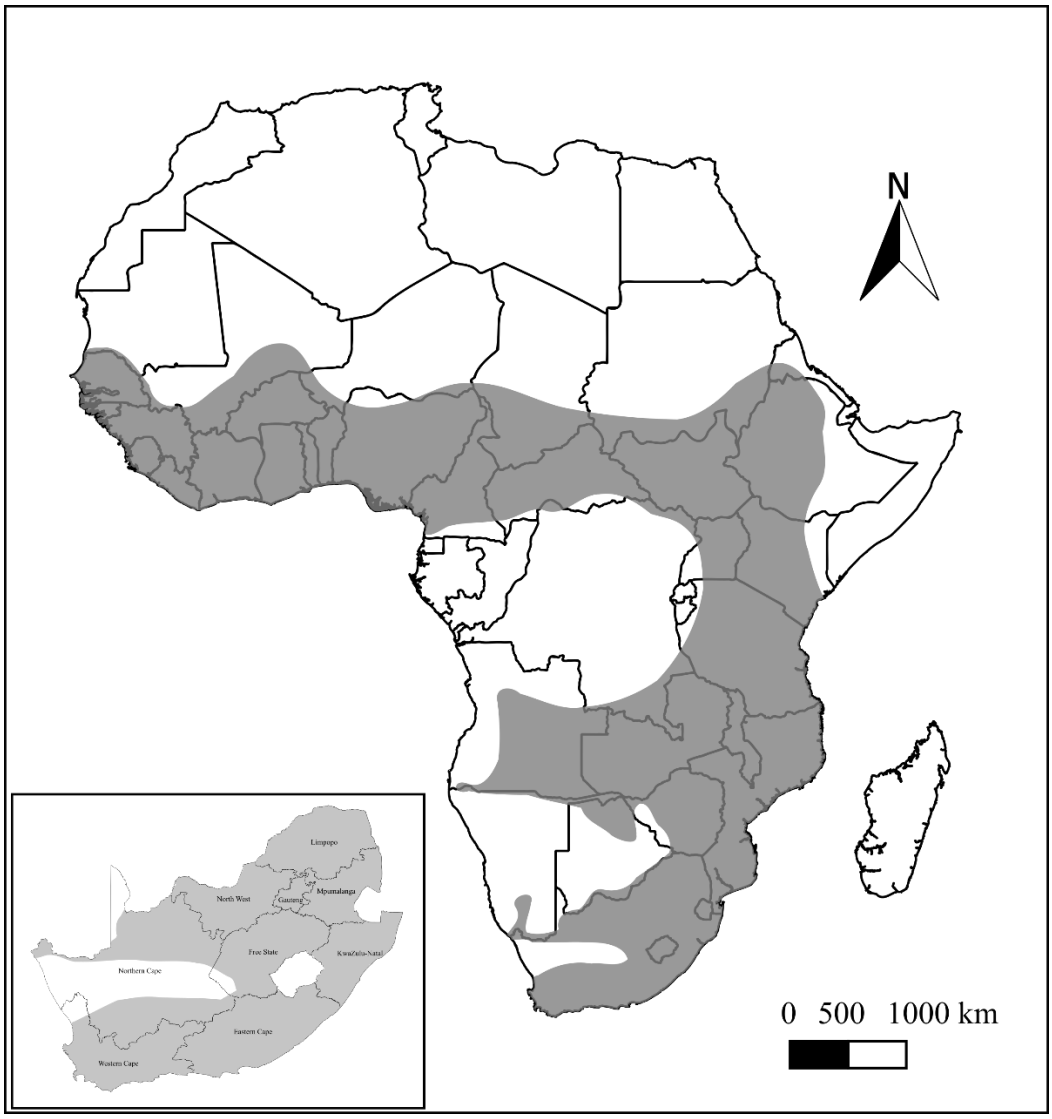


Figure 1.1 Distribution of *A. capensis*, South African range as inset (Jacques, Reed-Smith and Somers, 2015).

The African clawless otter is described as a crab eater, but has also been known to eat fish, frogs, insects, waterfowl and small mammals (shrews and

rodents) (Rowe-Rowe, 1977a; van der Zee, 1981; Ligthart and Nel, 1994; Somers and Purves, 1996; Perrin and Carugati, 2000a; Somers and Nel, 2003; Parker, Burchell and Bernard, 2005). The selection of secondary prey seems to be related to prey availability (Rowe-Rowe and Somers, 1998). Even though the otter is sometimes observed in groups, foraging is a solitary activity (Rowe-Rowe and Somers, 1998). The otter will use their forelimbs to search for crabs as they walk along the riverbed, occasionally submerging their head (Rowe-Rowe, 1977b; Somers, 2000a). In deeper water the otter will dive underwater and search the riverbed for prey (Rowe-Rowe, 1977b). Rowe-Rowe (1977b) measured dives to last up to 26 seconds, with an average dive time of 17.4 seconds. Upon capture, small fish and crabs are usually eaten in the water, but sometimes larger crabs and fish are taken to the riverbank and consumed using the forelimbs (Rowe-Rowe, 1997b; Somers, 2000). Usually the entire crab is consumed, including the hard shell of the carapace, although with very large crabs otters may not consume the pinchers, and otters will not eat the pinchers, carapace and sometimes the legs of crayfish (Somers, pers. comm.). Fish are eaten head first as opposed to *H. maculicollis* which eats fish from tail to head (Rowe-Rowe, 1977b).

The African clawless otter is predominantly a solitary animal, but can sometimes be found in pairs or small family groups (Somers and Nel, 2004a). Otter group sizes range from one to five individuals in KwaZulu-Natal and Tsitsikamma National Park respectively (van der Zee, 1981; Arden-Clarke, 1986). Females have been shown to be territorial, while males have home ranges that overlap with other individuals of both sexes (Somers and Nel, 2004a). Gestation lasts 60 to 64 days, with one to three pups born at varying times throughout the year. Pups are weaned at eight weeks and the average life expectancy is around 14 years (captivity; van der Zee, 1981; Arden-Clarke, 1986).

The most recent IUCN Red List assessment has listed *A. capensis* as ‘Near Threatened’ owing to habitat loss, water pollution and degradation of the associated ecosystems of *A. capensis* through increased presence of invasive species and agricultural activity along rivers. These impacts affect resources required by *A. capensis* such as vegetation for denning and resting sites, and prey present in rivers (Jacques, Reed-Smith and Somers, 2015).

Hydrictis maculicollis (common name: spotted-necked otter) is much smaller in size than *A. capensis* (♂♂ HB = 650–760 mm, ♂♂ WT = 4.5–6.6 kg; ♀♀ HB = 570–606 mm, ♀♀ WT = 3.8–4.7 kg; Perrin and d'Inzillo Carranza, 1999). The body is covered with a chocolate-brown to dark reddish coloured dense fur, with a white to off-white undercoat. This colouration is generally constant on all parts of the body, except for white mottling on the throat and neck (Skinner and Chimimba, 2005; D'Inzillo Carranza and Rowe-Rowe, 2013). The tail is long and dorsoventrally flattened (♂♂ T = 350–440 mm; ♀♀ T = 335–440 mm; D'Inzillo Carranza and Rowe-Rowe, 2013). Feet are webbed with five toes, each possessing a 10-mm creamy-white claw. The spraints of adults are 12–18 mm in diameter and are found within 10 m (usually 2–4 m) of water (Walker, 1996). If the otter's diet has consisted mostly of crab, the spraints will be dark brown fading to cream over time. If fish has been the predominant prey item, the spraints will be dark brown fading to light grey (Rowe-Rowe, 1992).

The spotted-necked otter is restricted to permanent freshwater in the tropic and sub-tropic regions of sub-Saharan Africa (D'Inzillo Carranza and Rowe-Rowe, 2013). Rowe-Rowe and Somers (1998) reported the range from Sierra Leone eastward to Chad, then from southern Central African Republic to south-western Ethiopia. On the western side of Africa, they occur up to the northern border of Namibia. To the east, they can be found from western Kenya and Tanzania to areas of Mozambique and eastern South Africa. An out of range sighting (1200 km from recorded distribution) on the lower Orange River was recorded by Power and Slater-Jones (2010) in the Ais/Ais Richterveld Transfrontier Park (Figure 1.2). *Hydrictis maculicollis* is not as widely distributed as *A. capensis* and is only found in freshwater (Rowe-Rowe and Somers, 1998). Mean area of core home range has been estimated to be 8.7 km^2 for males and 3.4 km^2 for females, with an overall mean of 6 km^2, and the length of river in this measured range was 14.8 km (Perrin *et al*., 2000). *Hydrictis maculicollis* requires dense vegetation or holes for refuge (Rowe-Rowe, 1992; Perrin and Carugati, 2000b). It is active in early morning and late afternoon and have been known to forage in the evening when sufficient moonlight is available (Perrin and D'Inzillo Carranza, 2000a). The spotted-necked otter also rolls on vegetation, the ground or

rocks after swimming to dry off, leaving definitive rolling spots of flattened vegetation known as rolling sites (Rowe-Rowe, 1992).

Hydrictis maculicollis has been observed foraging several times over a 24-hour period, and may sometimes forage as a group but cooperative hunting does not seem to occur (Reed-Smith *et al*., 2014). This species eats predominantly fish but can consume crabs, frogs, and insects and in rare cases birds, mammals and reptiles remains have been found in faeces (Rowe-Rowe, 1977a; Somers and Purves, 1996; Perrin and Carugati, 2000a). There does not seem to be any form of territoriality demonstrated by *H. maculicollis*, with home-range overlap occurring both intra- and inter-sexually (Perrin, D'Inzillo Carranza and Linn, 2000). Generally, individuals appear to be solitary but can occur in groups of up to 12 individuals, depending on the activity and members present. Groups usually consist of females and pups, adolescent groups, and same sex groups (Reed-Smith *et al*., 2014). Mating has been reported to occur from May through to August and gestation is estimated to be around 60–63 days with young being born toward the end of the dry season (reported by D'Inzillo Carranza and Rowe-Rowe, 2013). There are typically two pups in a litter, but occasionally three, with young being born in holes along riverbanks, rocky crevices or reed beds (Skinner and Chimimba, 2005).

The spotted-necked otter is classified as 'Near Threatened' on the IUCN Red List due to increasing conflict with humans over resources (water, land and fish), as well as a decrease in suitable habitat. Increase of water pollution (industrial and domestic waste introduced to rivers) and habitat degradation due to human activities have a profound impact on resources required by *H. maculicollis* (Reed-Smith, Jacques and Somers, 2015).

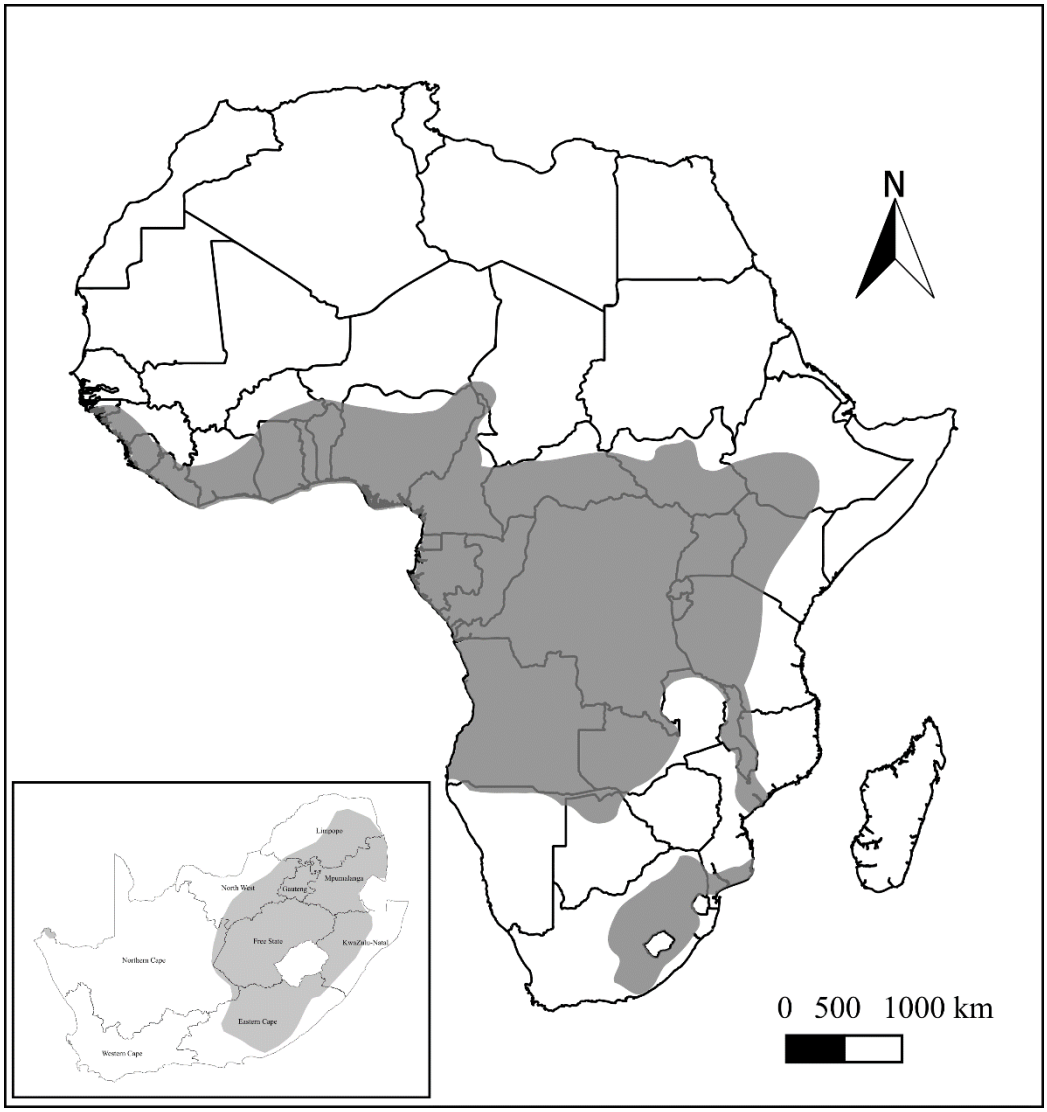


Figure 1.2 Distribution of *H. maculicollis*, South African range as inset (Reed-Smith *et al*., 2015).

1.5 Study area (Gauteng and rivers)

The province of Gauteng is located between 1081 m and 1899 m above sea level (GCRO, 2013) on the interior plateau of South Africa and receives most of its rainfall during the summer months. Average temperatures range from 14.8 °C to 26.3 °C in summer and 4.3 °C to 18.8 °C in winter. The mild and relatively dry (average 689.7 mm rainfall per year) climate supports a grassland biome with a small area of bushveld (SAWS, 2010).

Gauteng is considered the economic centre of the country, contributing 34% of South Africa's GDP (OECD, 2011). In the past, the province was economically reliant on agriculture until the discovery of vast mineral deposits in and around Johannesburg, subsequently leading to the economy becoming largely dependent on mining and mineral extraction services. While the mining and minerals sector is still important to the economy, secondary and tertiary sectors are the largest contributing factors to the economy which is attracting both local and international investors.

The great economic success of the province has drawn a large population of 13.2 million into a space of roughly 18 179 km^2 (GCRO, 2013; StatsSA, 2015). Two major municipalities are situated in Gauteng, Tshwane to the north and Johannesburg in the centre. The central region of Gauteng comprises mostly densely populated urban areas with extensive built-up sectors of residential, commercial and industrial buildings interlinked by extensive road networks. Surrounding this urbanised central region are expanses of natural land, small holdings and agricultural land (GDARD, 2014). Gauteng is divided into the Crocodile West Marico, Olifants, and Vaal catchments which are comprised of numerous rivers across the province (GCRO, 2013), from which 11 were chosen for this study, and include the following (see Figure 1.3):

a) Modderfonteinspruit begins in an industrial area to the northeast of Johannesburg and flows into the Jukskei River. Its journey to the Jukskei River flows through two more industrial areas, and receives effluent from two sewage works (van Veelen, 2002). The water has a high mineral content from the industrial areas as well as a power station occurring along the stream (Huizenga and Harmse, 2005) and contains sulphate, fluoride and ammonia from surrounding industrial areas (van Veelen, 2002). Several areas of the catchment are urbanised but most of it comprises open areas and agricultural holdings. Despite this and due to the high level of industrial effluent entering this stream, it is the most affected stream in the Jukskei catchment (van Veelen, 2002).

b) Sandspruit is a tributary of the Braamfonteinspruit that flows through an industrial area and suburban areas of northern Johannesburg. The catchment area surrounding the Sandspruit is completely urbanised. The stream bed is sandy, hence the name of this stream (van Veelen, 2002).

c) Braamfonteinspruit is a stream that begins in central Johannesburg, flows through the northern suburbs of the city and feeds into the Jukskei River. It is known for draining sewage and storm water runoff (Foster, 2009).

d) The Jukskei River, one of the main tributaries of the Crocodile River, flows predominantly through the city of Johannesburg. The upper portion of the river is diverted through underground culverts (van Veelen, 2002). Below the point of joining the Modderfonteinspruit, the river receives industrial and sewage effluent, resulting in higher levels of plant growth nutrients (nitrogen and phosphorus) and an increase in algal biomass (van Veelen, 2002). Studies have shown extremely high levels of *Escherichia coli* (van Veelen, 2002). The river has shown high concentrations of sodium, potassium, phosphate and nitrate, mostly likely attributed to the increase in population density in the Jukskei Catchment area (Huizenga and Harmse, 2005).

e) The Klein Jukskei River flows through the suburbs north-west of Johannesburg, a golf course and industrial area. Upper sections of the river are urbanised with areas downstream consisting of agricultural holdings. One of the streams feeding into the Klein Jukskei flows past another industrial area and a municipal waste disposal site (van Veelen, 2002). Despite the level of exposure to industrial areas, no evidence of water pollution has been reported in the river during the period of 1979-2002 (Huizenga and Harmse, 2005).

f) The Crocodile River joins the Jukskei below the major polluting discharges. This river receives runoff from the predominantly agricultural

surrounding land (Taylor *et al*., 2005) and contains nitrogen, phosphorus and organic matter from waste discharge (Walsh and Wepener, 2009). The river is exposed to minor contamination by mine water, specifically north of the confluence with the Hennops River, with of most the river not showing signs of water pollution (Huizenga, 2004).

g) Hennops River is a tributary of the Crocodile River which receives drainage from surrounding agricultural smallholdings and is exposed to high levels of pollution due to poor sanitation systems and overloaded sewerage systems from northern Johannesburg (DWAF, 2004). It shows signs of inorganic pollution, primarily from mine water and urbanisation (Huizenga, 2004).

h) The Wonderfonteinspruit/Mooi River Loop originates to the west of Johannesburg, flows past the mining areas of Carletonville and joins up with the Mooi River. The river receives contamination from various points as there are mine residue deposits of old mines around the point of origin of the river, while active gold mines are discharging process water into the water environment. Several growing human communities in the catchment contribute to the level of pollution in the river (DWAF, 2009).

i) Klip River was exposed to extensive gold mining activity in surrounding areas which may have led to potential polluting of ground and surface water. The upper section of the catchment area has several runoff points from old mines and mine residue deposits. Many settlements occur along this river, leading to urban runoff and the possibility of sewage entering the river (DWAF, 2003a; 2009). The upper reaches of the Klip River are also exposed to the effluent of the three southern Johannesburg Water Waste Water Treatment Works (WWTWs) and four WWTWs to the east. This pollution has led to high nutrient levels and pathogens affecting water quality (DWAF, 2009). The lower section of the river is exposed to agricultural land, a WWTW, and industrial areas (DWAF, 2009).

j) Blesbokspruit is a stream that flows through the eastern portion of Gauteng and supplies water to the Vaal Dam in southern Gauteng. The stream receives untreated acid rock drainage from the Grootvlei Mine, leading to high levels of iron and suspended solids being present in the stream (de Wet and Sidu, 2013).

k) Pienaars River is one of the several major tributaries feeding the Crocodile River, which receives treated sewage discharge from the Baviaanspoort Sewage Treatment Plant (Pieterse and Toerien, 1978; Satory, 1988; DWAF, 2004). The middle section of the river receives agricultural and industrial effluent (Satory, 1980; DWAF, 2004).

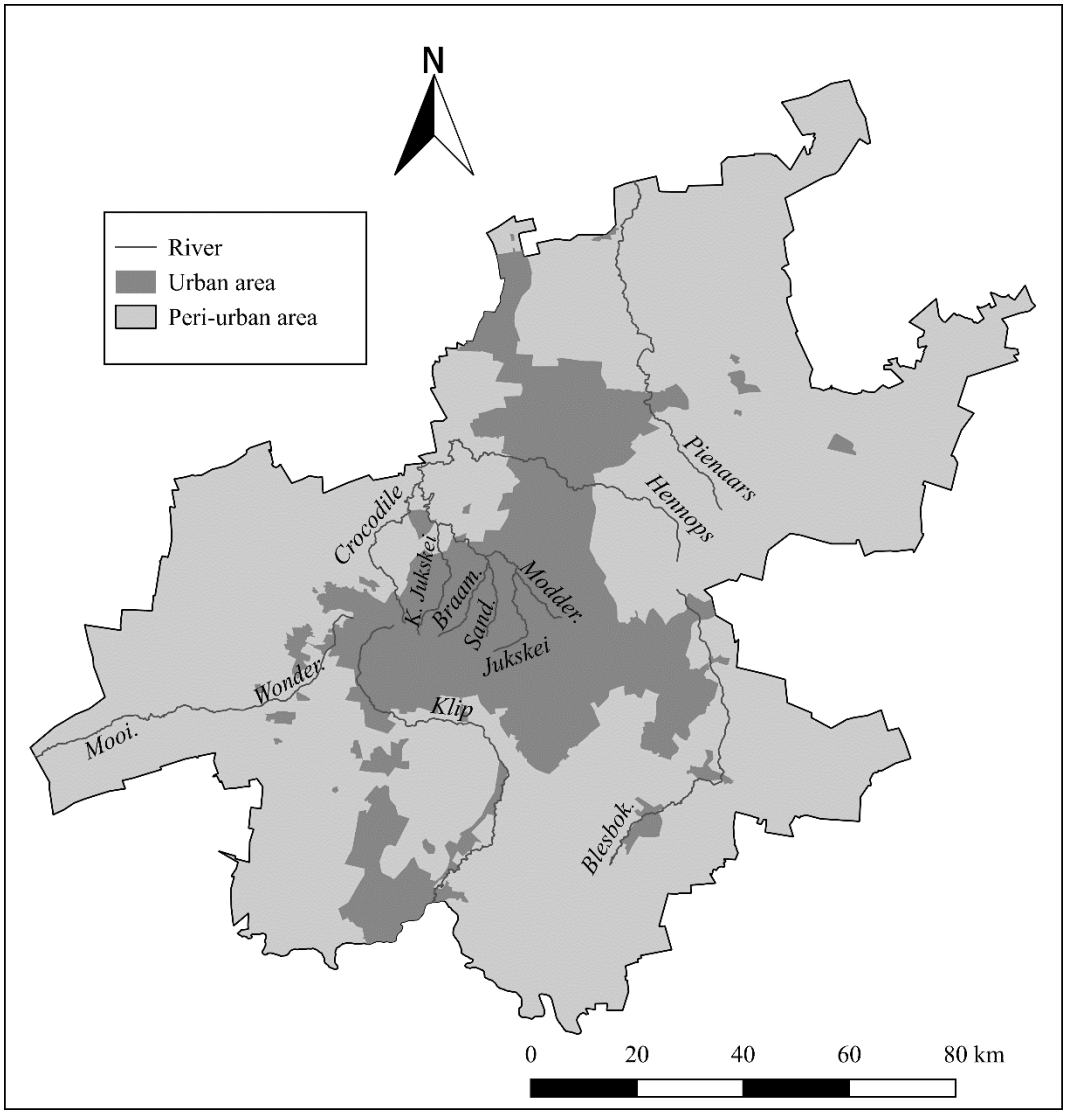


Figure 1.3 Eleven rivers selected for survey during this study. Urban areas within the province are indicated showing the relation between study rivers and these modified regions of Gauteng (GDARD, 2011). (Mooi. – Mooi River Loop; Wonder. – Wonderfonteinspruit; K. Jukskei – Klein Jukskei River; Braam. – Braamfonteinspruit; Sand. – Sandspruit; Modder. – Modderfonteinspruit; Blebok. – Blesbokspruit).

The expansion of urban areas in Gauteng, South Africa has been rapid and extensive over the past decade to accommodate the ever-increasing human population (GCRO, 2013). This greater level of development weighs heavily on the surrounding natural habitats that are replaced with novel urban elements such as roads and buildings. Assessing the impacts urban encroachment has on native animal species is relevant to ecologists due to the long-term effects of land

modification required in city development (McKinney, 2006). To understand how a species can successfully survive in an urban environment, from an ecological viewpoint, two otter species were selected for study. Distribution maps (see Jacques *et al*., 2015; Reed-Smith *et al*., 2015) of *A. capensis* and *H. maculicollis* indicate that both species are present in areas with high urban development in Gauteng, but no intensive ecological studies have been conducted to validate this claim. Several diet, habitat, distribution and movement behaviour studies have focused on *A. capensis*, and *H. maculicollis* to a lesser extent, in coastal regions of South Africa, with moderate to little human disturbance, over the past 40 years (see Rowe-Rowe, 1977a, 1977b; van der Zee, 1981; Arden-Clarke, 1986; Rowe-Rowe, 1992; Ligthart and Nel, 1994; Carugati, Rowe-Rowe and Perrin, 1995; Somers and Purves, 1996; Rowe-Rowe and Somers, 1998; van Niekerk *et al*., 1998; Perrin and D'Inzillo Carranza, 1999, 2000a, 2000b; Perrin and Carugati, 2000a, 2000b; Somers, 2000a, 2000b; Somers and Nel, 2003, 2004a, 2004b; Parker *et al*., 2005; Perrin and Carugati, 2006; Nel and Somers, 2007; Kubheka *et al*., 2013; Jordaan *et al*, 2015). These previous studies provide important knowledge that will be used to in the comparison the ecology of *A. capensis* and *H. maculicollis* in areas of varying levels of human disturbance.

1.6 Aims and objectives

The aim of this study was to establish whether two otter species, *A. capensis* and *H. maculicollis*, showed similar ecological patterns compared to conspecific individuals in areas with moderate to low levels of human disturbance. By comparing the ecology of *A. capensis* and *H. maculicollis* in a highly-impacted area to otters in less impacted environments, their ability to adjust and survive in an environment with high levels of human disturbance could be assessed. This study also provides new knowledge to the current body of work on *A. capensis* and *H. maculicollis*, and will aid in future work on these species.

1.7 Arrangement of thesis

Chapter 1 (current chapter) is an introduction into the effects urban areas have on wildlife. It also presents the study area and species that were examined in this study.

Chapter 2 determines the distribution (spatial arrangement) of *A. capensis* and *H. maculicollis* within urban and peri-urban (less developed areas surrounding the urban areas) areas of central Gauteng.

Chapter 3 investigates the correlation between habitat variables and presence of otter signs to understand the habitat use of otters in areas of varying human disturbance.

Chapter 4 is aimed at identifying and comparing the diet of otters in Gauteng to conspecifics elsewhere in South Africa. Cities present animals with novel resources which may lead to otters in urban areas consuming food that falls outside the trophic niche of conspecifics in less impacted areas.
The aim of Chapter 5 is to assess the genetic health of otters occurring in central Gauteng to provide insight to the current genetic variation of this population and to identify signs of possible barriers to gene flow.

Chapter 6 is a concluding chapter that discusses the findings from each experimental chapter and interprets the connectivity between distribution, habitat use, diet, and genetic health of otters in an area with varying levels of human disturbance. It also sets up hypotheses for further research focused on the two studied otter species.

The experimental chapters are written in the form of manuscripts intended for publication, and therefore a certain level of overlap occurs between the chapters with regard to referencing and methods. A single reference list for all chapters is

presented following Chapter 6. Figures and tables are numbered according to chapter, and page numbering is continuous throughout this thesis.

Appendix A is a short communication focused on the diet of *H. maculicollis* based on spraints found during the study. This work was conducted separately from the *A. capensis* diet analysis due to the small sample size of spraints found.

Appendix B is a published methodology article, which I co-authored, explaining the technique used to develop Cytochrome B (*Cyt B*) primers, used to accurately identify *A. capensis* and *H. maculicollis* based on DNA extracted from spraint samples. My contributions toward this article included, sample collection, DNA extraction from samples, shared laboratory workload of PCR and sequencing. I also conducted analysis of resulting sequence chromatograms and then shared the workload of further laboratory work required for the testing of developed partial *Cyt B* primers, as well as writing a large portion of the article.

CHAPTER TWO

Country Otter, City Otter: the distribution patterns of two otter species in an urbanised area of Gauteng, South Africa

Abstract

Current coarse-scale distribution maps of two African otter species, *Aonyx capensis* and *Hydrictis maculicollis*, indicate their occurrence in several urbanised areas of South Africa. There are however no fine-scale studies focusing on the actual distribution of these two species in urban areas. This study aimed to develop a more accurate representation of the distribution of *A. capensis* and *H. maculicollis* in the central region of Gauteng in South Africa which has an extensive river system that flows through both urban and peri-urban areas. Eleven rivers were surveyed for signs of otter occurrence (footprints, holts, spraints/scat and sightings). Species identification was based on analysis of DNA extracted from spraints and occurrence of positive signs such as holts and tracks, and the distribution of all signs was mapped. Signs were found along ten of the 11 rivers surveyed, but the mean percentage of sign occurrence was low (16.64%). In total, 247 positive signs of occurrence were recorded; three sightings were of the animals themselves, ten were footprint sightings and 234 were spraint samples. Of all the signs observed, 181 signs were of *A. capensis* and only 11 positive signs were found for *H. maculicollis*, while the remaining signs could not be allocated. Significantly more signs were found in the peri-urban areas for both *A. capensis* and *H. maculicollis*, than the urban areas. The occurrence of signs indicates both species are present in urban areas, but at a much lower frequency than in the peri-urban areas.

2.1 Introduction

The current distribution (geographical region in which a species can be found) of two African otter species, *Aonyx capensis* and *Hydrictis maculicollis*, indicate the ranges of both species cover aquatic habitats across sub-Saharan Africa. These species are found in almost every environment, excluding arid deserts (Skinner and Chimimba, 2005; D'Inzillo Carranza and Rowe-Rowe, 2013; Somers and Nel, 2013). Within South Africa, *A. capensis* is widespread and occurs in all nine provinces of the country, mainly along rivers but also along coastal waters close to freshwater sources (Skinner and Chimimba, 2005). *Hydrictis maculicollis* is restricted to the eastern regions of South Africa and has a much smaller reported distribution than *A. capensis*, and neither otter species are likely to occur in drier regions of the country (Skinner and Chimimba, 2005). Range determination was based on scientific research as well as anecdotal evidence, with some areas more intensively studied than others. *Aonyx capensis* home range measurements extend from 4.9 to 54.1 km with core home range (area of highest activity e.g. denning sites) length from 0.2 to 9.8 km (Somers and Nel, 2004a). Mean area of *H. maculicollis* core home range has been estimated to be 8.7 km^2 for males and 3.4 km^2 for females, with an overall mean of 6 km^2, and the length of river in this measured range was 14.8 km (Perrin *et al*., 2000). Within South Africa an extensive collection of published data relating to otter presence, diet and habitat use in provinces along the coastline of the country is available for Kwa-Zulu Natal (Rowe-Rowe, 1992; Perrin and D'Inzillo Carranza, 2000a; Perrin and Carugati, 2000b: Kubheka *et al*., 2013), the Eastern Cape (van der Zee, 1981; Arden-Clarke, 1986; Somers and Purves, 1996), the Western Cape (Ligthart and Nel, 1994; van Niekerk *et al*., 1998; Somers and Nel, 2004a, 2004b) and an out-of-range sighting in the Northern Cape (Power and Slater-Jones, 2010). To our knowledge research covering the central provinces of South Africa, including Gauteng, is limited, even though distribution maps (Figure 2.1) indicate the occurrence of both species in these areas. The most informative occurrence data set for otters in Gauteng is that of Rautenbach (1982), which was based on museum samples and sightings.

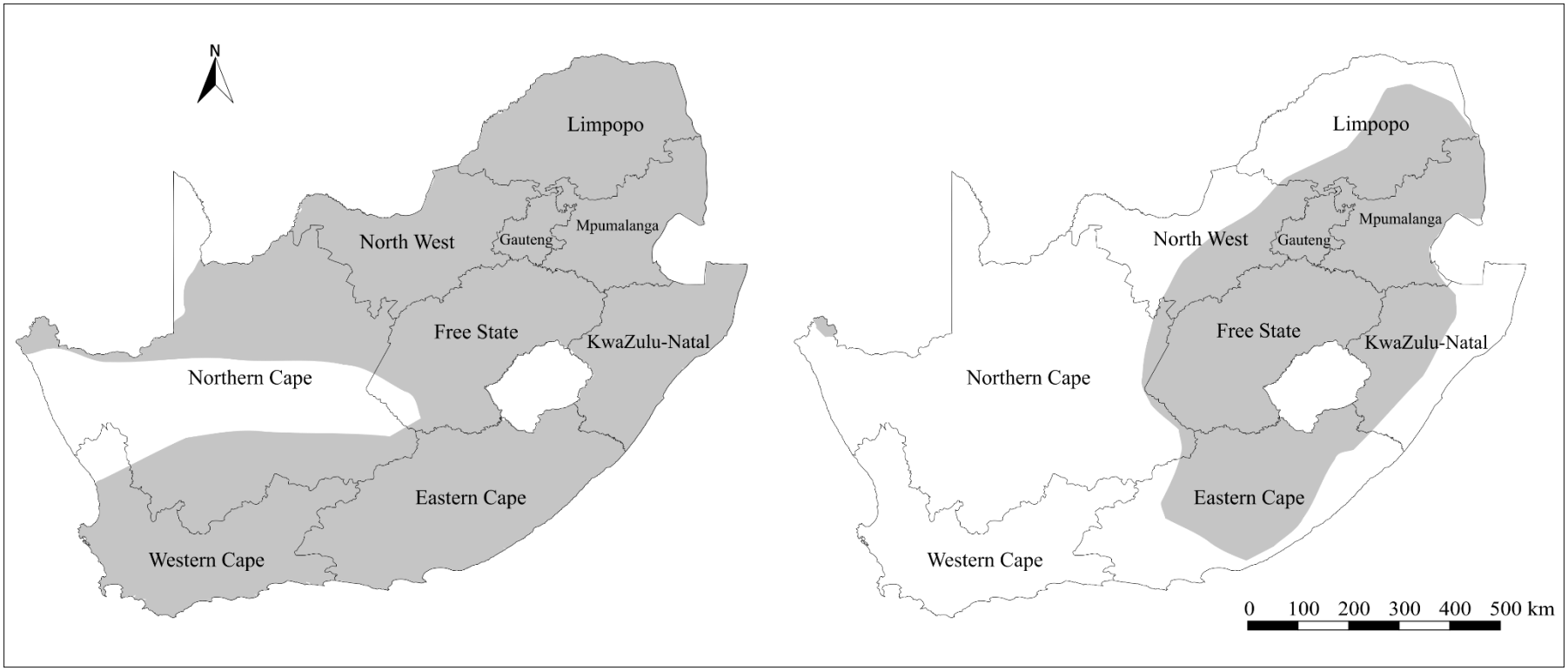


Figure 2.1 Distribution of two African otter species, *A. capensis* (left) and *H. maculicollis* (right), in South Africa (Jacques *et al.*, 2015; Reed-Smith *et al.*, 2015).

2.1.1 Occurrence of otters in urban areas

Otters are not restricted to undisturbed natural environments and occurrence has been reported in urban areas across the world. The Eurasian otter (*Lutra lutra*) is known to occur in urban areas in several countries, from Cork City, Ireland (Sleeman and Moore, 2005) to Daegu City, South Korea (Park *et al*., 2011). In Daegu City, otters have moved into urban areas previously uninhabited by the animals prior to the 2000s, when a clean-up initiative was started to address industrial runoff entering the local rivers (Park *et al*., 2011). *Lutra lutra* has been reported to recolonise urbanised areas in Italy, possibly due to a stabilisation of the area after the initial disturbance caused by expansion of the city (Marcelli and Fusillo, 2009). However, there are limitations to the level of human disturbance that *L. lutra* is able to tolerate, as was shown by Robitaille and Laurence (2002), with increases in human and road density driving down otter numbers.

In South Africa, most research on otters has occurred along rivers in nature reserves that are relatively undisturbed by human activity, or have low levels of human presence, such as along hiking trails and in agricultural areas (van der Zee, 1981; Arden-Clarke, 1986; Rowe-Rowe, 1992; Watson and Lang, 2003; Parker *et al*., 2005). Somers and Nel (2004a, 2004b) radio-tracked *A. capensis* along two rivers in the Western Cape, one of which was the Eerste River, which flows through the town of Stellenbosch in the Western Cape, where it was found that *A. capensis* occurred in disturbed areas along the Eerste River. However, when Kubheka *et al*. (2013) compared the number of spraint sites and otter observations along a stretch of the Mooi River flowing through a rural village and farmland in KwaZulu-Natal with two studies carried out in the 1970s and 1990s in the same area, a marked decrease in occurrence was found, which they suggested was linked to the increased human presence in the area.

2.1.2 Non-invasive sampling methods for cryptic species

In the past, various methods have been utilised to gather distribution data, ranging from telemetry and radio tracking (Arden-Clarke, 1986; Somers and Nel, 2004a, 2004b) to direct observation or video-recorded footage of activity (Guter *et al*.,

2008). These standard methods can be limiting or very difficult to apply, especially with species that are elusive, such as otters. Traditional methods are more effective when the researcher has a good knowledge of the movement patterns of the study species, allowing for traps to be set along known movement pathways or in locations that will guarantee high success rates of capture (Perrin and D'Inzillo Carranza, 1999). For these reasons, non-invasive methods are preferred in situations in which very little is known about the species or even whether the species frequents an area (Kruuk *et al*., 1986; Mason and Macdonald, 1987), especially if the study species population numbers are decreasing and the species is considered threatened. In the case of otters, these methods include identification of scat or tracks, as well as refugia or dens (holts). In recent studies, the utilisation of DNA extraction from scat samples has allowed for abundance determination of a *L. lutra* population (Hung, Li and Lee, 2004; O'Neill *et al*., 2013; Lehoczky *et al*., 2015; Pagacz, 2016), and the distinction between two otter species that cohabit a region (see Madisha *et al.*, 2015; Appendix B). In the current study, non-invasive sampling was used to collect otter spraints (scat), as well as record any footprints, dens, or sightings, along the riverbanks of 11 rivers in Gauteng to determine the distribution of *A. capensis* and *H. maculicollis* in the province.

2.2 Methodology

2.2.1 Study area

Gauteng is the smallest of the nine provinces of South Africa, but is the driving force behind the country's economy, with a large percentage of the country's population living in the province's two major municipalities, Johannesburg and Tshwane (Pretoria). Human development is continually encroaching on natural land.

A geographic information system (GIS) shape file from the Gauteng Department of Agriculture and Rural Development's (GDARD) Gauteng Conservation Plan Version 3.3 (C-PLAN 3.3) (GDARD, 2011) was used to differentiate between urban and peri-urban areas. This shape file represents the

‘urban area’ which is defined as densely populated areas with extensive built-up regions of residential, commercial and industrial buildings as well as an extensive network of roads. Peri-urban areas are defined as regions comprising natural land, small holdings, and agricultural land (as per GDARD, 2014). Eleven rivers within Gauteng that flow through natural, suburban, commercial, and industrial areas were selected for surveying (Figure 2.2). The full length of the chosen rivers was measured in Google Earth (Google, 2012) and the length of each river occurring in the delineated urban and peri-urban areas was also measured. These measurements were used to determine the length of the river occurring in either urban or peri-urban areas (Table 2.1). Sampling sites were identified at 5 km intervals along each river from the source of the river to the point at which the river meets the provincial boundary. Along both riverbanks of study rivers, at every 5-km site, a 400 m by 10 m transect was assessed on foot. Signs of otter occurrence (actual observation of otters, footprints, holts, sprainting sites and individual spraints) were searched for within each transect and recorded upon detection. Each 5-km site was visited once during the study, and a total of 71 sites were surveyed.

Table 2.1 Length of study rivers and the lengths of each river in peri-urban and urban areas of Gauteng, South Africa. Number of 5-km sites surveyed per urban and peri-urban areas along each river is indicated in parentheses.

River	Total length (km)	Peri-urban length (km)	Urban area length (km)	Proportion in urban area
Blesbok.	97.81	85.23 (7)	12.58 (0)	0.129
Braam.	20.36	0	20.36 (4)	1
Crocodile	42.75	33.66 (7)	9.09 (1)	0.213
Hennops	72.28	51.67 (6)	20.61 (2)	0.285
Jukskei	52.47	13.51 (2)	38.96 (5)	0.743
K. Jukskei	27.44	6.08 (1)	21.36 (4)	0.778
Klip	90.58	48.01 (4)	42.57 (7)	0.47
Modder.	13.41	0	13.41 (4)	1
Mooi.	78.88	72.59 (7)	6.29 (0)	0.08
Pienaars	85.57	79.07 (6)	6.50 (0)	0.076
Sand.	18.11	0	18.11 (4)	1

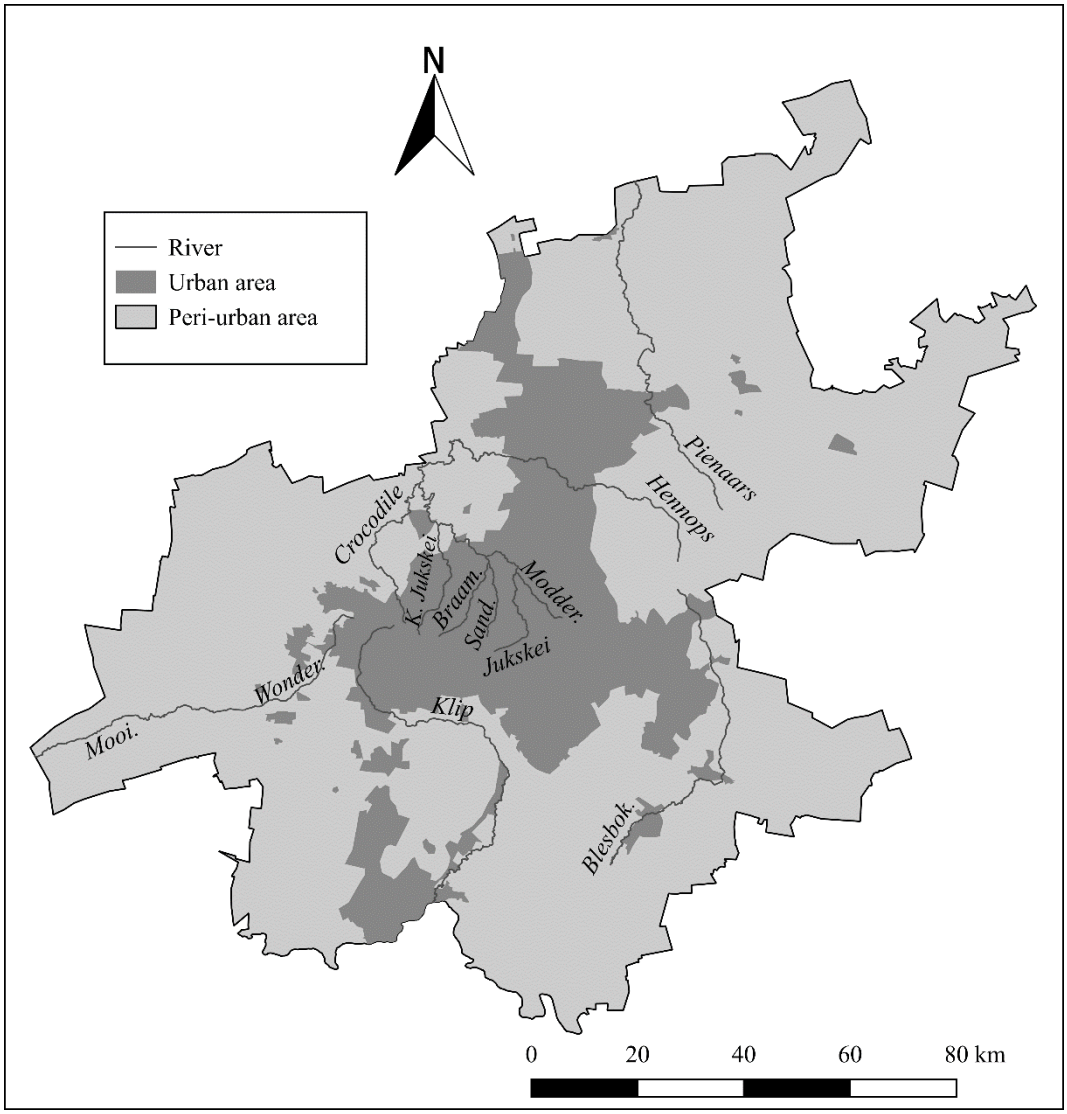


Figure 2.2 Locality of study rivers across Gauteng, South Africa (GDARD, 2011). Mooi. – Mooi River Loop; Wonder. – Wonderfonteinspruit; K. Jukskei – Klein Jukskei River; Sand. – Sandspruit; Braam. – Braamfonteinspruit; Modder. – Modderfonteinspruit; Blesbok. – Blesbokspruit.

2.2.2 Otter sign detection

Riverbanks of selected study rivers were searched for the presence of otter signs (footprints, spraints, dens, and otter sightings) between June 2012 and September 2014. Fieldwork was restricted to the dry autumn and winter seasons (mid–May to mid–September), as this is the time of year were otter sprainting rate variation is small and the distribution of spraint sites is the most even (Rowe-Rowe, 1992; Perrin and Carugati, 2006; Kubhenka *et al*., 2013), to reduce biasing due to

monthly variation. The surveys were also conducted in the dry months to reduce the chance of spraints and footprints being washed away due to river flooding, which is a common occurrence in the wet months. If there was rainfall during the surveying period, at least two days were given before surveying resumed, to allow time for fresh spraints to be deposited. This methodology seemed effective at preventing the loss of spraints due to rainfall or flooding, as all sites did not present signs of flooding (vegetation flattening, loss of riverbank sediment) which is observed during the wet season and fresh spraints were present following rainfall (Ponsonby, pers. obs.). Upon discovery of otter signs, coordinates were recorded using a hand-held GPS (Garmin® eTrex VistaCX GPS) device. When spraints were found, regardless of age (except for extremely weathered spraints that had deteriorated significantly), they were collected using individual inverted re-sealable plastic bags to prevent contact with human skin, thereby avoiding cross-contamination. Spraint sites were identified as areas with multiple spraints present, and spraints were only collected from spraint sites if they were a minimum distance of 30 cm from the nearest adjacent spraints, to reduce possible contamination. Several spraints were taken from spraint sites as there have been instances of both species, as well as several conspecifics, using the same spraint sites (Rowe-Rowe, 1992). Spraint collection bags were labelled with survey site number, the GPS coordinates of the site at which they were found and the date. Upon reaching 400 m from the starting point the search was terminated. Collected spraints were stored in a refrigerator at the University of the Witwatersrand, Johannesburg at -10°C until further analysis of the spraints was conducted.

2.2.3 Genetic analysis and species identification

DNA was extracted from each spraint sample using a QIAGEN® QIAamp® DNA Stool Mini Kit (QIAGEN, 2010). Species identification was conducted using developed partial *Cyt B* primers and associated PCR methodology (see Madisha *et al.*, 2015). The National Center for Biotechnology Information (NCBI) BLAST function was used to identify all possible GenBank reference samples (Eurasian otter, *L. lutra* EF689067; Cape clawless otter, *A. capensis* AF057118 and the spotted necked otter, *L. maculicollis* AF057125). Ten microsatellite markers

developed for studies of *L. lutra* (Dallas and Piertney, 1998) were used for genotyping analysis: Lut435, Lut453, Lut457, Lut604, Lut615, Lut701, Lut715, Lut782, Lut818, and Lut832 (Chapter 5 contains a detailed description of microsatellite methodology used). Genetic analyses were conducted at the National Zoological Gardens of South Africa's genetics unit. Once the species was identified, all positive observations were plotted on a 1:10 km digital map of Gauteng which also illustrated the urban boundary and major rivers studied, using the GIS software package QGIS 2.18.3 (QGIS Development Team, 2009).

2.2.4 Data analysis

A modified version of the analysis applied by Prigioni *et al.* (2005) was used for this study, as follows. For each river, the overall occurrence of signs was shown by the number of positive sites (those at which signs of otter occurrence were found) divided by the total number of sites along the river. In order to determine if the peri-urban or urban area may have been a predictor of otter presence, the number of all signs found (per species) was compared between peri-urban and urban areas using a chi-squared test. The 'expected' value was calculated by multiplying the total number of positive sites for peri-urban and urban areas by the total number of sites (positive and negative) per area. This value was then divided by the total number of sites surveyed (McHugh, 2013). For each study river, the density of signs for each species (total number of signs per total distance of riverbank surveyed) was calculated for urban and peri-urban areas and compared using independent-samples t-tests. A Pearson's correlation was used to analyse the relationships between the proportion of river length in urban areas and the number of *A. capensis* and *H. maculicollis* signs observed; the data met all of Pearson's correlation assumptions. All data analyses were conducted using STATISTICA version 10 (StatSoft, 2011). All tests were 2-tailed and significance levels of tests were set at $p < 0.05$.

2.3 Results

Out of the 71 5-km survey sites visited, 40 sites were positive for the presence of otters with a mean occurrence percentage of 56.34 ± 0.06 % across all 5-km sites. The rivers with the highest success rate were the Crocodile, Klip and Pienaars rivers (100% success). In total, 247 positive signs of occurrence (sightings of animals, footprints, and spraints) were recorded. Of these, three sightings were of *A. capensis*, ten were footprint sightings, 52 were spraint sites (mean number of spraints per site = 3.58) and 43 were solitary spraints, totalling 234 spraint samples. One hundred and eighty-one signs (spraints, footprints, and sightings) were of *A. capensis* and only 11 signs (footprints and spraints) were found for *H. maculicollis.* No signs were found along the Sandspruit. Nine of the spraint samples were too far degraded to extract DNA, and all samples found along the Modderfonteinspruit did not yield DNA for analysis; the reason for this is unclear (refer to Figure 2.3 for the location of signs occurrence). Due to possible degraded DNA, analyses were unable to identify individuals and therefore home ranges could not be measured. No holts (refugia/dens) were found during the surveys. Sections of the Hennops River and Mooi River Loop/Wonderfonteinspruit have been channelled underground and could not be accessed, these sites were therefore excluded from any analyses.

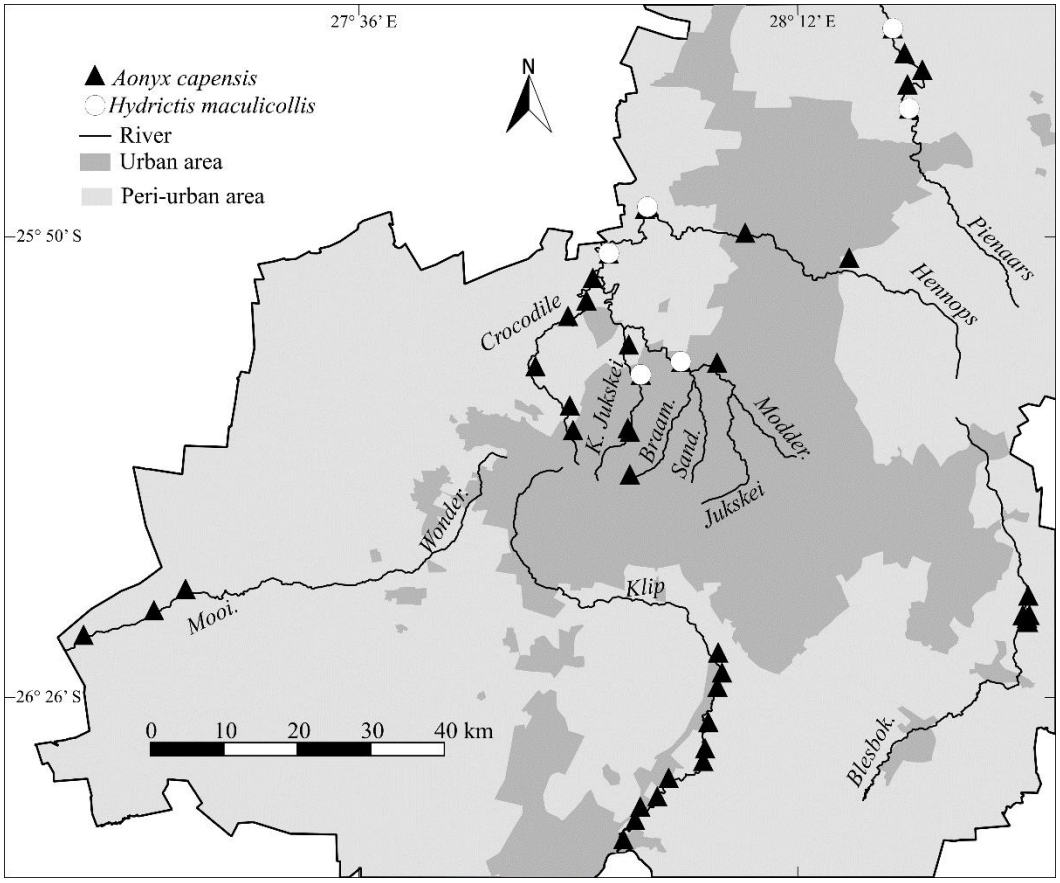


Figure 2.3 Occurrence of *A. capensis* and *H. maculicollis* signs along 11 rivers in Gauteng, South Africa. Mooi. – Mooi River Loop; Wonder. – Wonderfonteinspruit; K. Jukskei – Klein Jukskei River; Braam. – Braamfonteinspruit; Sand. – Sandspruit; Modder. – Modderfonteinspruit; Blesbok. – Blesbokpruit.

A comparison between the total number of A. capensis sign found in peri-urban and urban areas yielded no difference; $\chi 2$ (1, N = 71) = 0.083, p = 0.812) (Table 2.2). Similarly, no difference was observed between the number of H. maculicollis sign found in urban and peri-urban areas; $\chi 2$ (1, N = 71) = 0.102, p = 0.812) (Table 2.3). The density of all A. capensis signs found was 2.76 ± 0.36 signs/km of riverbank, with 1.76 ± 0.42 signs/km found in peri-urban areas and 1.69 ± 0.66 signs/km in the urban areas. No statistical difference was found between the densities in the two areas (t(11) = 0.395, p = 0.7). Comparison of A. capensis spraint site densities between urban and peri-urban areas showed no significant difference: t(8) = 0.614, p = 0.556; Table 2.2). The overall density of H. maculicollis signs found in the peri-urban areas and the urban areas did not differ (t(3) = 0.11, p = 0.92; Table 2.3). Comparison between the number of H. maculicollis spraint sites found in the peri-urban area and the urban area was not conducted as only one site was found in the urban area. Comparing the density of signs between the species indicated a difference, with more A. capensis signs present than H. maculicollis (t(16) = 2.262, p = 0.038). The occurrence of spraint sites was not significantly different between species, with 0.82 ± 0.25 A. capensis spraint sites/km, while for H. maculicollis 0.09 ± 0.06 spraint sites occurred per km (t(11) = 1.679, p = 0.121).

An analysis of the number of A. capensis signs observed and the proportion of river within the urban areas resulted in a negative correlation (r(10) = -0.606, p = 0.047; Figure 2.4). A non-significant decrease in the number of H. maculicollis signs found was observed as the proportion of river flowing through urban areas increased (r(10) = -0.426, p = 0.191).

Table 2.2 ***Aonyx capensis*** **signs found along riverbanks in peri-urban (a) and urban (b) areas of Gauteng, South Africa.**

(a)

River	Distance surveyed (km)	Spraint sites	Solitary spraints	Footprints	Otters sighted	Total signs	Total signs/km	Spraint sites/km
Blesbok.	5.4	2	8	0	3	13	2.41	0.37
Braam.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Crocodile	4.3	11	2	0	0	13	3.02	2.56
Hennops	5.85	0	2	3	0	5	0.85	0
Jukskei	1.8	0	0	0	0	0	0	0
K. Jukskei	0.9	0	0	1	0	1	1.11	0
Klip	5.25	5	3	2	0	10	1.90	0.95
Modder.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mooi.	4.25	2	2	0	0	4	0.94	0.47
Pienaars	4.45	11	6	0	0	17	3.82	2.47
Sandspruit	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TOTAL	32.20	31	23	6	3	63		
MEAN	4.03 ± 0.58	3.88 ± 1.56	2.88 ± 0.93	0.75 ± 0.39	0.38 ± 0.35	7.88 ± 2.06	1.76 ± 0.42	0.85 ± 0.36

(b)

River	Distance surveyed (km)	Spraint sites	Solitary spraints	Footprints	Otters sighted	Total signs	Total signs/km	Spraint sites/km
Blesbok.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Braam.	3.65	1	1	0	0	2	0.548	0.274
Crocodile	0.9	1	0	0	0	1	1.111	1.111
Hennops	3.15	0	1	0	0	1	0.317	0
Jukskei	4.5	5	2	0	0	7	1.556	1.111
K. Jukskei	3.6	4	0	0	0	4	1.111	1.111
Klip	2.5	4	8	1	0	13	5.2	1.6
Modder.	3.6	0	0	0	0	0	0	0
Mooi.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pienaars	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Sandspruit	3.6	0	0	0	0	0	0	0
Total	25.5	15	12	1	0	28		
Mean ± SE	3.19 ± 0.36	1.88 ± 0.69	1.5 ± 0.9	0.13 ± 0.12	0	3.5 ± 1.49	1.69 ± 0.66	0.65 ± 0.21

Table 2.3 ***Hydrictis maculicollis*** **signs found along riverbanks in peri-urban (a) and urban (b) areas of Gauteng, South Africa.**

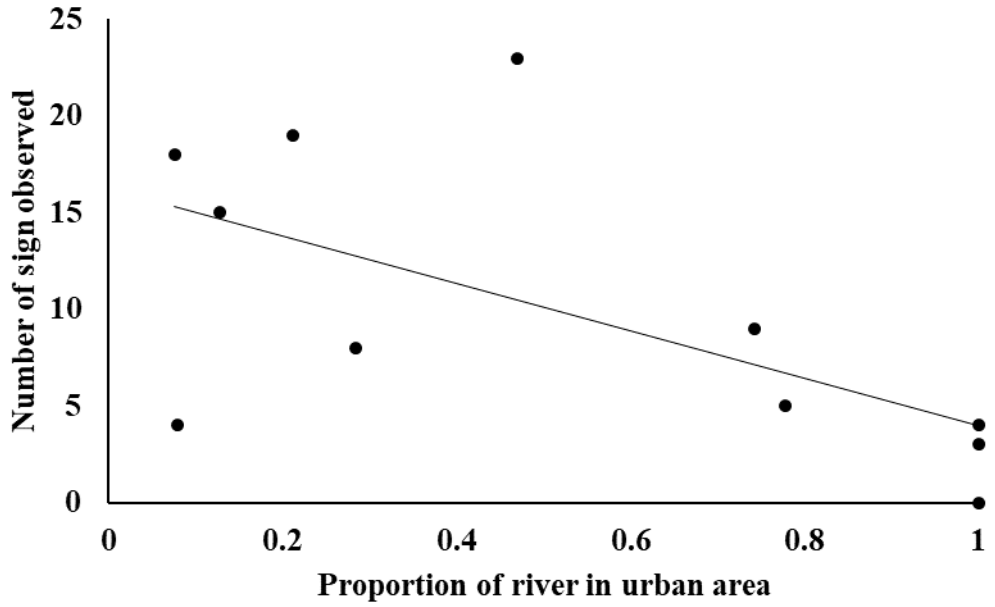
(a)

River	Distance surveyed (km)	Spraint sites	Solitary spraints	Footprints	Otters sighted	Total signs	Total signs/km	Spraint sites/km
Blesbok.	5.4	0	0	0	0	0	0	0
Braam.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Crocodile	4.3	1	0	0	0	1	0.23	0.23
Hennops	5.85	0	0	2	0	2	0.34	0
Jukskei	1.8	0	0	0	0	0	0	0
K. Jukskei	0.9	0	0	0	0	0	0	0
Klip	5.25	0	0	0	0	0	0	0
Modder.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mooi.	4.25	0	0	0	0	0	0	0
Pienaars	4.45	3	0	0	0	3	0.67	0.67
Sandspruit	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TOTAL	32.20	4	0	2	0	6		
MEAN	4.03 ± 0.58	0.5 ± 0.35	0	0.25 ± 0.23	0	0.75 ± 0.39	0.16 ± 0.08	0.11 ± 0.08

(b)

River	Distance surveyed (km)	Spraint sites	Solitary spraints	Footprints	Otters sighted	Total signs	Total signs/km	Spraint sites/km
Blesbok.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Braam.	3.65	0	0	0	0	0	0	0
Crocodile	0.9	0	0	0	0	0	0	0
Hennops	3.15	0	0	0	0	0	0	0
Jukskei	4.5	0	0	1	0	1	0.22	0
K. Jukskei	3.6	1	0	0	0	1	0.56	0.28
Klip	2.5	0	0	0	0	0	0	0
Modder.	3.6	0	0	0	0	0	0	0
Mooi.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pienaars	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Sandspruit	3.6	0	0	0	0	0	0	0
Total	25.5	1	0	1	0	2		
Mean ± SE	3.19 ± 0.36	0.13 ± 0.12	0	0.13 ± 0.12	0	0.25 ± 0.15	0.1 ± 0.07	0.03 ± 0.03

(a)



(b)

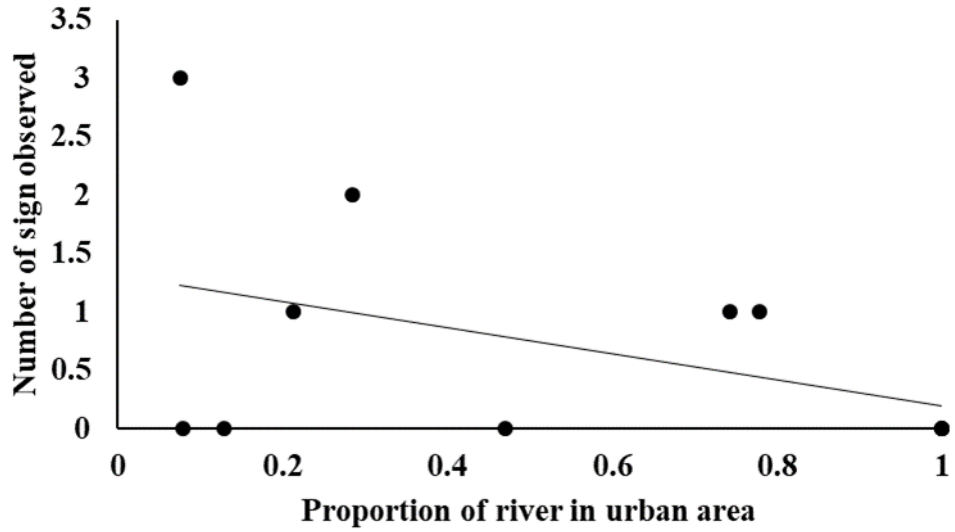


Figure 2.4 Correlation between the proportion of river length occurring in urban areas and the number of *A. capensis* (a) and *H. maculicollis* (b) signs. Both graphs illustrate a decrease in the number of otter signs found as proportion of river length flowing through urban areas increase.

2.4 Discussion

The aim of this study was to establish the distribution of two otter species (*A. capensis* and *H. maculicollis*) in an area with varying levels of human disturbance. Predominantly more *A. capensis* signs were found than *H. maculicollis* signs, raising concerns over the conservation status of *H. maculicollis* in Gauteng. Previous studies have shown great variance in the density of *A. capensis* along riverbanks, with the number of otters per kilometre of river ranging from one otter per 1.25–2.5 km (Perrin and Carugati, 2000b) to one otter per 8–10 km of river (Butler and du Toit, 1994). A wide variation in estimations of density along riverbanks has been documented for *H. maculicollis* too, ranging from one otter per 1–2 km of river (Perrin *et al.*, 2000), down to one otter per 8–11 km in disturbed areas (Rowe-Rowe, 1992). These previous studies used the number of holts to estimate the number of otters per kilometre of the river, which could not be achieved in the current study as no holts were found. The number of spraint sites present per kilometre was also used as an indicator in the past, with Rowe-Rowe (1992) finding 4.0–5.2 spraint sites/km for *A. capensis* and 1.7–3.3 spraint sites/km for *H. maculicollis*. Ardent-Clarke (1986) found 4.3 spraint sites/km for *A. capensis*, and more recently Perrin and Carugati (2006) observed 1.8–8.9 spraint sites/km for *A. capensis* and 2.5–6.5 spraint sites/km for *H. maculicollis*. The densities of spraint sites of both species observed during my study were lower than previous studies, with *Aonyx capensis* spraint sites ranged from 0.37–2.56/km (peri-urban) and 0.2.7–1.6/km (urban). The number of *H. maculicollis* spraint sites occurred at 0.23–0.67/km (peri-urban) and 0.28 spraint sites/km (urban). One hypothesis is that disturbance due to human activities along the studied riverbanks reduces time otters spend on riverbanks, thus resulting in lower densities of spraint sites observed during my study compared to previous studies. The higher level of human disturbance along the riverbanks in urban areas may influence the behaviour of otters, similar to the decrease in otter presence seen by Kubheka *et al*. (2013) with an increase in the number of humans present in the area as well as changes to the surrounding land for agricultural purposes.

The number of both individual spraints and spraint sites did not show any difference between the urban and peri-urban areas for *A. capensis*, indicating no change in sprainting behaviour in both of the areas. Very few *H. maculicollis* spraint sites were observed with no individual spraints found for both areas. To date, the only records of otter occurrence in Gauteng were based on old museum samples and sightings (Rautenbach, 1982), a challenging prospect when setting out to study an elusive species in a densely populated urban area. A low percentage of positive sites was expected for the urban areas but was not expected for the peri-urban areas, as it was predicted that otters would spend more time in areas with little to no human activity (Perrin and Carranza, 2000b). Less spraints in the urban areas may also relate to the home range of the otters, with the urban area occurring in the furthest extents of the otters' home range, and the core home range (area containing denning sites) occurring more in the peri-urban areas. High numbers of spraints are usually found around holts which occur in the core range (Perrin *et al*., 2000; Somers & Nel, 2004b). This may also be the reason for the lack of holts observed and a low number of spraints found during the study and would require further study of the otters' home range in this and surrounding regions to establish the true extent of the range and determine core range of the otters' distribution. Somers and Nel (2004b) calculated *A. capensis'* total home range length to vary from 4.9 to 54.1 km with a core home range length of 0.2 to 9.8 km, so there is a possibility that otters occurring in Gauteng have a core area with holts further from urban areas and only venture into the city in search of food or other resources. The presence of single spraints may suggest that urban areas occur in the furthest extent of an otter's territory, or home range. The river otter (*Lontra canadensis*) has been shown to utilise two forms of spraint marking depending on social group size, with social groups marking in large close to food sources, while solitary otters use less spraint at higher frequency across larger areas to indicate territory boundaries (Ben-David *et al*., 2005).

A lack of holts and the usual high level of spraints in close proximity to these otter refugia raise questions with regards to the movement and usage of the riverbanks in this area. Otters make use of several holts within their home range as places of safety during periods of rest (Kruuk, 2006). Perrin and Carugati

(2000b) observed a decrease in *H. maculicollis* resting periods and a lack of holts along a stretch of river flowing next to a village which was used heavily by humans throughout the day. A similar pattern may be occurring along Gauteng rivers with the otters not using holts in areas with high human presence and simply moving through these areas to reach more suitable and less disturbed areas. This may therefore be an indication that the urban region within Gauteng occurs at the outer extremities of the species' home ranges, with the core range occurring outside of the province along larger rivers. This may be a reasonable explanation as the headwaters of the studied rivers occur in central Gauteng, with rivers flowing outwards where they meet other tributaries of larger rivers such as the Vaal and Crocodile Rivers (DWAF, 2004; Swanepoel, 2009). These larger rivers may provide more suitable habitats for otters such as deeper waters with higher fish populations (Chapter 4) and possibly lower levels of disturbances from human activities as indicated by land use maps (GTI, 2012). Somers and Nel (2004) showed that the species in the Western Cape is more likely to occur in large rivers and rarely use the tributaries. Based on these findings it is possible that other factors are influencing the distribution of the species.

A noticeable finding was the extremely low number of *H. maculicollis* signs found in Gauteng. This may be due to its shy nature, rarity, and the presence of human disturbance, which has been shown to affect the distribution of *H. maculicollis* (Kubheka *et al*., 2013). The negative correlation between the number of signs detected and the proportion of river length within urban areas suggests that the level of urbanisation present along these rivers may have an adverse effect on the distribution of this species, or at least the sprainting activity of the animals. Another aspect to consider is prey availability in Gauteng rivers, as *H. maculicollis* has been found to consume fish more frequently than *A. capensis* (Perrin and Carugati, 2000a), and it is possible that fish numbers are lower than necessary to sustain the otter population, especially in the heavily impacted urban rivers (Kleynhans *et al.* 2007). These two otter species have been shown to coexist in the same area as each species relies on different prey types (Perrin & Carugati, 2000a), but if fish numbers are too low, *H. maculicollis* would have to

resort to consuming crustaceans to survive (Chapter 4), impacting on the food source of *A. capensis*, which may lead to competition between the two species.

Results from this study confirmed the presence of African clawless otters (*A. capensis*) and spotted-necked otters (*H. maculicollis*) in urban areas of Gauteng, based on the occurrence of signs. Compared to previous studies fewer signs were found than in natural areas, suggesting a reduced presence of otters on riverbanks possibly to avoid confrontation with humans/threats present along rivers. The lack of holts and reduced density of spraint sites further suggest the core home range of the animals fall outside of the region the study covered. Further intensive sampling is needed with longer transects surveyed along riverbanks to improve the chance of finding den sites.

CHAPTER THREE

Habitat correlates of the African clawless otter signs in areas affected by human disturbance in Gauteng, South Africa

Abstract

Urban development has resulted in the decrease of natural resources but has led to the creation of novel environments, which provide a matrix of altered and unaltered habitats providing patches of suitable habitat that may be exploited by opportunistic species. The aim of this study was to establish the effect areas of varying human disturbance had on the habitat use of otters. The presence of signs (spraints, footprints, holts, sightings of animal) was used to assess the habitat use of African clawless otters (*Aonyx capensis*) along 11 rivers in peri-urban and urban areas of Gauteng, South Africa. Habitat resource availability was surveyed along riverbanks and the presence of resources at sites lacking clear otter occurrence were compared to sites with observed otter activity (sprainting sites, footprints and otter observations). Differences were observed between riverbank characteristics and plant species present in peri-urban and urban study sites, leading to separate evaluations of each area. Binomial logistic regressions showed the presence of tree and reed cover, as well as two grass species (*Aristida junciformis* subsp. *junciformis* and *Hyparrhenia hirta*), were associated with otter activity in urban areas and the presence of buildings near rivers decreased the occurrence of otters. Several variables were associated with positive signs for otters in the peri-urban areas, namely reed beds, grass cover, depth of the river, and slope of the riverbank. Two tree species (*Rhus lancea* and *Acacia dealbata*) were more common at sites of otter presence, while fewer signs of otter were present near *Celtis africana*. Roads had mixed effects on otter signs, with roads less than 100 m from the survey site selected over roads further away. Less variability in habitat use in urban areas may suggest otters select specific patches for sprainting that provide adequate shelter from threats. This demonstration of

habitat use between two areas of varying disturbance suggests otters are able to utilise habitat affected by urban development.

3.1 Introduction

Available habitat has a profound impact on the movement, diet, and denning ability of an animal (Zabala *et al*., 2002; Bhattacharyya *et al*., 2015). Therefore, disturbance to natural environments through human activity would impact on animal behaviour. This becomes more problematic as urban development tends to result in homogeneous environments (McKinney, 2008), with species able to cope with these environmental changes demonstrating adjustments in habitat use (Tuomainen and Candolin, 2011). Individuals able to adjust their habitat use possibly stand a greater chance of survival in changing environments (Tuomainen and Candolin, 2011; Ahlers, 2015; Díaz-Ruiz *et al*., 2015). Several studies have focused on animal species adapting to urban environments through various alterations in their behaviour, from movement patterns (Riley *et al*., 2003; Herr, Schley and Roper, 2009; Gese *et al*., 2012), time of activity (Díaz-Ruiz *et al*., 2015), and denning activity (Parris and Hazell, 2005; Herr *et al*., 2010).

A combination of urban areas and surrounding less impacted habitat types can be found in Gauteng, the smallest and most densely populated province in South Africa with an average population increase of 2.7% annually from 2001 to 2011, which drives the demand for urban expansion to accommodate the population (GCRO, 2013). This expansion has resulted in the transformation of natural land to facilitate human requirements and degraded available water bodies through poor development planning, disregard for legislation and failing infrastructure (GCRO, 2013). The degradation of riparian areas in cities is common as rivers are modified to meet human demands, along with the inevitable use of rivers as a means of expelling both domestic and industrial waste products (Grimm *et al*., 2008). These modified urban rivers tend to result in reduced biodiversity, having cascade effects on all associated species (Paul and Meyer, 2001; Walsh *et al*., 2005).

Aonyx capensis are medium-sized semi-aquatic mammals with an extensive distribution covering most of Africa, except for arid regions (Skinner and Chimimba, 2005). This species is reliant on freshwater and occurs along rivers and other water bodies such as lakes and dams. Previous studies on *A. capensis* found the species more likely to use dense reed beds and rocky substrate in riparian habitat (Nel and Somers, 2007). *Aonyx capensis* has also been shown to use holts protected by dense vegetation cover (specific vegetation, namely *Merxmuellera* sp. and *Leucosidea* was observed by Rowe-Rowe, 1992). *Aonyx capensis* sprainting sites have been found in the grass above riverbanks, near fairly deep water (0.5 – 1 m deep) and near holts (Rowe-Rowe, 1992). Due to the cryptic nature of otters, it is often difficult to make predictions about habitat utilisation based solely on observations of daily activity patterns (Day *et al*., 2016). Even though otters are difficult to study owing to their cryptic nature, valuable information can be gathered from their sprainting activity. Previous studies on *L. lutra* have found individuals to leaves spraints at conspicuous sites, and at sites of fresh water and dens highlighting the use of spraints as indicators of resources (Kruuk, 1992). The intensity of *L. lutra* sprainting increases at points where different male territories meet, in which case spraint deters other males and reduces conflict over resources and mates (Erlinge, 1968). Spraint marking is also adjusted depending on social activity in *L. canadensis*, which utilises two forms of spraint marking depending on social group size. Family groups mark frequently in a small area close to food sources, while solitary otters use small deposits of spraints across large areas to indicate territorial boundaries (Ben-David et al., 2005). By evaluating the habitat type where spraints occur and where it is absent, it becomes easier to predict where otters could be found (Angelici *et al.*, 2005). However, the reliability of using spraint sites alone to determine otter habitat use has often been disputed (Kruuk and Conroy, 1987; Mason and Macdonald, 1987). As this is an exploratory study in an area which has no available ecological data for *A. capensis*, it is with reservation and caution that this method be applied to provide baseline data for future work on this otter species. The aim of this study was to understand habitat use of otters in areas of varying human disturbance. This was achieved by identifying habitat variables and plant species present at

sites containing signs of otter presence. It was hypothesised that urban areas would have little to no presence of otter signs, due to a lack of suitable habitat variables resulting from human development. It was predicted that *A. capensis* signs of occurrence would be present along riverbanks with dense grass and shrub patches, reed beds and rocky substrate next to deep water.

3.2 Methodology

3.2.1 Study area

The study was conducted between June 2012 and September 2014 and fieldwork was restricted to autumn and winter seasons to avoid the loss of spraint samples due to riverbank flooding, which is common during the wet seasons (spring and summer) (SAWS, 2010). Gauteng, located in the north-eastern part of South Africa, is the economic powerhouse of the country, contributing 34% of South Africa's GDP (OECD, 2011). The great economic success of the province has drawn a large population of 13.2 million into a space of roughly 18 179 km^2 (GCRO, 2013; StatsSA, 2015). Gauteng lies between 1081 m and 1899 m above sea level (GCRO, 2013) and experiences a mild climate with most rainfall occurring in summer. Average temperatures range from 14.8 °C to 26.3 °C in summer and 4.3 °C to 18.8 °C in winter. The mild and relatively dry (average 689.7 mm rainfall per year) climate supports a grassland biome with a small area of bushveld (SAWS, 2010). Two major cities are situated in Gauteng, Tshwane (Pretoria) to the north and Johannesburg in the centre.

The Gauteng Department of Agriculture and Rural Development (GDARD) Gauteng Conservation Plan Version 3.3 (C-PLAN 3.3) shapefile (GDARD, 2011), which indicates the proposed boundary between urban and peri-urban/rural areas in Gauteng, was used to identify suitable rivers for sampling. Urban areas are defined as densely populated areas with extensive built-up regions of residential, commercial and industrial buildings as well as an extensive network of roads. Peri-urban areas are defined as regions comprising natural land, small holdings, and agricultural land (as defined by GDARD, 2014). I measured the length of 11 rivers flowing through both urban and peri-urban areas of

Gauteng using Google Earth (Google, 2012) and sampling sites were identified at 5 km intervals along each river (Figure 3.1). All GIS work was conducted in the GIS software package QGIS 2.18.3 (QGIS Development Team, 2009). These rivers were chosen to represent all four cardinal directions from Johannesburg, as this would cover an extensive area of Gauteng and would give an adequate representation of the overall condition of rivers in the province.

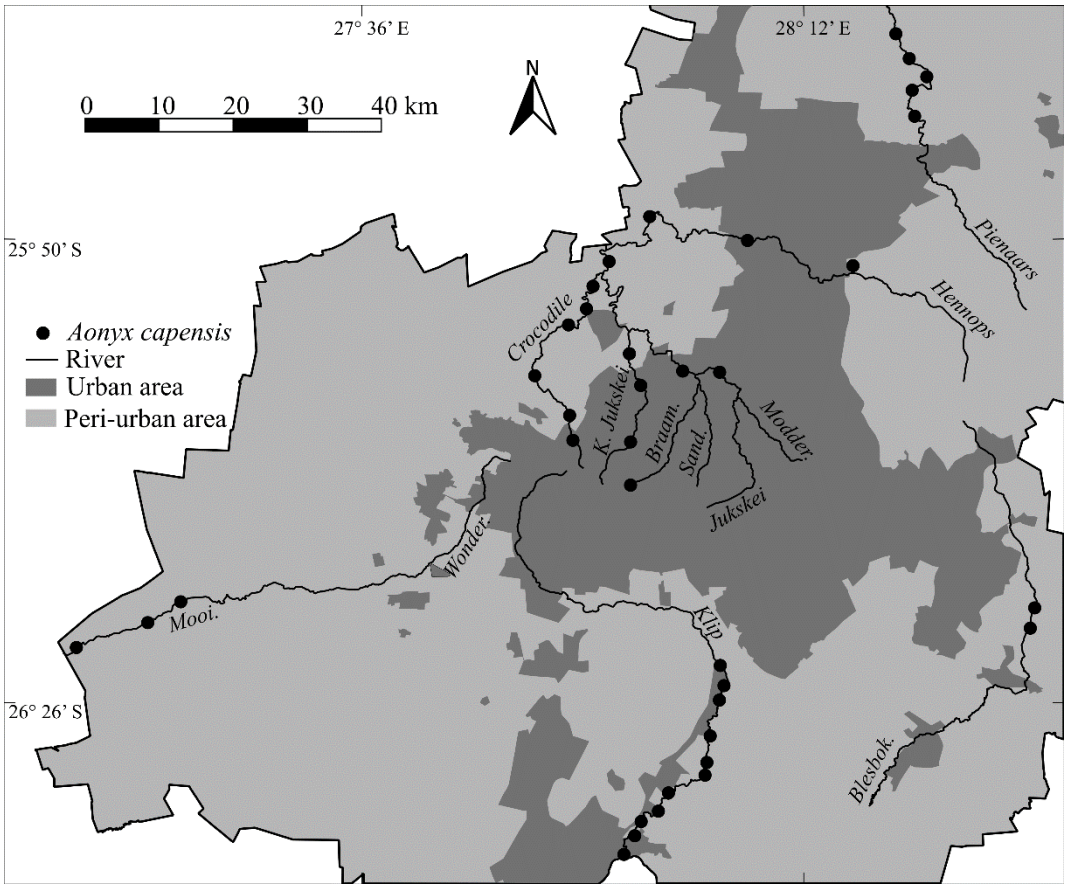


Figure 3.1 Study rivers and survey sites positive for *A. capensis* signs within Gauteng, South Africa (GDARD, 2011). Mooi. – Mooi River Loop; Wonder. – Wonderfonteinspruit; K. Jukskei – Klein Jukskei River; Braam. – Braamfonteinspruit; Sand. – Sandspruit; Modder. – Modderfonteinspruit; Blesbok. – Blesbokspruit.

3.2.2 Habitat surveying

Each 5-km site was visited once during the study period. At each 5-km site along the study rivers a 400 m by 10 m transect was surveyed on both riverbanks, where

possible. Sites at which access was denied, or the study river was channelled underground, were excluded from the study. Every 50 m during the 400 m transect, a survey was conducted in a 100 m^2 area from the water edge, this survey will be referred to as a Habitat Survey Site (HSS). In each HSS a 1 m^2 quadrat was randomly thrown out 10 times and the substrate and vegetation variables present in the quadrat were recorded. Substrate cover was divided into groups of cover type: soil (particle size < 2 mm max. diameter), gravel (3–20 mm), stone (21–150 mm), and boulder (> 150 mm) (Durbin, 1993). Vegetation type present was broadly classified as grass, shrub, reed, and tree cover. Percentage of each substrate and vegetation cover type was calculated for each HSS. As per Durbin (1993), who assigned all measured variables to categorical classes for analysis, the following scoring procedures were used: Each percentage of substrate occurrence was assigned to a class using midpoint scores: 0 = absent; 3 = 1–5%; 13 = 6–25%; 35 = 26–50%; 73 = 51–95%; 98 = 96–100%. The distance from the survey site to roads was measured and scaled as follows: absence = 0; < 100 m = 1; 100 m–500 m = 2; > 500 m = 3. Buildings and arable land were regarded as present if within 500 m of the HSS. Physical characteristics of the river was measured as followed: for depth, a graduated 2 m measuring pole was placed on the riverbed as close as possible to the middle of the river. Depth was categorised as 1 = 0–0.49 m; 2 = 0.5–0.9 m; 3 = 1–1.4 m; 4 = 1.5–2 m. A handheld digital thermometer was used to record the water temperature at each HSS and was scaled as follows: 1 = 9–12 °C; 2 = 12–15 °C; 3 = 15–18 °C; 4 = 18–21 °C; 5 = 21–24 °C. Width of the river at each HSS was measured using a 100 m measuring tape extended across the river, or estimated using Google Earth (Google, 2012) in instances where the river/water body was too large to measure physically: 1 = 1–10 m; 2 = 10–20 m; 3 = 20–30 m; 4 = 30–40 m; 5 = 40–50 m; 6 = 50–60 m; 7 = 60–120 m; 8 = > 120 m. Categorisation of continuous data was used to simplify the analysis of data by creating easily assessible categories for each measured variable. Numerous groups of small intervals were created as this reduces the amount of information lost and does not reduce the quality of the outcome of the analysis (Altman, 2005). Bank slope and flow rate were described using a categorical method based on visual assessment; bank slope was classed as mild

(0° to 15°), medium (16° to 40°) or steep (41° to 90°). The flow rate was classed as still (no water movement observed), slow (slight water movement observed), medium (slight rolling of water surface but no tumbling/white water present) or fast (water surface very rough and white-water present). Grass, tree, and shrub species present in each HSS were identified to species level and recorded. During the 200-m survey, positive otter signs (footprints, spraints, holts, sightings of animals) were recorded along with associated habitat features, as described above.

Table 3.1 Plant species identified along riverbanks of study rivers in Gauteng, South Africa

Plant type	Species name
Tree	*Acacia dealbata*, *Acacia karoo*, *Celtis africana*, *Eucalyptus camaldulensis*, *Ligustrum lucidum*, *Melia azedarach*, *Morus nigra*, *Populus alba*, *Quercus palustris*, *Rhus lancea*, *Salix babylonica*, *Solanum mauritianum*
Shrub	*Bidens pilosa*, *Canna indica*, *Lobularia maritima*, *Petroselinum crispum*, *Plectranthus hereroensis*, *Pyracantha koidzumii*, *Ricinus communis*, *Tagetes minuta*
Grass	*Aristida diffusa*, *Aristida junciformis* subsp. *junctiformis*, *Cynodon dactylon*, *Digitaria eriantha*, *Eleusine coracana* subsp. *africana*, *Hyparrhenia hirta*, *Leptochloa fusca*, *Setaria megaphylla*

3.2.3 Data analysis

Spearman's rank correlation was used to identify relationships between riverbank characteristic variables as categorical data were being analysed. Plant species, presence of arable land and buildings data were excluded from the Spearman's rank correlation analysis as these data are binary. Variables with correlation coefficients of 0.7 or greater were removed as collinearity between variables can severely impact results of the regression analysis (Manly *et al*., 2007). Riverbank characteristic variables used in the Spearman's rank correlation are displayed in Table 3.2.

Table 3.2 Riverbank characteristic variables used for Spearman's rank correlation analysis.

Variable group	Riverbank characteristic variables
Ground cover	Soil, gravel, stone, boulder
Vegetation cover	Tree cover, grass cover, shrub cover, reed cover
Anthropogenic features	Distance from road
River characteristics	River width, river depth, water temperature, river flow rate, riverbank slope

Differences between variables present in the urban and peri-urban areas were establish using the Mann-Whitney U test (data were not normally distributed), alpha value was set at 0.001 for the analyses to reduce the chance of type 1 errors. As several differences between the peri-urban and urban areas were detected, the data for urban and peri-urban areas were analysed separately using binomial logistic regression. The binomial logistic regression determines the odds of the dependent variable occurring with relation to the independent variable. Coefficient values calculated for the independent values are commonly only used as an indication of the relationship between the dependent and independent variables, with a negative value resulting in a decrease of Y in relation to X, whereas a positive value causes an increase in Y as X increases. The odds ratio calculated for each independent variable indicates the strength of the relationship between the dependent and independent variable, with values greater than zero increasing the likelihood of the dependent variable occurring, and an odds ratio value less than zero decreasing the chance of the dependent variable occurring in relation to the independent variable (Peng, Lee and Ingersoll, 2002; Manly *et al.*, 2007; IDRE, 2017). Separate analyses were conducted to establish if high levels of habitat disturbance present in urban areas may relate to otters using different habitat variables in urban areas compared to peri-urban areas (as per Manly *et al*., 2007). Riverbank land cover and characteristics were analysed separately from vegetation species data as collinearity occurred between vegetation cover categories and several plant species, which is to be expected. This resulted in four separate binomial logistic regression analyses being conducted; Model 1)

presence/absence of otter signs (dependent variable) in relation to peri-urban riverbank characteristics (independent variables); Model 2) presence/absence of otter signs (dependent variable) in relation to urban riverbank characteristics (independent variables); Model 3) presence/absence of otter signs (dependent variable) in relation to peri-urban plant species (independent variables); and Model 4) presence/absence of otter signs (dependent variable) in relation to urban plant species (independent variables). All riverbank characteristics variables except river width were used for Model 1 and Model 2, and all vegetation species were used in Model 3 and Model 4. Differences within each riverbank characteristic were assessed using Fisher LSD post hoc tests to determine the influence each level of a category has on the occurrence of otter sign at a survey site. The number of peri-urban HSS positive for otter presence was 66 and 530 peri-urban HSS did not have signs of otter present. Within urban areas, 22 HSS were positive for otter signs and 337 had no signs of otter present. The Hosmer-Lemeshow goodness-of-fit statistic was used to evaluate how well the model represents the data. This test has been shown to be very effective in testing binomial logistic regression models which do not fit normal distributions and therefore cannot effectively be tested with standard methods (Sweet and Grace-Martin, 2008; Sarkar and Midi, 2010). All analyses were conducted using the SPSS (version 21.0.0) software package.

3.3 Results

In total 359 urban HSS and 597 peri-urban HSS were assessed, with 22 urban HSS positive for otter signs and 66 HSS containing otter signs in peri-urban areas. Results of the Spearman's rank correlation indicated a strong correlation occurred between river width and river depth, therefore river width was removed from further analysis. Comparisons of variables (irrespective of otter signs) between the urban and peri-urban areas identified several differences between the occurrence of substrate and vegetation variables (Tables 3.3 and 3.4).

Table 3.3 Mann-Whitney U test results highlighting riverbank characteristics that vary significantly between urban and peri-urban habitat survey sites along study rivers in Gauteng, South Africa. Alpha set at 0.001.

Variables	$U_{(359)}$	Z	p-value
Trees	87467.5	-4.889	2.68×10^{-7}
Grass	91379	-3.852	0.0002
Shrubs	85482	-6.388	1.12×10^{-6}
Reeds	72424	-9.484	5.28×10^{-24}
Arable land	98691	-5.311	4.84×10^{-8}
Building	73468.5	-12.44	3.85×10^{-36}
River depth	75292	-7.726	1.1×10^{-14}
Flow (Medium)	86915.5	-6.239	1.41×10^{-9}
Flow (Fast)	88135	-5.54	8.64×10^{-8}
Slope (Mild)	84141.5	-6.903	1.79×10^{-11}
Slope (Steep)	91804	-4.29	4.02×10^{-5}

Table 3.4 Results of Mann-Whitney U analysis indicating significant differences in plant species present between urban and peri-urban habitat survey sites along study rivers in Gauteng, South Africa. Alpha set at 0.001.

Variables	$U_{(359)}$	Z	p-value
Rhus lancea	95657.5	-4.718	2.2×10^{-6}
Quercus palustris	103164	-3.643	0.0003
Morus nigra	93065	-4.44	9.65×10^{-6}
Ligustrum lucidum	87059	-8.112	5.46×10^{-16}
Acacia dealbata	91183.5	-6.311	2.98×10^{-10}
Aristida diffusa	93107.5	-4.938	8.43×10^{-7}
Cynodon dactylon	96016	-4.859	1.24×10^{-6}
Eleusine coracana subsp. *africana*	90553.5	-4.64	3.92×10^{-6}
Tagetes minuta	98581.5	-3.987	0.00007
Bidens pilosa	89402.5	-6.265	4.05×10^{-10}
Ricinus communis	103759	-4.103	0.00004

3.3.1 Peri-urban area analysis

In the peri-urban areas, no signs of otter presence were found at HSS with 100% reed cover. Results of the binomial logistic regression for substrate and river characteristic variables influencing otter sign occurrence in peri-urban areas were significant ($\chi^2_{(49)} = 101.45$, $p = 0.000016$). This model (Model 1) explained 31.2% (Nagelkerke R^2) of the variance in the occurrence of otter signs. Several variables (presence of grass, presence of reed beds, distance from road, river width, river depth, and riverbank slope) (Table 3.5) were highlighted as having a significant impact on the occurrence of otter signs.

Fisher LSD post hoc results indicate the likelihood of finding otter signs was significantly greater on mild ($p = 0.0014$) or medium ($p = 0.001$) sloped banks than those with a steep gradient. Riverbank along deeper water (1 – 2 m) had a higher number of signs present than shallow water (0 – 0.5 m), however post hoc tests did not indicate a significate difference between the number of otter signs found at each categorical level. Banks with between 26% and 50% of reed

bed cover and 1 - 5% grass cover had the highest occurrence of signs. Reed bed cover between 26% and 50% was significantly different from all other levels of reed bed cover: 0% (p = 0.000065), 1 – 5% (p = 0.033), 6 – 25% (p = 0.03) and 51 – 95% (p = 0.00014). Grass cover (1 – 5%) had a significantly higher likelihood of having otter signs present than 0% (p = 0.03), 26 – 50% (p = 0.024) and 51 – 95% (p = 0.037). An interesting result was that of the distance from the river to surrounding roads, with a greater number of signs found along rivers that were < 100 m from a road, but less likely if roads were further away (> 500 m). Significantly less otter signs were found at sites where roads were further than 500 m away than if roads were less than 100 m away (p = 0.00047) and if roads occurred between 100 m and 500 m (p = 0.00004). For Model 1 the Hosmer-Lemeshow test was not significant indicating the model did not deviate significantly from the data and can be considered an effective model (HL $\chi^2_{(8)}$ = 7.927, p = 0.441).

The binomial logistic regression using the presence of vegetation species at peri-urban HSS (Model 2) was significant ($\chi^2_{(23)}$ = 44.22, p = 0.005) but only explained 14.3% (Nagelkerke R^2) of variance of samples. Significant variables detected in Model 2 (peri-urban area vegetation species) were karee (*Rhus lancea*), white stinkwood (*Celtis africana*) and silver wattle (*Acacia dealbata*) trees. The presence of silver wattle and karee resulted in a greater chance of finding otter signs, whereas a decrease in chance occurred if stinkwood trees were present (Table 3.5). Validation of Model 2 showed that the model did not deviate significantly from the dataset and is therefore a valid model (HL $\chi^2_{(8)}$ = 8.986, p = 0.343).

Table 3.5 Binomial logistic regression results for riverbank characteristics (Model 1) and vegetation species (Model 2) variables influencing the occurrence of otter sign on riverbanks in peri-urban areas.

Model 1

Variable	Coefficient	S.E.	Odds ratio	*p*-value
Grass (1–5%)	1.82	0.93	6.17	0.049
Reed (26–50%)	1.65	0.75	5.19	0.027
Road (< 100 m)	1.05	0.5	2.84	0.037
Road (< 500 m)	-2.13	1.07	0.12	0.046
Depth (1–1.5 m)	2.02	0.66	7.56	0.002
Depth (1.5–2 m)	1.49	0.73	4.44	0.04
Slope (Mild)	1.68	0.51	5.34	0.001
Slope (Medium)	1.71	0.49	5.51	0.0005
Constant	-4.74	1.64	0.01	0.004

Model 2

Variable	Coefficient	S.E.	Odds ratio	*p*-value
Karee	1.16	0.41	3.19	0.005
W. stinkwood	-1.42	0.5	0.24	0.005
Silver wattle	1.46	0.54	4.29	0.007
Constant	-2.10	0.24	0.12	0.0001

3.3.2 Urban area analysis

No signs of otter presence were found at HSS with more than 25% stone substrate, more than 25% reed bed cover, or near agricultural land. Model 3 was significant ($\chi^2_{(34)} = 59.04$, $p = 0.005$) and explained 41.1% (Nagelkerke R^2) of variance of samples (Table 3.6). The binomial logistic regression using vegetation species (Model 4) was significant ($\chi^2_{(22)} = 71.1$, $p = 0.0001$) and explained 48.6% (Nagelkerke R^2) of variance. Several variables were found to have a significant effect on the presence of otter signs in urban areas. Signs were more likely to

occur along riverbanks with 6–25% tree cover and 1–5% reed bed cover, while the presence of buildings near the riverbank greatly reduced the chance of finding otter signs. Fisher LSD post hoc tests revealed presence of otter signs at sites with 6 – 25% tree cover was only significantly different from sites with 26 – 50% tree cover (p = 0.021), and no significant difference occurred between the different levels of reed bed cover. Post hoc results also indicated significantly less otter signs were present near buildings (p = 0.016). Results of the Hosmer-Lemeshow test did not indicate a difference between Model 3 and the data (HL $\chi^2_{(8)}$ = 11.378, p = 0.181). Two grass species, wire grass (*Aristida junciformis* subsp. *junciformis*) and thatch grass (*Hyparrhenia hirta*), had the highest association with the occurrence of otter signs (Table 3.6). No deviation of the model from the data was detected with Model 4 (HL $\chi^2_{(8)}$ = 1.637, p = 0.99).

Table 3.6 Significant riverbank characteristics (Model 3) and vegetation species (Model 4) variables influencing the occurrence of otter sign along riverbanks in urban areas.

Model 3

Variable	Coefficient	S.E.	Odds ratio	*p*-value
Tree (6–25%)	2.67	1.28	14.4	0.037
Reed (1–5%)	3.79	1.54	44.11	0.014
Building	-3.07	1.23	0.05	0.012
Constant	-12.86	5.94	0	0.03

Model 4

Variable	Coefficient	S.E.	Odds ratio	*p*-value
Thatch grass	2.33	0.89	10.24	0.009
Wire grass	3.62	0.8	20.44	0.0001
Constant	-4.00	1.01	0.02	0.0001

3.4 Discussion

Signs of otter presence were observed along rivers in both urban and peri-urban areas in Gauteng, South Africa. There were, however, clear differences between both river characteristics and riverbank vegetation of the two areas. These differences presumably stem from the level of modification present in the urban areas. Despite this high level of disturbance *A. capensis* has been able to utilise habitat features in the urban environment and it follows trends seen by individuals in less disturbed environments (see Rowe-Rowe, 1992; Perrin and Carugati, 2000b; Somers and Nel, 2004a). Previous habitat studies found *A. capensis* more likely to use dense reed beds and rocky substrate (Nel and Somers, 2007), and have also been shown to use holts protected by specific vegetation, namely mountain wire grass (*Merxmuellera* sp.) and oldwood shrub (*Leucosidea sericea)* (Rowe-Rowe, 1992). Similarly, in the current study otter signs were observed at sites with reed beds present, at varying densities for the two areas, 6–25% in urban and 26–50% in peri-urban areas. The occurrence of otter signs was disproportionally higher at sites with two tall grass species, namely wire grass (*Aristida junciformis* subsp. *junciformis*) and thatch grass (*Hyparrhenia hirta*). It is interesting that signs were not present near very dense reed beds considering the excellent cover reeds provide. The close proximity of reeds in dense beds could hinder the movement of otters, making it difficult to move through or defecate in dense reed beds.

Tree cover (6 – 25%) was identified as being important in relation to otter presence in urban areas, which may serve two purposes, namely shelter and territorial marking. Otters have been shown to mark conspicuous landmarks such as large trees and stumps on the riverbank (Kruuk, 2006), as well as tree roots and stumps providing shelter and possible denning locations for otters (Rowe-Rowe, 1992; Perrin and Carugati, 2000b). However, this level of tree cover is likely more important to plant species growing underneath tree canopies, with dense canopies reducing the level of light and rainfall reaching these sub-canopy plant species (Dohn *et al*., 2013). With a lower canopy cover more sunlight and rainfall will penetrate down to grasses and shrubs on the ground, increasing growth. The

intricate relationship between tree and grass growth is complex and would influence otter presence in an area, as it affects the density and type of grasses able to grow underneath certain tree species, providing adequate cover for otters assuming grass is tall enough to conceal otters.

As Perrin and Carugati (2000b) showed vegetation cover was important for otter presence, the relationship between *R. lancea* and *A. dealbata* and sub-canopy grass and shrub species is a possible reason for the higher occurrence of otter signs near these tree species. It is important to note *R. lancea* has thin elongated leaves which allow more light and rain to reach plant species growing below the tree canopy, resulting in a higher density of vegetation along the ground (pers. obs.), providing ideal cover for otters moving along the riverbanks, based on observations in previous studies (Perrin and Carugati, 2000b; Nel and Somers, 2007). *Acacia dealbata* also possesses fine-leaf structure influencing light and water able to penetrate through the canopy to plant species below these trees, influencing vegetation density available to otters. *Celtis africana* (white stinkwood) is a tall deciduous tree occurring across many different land types (Palgrave, 1981). During the study the ground underneath *C. africana* was bare soil at most survey sites, possibly due to the dense shade provided by the tree when full foliage is present (Ludwig *et al*., 2001), reducing the available cover for otters with the lack of grass or shrubs.

Rhus lancea is a common evergreen tree found along rivers, reaching an average of 8 m in height (Palgrave, 1981) and would serve as a conspicuous landmark clearly seen along a riverbank, suggesting why the occurrence of signs near this tree species was so high, possibly indicating a form of territorial marking. Kruuk (1992) describes how *L. lutra* leaves spraints at conspicuous sites, and at sites of fresh water and dens highlighting the use of spraints as indicators of resources. Increased intensity of sprainting has been observed at points where different male otter (*L. lutra*) territories meet, in which case spraint deters other males and reduces conflict over resources and mates (Erlinge, 1968). *Lontra canadensis* utilises two forms of spraint marking depending on social group size, with social groups marking intensively close to food sources, while solitary otters

mark at higher frequency across larger areas to indicate territory boundaries (Ben-David *et al*., 2005).

Riverbanks with a mild to medium gradient had the highest occurrence of spraints present, possibly as these gradients are more suited to otters entering and exiting the water as a greater incline would be difficult to climb. The depth of water near sites of otter sign is similar to that described by Nel and Somers (2007) but differed from other studies in which presence was higher in shallow water due to catching of crab (Perrin and Carugati, 2000b; Somers and Nel, 2004b). Human disturbance had a significant effect on the occurrence of otter signs in the urban areas, with far fewer signs being observed along riverbanks near buildings. Avoidance of humans is the most plausible conclusion to draw from this, as Nel and Somers (2007) showed fewer signs were found near areas with human activity along the water. No signs of otter presence were found near agricultural land in the urban area either, similar to Nel and Somers (2007) who found less scat near areas exposed to agricultural pollution. However, as Somers and Nel (2004b) showed, otters were present near areas of human disturbance but did not seem to defecate or den in those areas. As previously discussed in Chapter 2, no difference was observed between the frequency of otter signs occurring in urban or peri-urban areas, indicating that human impact affects otter presence at different ecological scales. At the broader scale otters are moving through urban areas unobstructed, but on a more localised scale certain types of urban development (e.g. buildings), which can be associated with the high presence of human activity, seem to be avoided by otters. In the urban areas studied, otters could be moving through modified areas but not remaining for extended periods of time to avoid detection. A curious situation arises in the peri-urban environment when the proximity of signs to roads is considered. The regression model indicated the presence of signs near roads closer than 100 m to the survey site, but signs were less common at sites further than 100 m. On closer inspection, the roads in peri-urban areas were often dirt roads seldom used by cars and spraints were found on some of the roads, indicating the possibility that otters use the roads for movement across land, which has been observed in several other mammal species (reviewed in Coffin, 2007). The uniform nature of these roads and lack of obstruction may

provide an unhindered route of travel that is not obstructed by vegetation, allowing for a greater field of view for otters. Even though differences were measured in the available and actual habitat present for the peri-urban and urban areas surveyed during this study, it is evident that *A. capensis* can move through both areas. In both instances, dense vegetation cover is necessary, albeit at a lower percentage cover in the urban area than in the peri-urban area. This is possibly because of the manner in which the natural resources in the urban environment have been altered, leaving only sparse patches of natural unmaintained vegetation.

The limitations of making accurate assessments of habitat use based solely on the presence of otter signs are understood, and therefore all relationships between habitat variables and signs are cautious estimations made as an exploratory study as no work on otters has been conducted in the area before. The presence of signs only indicates an otter has moved through an area, but this does not provide information about time spent in areas or behaviours (foraging, conspecific interactions, resting) carried out at these sites, which is important for establishing core ranges and home ranges of animals. Spraints are important for communication in otters and are used to indicate resources as well as territory boundaries, necessary for the avoidance of conflict between conspecifics. Future studies using radio telemetry to accurately assess otter movement through the urban environment will greatly improve upon the data gathered during this study. This study established differences between riverbank characteristics and plant species variables associated with otter signs in peri-urban and urban study sites. Despite habitat differing between urban and peri-urban areas, *A. capensis* is able to move through and establish spraint sites in both areas. This demonstration of habitat use between two areas of varying disturbance suggests otters are able to utilise habitat affected by urban development.

CHAPTER FOUR

Diet composition of the African clawless otter (*Aonyx capensis*) in urban Gauteng, South Africa[1]

Abstract

The diet of the African clawless otter (*Aonyx capensis*) was studied in urban and peri-urban areas of Gauteng, South Africa. To date, research on *A. capensis* has focused on the coastal areas of South Africa, specifically KwaZulu-Natal and the Western and Eastern Cape provinces, with moderate to little human disturbance. The aim of this study was to investigate the diet of *A. capensis* in a densely urbanised area and to compare findings to data obtained from studies in less disturbed areas. Eleven rivers spanning the majority of Gauteng and flowing through varying levels of urbanisation were surveyed. Spraint (faecal) samples were collected along the riverbanks and diet analysis was conducted, grouping prey by class. It was found that crustaceans were the most common prey consumed by *A. capensis*, which was expected. The diet was not limited to crab alone, as remnants of birds, small mammals, fish and insects were also discovered in the spraints. A wide selection of prey similar to this has been identified in previous studies whereby otters are less selective over prey and demonstrate a broader trophic niche when availability is low. These results confirm that *A. capensis* is a generalist feeder and is not restricted to specific prey, a useful trait for the survival of species occurring in disturbed areas.

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4.1 Introduction

As the ever-expanding urban sprawl requires more land to sustain human population growth, natural land is at risk of degradation as new urban spaces are developed (Grimm *et al*., 2000). This use of natural land for development ultimately results in the disruption of established animal and plant populations (McKinney, 2006, 2008). Environmental changes due to urbanisation lead to land degradation, pollution, erosion, and river alteration (Grimm *et al*., 2000; Paul and Meyer, 2001). Urban development tends to increase the homogeneity of the environment (McKinney, 2008), decreasing the diversity of species able to survive in the urban area (Willis, Winemaker and Lopez-Fernandez, 2005). This does not only affect terrestrial habitats, as the ability of rivers to sustain numerous taxa is also reduced as overall river health declines (reviewed in Paul and Meyer, 2001) or riverbank vegetation becomes homogenous (Huston, 1994; Willis *et al*., 2005). Homogenisation of riverbanks (and other natural spaces) can be brought about by the simplification of natural vegetation through landscaping of urban areas to appeal to humans (Marzluff and Ewing, 2001), and the increase in non-pervious ground covering such as pavements, roads and buildings (Blair and Launer, 1997).

These environmental changes in urban areas affect wildlife in many ways, as available habitats decline or deteriorate, and prey populations decline (McKinney, 2006). With a decrease in prey numbers, predators dependent on these species need to respond either by moving to areas where prey is still abundant or by feeding on new prey (Morey, Gese and Gehrt, 2007). Carnivorous species living in urban areas would thus have a broad diet to improve their success in the changing environment of urban areas, as land transformation alters habitat and food availability (McKinney, 2006; 2008). For example, opportunistic carnivores such as coyotes (*Canis latrans*) in metropolitan areas showed variation in diet depending on temporal and spatial factors affecting food availability (Morey *et al*., 2007).

The distribution of the African clawless otter (*A. capensis*) within South Africa has been shown to include rivers that flow through, or are in close

proximity to, urban development (Skinner and Chimimba, 2005). As urbanisation tends to lead to homogeneity of species, prey availability for the otter may be affected. One of the greatest risks to urban river systems is the disposal of waste, leading to high levels of pollutants in the water and surrounding soil. Due to the intricacy of natural systems, the introduction of harmful chemicals often destabilises a system, resulting in the loss of species. These pollutants disrupt ecosystems by changing the water chemistry, causing a cascade effect for water fauna and flora (Walsh *et al*., 2005; Bernhardt *et al*., 2008). A common example is that of phosphates from agriculture or sewage effluent entering a river, resulting in increased algal growth and anaerobic conditions (Jarvie, Neal and Withers, 2006). These eutrophic conditions are not suitable for oxygen-dependent species such as fish or crabs (Biggs, 2000; Smith, Joye and Howarth, 2006). Otters relying on fish and crab for food would be directly affected by this change in prey availability. A constant decline in accessible food sources may lead to intra- and inter-specific competition causing species to pursue less preferred prey options (e.g. birds being eaten by otters), but is more likely to lead to range expansion and possible localised extinction (Bino *et al.*, 2010). Consequently, species with a broader dietary range are likely to be more successful in adverse conditions than specialist feeders restricted to the consumption of a single prey type (Abrams, 2010; Dennis *et al*., 2011). The establishment and survival of hardy species that display opportunistic feeding behaviour are common in urban areas exposed to land degradation and increased levels of pollutants (McKinney, 2006; Lowry *et al*., 2013).

Unfortunately, very little is known about the effects urban sprawl has on *A. capensis*, which raises concerns regarding its conservation. The species is listed as ‘near threatened’ on the IUCN Red Data List (2016) due to increasing threats of habitat loss, exploitation/direct loss and accidental mortality through fish traps (Jacques *et al*., 2015). The Red Data List assessment may have to be amended if urban development continues at its current rate, as industrial pollution has been found to have a direct negative impact on the presence of otters in Europe, while agricultural pollution and urbanisation negatively affect otter density by affecting prey populations forming part of the diet of otters (Roos *et al*., 2015). Several

rivers within Gauteng, South Africa in the distribution range of *A. capensis* have been shown to be heavily polluted, likely due to the close proximity of rivers to industrial activities and mining areas (DWAF, 2003a, 2003b; Huizenga, 2004), which may be affecting prey species (fish and crab) eaten by *A. capensis* (see Walsh *et al*., 2005; Mangadze, Bere and Mwedzi, 2016).

Given the high level of habitat degradation in urban areas across South Africa, this study aimed to investigate how this would impact the diet of *A. capensis* present in the central area of Gauteng, South Africa. Specifically, to identify any possible differences in *A. capensis* diet compared to data reported in previous studies, as preferred prey may not be available due to high pollution levels in the selected rivers. Previous dietary studies suggest that in general, crab and fish make up the majority of the diet of *A. capensis*. Based on the findings of Perrin and Carugati (2000a), the diet of *A. capensis* was predicted to predominantly comprise of crab with a much lower level of fish remains. Diet was also compared between urban and peri-urban areas to determine differences in prey consumed in the two areas, as I predict less preferred prey (i.e. birds, mammals, insects) being consumed in urban areas. The percentage of each prey type group eaten was compared between study rivers to identify possible differences in the preferred prey per river.

4.2 Methodology

4.2.1 Study area

Gauteng is located in the north-eastern part of South Africa and is the economic powerhouse of the country, contributing 34% of South Africa's GDP (OECD, 2011). The great economic success of the province has drawn a large population of 13.2 million into a space of 18 179 km^2 (GCRO, 2013; StatsSA, 2015). Gauteng is between 1081 m and 1899 m above sea level (GCRO, 2013) and experiences a mild climate with most rainfall occurring in summer. Average temperatures range from 14.8 °C to 26.3 °C in summer and 4.3 °C to 18.8 °C in winter. The mild and relatively dry (average 689.7 mm rainfall per year) climate supports a grassland biome with a small area of bushveld (SAWS, 2010). Two

major municipalities are situated in Gauteng, Tshwane (Pretoria) to the north and Johannesburg in the centre.

A GIS shapefile developed by The Gauteng Department of Agriculture and Rural Development (GDARD) for the Gauteng Conservation Plan Version 3.3 (C-PLAN 3.3) (GDARD, 2011) was used to identify the boundary between urban and peri-urban/rural areas in Gauteng. As per GDARD (2014) urban areas are defined as densely populated areas with extensive built-up regions of residential, commercial and industrial buildings as well as an extensive network of roads. Peri-urban areas are defined as regions comprising natural land, small holdings, and agricultural land. Eleven rivers that were present in peri-urban and urban areas indicated by the GDARD shapefile were chosen to represent all four cardinal directions from Johannesburg, as this would cover an extensive area of Gauteng and would give representation of the overall condition of rivers in the province. The length of these 11 rivers flowing through both urban and peri-urban areas of Gauteng was measured using Google Earth (Google, 2012) and sampling sites were identified at 5 km intervals along each river (Figure 4.1).

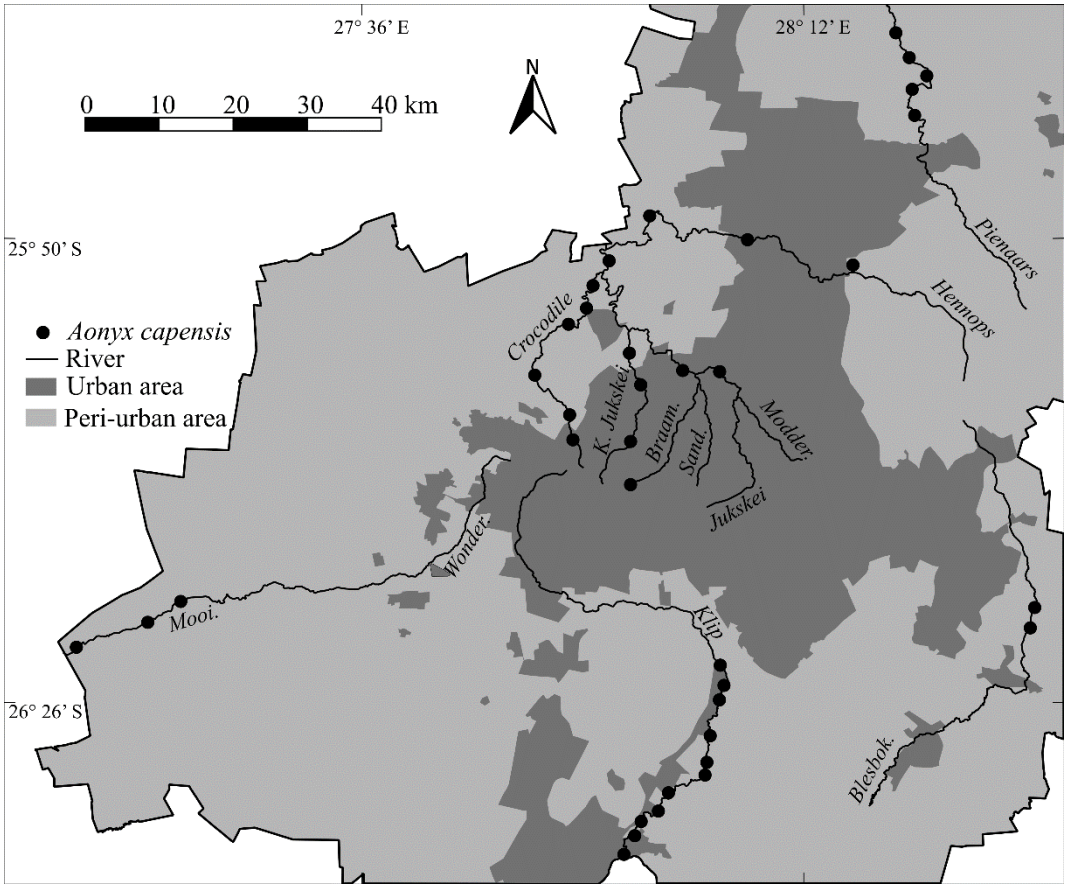


Figure 4.1 Rivers surveyed and sites of spraint collections in relation to urban and peri-urban areas of Gauteng. Sources of all the rivers occurs in the centre of the province, with rivers flowing toward the provincial boundary (GDARD, 2011). Mooi. Loop – Mooi River Loop; Wonder. – Wonderfonteinspruit; K. Jukskei – Klein Jukskei River; Braam. – Braamfonteinspruit; Sand. – Sandspruit; Modder. – Modderfonteinspruit; Blesbok. – Blesbokspruit.

4.2.2 Prey availability

No trapping or electrofishing was conducted to establish the prey species available in each of the study rivers. However, the National River Health Programme (RHP) (recently renamed the River Ecostatus Monitoring Programme – REMP) provided data on fish and macroinvertebrate assemblages of the Crocodile West Water Management Area (WMA) from 2013–2015. Assessment of fish in the Upper Crocodile River indicate a decrease in fish Present Ecological State: Ecological Category (PES: EC) from 2013 to 2015, indicating unfavourable conditions for most fish species. Conditions along the Hennops River are

considered adequate for fish but this is only based on visual observations over the three years, and several conditions such as pollution and flow modification reduce the likelihood of certain fish species being present. A negative trend is observed in the Jukskei River due to urban runoff, with species given a low frequency of occurrence due to siltation and enrichment. Visual observations were only conducted for the Jukskei due to high water levels for the 3 years and an instance of sewage entering the water in 2015. Along the Pienaars River, high flow levels and sewage in the water prevented electrofishing and only visual assessments are available. Urban runoff and pollution are cited as reducing the occurrence of fish species (Singh and Todd, 2015). Recent data on the frequency of occurrence of fish in the upper Vaal catchment area are not available and therefore data from 2007 were used. These data indicated a relatively high frequency of fish species in the Klip River (Kleynhans, Louw and Moolman, 2007). A total of 29 frog species have been shown to occur in Gauteng, with 14 of those species occurring in suburban areas and agricultural land (du Preez and Carruthers, 2009). Three crab species *Potamonautes sidneyi*, *Potamonautes unispinatus* and *Potamonautes warren*) are reported in Gauteng, but crab population densities are reported to be low, with individuals growing to smaller sizes due to high levels of pollutants in the water (de Kock, 2001; Cumberlidge and Daniels, 2009). From the Animal Demography Unit's Coordinated Waterbird Counts (CWAC) data a mean of 70 waterbird species present in Gauteng based on observations from 58 sites was estimated (CWAC, 2017). Common waterbird species in Gauteng are red-knobbed coot (*Fulica cristata*), cattle egret (*Bubulcus ibis*), yellow-billed duck (*Anas undulata*), African sacred ibis (*Threskiornis aethiopicus*) and the common moorhen (*Gallinula chloropus*) (CWAC, 2017).

4.2.3 Spraint collection and analysis

The study was conducted between June 2012 and September 2014 and fieldwork was restricted to late autumn, winter and early spring to avoid riverbank flooding, which is common during the wet seasons (late spring and summer) (SAWS, 2010). The wet season was avoided as spraints have been shown to degrade quicker when exposed to rain (Rowe-Rowe, 1992), while flooding of riverbanks

was likely to wash away spraints. If there was rainfall during the surveying period, at least two days were given before surveying resumed, allowing otters time to deposited fresh spraints. This methodology seemed effective at preventing the loss of spraints due to rainfall or flooding, as all sites did not present signs of flooding (vegetation flattening, loss of riverbank sediment) and fresh spraints were present following rainfall (Ponsonby, pers. obs.). At each 5-km sampling site, a 400 m by 10 m transect on both riverbanks was surveyed by foot. During the survey of the transects, any spraints found, regardless of age were collected (except spraints that were very old and degraded). Spraint sites were identified as areas with multiple spraints present, and spraints were only collected from spraint sites if they were a minimum distance of 30 cm from the nearest adjacent spraints, to reduce possible contamination. Several spraints were taken from spraint sites as there have been instances of both species, as well as several conspecifics, using the same latrine (Rowe-Rowe, 1992). Re-sealable plastic bags were inverted to collect spraints individually to avoid DNA contamination (for analyses in concurrent studies). Collection bags were sealed and labelled with the GPS coordinates (using a hand-held Garmin eTrex Vista Cx GPS device) and the date. Each 5-km site was visited once during the period of this study.

In total, 211 spraint samples were collected for the duration of the study. Each individual grouping of spraints collected from spraint sites was considered to be a unique representation of prey consumed by an otter, thus a combined representative mean for each spraint site was not calculated for comparison between other spraints found during the study, and for the comparison of findings from previous studies. Collected spraints were stored in a refrigerator at the University of the Witwatersrand, Johannesburg at -10 °C until further analysis of the spraints was conducted. Prior to conducting the diet analysis, each spraint was individually swabbed for DNA to be used to identify the species of the otter that produced the spraint (see Madisha *et al*., 2015; Appendix B for detailed methods). DNA analysis concluded that 171 samples came from African clawless otters, eight samples came from spotted-necked otters (*Hydrictis maculicollis*) and 32 samples did not yield results.

A method similar to that proposed by Perrin and Carugati (2000a) was used to isolate digested prey remains from the rest of the spraint. Each spraint sample was individually washed with water through a 1 mm sieve to collect all the larger remains of prey, which were placed on newspaper and allowed to air dry for 24 hours. Once a sample was dry, it was placed in a petri dish and examined under a stereoscopic microscope at 10X magnification. To determine the percentage composition of prey remains contained per spraint, a piece of graph paper (grid squares measure 5 mm x 5 mm) was placed under the petri dish containing the remains from one spraint. The total number of squares on the graph paper covered by each type of prey was counted and the percentage of the prey item that was contained in the spraint was calculated. Identification of prey species was carried out visually and remains were identified to class level, and where possible to order level of classification using photographs of remains and museum specimens of animals and skeletons. Birds were identified by the presence of feathers and general shape and hollow characteristic of the bones, while crab remains were identified by colour, shape and texture of exoskeleton fragments. The presence of scales and thin bones were the common identifying feature of fish; very small distinctive exoskeleton limbs were used to distinguish insects and the occurrence of fur or hair along with jaw bones and limb bones were used to identify mammals. Care was taken not to confuse otter hair with hair from prey mammals; otter hair is occasionally found in spraints as otters spend extensive periods of time grooming and hair is often ingested (Kruuk, 2006); this was achieved by comparing scale patterns of hair found in spraints to photographs of species hair samples (Keogh, 1979). The presence of any foreign objects such as refuse (plastic, etc.) or any vegetation was also recorded.

The percentage occurrence (PO) and relative occurrence (RO) was calculated for each prey type in peri-urban and urban areas, as well as per study river. These two values are calculated to account for over estimation of prey with hard remains that tend to be more common in spraint, and under estimation of soft prey remains (Carss and Parkinson, 1996). Equations for PO and RO are as followed:

$$\text{PO} = \frac{\text{Number of spraints containing certain prey type}}{\text{Total number of spraints}} \text{ X } 100$$

$$\text{RO} = \frac{\text{Total number of certain prey type in all spraints}}{\text{Total number of all prey types in all spraints}} \text{ X } 100$$

PO and RO for all spraints were used for comparison with previous studies that utilised the same method of evaluating occurrence of prey in the diet of *A. capensis*. Comparisons between mean occurrence of prey types from this study and previous studies (Lighart *et al*., 1994; Somers and Purves 1996; Perrin and Carugati, 2000; Somers and Nel, 2003) were conducted using a two-tailed z-test.

The PO of each prey type for each spraint sample was arcsine transformed prior to running statistical tests as the data did not follow a normal distribution (see McDonald, 2014). A multivariate general linear model (GLM), using a repeated measures design for arcsine transformed PO of each prey type per spraint sample, was used due to the multiple dependent variables (prey types) associated with a single sample (Carey, 2013). The GLM was used to identify whether study rivers, areas (peri-urban and urban), and combination of the two were significant predictors influencing the number of each prey type present per spraint sample. Area, study river, area*river were entered into the GLM as independent variables, and PO of each prey type per spraint sample as dependent variables. The GLM analysis was followed by Fisher LSD (least significant difference) post hoc tests to determine any significant differences in the PO of each prey items present between urban and peri-urban areas, between rivers, and between the combination of river and area. All statistical analyses were conducted using STATISTICA 10© (StatSoft, Inc., 2011), and the significance level was set at $p < 0.05$.

4.3 Results

As predicted, crab made up the majority of the diet of *A. capensis* (88.3 ± 0.02% of all samples contained crab remains), while fish contributed much less than

expected (20.47 ± 0.03% of all samples contained fish remains) (Table 4.1). Bird feathers and bones were also present in the *A. capensis* spraints (27.49 ± 0.03% of all samples contained bird remains). Several pieces of plastic and glass were found in some spraint samples from both areas.

Table 4.1 Diet comparison of *A. capensis* in areas of differing levels of urbanisation in Gauteng, South Africa. (X = number of samples with prey type present; PO = percentage of occurrence of prey type; RO = relative percentage of occurrence of prey type).

	Peri-urban area (n = 116)			Urban area (n = 55)		
Prey	X	PO (%)	RO (%)	X	PO (%)	RO (%)
Crab	103	88.79	45.98	48	87.27	42.48
Insect	34	29.31	15.18	11	20	9.73
Bird	30	25.86	13.39	17	30.91	15.04
Mammal	26	22.41	11.61	12	21.82	10.62
Fish	13	11.21	5.80	22	40.00	19.47
Vegetation	13	11.21	5.80	0	0	0
Pollution	5	4.31	2.23	3	5.45	2.65

Several differences were observed between *A. capensis* samples found in Gauteng compared to those analysed in previous studies (Table 4.2). The most striking is the lack of amphibian remains in the Gauteng samples. The proportion of bird remains was considerably higher than all the previous studies ($p = 0.0001$), while the occurrence of crab and fish were moderately lower in some instances and significantly lower in others.

Table 4.2 Comparing the current study percentage occurrence (PO) and relative occurrence (RO) of prey types to PO and RO measured in previous studies of *A. capensis* diet in areas with lower human impact using a pairwise Z test. A = current study; B = Somers and Nel (2003), Oliphants River; C = Somers and Nel (2003), Eerste River; D = Perrin and Carugati (2000a); E = Somers and Purves 1996; F = Lighart *et al*. 1994; $\alpha = 0.05$. Symbols following values indicate: significance difference of $p < 0.05$ (*) and significance difference of $p < 0.001$ (**).

Prey type (%)	A (n=171)	B (n=824)	C (n=362)	D (n=735)	E (n=66)	F (n=105)
Amphibian	0/0	10.9**/5.7**	6.6**/4.3*	26**/16**	18.2**/9.2**	7**/3*
Bird	27.5/13.9	0.7**/0.4**	4.7**/3.1**	0.4**/0.2**	0**/0**	2**/1**
Crab	88.3/44.8	96.4**/50	93.9*/61.3**	90/57*	100*/50.8	96*/49
Fish	20.5/10.4	50**/25.9**	32.6*/21.3**	15/10	36.4*/17.7	19/10
Insect	26.3/13.4	10.4**/5.4**	11**/7.2**	22/14	37.9/19.2	33/17
Mammal	22.2/11.3	0**/0**	2.5**/1.6**	0.1**/0.4**	0**/0**	31/16

A comparison of spraint samples from urban and peri-urban areas showed that area was a moderate predictor in prey type presence in spraints ($F_{6, 169} = 2.337$; $p = 0.030$). Post hoc tests indicate that crab was the highest occurring prey type in both areas, and the occurrence of crab was higher in peri-urban than urban areas. A significant difference was also detected between the number of fish consumed in peri-urban and urban areas (Table 4.3).

The surveyed rivers were significant predictors for the prey type eaten ($F_{48, 972} = 2.628$; $p = 0.0000002$; Table 4.4). The highest occurrence of bird remains was found in samples along the Braamfonteinspruit. The presence of fish remains in samples from the Klip River was greater than the other rivers. Spraints collected along the Klein Jukskei River had significantly higher proportions of crab remains, while the presence of vegetation was highest in samples from the Hennops River. Refuse was highest in samples from the Braamfonteinspruit (Table 4.4) and included pieces of plastic and glass. The levels of insects and mammals present in samples showed no difference between the rivers. Results of the 2-factor analysis GLM (area*river) found no combination of these factors influenced prey consumed by otters ($F_{6, 960} = 0.835$; $p = 0.543$).

Table 4.3 Comparison of prey types present in *A. capensis* spraint samples between peri-urban (P) and urban (U) areas using Fisher LSD post hoc testing. Bold values indicate significant differences. <0.0001 indicate extremely small values. Alpha set at p = 0.05.

	Bird (P)	Crab (P)	Fish (P)	Insect (P)	Mammal (P)	Vegetation (P)	Pollution (P)
Bird (U)	0.729	**<0.0001**	0.276	0.143	0.928	0.082	**0.019**
Crab (U)	**<0.0001**	**0.010**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Fish (U)	0.132	**<0.0001**	**0.003**	**0.001**	0.078	**<0.0001**	**<0.0001**
Insect (U)	**0.014**	**<0.0001**	0.300	0.510	**0.027**	0.698	0.820
Mammal (U)	0.991	**<0.0001**	0.155	0.072	0.807	**0.038**	**0.007**
Vegetation (U)	**0.005**	**<0.0001**	0.175	0.327	**0.011**	0.478	0.925
Pollution (U)	**0.006**	**<0.0001**	0.194	0.357	**0.013**	0.516	0.972

Table 4.4 Relative occurrence (RO) of prey types from A. capensis spraint samples found along study rivers in Gauteng. Bold values indicate the highest RO of a prey type out of all the rivers. Asterisks highlight the RO values that differ significantly from the highest RO for that specific prey type. Symbols following values indicate: the highest occurrence (^), significance difference of p < 0.05 (*), and significance difference of p < 0.001 (**). Fisher LSD post hoc testing was used to test difference between rivers.

Prey type (%)	Blesbok.	Braam.	Crocodile	Hennops	Jukskei	K. Jukskei	Klip	Mooi.	Pienaars
Bird	27.5	**33.33** ^	12.37 *	16.67 *	14.29 *	11.53 **	7.81 **	8.33 **	10.91 **
Crab	35 *	33.33	47.42	50	42.86 *	**61.53** ^	43.75 *	50	43.64
Fish	5 *	16.67	4.12 **	0	8.57 *	11.53	**28.13** ^	0	7.27 *
Insect	12.5	0	17.53	16.67	11.43	3.84	9.38	16.67	18.18
Mammal	12.5	0	11.34	0	22.86	7.69	7.81	25	9.09
Vegetation	7.5 *	0	3.09 **	**16.67** ^	0 *	0 *	1.56 **	0	9.09
Pollution	0 *	**16.67** ^	4.12	0	0 *	3.84 *	1.56 **	0	1.82 **

4.4 Discussion

The introduction of chemical and physical pollutants to natural water courses coupled with habitat fragmentation have resulted in decreasing population sizes of species that are unable to cope with these changes in urban environments, which in turn affects other species present in the associated food web (Madsen and Prang, 2001; Bonier *et al*., 2007). This study aimed to determine the diet of the African clawless otter (*A. capensis*) in areas with varying levels of human disturbance, and to what extent it differed with findings from previous studies in less disturbed areas of the country. Overall, crab was the main dietary component for *A. capensis* which agrees with previous studies (Rowe-Rowe, 1977a; van der Zee, 1981; Ligthart *et al*., 1994; Somers and Purves, 1996; Perrin and Carugati, 2000a; Somers and Nel, 2003). Remains of birds, fish, mammals and insects were also found, concurring with previous studies (Ligthart *et al*., 1994; Somers and Purves, 1996; Perrin and Carugati, 2000a; Somers and Nel, 2003). It is of interest to note the lack of amphibian remains in the spraints from Gauteng samples, as several previous studies confirmed amphibian remains in *A. capensis* spraints (Ligthart *et al*., 1994; Somers and Purves, 1996; Perrin and Carugati, 2000a; Somers and Nel, 2003). Several amphibian species are reported to exist in Gauteng, but poor water quality (Howe, Gillis and Mowbray, 1998) and habitat loss (Hamer and McDonnell, 2008) may be impacting their presence in the heavily impacted urban areas of Gauteng.

While it was expected that the proportion of crab being consumed would be much greater than any of the other prey type, the rather small proportion of fish in the diet was surprising. According to a study of the frequency and abundance of fish per river by Kleynhans *et al.* (2007), it appears that more fish are available in the Klip River than any of the other study rivers. More fish present in the Klip River supports the higher occurrence of fish remains found in spraints occurring along this river. This might strengthen the argument that crab and other terrestrial prey such as birds and mammals are more easily accessible than fish for the otters. Results from river assessments of the Crocodile, Hennops, Jukskei and Pienaars rivers by the River Ecostatus Monitoring Programme (REMP) conducted from

2013 to 2015 further indicate that the frequency of fish in these rivers is lower than the reference species and numbers are expected to be decreasing due to the poor conditions of these rivers (Singh and Todd, 2015).

Several of the rivers surveyed are heavily polluted, likely due to the rivers flowing through areas of high urbanisation (industrial activities and mining areas) exposing the water to significant levels of pollutants, waste disposal and mine drainage contamination (van Veelen, 2002; DWAF, 2003a, 2003b; de la Rey *et al*., 2004; Huizenga, 2004; Swanepoel, 2009; Matowanyika, 2010), which may have resulted in a decline in fish numbers of species sensitive to pollutants (see Walsh *et al*., 2005; Mangadze *et al*., 2016). This theory has some validity as data from the Department of Water and Sanitation (DWS) show high levels of aluminium (Al) and copper (Cu) as well as low pH values in the Jukskei River which flows through both urban and peri-urban areas (DWS, 2016) and presents a very low number of fish species present (Kleynhans *et al*., 2007). The Blesbokspruit, which flows through far less urbanised areas than the Jukskei River, has instances of high Al and Cu levels and low pH, albeit at fewer monitoring stations than along the Jukskei River (DWS, 2016). Measurements show high levels of aluminium in the Klip River, but the pH is neutral, which is favourable for fish as embryonic and larval stages are negatively affected by Al in low pH water (Duis and Oberemm, 2001). Aquatic species have been shown to demonstrate behavioural changes in the presence of varying levels of pollutants in water, indicating the ability to detect the presence of harmful substances and react in an avoidance manner in an attempt to prevent uptake and resulting damage from the detected pollutant (Hering *et al*., 2006; Sih, 2013; Lanctôt *et al*., 2016). Fish die off or avoidance of unfavourable conditions may be occurring in fish of Gauteng rivers with unsuitable levels of pollutants in the water, whereas the less motile crab may remain in a span of the river that is unfavourable. This may be the case in the Jukskei River, which has a significantly lower percentage of fish compared to the Klip River. The South African Scoring System version 5 (SASS5) indicates Potamonautidae crab as being relatively tolerant of polluted water (Dickens and Graham, 2002), which could also be an explanation for the higher proportions of crab remains in the otter spraints. Fishing along rivers in

Gauteng has been shown to be a source of food for some households (OECD, 2011), which may further reduce the number of fish present in the rivers.

Spraint samples analysed from the Braamfonteinspruit had significantly fewer crab remains than those from the other rivers surveyed. This may indicate a lower number of crabs present in the Braamfonteinspruit, requiring the otters to eat other prey items such as birds, which is evident in the higher proportion of bird remains in the samples from the Braamfonteinspruit. With a higher presence of birds, otters may be choosing birds as a food source because of availability. The presence of litter in spraints was also highest in samples found along the Braamfonteinspruit, this coupled with personal observation of high levels of refuse and possible chemical pollution (presence of dense foam in some parts of the river), suggesting that the low presence of crab remains may be due to possible low water quality of the river. However, this may not be the only factor affecting crab numbers, as levels of contamination vary along the lengths of rivers (Liu, Wu and Zhang, 2005; Bu *et al*., 2010).

Previous studies have shown that *A. capensis* and *H. maculicollis* are able to coexist along the same stretch of river as the associated diet of each otter species does not lead to competition as different prey types are favoured, i.e. *A. capensis* prefers crab, whereas *H. maculicollis* selects fish (Somers and Purves, 1996; Perrin and Carugati, 2000a). This may not be the case in the urban environment as fish numbers may be lower than in pristine environments, resulting in additional interspecific conflicts if both otter species depend on crabs as their main source of food in urban areas (see Wauters, Gurnell and Tosi, 2002; Caro and Stoner, 2003). The noticeable variation in the diet of *A. capensis* along all studied rivers suggests a wider diet breadth and exploitation of available food sources which may be of benefit in areas affected by habitat degradation and reduced water quality.

Another problem these otter species encounter is that of decreasing heterogeneity of habitat in urban areas which can have an impact on resource availability, further increasing the chances of conflict occurring between the species (Wauters *et al*., 2002). A problematic situation may arise if both species continue to rely on crab, as this may lead to the overconsumption and collapse of

the crab population in the area, causing possible localised extinction and the need for range expansion by otters to find alternative sources of food. However, *A. capensis* does demonstrate the ability to prey on a wider selection of species than expected. This wider breadth in the diet possibly increases species survival in this highly-disturbed environment as mammals and birds may be used as sources of food. Studies have shown that otters are able to alter their diet in accordance with fluctuations in prey numbers, for example during the spawning periods of fish (Crowley, Johnson and Hodder 2013).

Otters in urban areas appear to be foraging successfully and utilising less preferred prey types outside of what is often considered to be their main sources of food, i.e. crabs and fish. A level of adaptability is evident in the foraging behaviour of *A. capensis* that may allow for survival in an area that is considered unsuitable for medium-sized mammals. The possible dependency on crab as a means of nutrition for *A. capensis* may pose a problematic situation in the future if crab numbers decrease due to pollution entering the rivers. Future research into the health of the rivers is required to draw conclusions regarding the crab and fish population numbers present in the rivers.

CHAPTER FIVE

Genetic diversity of African clawless otters (*Aonyx capensis*) occurring in urbanised areas of Gauteng, South Africa[2]

Abstract

Genetic diversity is the basis of the evolutionary potential of species to respond to environmental changes. However, restriction to the movement of species can result in populations becoming less connected which can reduce gene flow and can subsequently result in a loss of genetic diversity. Urban expansion can lead to the fragmentation of habitats which affects the ability of species to move freely between areas. In this study, the genetic diversity of the African clawless otter (*Aonyx capensis*) in Gauteng (South Africa) was assessed using non-invasive sampling techniques. DNA was extracted from spraint (faecal) samples collected along nine rivers and genotyped using ten microsatellites to assess population structure and genetic diversity. Samples were grouped based on locality and by catchment to determine whether isolated subpopulations exist. Genetic diversity of *A. capensis* in Gauteng was found to be low ($H_o = 0.309$). Analysis of genetic structure provides support for the otter populations being panmictic with high gene flow between populations from different rivers. Results from the study indicate that *A. capensis* movement is not being affected by physical barriers in urbanised areas. However, as the genetic diversity of the species in the study area is low, these animals may not be able to cope with future environmental changes.

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5.1 Introduction

Over the past eight decades, urban areas have expanded into surrounding natural environments at a significant rate. This development into previously undisturbed areas has resulted in species being driven out of their habitats in search of suitable environment with less human disturbance. In some cases, species will remain in the urban areas and utilise the novel environment (McKinney, 2002). Many cities include open spaces such as parks and sports fields which provide new habitat for wildlife. However, not all species are suited to living in cities (Maciusik *et al*., 2010; Gese *et al*., 2012) and thus, a decrease in biotic diversity may occur, whereby only the more resilient (able to withstand or recover from adverse conditions) species survive as they possess characteristics allowing them to tolerate the urban setting (McKinney, 2002). Species in urban areas can occur across a broader habitat range which reduces restriction to one specific habitat type, allowing them to move to another area if conditions become unfavourable (Herr *et al*., 2008; Bino *et al*., 2010; Gosselink *et al*., 2010; Gese *et al*., 2012). However, barriers such as roads and fences may prevent movement of some animals between suitable habitats, limiting their movement and reducing their chances of finding shelter, food and mates. A reduction in interactions with unrelated individuals of the same species (Bascompte and Solé, 1996) can result in lower genetic diversity and an increase in the level of inbreeding (Amos *et al*., 2001).

Two otter species occur in South Africa, the African clawless otter (*Aonyx capensis*) and the spotted-necked otter (*Hydrictis maculicollis*). The distributions of both species range across most of South Africa and include inland and coastal areas as well as large urbanised areas (including the current study area), with *A. capensis* having a much greater distribution range than *H. maculicollis* (Skinner and Chimimba, 2005). The IUCN Red List (2016) has categorised both otters as near-threatened, with habitat degradation posing the highest threat to freshwater environments used by the otters (Jacques *et al*., 2015; Reed-Smith *et al*., 2015). Kubheka *et al*. (2013) demonstrated that there has been a decrease in abundance of both otter species along a stretch of the Mooi River in South Africa that has

experienced an increase in human activity along its banks in recent years, lending to the urgency to better understand anthropogenic effects on otters. Somers and Nel (2004b) reported that *A. capensis* has a home range size that varies from 4.9 km to 54.1 km and a core length from 0.2 km to 9.8 km. Their study also indicated that male ranges overlapped with those of other males and females, while females possibly demonstrated territoriality. However, there is a possibility that the ability of otters to travel great distances in dense urban areas may be hampered due to barriers such as buildings, roads, fences and high levels of human activity, which in turn would impact on intraspecific encounters. To date no studies have focused on the genetic diversity of either of the two otter species, making it impossible to draw conclusions regarding the general genetic health and risks facing these otter species in the future.

The aim of this study was to assess the population genetics of *A. capensis* in a region exposed to varying levels of human disturbance. It was hypothesised that population structuring would reflect division of the local population caused by geographical separation from catchment areas, as well as restriction of movement due to areas of heavy urban development. It was also hypothesised that low genetic diversity and inbreeding would be evident as unrelated individuals may not be able to interact and reproduce successfully in urban Gauteng.

Spraint samples were collected and examined from river catchments in Gauteng (South Africa) to determine the level of genetic diversity and structure of the *A. capensis* population, using ten microsatellite primers developed for the *L. lutra*). The use of cross-species primers has been conducted successfully for amplification of alleles in numerous otter and other animal species in situations where species specific primers have not yet been developed (Beheler *et al*., 2005; Pickles *et al*., 2009; Guertin *et al*., 2012; Brzeski, Gunther and Black, 2013; Trinca, Jaeger and Eizirik, 2013). Gauteng was selected as it represents a complex landscape comprised of urban areas surrounded by less transformed peri-urban areas. There are numerous interconnecting rivers within Gauteng that flow through varying levels of urbanisation (residential suburbs, industrial, mining and commercial areas) and natural environments.

5.2 Methodology

5.2.1 Study area and sample collection

The Gauteng province of South Africa is comprised of 3 river catchments; Crocodile River West catchment (A), Olifants River primary catchment (B) and the Vaal River primary catchment (C). These catchments contain the headwaters of several major river systems (GDARD, 2014). The study focused on nine rivers in the province which occur in two of the three catchments - Pienaars, Hennops, Jukskei, Klein Jukskei (referred to as K. Jukskei henceforth), Crocodile rivers (catchment A) and Braamfonteinspruit (Braam.), Mooi River Loop/ Wonderfonteinspruit (Mooi.), Klip River and Blesbokspruit (Blesbok.) (catchment C). Sampling was conducted from June 2012 to October 2014 and was restricted to the autumn and winter seasons as these seasons have much lower rainfall levels, reducing the chance of spraints deteriorating due to rain or being washed away by flooded rivers (Rowe-Rowe, 1992), which is a common occurrence in summer. Google Earth (Google, 2012) and the Resource Quality Information Services river coverage data for South Africa (RQIS, 2012) were used to measure the full length of the chosen rivers (Figure 5.1) and sampling sites were identified at 5 km intervals along each river. Sites were selected at 5 km intervals as this is the shortest home range length of *A. capensis* found by Somers and Nel (2004a), but due to possible DNA degradation individuals could not be identified, preventing the estimation of home ranged based on occurrence of multiple spraints from the same individual. A 400 m by 10 m transect was surveyed at each 5-km point along both sides of the river, actively searching for signs of otter presence (footprints, spraints and sightings of animals), with each 5-km site being surveyed once. Spraints occurred in various forms: small deposits of anal jelly, a single cigar-shaped faecal deposit, a solitary pile of faeces (comprising 3 or 4 cigar-shaped faeces), or a spraint site with numerous piles of faeces. Otter spraints can be easily identified based on a pungent fishy smell that can be detected several metres away, as well as the characteristic presence of pieces of crab carapace in the spraints (as described by Rowe-Rowe, 1992). Each spraint sample (anal jelly, single cigar-shaped faeces, or solitary pile of faeces) was collected

separately in re-sealable plastic bags, and a solitary pile of spraints was considered one sample. At spraint sites care was taken to select spraint piles (each one collected separately) that were at least 30 cm from the nearest neighbouring spraint piles. Multiple spraints were collected separately from spraint sites, as previous studies have shown that multiple individuals (Rowe-Rowe, 1992; Ben-David *et al*., 2005), as well as both otter species (*A. capensis* and *H. maculicollis*), use the same spraint sites on occasion (Rowe-Rowe, 1992). Regardless of age (except for extremely weathered spraints that had deteriorated significantly), spraints were collected. The GPS coordinates were recorded at every location where spraints were found using a handheld Garmin® eTrex VistaCX GPS device. (see Figure 5.1 for positive sign localities). Samples were stored at -10 °C prior to DNA extraction.

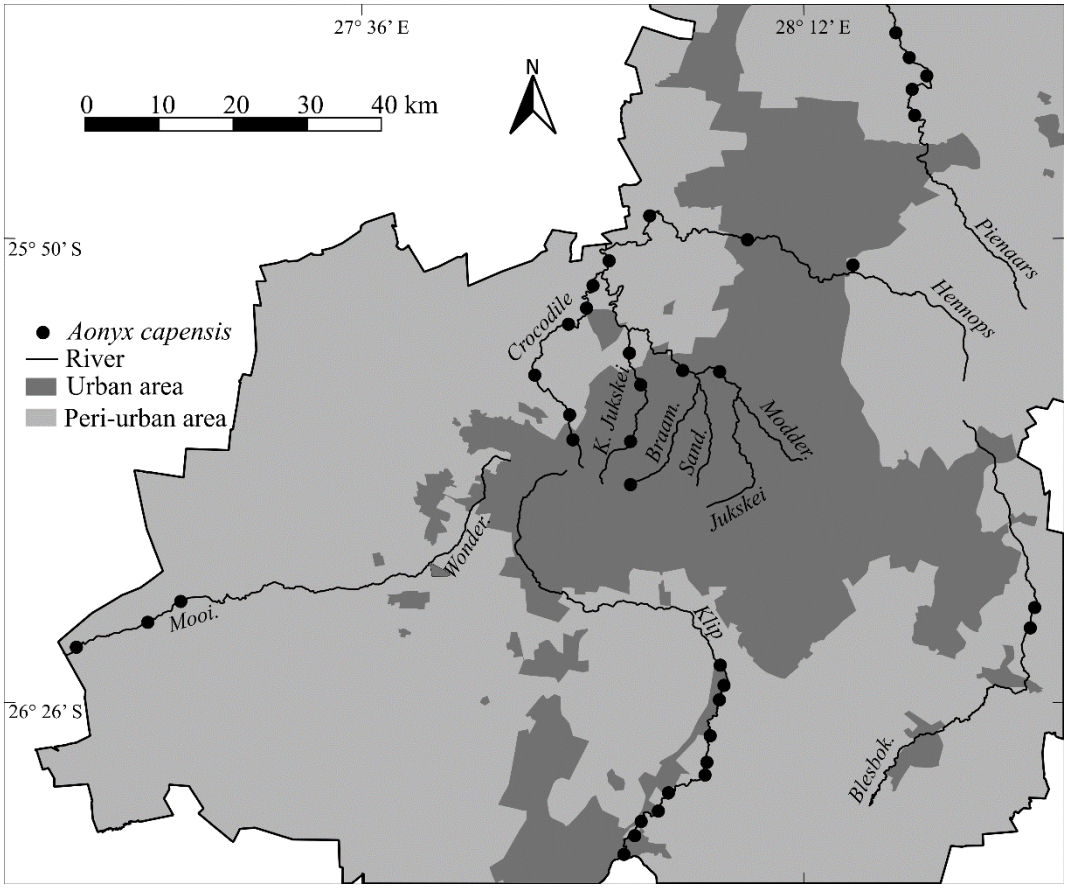


Figure 5.1 Rivers surveyed and collection sites of *A. capensis* spraints in relation to peri-urban and urban areas. Mooi. – Mooi River Loop; Wonder. – Wonderfonteinspruit; K. Jukskei – Klein Jukskei River; Braam. – Braamfonteinspruit; Sand. – Sandspruit; Modder. – Modderfonteinspruit; Blesbok. – Blesbokspruit.

5.2.2 DNA extraction and species identification

DNA was extracted from 211 spraint samples using the QIAGEN® QIAamp® DNA Stool Mini Kit (QIAGEN, 2010) according to the manufacturer's instructions for isolation of DNA from stool for human DNA analysis (see McElwee, 2008). Species identification was conducted using developed partial *CytB* primers (see Appendix B; Madisha *et al*. 2015). A homology search was done on all sequences obtained using the BlastN function on the National Center for Biotechnology Information (NCBI) online database. Control sample DNA for *A. capensis* and *H. maculicollis* was extracted from reference tissue samples obtained from the National Zoological Gardens Biomaterials Bank (Biobank).

These reference samples were collected from roadkill specimens from various locations across South Africa.

5.2.3 Amplification and genotyping

Ten microsatellite markers developed for studies of *L. lutra* (Dallas and Piertney, 1998) were used for genotyping analysis: Lut435, Lut453, Lut457, Lut604, Lut615, Lut701, Lut715, Lut782, Lut818, and Lut832. These markers have been shown to be polymorphic in up to six different otter species (including *A. capensis*), but not in other carnivore species (Dallas and Piertney, 1998). Optimisation of the primers was conducted testing various quantities of reagents and temperatures at which to conduct PCR amplification, with the following method deemed the most effective based on number of successful amplifications. Amplification was carried out using a 15 µl reaction volume containing 7.5 µl of Platinum master mix (1X), 3 µl of forward and reverse primer (10 pmol), 2.5 µl of double distilled water and 2 µl template DNA (20 ng). The cycling conditions for PCR amplification were as follows; 5 minutes (min) at 95 °C initial denaturation, 38 cycles for 30 seconds (sec) at 95 °C, 30 sec at 45 – 52 °C and 30 sec at 72 °C, followed by extension at 72 °C for 20 min. The PCR reaction was carried out in the Bio-Rad Thermal Cycler (Bio-Rad Laboratories, Inc.). PCR products were run against GenescanTM 500 LIZ^{TM} (Applied Biosystems, Inc.) internal size standard on an ABI 3130 Genetic Analyzer. Samples were genotyped using GeneMapper v.4.0 (Applied Biosystems, 2005). All PCR reactions were repeated five times, and genotyping was conducted using a comparative method in which alleles obtained for each sample were compared, and the most frequently observed alleles for each locus were selected for each sample. Allelic peaks were scored based on height and occurrence in prescribed binning areas based on the range of each marker. In cases presenting multiple allelic peaks, the highest peak was chosen as the first allele. The second allele was selected if it was no less than half the height of the first allele, fell into a prescribed bin, and was of a reasonable distance of base pairs apart from the first allele selected (see Appendix Figures for visual representation of genotype scoring).

5.2.4 Microsatellite analysis

In order to exclude possible errors, MICROCHECKER version 2.2.3 (van Oosterhout *et* al., 2004) was used to detect the presence/absence of null alleles and allelic dropout. GenAlEx 6.5 (Peakall and Smouse, 2006; 2012) was used to test for deviations from Hardy-Weinberg Equilibrium (HWE). Linkage disequilibrium was determined using the online version of Genepop 4.2 (Genepop on the web; Rousset, 2008). Duplicate samples of individuals were identified using the probability of identity in GenAlEx 6.5 (Peakall and Smouse, 2006; 2012). Matching profiles indicating duplicate sampling of the same individual were excluded from further analysis to prevent redundancy. However, as genetic profiles are dependent on the quality of DNA extracted which can be compromised in non-invasive samples. As some samples collected were older than others, this may have impacted the quality of DNA extracted. If an individual defecated several times along a study river, the presence of null alleles due to low quality DNA would greatly affect the genetic profile obtained for that individual. A single locus difference will render a genetic profile unique, requiring multiple repeats to be conducted increase the accuracy of allele detection. Genetic diversity was assessed using GenAlEx 6.5 (Peakall and Smouse, 2006; 2012) to determine the number of alleles, expected heterozygosity (H_e) and observed heterozygosity (H_o).

5.2.5 Population structure

Due to areas of heavy urbanisation, and large distances between study rivers restricting movement of otters, each river was defined as a potential population, resulting in nine theoretical populations. As the study rivers occur in two catchment areas, these were considered individual populations for the Catchment analysis portion of this study. GenAlEx 6.5 (Peakall and Smouse, 2006; 2012) was used to determine population differentiation (F_{ST}) and for Analysis of Molecular Variance (AMOVA). In order to assess the genetic partitioning across Gauteng river otter populations, two different approaches were used based on multilocus genotypes. A Bayesian clustering analysis was conducted using the statistical program STRUCTURE version 2.3.4 (Pritchard, Stephens and

Donnelly, 2000) for the assignment of individuals to groups based on genetic similarity. STRUCTURE was run with and without LOCPRIOR using 100 replications at each value for *K* (*K* = 1 - 12) for the 'per river' analysis and for the Catchment analysis (K = 1 - 4). The values used for *K* for 'per river' analysis took into account the possible nine populations designated to each study river, and an extra three population in case more than nine independent populations occurred. If 12 populations were detected, the value for *K* would be increased further to accurately detect the possible number of populations. The *K* value for Catchment analysis included the two actual catchments and two extra potential populations as the large areas of the catchments may be occupied by more than two populations. The runs were conducted with a burn-in period of 100 000 repeats followed by 1 000 000 repeats of the Markov chain Monte Carlo (MCMC). The result files from each run (with LOCPRIOR and without LOCPRIOR) were uploaded to the web-based STRUCTURE-HARVESTER (Earl and vonHoldt, 2012) programme which uses likelihood methods to assume the correct number of genetic clusters (K). In addition, the genetic distance was calculated and a Principal Coordinates Analysis (PCoA) was conducted for the data using GenAlEx version 6.5 (Peakall and Smouse, 2006; 2012).

5.2.6 Relatedness analysis

Pairwise relatedness was calculated between all samples per river, with each river considered as a separate population, using GenAlEx version 6.5 (Peakall and Smouse, 2006; 2012). Results were obtained for three different relatedness tests; Ritland (1996) estimator, Lynch and Ritland (1999) estimator, and Queller and Goodnight (1989) estimator. The mean of the three results obtained for each pairwise comparison and used to create a box and whisker plot for each river. This analysis was restricted to the Blesbok., Crocodile, Jukskei, K. Jukskei, Klip, and Pienaars rivers as the Braam., Mooi. and Hennops River did not have sufficient samples for an adequate analysis. The same procedure was conducted for the comparison between catchments, with Catchment A comprising of samples from the Pienaars, Jukskei, K. Jukskei, and Crocodile rivers. While Catchment C included the Blesbok. and Klip River. If the median occurs at or below zero

individuals within the population are not highly related, whereas if the median is above zero individuals in the population are considered related.

5.3 Results

Of the total 211 samples collected, 171 spraint samples were identified as *A. capensis*, while eight were identified as *H. maculicollis*. A total of 32 samples remained unidentified to species, possibly due to sample DNA being too degraded for successful amplification or an actual lack of DNA present in the sample. Due to the low number of samples identified as *H. maculicollis*, population genetic assessments were conducted only for *A. capensis* in the study presented here.

5.3.1 Marker assessment

Aonyx capensis samples from two catchments in Gauteng (South Africa) were genetically analysed using ten microsatellite markers. Sample distribution included 14 samples from Blesbok., two samples from Braam., 48 samples from Crocodile River, three samples from Hennops River, 16 samples from Jukskei River, 15 samples from K. Jukskei River, 37 samples from Klip River, six samples from Mooi. and 30 samples from Pienaars River. Braam., Mooi. and Hennops results are excluded due to small sample size. The sample collection comprised the first genetic analysis of *A. capensis* in South Africa. Genotypes obtained were corrected using MICROCHECKER and three markers (Lut604, Lut782 and Lut818) showed a high presence of null alleles (mean > 0.45) and were thus excluded from further analysis (Table 5.1). Using probability of identity, no matching profiles were identified, because of this the home range of individuals could not be estimated. All markers appeared to be significantly linked based on Genepop analysis, which may be due to the presence of non-amplified alleles (null alleles). Markers from Blesbok., Crocodile, Jukskei, K. Jukskei, Klip, and Pienaars rivers deviated from the Hardy-Weinberg equilibrium (Table 5.2). The observed deviations may be due to null alleles, low levels of observed heterozygosity at all loci and/or differences in sample sizes between rivers.

Table 5.1 Results of genotype assessment conducted using MICROCHECKER version 2.2.3 (van Oosterhout *et* al., 2004). Markers with a high occurrence of null alleles are displayed in bold.

Marker	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2	Mean
Lut435	0.29	0.41	0.28	0.40	0.345
Lut453	0.31	0.45	0.30	0.60	0.415
Lut457	0.32	0.49	0.32	0.44	0.393
Lut604	**0.34**	**0.53**	**0.32**	**0.60**	**0.458**
Lut615	0.30	0.43	0.29	0.49	0.378
Lut701	0.34	0.52	0.32	0.52	0.425
Lut715	0.26	0.36	0.25	0.57	0.358
Lut782	**0.33**	**0.51**	**0.32**	**0.76**	**0.478**
Lut818	**0.41**	**0.76**	**0.38**	**0.77**	**0.579**
Lut832	0.32	0.50	0.32	0.55	0.423

Table 5.2 Number and range of alleles detected in *A. capensis* using ten microsatellite markers developed for *L. lutra*. Hardy-Weinberg Equilibrium (HWE) probability values are given for each marker in each river population group. Values marked with * are in HWE.

Locus	Number of alleles	Allele range	Blesbok. p-value	Crocodile p-value	Jukskei p-value	K. Jukskei p-value	Klip p-value	Pienaars p-value
Lut435	9–23	103–175	0.00008	1.25×10^{-16}	3.07×10^{-07}	0.02	0.00001	3.56×10^{-11}
Lut453	5–24	84–210	0.007	1.79×10^{-17}	2.22×10^{-08}	**0.05***	7.92×10^{-09}	1.68×10^{-12}
Lut457	9–21	120–198	0.002	2.15×10^{-30}	0.0002	0.0003	3.77×10^{-35}	2.26×10^{-11}
Lut604	7–17	100–185	0.007	6.34×10^{-17}	0.002	0.0034	9.00×10^{-21}	8.75×10^{-12}
Lut615	8–27	122–290	**0.178***	1.78×10^{-20}	0.0004	0.001	6.12×10^{-17}	1.87×10^{-07}
Lut701	7–18	181–247	**0.062***	1.67×10^{-37}	0.00007	0.027	2.65×10^{-20}	1.07×10^{-14}
Lut715	8–17	139–225	**0.332***	1.51×10^{-28}	0.007	0.013	5.10×10^{-13}	1.84×10^{-08}
Lut782	4–13	162–218	**0.092***	2.41×10^{-15}	**0.256***	0.009	2.06×10^{-18}	0.016
Lut818	5–7	143–179	0.002	2.00×10^{-15}	3.40×10^{-08}	0.00001	0.00002	7.67×10^{-09}
Lut832	6–19	134–248	0.023	4.59×10^{-22}	0.024	0.0001	2.02×10^{-25}	3.87×10^{-19}

5.3.2 Genetic analysis: populations defined by river

Genetic assessments were then carried out for each river referred to here as 'per river' analyses. All loci were polymorphic with the number of alleles ranging from 4 to 27, and averaging nine alleles per locus. Genetic diversity estimates by observed and expected heterozygosity and the number of alleles within each river were moderate to high. The mean expected heterozygosity (H_e) was 0.730 with a mean observed heterozygosity (H_o) of 0.344 (Table 5.3). In all instances, H_o was lower than H_e and values for H_o varied per river with Klip River being the lowest (H_e = 0.266) and Blesbok. being the highest (H_o = 0.406). Upon using each river as a potential population, STRUCTURE HARVESTER identified K = 3 (Figure 5.2 and Figure 5.3) as the most likely number of subpopulations, although no significant population structure was observed. Similarly to STRUCTURE, the PCoA did not reveal any definite clusters using genetic distance (Figure 5.4). A low mean genetic differentiation (Fst = 0.037) between all rivers is shown in Table 5.4, with populations along the Crocodile and Pienaars rivers showing the lowest differentiation (Fst = 0.014). Populations from all rivers displayed private alleles at all loci, with the Crocodile and Pienaars River populations showing the highest number with eight private alleles each.

Table 5.3 Genetic variation estimates for (A) - 'per river' analysis and (B) – Catchment analysis: H_o = mean observed heterozygosity; H_e = mean expected heterozygosity; N_a = mean number of alleles per locus*; and N_e = mean number of effective alleles along nine rivers in Gauteng, South Africa. The standard error is indicated. All analyses were conducted in GenAlEx version 6.5 (Peakall and Smouse, 2006; 2012). Sample size for river and catchment represented in parentheses.**

(A)

Rivers	H_o	H_e	N_a	N_e
Blesbok. (14)	0.406 ± 0.044	0.753 ± 0.027	6.9 ± 0.526	4.47 ± 0.449
Crocodile (48)	0.312 ± 0.051	0.879 ± 0.026	17.4 ± 1.796	10.388 ± 1.279
Jukskei (16)	0.289 ± 0.044	0.805 ± 0.028	8.6 ± 0.806	6.107 ± 0.805
K. Jukskei (15)	0.319 ± 0.05	0.854 ± 0.013	10.0 ± 0.715	7.460 ± 0.767
Klip (37)	0.266 ± 0.033	0.879 ± 0.015	14.4 ± 1.185	8.989 ± 0.702
Pienaars (30)	0.292 ± 0.042	0.888 ± 0.019	16.3 ± 2.05	11.205 ± 1.614
Mean ± SE	0.314 ± 0.044	0.843 ± 0.021	12.267 ± 1.18	8.103 ± 0.936

(B)

Catchment	H_o	H_e	N_a	N_e
A (114)	0.311 ± 0.033	0.905 ± 0.016	25.5 ± 2.684	12.938 ± 1.792
C (57)	0.308 ± 0.026	0.873 ± 0.014	16.8 ± 1.191	8.65 ± 0.824

* N_a = sum of all alleles detected per river/ number of loci

** N_e = 1/ (sum of squared population allele frequencies)

Table 5.4 Genetic differentiation between otter populations grouped according to the river along which spraint samples were collected (Weir and Cockham, 1984).

	Blesbok.	Crocodile	Jukskei	K. Jukskei	Klip
Crocodile	0.038				
Jukskei	0.063	0.043			
K. Jukskei	0.051	0.028	0.058		
Klip	0.039	0.015	0.047	0.032	
Pienaars	0.043	0.014	0.041	0.029	0.020

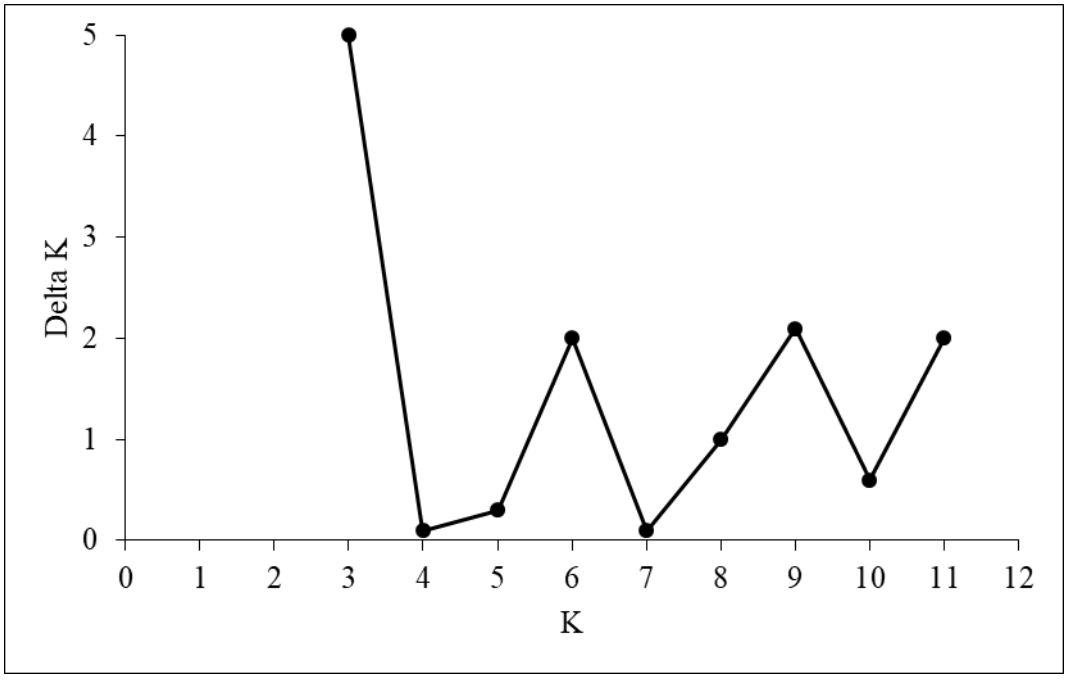


Figure 5.2 Delta K values obtained from STRUCTURE HARVESTER (Earl and vonHoldt, 2012) indicating the greatest likelihood of number of clusters present in the 'per river' sample group is three.

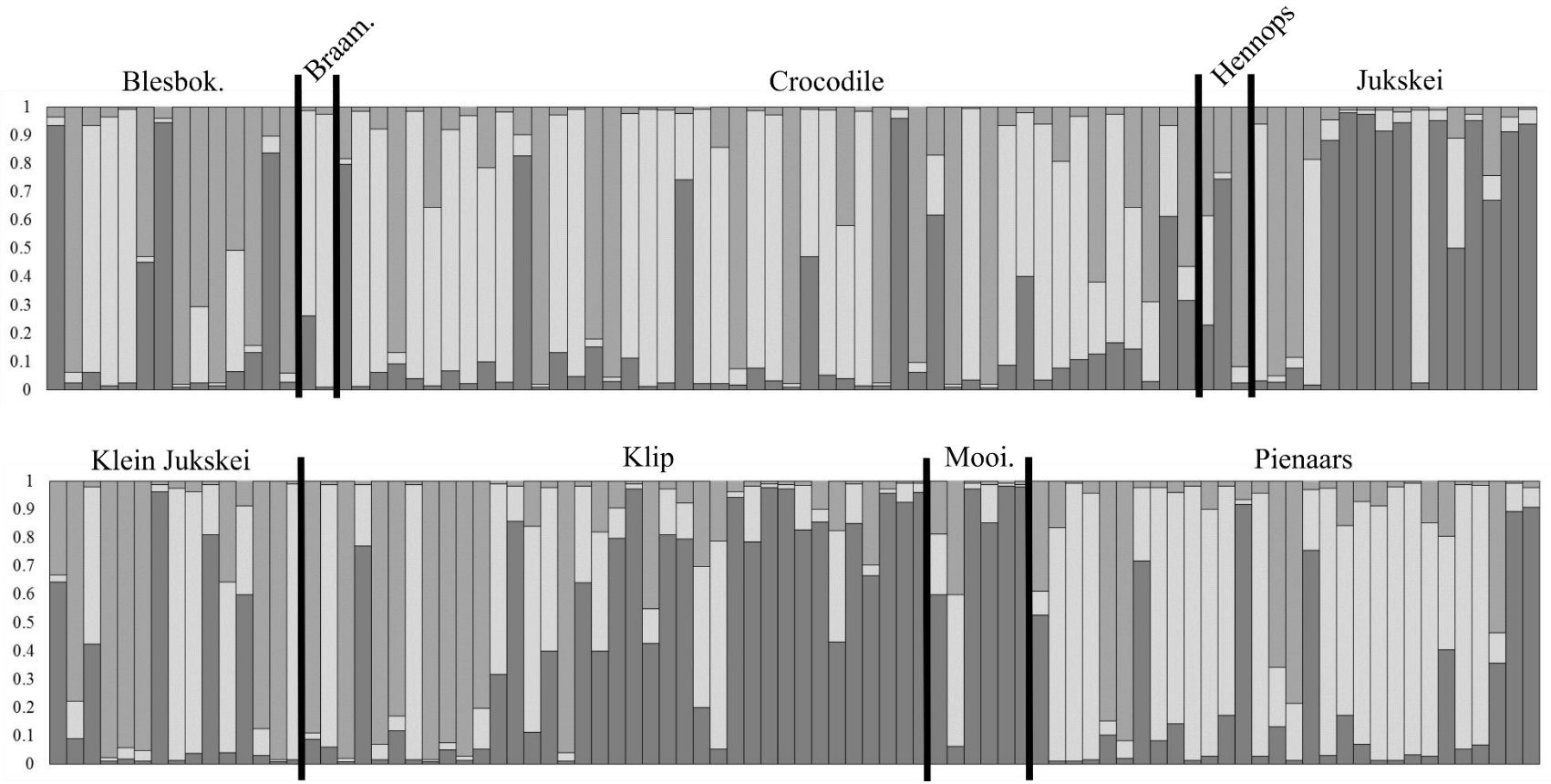


Figure 5.3 Plots of assignment probabilities from STRUCTURE for the 'per river' analysis showing the posterior probability of assigning each individual to each of the inferred clusters. Each individual is represented by a vertical bar and the colours refer to the different clusters. Average cluster membership for K = 3.

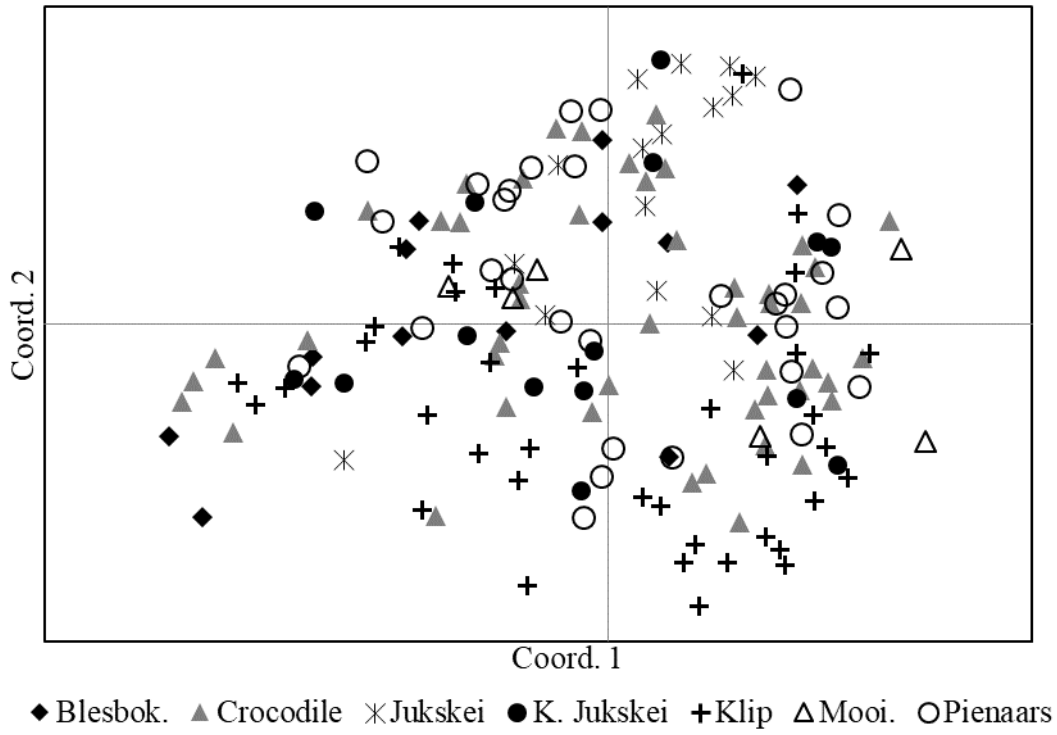


Figure 5.4 Principal Coordinates Analysis (PCoA) for microsatellite data for each river surveyed.

5.3.3 Relatedness: populations defined by river and catchment

Pairwise relatedness comparisons between individuals within each river population indicated that the mean relatedness for each river is low as the median of the box and whisker plots for each river falls below zero. However, the Blesbokspruit had two maximum outliers, which may be the result spraints from the same individual being collected. Overall relatedness of individuals in both Catchment A and C was low based on the box and whisker plot (Figure 5.5).

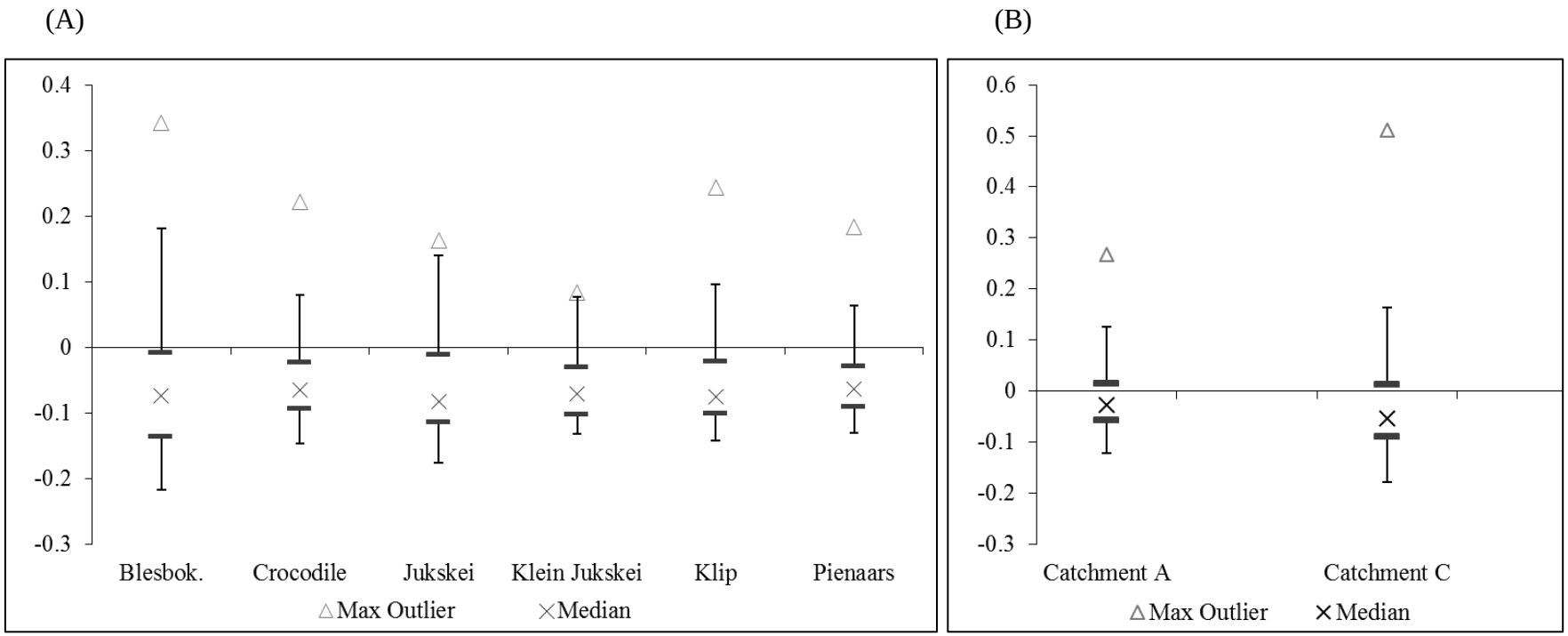


Figure 5.5 Box and whisker plots depicting the relatedness within each (A) river population and (B) catchment population. Overall relatedness is low for all populations indicating interbreeding is not considerably high in the populations.

5.3.4 Genetic analysis: populations defined by catchment area

Due to sample size differences (small sample size of Braam., Mooi., and Hennops River), which resulted in limitations, some analyses could not be performed in the 'per river' analyses. These rivers were subsequently clustered depending on the water catchment to which they belong (Catchment A - Pienaars, Hennops, Jukskei, K. Jukskei, Crocodile rivers; Catchment C – Braam., Mooi., Klip River and Blesbok.) and genetic assessments for each of the two catchments (A and C) were conducted. When separated into catchment areas, all loci were polymorphic with the number of alleles ranging from 9 to 39, and averaging 21 alleles per locus. Expected heterozygosity within all groups (H_e) was 0.889 and observed heterozygosity (H_o) was 0.309, this difference may be due to the use of non-species specific markers or genotypic error (Table 5.1). There was a significant deviation from the Hardy-Weinberg equilibrium ($p < 0.001$) for all the markers, which may indicate genotyping error and resulting underestimation of heterozygosity. However, genetic differentiation ($F_{ST} = 0.01$) between the two catchments (A and C) was low and non-significant ($p \geq 0.05$).

Results from STRUCTURE Harvester identified K = 2 as the most likely number of genetic clusters for the catchment analysis (Figure 5.6, Figure 5.7 and Figure 5.8). No significant population sub-structuring could be observed with allele frequencies being somewhat similar, although more frequent in other rivers. This could be attributed to high gene flow but shows that some rivers may be favoured more than others. Although STRUCTURE supports the presence of two subpopulations, this was not observed in the PCoA, which clearly illustrates no significant clustering occurring (Figure 5.9). Private alleles were observed in all ten loci for Catchment A, with 100 private alleles occurring in the Catchment A population. Seven out of the ten loci had private alleles, totalling 13 alleles for the Catchment C population.

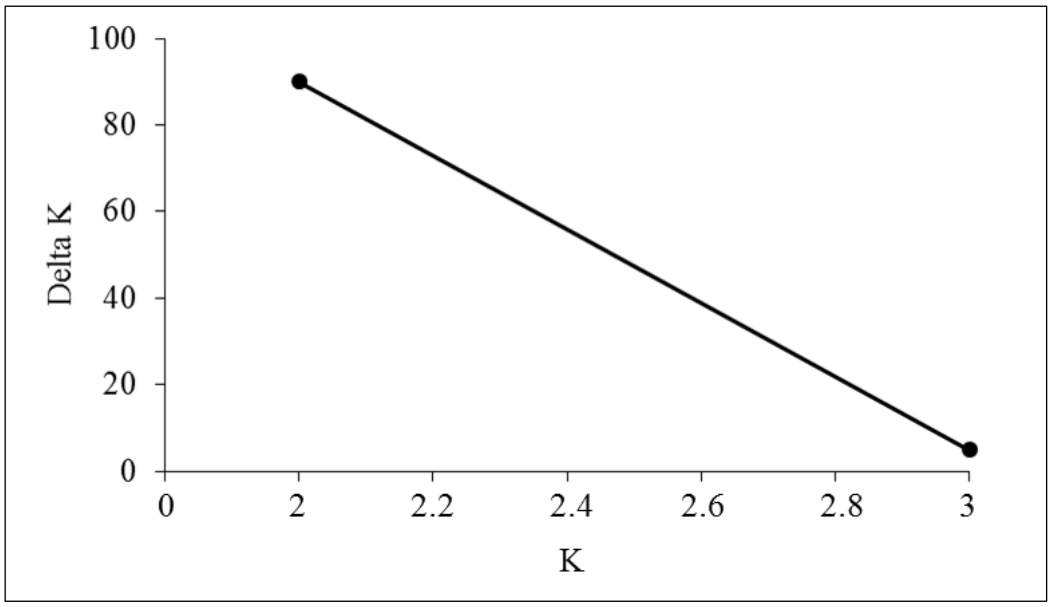


Figure 5.6 Delta K values obtained from STRUCTURE HARVESTER (Earl and vonHoldt, 2012) indicating the greatest likelihood of number of clusters present in the Catchment sample group is two.

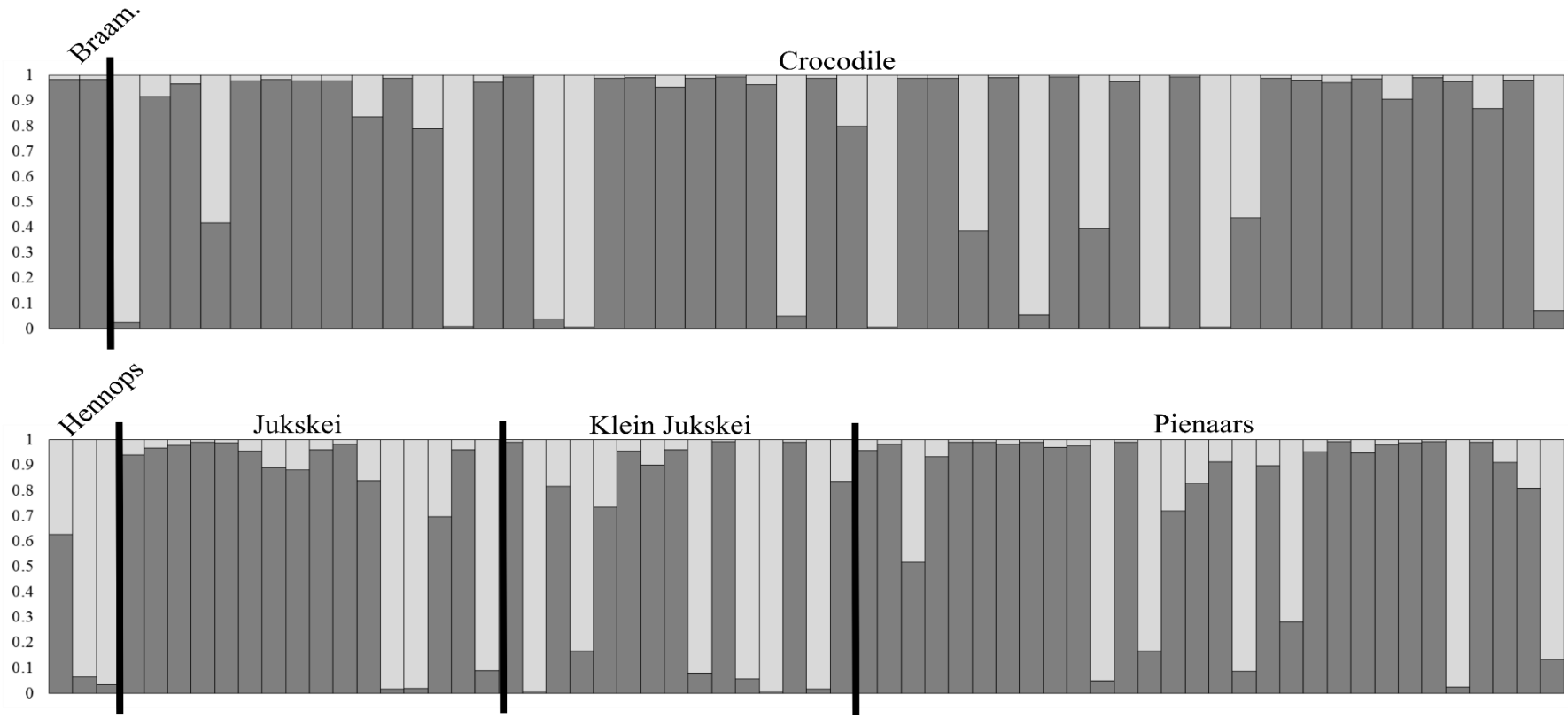


Figure 5.7 Bayesian assignment probabilities for Catchment A samples at K = 2. Each vertical bar represents an individual, which is divided into K colours representing estimated membership fractions in K clusters. The black vertical lines separate individuals into the nine rivers along which samples were found. No definite structure appears to occur within the overall provincial population.

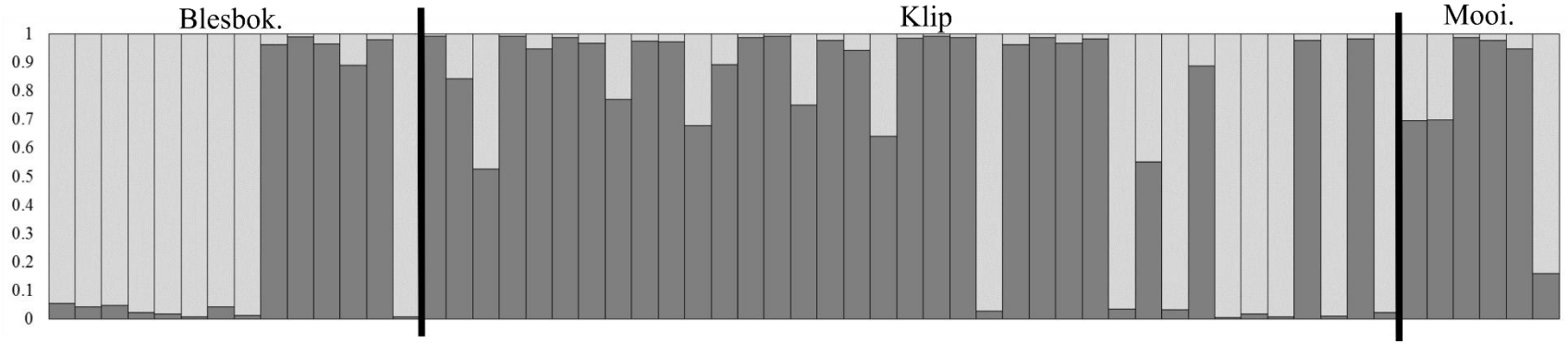


Figure 5.8 Bayesian assignment probabilities for Catchment C at K = 2. Each vertical bar represents an individual, which is divided into K colours representing estimated membership fractions in K clusters. The black vertical lines separate individuals into the nine rivers along which samples were found. No definite structure appears to occur within the overall provincial population.

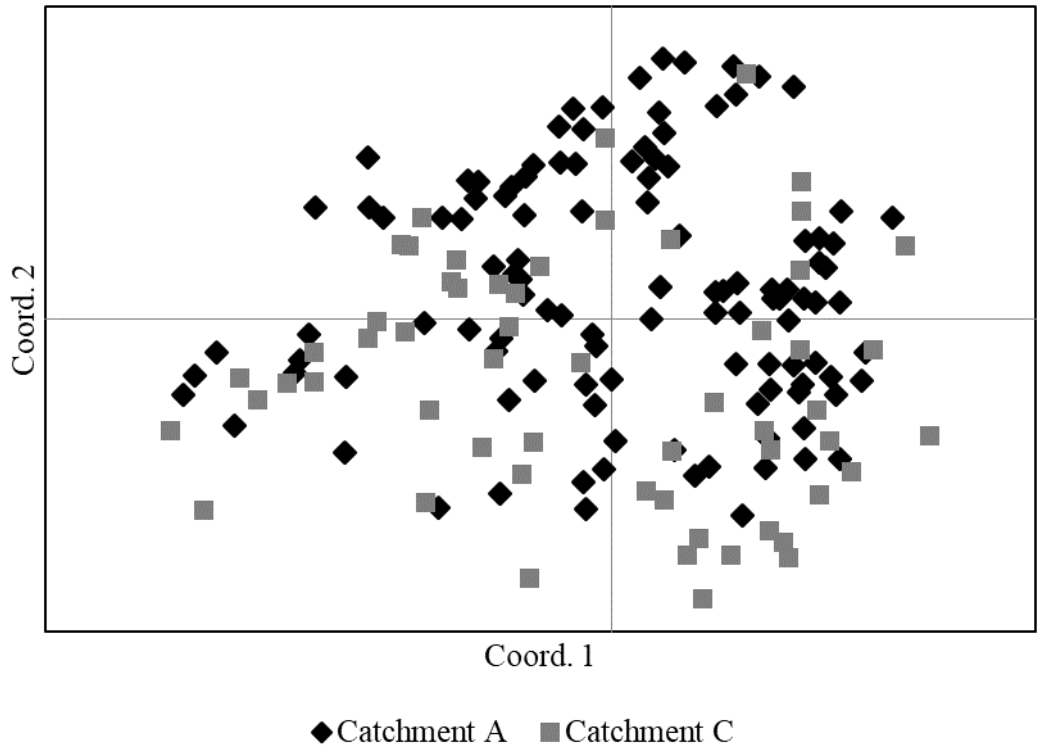


Figure 5.9 Principal Coordinates Analysis (PCoA) for the two water catchment areas assessed in Gauteng, South Africa.

5.4 Discussion

A relatively high level of genetic diversity is considered fundamental for species survival (Allendorf and Luikart, 2007). To achieve this diversity, high levels of gene flow within a population is required. This can be difficult in cases where the landscape presents barriers such as cities, mountain ranges, valleys and large rivers (Serrouya *et al*., 2012). Thus, human-mediated activities may have had an effect on patterns of genetic structure and diversity in *A. capensis* samples from two catchments in the Gauteng Province (South Africa). The genetic diversity across all the sampled river populations was low for observed heterozygosity (H_o = 0.309), with a high expected heterozygosity (H_e = 0.889). Historical data for the otter populations in Gauteng is however not available for direct comparison to assess whether genetic diversity has increased or decreased. Low genetic diversity introduces several negative effects for a population such as inbreeding, susceptibility to diseases, and reduced genetic fitness. All these factors combined

can eventually lead to population decline. Previous studies have shown an increase in diversity after species were reintroduced into the areas, or due to repatriation or ingress of species from adjacent areas, following initial declines (Williams and Scribner, 2010; Pickles *et al*., 2012; Zigouris *et al*., 2012). Thus, the observed low genetic diversity in the Gauteng *A. capensis* population may be due to a history of extirpation and recolonisation, as has been identified in other mustelid species (Larson *et al*., 2012; Hapeman *et al*., 2011). This is the most logical answer as there is no evidence in the literature indicating reintroduction of *A. capensis* to the area. It has been reported that genetic diversity decreases along a path of range expansion (Excoffier and Ray, 2008; Latch *et al*., 2009). A similar pattern has been observed in the Minnesota river otter population from Central North America where a decrease in heterozygosity was observed from the core population (Brandt *et al*., 2014).

The assessment of the population genetic structure of the otters occurring in Gauteng revealed no sub-structuring between the two populations/groups sampled within the two catchments as supported by a non-significant genetic differentiation (F_{ST} = 0.01). These results provide evidence of high levels of gene flow between groups sampled in Gauteng which is further supported by the low relatedness coefficient value (0.048). Although STRUCTURE identified three genetic clusters for the populations defined by river analysis and two genetic clusters for the populations defined by catchment analysis, this does not seem to be the case when considering the genetic distance between individuals within the two catchments (Figure 5.8). Cluster analysis programs such as STRUCTURE tend to introduce uncertainty to results obtained in situations where the study groups present low levels of divergence (Manel, Gaggiotti and Waples, 2005). The minimum number of genetic clusters that can be assigned by STRUCTURE is two, resulting in one homogenous population being labelled as K = 2, or two different groups. Lack of sub-structuring between the populations/groups was supported by the PCoA. Although F_{ST} values were moderate for the populations defined by river analysis (F_{ST} = 0.13), this value may be overestimated due to the presence of null alleles.

The presence of null alleles may have influenced the overall outcome of the study and could be attributed to the use of non-species specific primers and possible degraded DNA from faecal samples. Null alleles refer to alleles at any given locus that constantly fail to amplify and as such cannot be detected by PCR or subsequent analysis. They usually occur due to mutations in the flanking regions where the primers anneal for amplification, resulting in poor or no amplification at the affected locus (Dakin and Avise, 2004). The presence of null alleles does not necessarily impact the outcomes of population genetic analyses: their presence tends not to have significant consequences in analyses that use average probabilities (as opposed to individual parentage analyses), but they may cause overestimation of F_{ST} and genetic distances, as well as underestimation of observed heterozygosity, and slightly lower the power of assignment tests (such as STRUCTURE) (Dakin and Avise, 2004; Chapuis and Estoup, 2007; Carlsson, 2008).

The overall low genetic diversity of the Gauteng otter population is possibly linked to the rapid expansion of urbanised areas outward into previously undisturbed environment at an exponential rate due to a human population increase. The rapid expansion would have affected the established riparian habitats scattered throughout the province, driving species outward to less disturbed habitats, or possibly resulting in the extirpation of more sensitive species (McKinney, 2006). The emigration of species from the area would result in more resources becoming available for opportunistic species able to adapt to the novel urban environment, which could lead to conflict over resources with native species remaining in delineated (Matthysen, 2005; Bonier *et al*., 2007; Lowry *et al*., 2013).

Another explanation for the low genetic diversity could be related to the home range of otters, which can be extensive, ranging from 4.9 - 54.1 km (Somers & Nel, 2004), and may be even larger. Coyotes in developed areas have been found to possess home ranges double that of individuals in less developed areas as well as having dens in less developed forested areas (Gese *et al*., 2012), and it is possible that otters in urban areas may also be increasing the size of their home range to improve chances of finding food and mates. *Aonyx capensis* present in

Gauteng may have core ranges (areas with increased frequency of activity, usually where refugia are located) outside of the province from which the animals venture into Gauteng to forage. This is a practice seen in urban mammals that can navigate and utilise matrix habitats like those seen in urban areas (discussed in Baker and Harris, 2007). The lack of holts (otter refuges) observed during surveys is possible evidence of this being the situation with *A. capensis* in Gauteng. A larger breeding population may occur further north along the Crocodile River, which may have undergone range expansion into the Hennops River and subsequent tributaries with headwaters occurring in the city of Johannesburg (Brandt *et al*., 2014). This range expansion could explain the lower levels of genetic diversity in the tributary rivers (Jukskei River and K. Jukskei River). The low genetic differentiation between samples from Pienaars River and Crocodile River is interesting as the rivers are a considerable distance apart in Gauteng, but they share a confluence to the north of Gauteng. This may be considered further evidence for a larger breeding population further north along the Crocodile River which has divided and moved into Gauteng. Otter movement does not seem to be hindered by physical barriers as there was no evidence of sub-structuring occurring, relatedness was low, and there is evidence of high gene flow. These results suggest that urbanisation has not led to fragmentation of the population due to disruption of gene flow, which may indicate the otter population is successful (surviving and being able to reproduce viable offspring) in Gauteng. However, further sampling must be conducted to confirm that the genetic health is improving.

This analysis represents the first genetic analysis of a South African otter species to date and additional studies in the future would be required to assess changes in genetic diversity and differentiation. In addition, future studies should be conducted throughout their distribution range. This is imperative to assist in the assessment of the otter population and the effect urbanisation has on the ecology of the otters. Future studies could provide evidence of a recovering population with good genetic health, which could support the hypothesis that otters can adapt to urbanisation and associated human activity.

Appendix

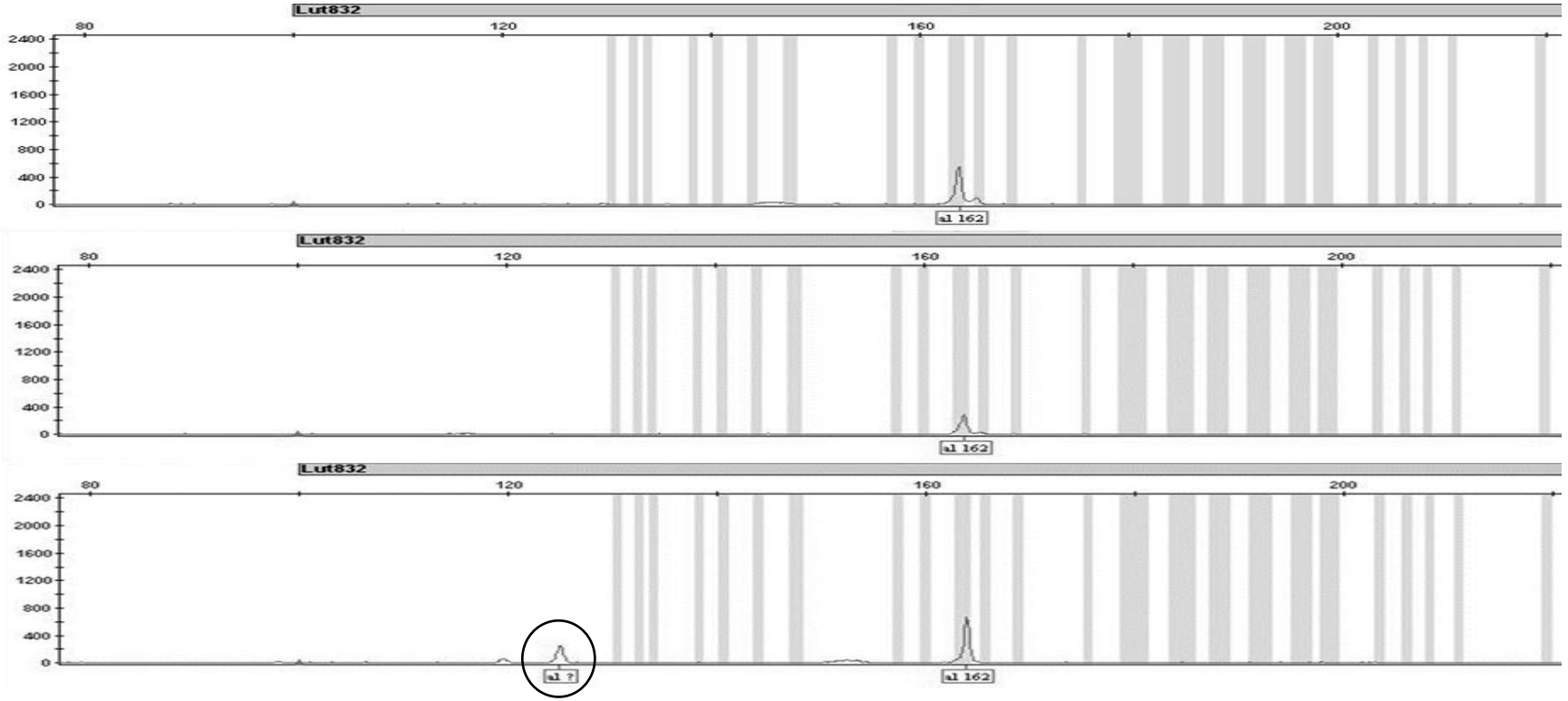


Figure 1 Multiple repeats of the same sample indicating the presence of a homozygous allele. Allele was scored as 162 based on the repeated detection of the allele, the occurrence of the allele in a prescribed bin, and the height of the allele indicating successful amplification. The circled allele in the bottom chromatograph was rejected as it only appeared once out of five repeats of the sample, and the allelic peak fell outside of the prescribed range of marker Lut832.

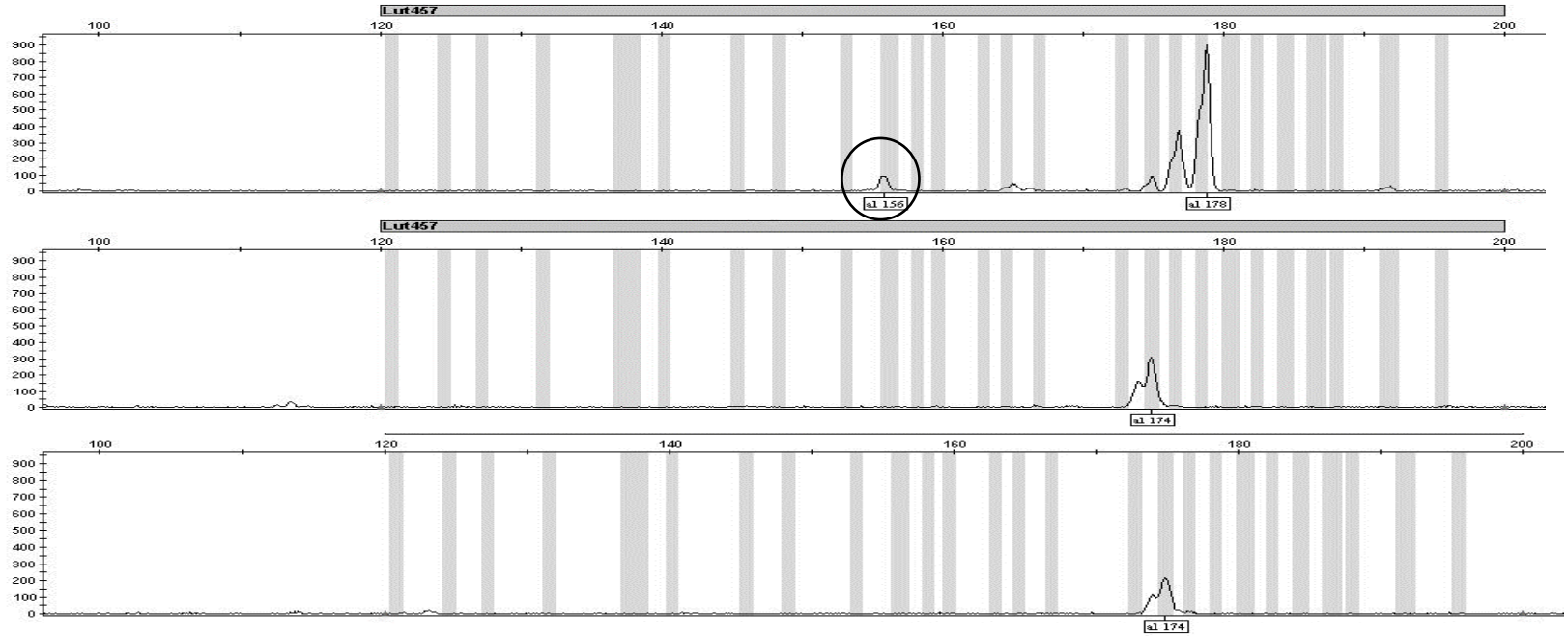


Figure 2 This sample was considered to be heterozygous with alleles 174 and 176 being scored as the correct genotypes. In the top image allele 156 (circled) was rejected as it occurred before the highest allelic peak (176) and did not appear in subsequent analyses. Allele 174 was selected as it fell in a prescribed bin in the accepted range of marker Lut457 and occurred more than once. Allele 174 and 176 are also an acceptable distance of base pairs apart.

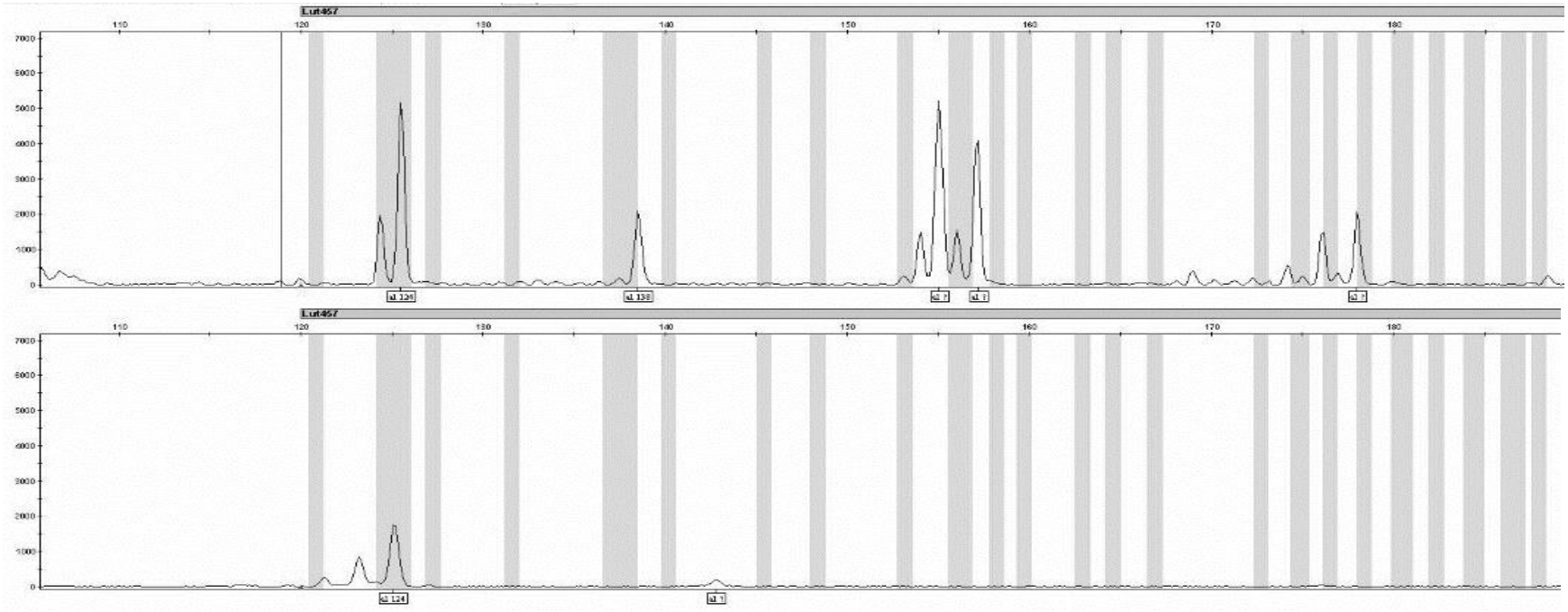


Figure 3 Initial amplification resulted in several allelic peaks with only one allele (124) falling into a prescribed bin. Repeat amplification yielded a more conclusive result with allele 124 present in a prescribed bin and being of an acceptable height. This sample was considered to be homozygous.

CHAPTER SIX

GENERAL DISCUSSION

6.1 The influence of environmental factors on evolution

In order to fully appreciate the manner in which a species is able to survive in an environment, many aspects need to be considered. Numerous environmental variables need to be evaluated, and the way these variables interact needs to be understood to explain survivability of a species. Eco-evolutionary dynamics involve the relationship between ecological factors affecting a species, and the evolutionary changes that result from these factors (Ezard *et al*., 2009; Pelletier *et al*., 2009). However, to fully understand this feedback relation one needs an extensive amount of data relating to the various aspects influencing a species to successfully determine how behavioural and physiological traits are evolving to adjust to ecological stressors (Ezard *et al*., 2009; Post and Palkovacs, 2009). Once a firm understanding of the feedback system is acquired, it is possible to make predictions relating to the survivability of a species going forward and how the species might be able to adapt to possible changes in the environment, which may result from impacts such as climate change or increased human disturbance in an area (Johnson *et al*., 2009). Due to the inherent ability to survive in a specific area, exposure to drastically different environmental conditions from their original habitat would most likely result in local extinction of the species (Hofmann and Todgham, 2010). It is very unlikely that the habitat of a species will dramatically change instantaneously, but populations do face a somewhat rapid environmental change through human activity (Sih, 2013). Habitat degradation and disturbance are on the increase with the exponential growth of the human population requiring more space, resulting in an expansion of cities into pristine natural environments to acquire space and resources for human development (Sutherland, 1998; Grimm *et al*., 2000).

This rapid change in environmental conditions of areas impacted by anthropogenic development drives the need for rapid changes in behaviour for animals to survive, or the use of pre-existing behavioural traits to cope with environmental change. Many carnivores have become successful exploiters of urban areas, consuming novel food sources such as refuse which is readily available in cities, to denning in people's homes to stay protected during colder months (Herr *et al*., 2010; reviewed in Bateman and Fleming, 2012). Animals are more likely to make use of, and be successful in anthropogenic (novel) environments if they express bold behaviour patterns (Evans *et al*., 2010; Atwell *et al*., 2012; Sinn *et al*., 2014). Bold individuals tend to be exploratory and take risks, usually in novel situations (Wilson *et al*., 1994). The bold or reserved nature of an animal plays heavily on the utilisation of novel resources, and resource use in general, with respect to resources selected for shelter or food. Bolder individuals may select habitats with greater food availability over shelter from human presence/predators, while less bold individuals will favour habitats presenting greater cover from human presence/predators (Pinter-Wollman, 2009; Evans *et al*., 2010; Pruitt *et al*., 2011). Avoidance behaviour has been documented in animals exposed to high levels of human activity, involving changing times of activity to increasing home ranges and movement patterns to avoid conflict with humans (Gese *et al*., 2012). Habituation to the presence of humans can be advantageous in certain instances such as increased duration of feeding/foraging time before fleeing approaching humans (McLeod *et al*., 2013; Bateman and Fleming, 2014). Habituation toward human activity can also have negative effects, by increasing the chance of human-animal conflict and possible mortality through road accidents (Rauer *et al*., 2003; Coffin, 2007). Successful adaptation to novel environmental conditions will ultimately lead to future generations gaining adaptive traits such as crypsis or boldness through inheritance of traits or learning from parents, enabling the new generations to adapt to further changes posed by urban areas (Sih, 2013).

6.2 Wildlife and the impact of urban areas

Expansive anthropogenic development of urbanised areas has become a necessity to accommodate the influx of people moving to cities, with a predicted 66% of the world's population residing in urban areas by 2050 (United Nations, 2015). The increasing size of urban landscapes has resulted in the modification or replacement of natural habitats for environments suited to human occupancy. These urbanised landscapes present animals with novel conditions which can be advantageous for certain species, but pose a severe threat to other species. For an animal to survive in its environment, it possesses phenotypic traits that help the animal withstand environmental pressures (e.g. extreme temperatures, reduced rainfall). Advantageous characteristics increase the likelihood of survival, thus improving an animal's fitness, as it will be able to reproduce, passing on the advantageous trait. Conspecifics that do not express useful traits are less likely to survive increased environmental stressors, reducing their chance of reproducing, and the less beneficial trait will be removed from the population (Conner and Hartl, 2004). This process of natural selection may result in future generations coping with environmental change, if genes are inherited that enable individuals to tolerate multiple different environments. This possibly improves the chances of survival in habitats affected by urban development and associated habitat degradation.

An animal with a broader trophic niche is more likely to survive habitat degradation, and loss of resources, than one with a much narrower diet (McKinney, 2002). Studies have found certain mammals with broad trophic and habitat niches are able to utilise novel resources (e.g. refuse and human domiciles) in urban areas (Bateman and Fleming, 2012). Examples include, but are not limited to, badgers, wolves, coyotes, red foxes, and even brown bears (Rauer *et al*., 2003; Bateman and Fleming, 2012). These species are considered to be urban 'adapters' or 'exploiters', and have been able to benefit from the establishment of urban environments (McKinney, 2006). The ability of urban wildlife to adjust to the novel challenges of urban environments (i.e. disturbance through human activities and unique resources) ultimately improves their chance of survival

(Lowry *et al*., 2012). Due to the high level of surfaces such as concrete and roads, which are able to absorb heat from the sun, and the presence of buildings acting as wind barriers, the average ambient temperature of urban areas is higher than surrounding natural areas (Luniak, 2004). Buildings also create new habitats for organisms to occupy providing them with shelter and protection from predators. Suburban homes sometimes have gardens with fruiting trees, plants, and vegetables that receive water in periods that are generally dry, allowing animals access to water, and potentially food, all year round. People also discarded refuse, and put out food to attract animals to their gardens, both events increase the likelihood of finding resources and reduces energy exerted searching for food (Davison *et al*., 2009; Bateman and Fleming, 2012; Sol *et al*., 2013). However, there is a downside to living in urban areas, and these can be detrimental to animals. Various forms of pollution are present (light, noise, chemical, and physical), which can have adverse effects on physiological and behavioural processes in animals. Roads introduce the dangers of collision with vehicles. Wildlife can be considered nuisance to humans, when homes are denning sites, pets are considered prey, and disease spread.

The effects of urban areas and human activities on numerous otter species have been studied. One of the greatest problems facing otters is the presence of environmental contaminates introduced to freshwater sources. High concentrations of man-made chemicals and heavy metals have been found in prey species consumed by otters as well as accumulating in otters, which can have detrimental effects on the animals and offspring (Mason and Macdonald, 1994; Grove and Henny, 2008; Boscher *et al*., 2010; Roos, 2013; Nelson *et al*., 2015). Habitat degradation (agriculture, forestry, infrastructure development such as dams) also impacts on the riverbanks used by otters, and associated prey, for denning and movement, resulting in a decrease in suitable habitat and food for these animals (Ruiz-Olmo, López-Martín and Palazón, 2001; Medina-Vogel *et al*., 2003; Strayer *et al*., 2003; Prakash *et al*., 2012). Direct conflict with fishermen and entanglement in fish nets (Rosas-Ribeiro *et al*., 2012; Roos, 2013; Akpona *et al*., 2015. All of these factors affecting otters have resulted in all species being placed on the IUCN Red List (IUCN, 2017).

Two such otter species, *A. capensis* and *H. maculicollis* are endemic to the African continent, and both species are listed as 'Near Threatened' on the IUCN Red List (Jacques *et al*., 2015; Reed-Smith *et al*., 2015). Their heavy reliance on freshwater raises concerns over the wellbeing of both otter species, as many rivers and other water bodies are impacted by urban development and associated activities. Increasing levels of pollution entering rivers, destruction of riverbank vegetation and modification of river structure through the introduction of dams and man-made channels are all factors threatening the survival of *A. capensis* and *H. maculicollis* (Jacques *et al*., 2015; Reed-Smith *et al*., 2015). The overarching aim of this study was to establish whether *A. capensis* and *H. maculicollis*, showed similar ecological patterns to conspecific individuals in areas with moderate to low levels of human disturbance, by concentrating on distribution (spatial arrangement), diet, and habitat use in an urban environment. This study was focused on better understand the ecology of otters in areas of high human presence to improve the current knowledge relating to animals affected by urbanisation.

6.3 Ecology of *A. capensis* and *H. maculicollis*

Aonyx capensis is reported to have a far greater distribution range than *H. maculicollis* (Jacques *et al*., 2015; Reed-Smith *et al*., 2015), and is the most likely reason for a higher occurrence of *A. capensis* signs than *H. maculicollis* in both the urban and peri-urban areas of central Gauteng. Similar to previous studies which showed otters tend to avoid human activity (Perrin and Carugati, 2000b; Madsen and Prang, 2001), a higher number of otter signs were found in peri-urban areas, as was predicted. The discrepancy between the number of otter signs found in peri-urban and urban areas may be related to available habitat and food resources. Past studies have related intensity of otter sprainting and time spent in areas to the presence of certain resources, as well as territory boundaries. *Lutra lutra* has been shown to defecate at conspicuous sites, and sites of freshwater and dens, highlighting the use of spraints as indicators of resources (Kruuk, 1992).

Higher intensity of sprainting activity by *L. lutra* has also be observed at points were male territories meet, in which case spraint acts as a deterrent, reducing conflict over resources and mates (Erlinge, 1968). Two differing forms of sprainting was recorded for *L. canadensis* relating to social group size. It was found that social groups (adults and young) concentrated sprainting activity close to food sources, while solitary otters mark at higher frequency to indicate territory boundaries (Ben-David *et al*., 2005). Several instances of *A. capensis* spraint sites occurring near resting sites (couches) have been observed (Verwoerd, 1987; Arden-Clarke, 1983; van Niekerk *et al*., 1998), and resting and denning sites are usually associated with specific habitat cover, namely dense vegetation (Perrin and Carugati, 2000). Regular use of specific sites for rolling (in order to dry off after swimming) and consuming large prey have been noted in *A. capensis* and *H. maculicollis* (Rowe-Rowe, 1977b). From these examples, both otter species have habitat requirements necessary for obtaining and consuming prey, establishing protected dens and resting sites, and marking territorial boundaries.

Past studies have shown a disproportionate occurrence of otters in habitats consisting of specific variables, most notably dense vegetation cover, reed beds and rocky substrate (Carugati *et al*., 1995; Perrin and Carugati, 2000b; Nel and Somers, 2007). These variables align with specific requirements of *A. capensis* with respect to concealment of holts and spraint sites by dense vegetation (Perrin and Carugati, 2000b; Nel and Somers, 2007), and higher occurrence of crab in reed beds and rocky substrate (Somers and Nel, 2004a). Results from this study showed a difference in habitat variables between urban and peri-urban areas, but signs of otter were still present in peri-urban and urban areas. This indicates otters are able to exploit urban areas, which appear to be providing an adequate matrix of habitats suited for otter presence. As this study focused on presence of sign, and did not measure intensity of habitat use by otters, it is not possible to conclude the extent at which otters are utilising urban areas. The lack of holts near sprainting sites observed during the study also highlight various questions regarding the use of habitat by otters in Gauteng, and suggests sprainting used to indicate available food resources, and demarcate territorial boundaries. This leads to the hypothesis that otters are marking locations of higher prey availability as a

means of signalling good foraging sites to members of family groups, and to prevent competition with non-related conspecifics entering another otter's territory.

The diet of *A. capensis* in Gauteng seems to be conservative, and is incorporating prey not usually preferred in other parts of their range, but nevertheless do form part of the reported diet of conspecifics in other parts of South Africa. Within Gauteng, the only significant difference in prey consumed between urban and peri-urban areas was that of fish, with more fish remains in spraints from urban areas. Crab was the most abundant prey detected in spraints found in peri-urban and urban areas, which corresponds with *A. capensis* diet studied elsewhere in South Africa. This is the likely explanation of spraints found near reed beds which provides a sheltered habitat for crabs (Somers and Nel, 2004). From the results of the study it appears *A. capensis* is able to move through Gauteng without hindrance from human disturbance to the environment, which is further justified by the panmictic population genetic structure of *A. capensis*. These results are good indicators that *A. capensis* is able to exploit areas affected by high levels of human disturbance, despite perceived negative impacts (pollution, habitat degradation, human activity) associated with rivers in Gauteng. Results from experiments conducted during this study do not provide evidence of individuals adapting to human disturbance, but show otters are behaving similarly to conspecifics (exposed to less habitat disturbance) in other regions of South Africa. This evidence of otters coping with high levels of human disturbance may be a positive sign for future conservation of otters, but further studies are required to determine the actual health and longevity of otters in urban areas. Bioaccumulation of heavy metals and organochlorides resulting from polluted river water is of major concern, as this was not examined and could not be deducted from the current experiments. The lack of holts observed during the study raises concern surrounding reproduction of both species, and may indicate otters are not breeding in urban areas, but in less disturbed areas much further away and are only foraging in urban areas.

6.4 Final assessment and future research

The current study provides baseline data relating to the ecology of *A. capensis*, and to a lesser degree *H. maculicollis*, in an area previously unstudied. It did however raise more questions relating to otter ecology, and the animal's associated environment, particularly from an urban ecological standpoint. Presence of otter signs can be informative to some extent, but information obtained can be very limited, requiring detailed studies into otter movement and home range sizes in urban areas, which would provide further insight into the ability of otters to navigate their environment. Quality home range and core range data are needed to assess population viability in urban areas, as it is unclear where otters are reproducing and raising young in Gauteng, and whether habitat degradation is affecting this period of the otter life cycle. From personal observations of a majority of the rivers studied, pollution is a serious problem and detailed water chemistry studies should be conducted to identify all possible contaminates, which could help identify parties responsible for introducing pollutants into the water. Relating to water health, studies into physiology of species reliant on these rivers would also be beneficial for trophic studies. Biodiversity studies should be conducted along all rivers in Gauteng as there appears to be a gap in current assemblages along the rivers, necessary for responsible and sustainable future urban development and wildlife conservation. As mentioned in Chapter 5, species specific genetic primers are being developed which will provide more accurate assessments of genetic health of both otter species. A national assessment of otter genetics should be conducted to better understand the current health situation of the populations, allowing for more conclusive conservation requirements being identified for *A. capensis* and *H. maculicollis*. Improved genetic techniques may provide accurate identification of individuals, creating an alternative method of detecting home range and habitat use of individuals.

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APPENDIX A

Diet of the spotted-necked otter, *Hydrictis maculicollis*, in urban Gauteng, South Africa[3]

Introduction

The vast majority of the human population is moving into metropolitan areas, resulting in the need to expand cities to accommodate the influx of citizens, meaning more natural surrounding environments are converted to urban spaces (Grimm *et al*., 2000). The development of natural land for human habitation destabilises habitats occupied by native fauna and flora species, often driving these species out of the area (McKinney, 2008), decreasing the biodiversity of these urban areas (Willis, Winemiller and Lopez-Fernandez, 2005). This decline in biodiversity is not only limited to terrestrial habitats as rivers in these impacted areas are more exposed to pollutants and channel modification, greatly reducing the number of species present in rivers (reviewed in Paul and Meyer, 2001). A decrease in biodiversity has a cascading effect on species able to tolerate habitat disturbance, as this brings about a reduction in available prey species for consumption (McKinney, 2006). Predators are forced to adapt to a decline in prey by relocating to more stable areas able to sustain more prey, or by capturing new prey species (Morey, Gese and Gehrt, 2007). This may not pose a problem, but the severity of the situation increases if several predatory species are reliant on the same reduced group of prey items (Caro and Stoner, 2003).

[3] This chapter has been submitted to African Journal of Ecology as: Ponsonby, D.W. and Schwaibold, U. Diet of the spotted-necked otter, *Hydrictis maculicollis*, in urban Gauteng, South Africa.

Two African otter species, the spotted-necked otter (*Hydrictis maculicollis*) and African clawless otter (*Aonyx capensis*), coexist along the Bushmans River (Somers and Purves, 1996), and the Mooi River, with *H. maculicollis* demonstrating a wider dietary breadth than *A. capensis* (Perrin and Carugati, 2000). The ability to successfully share the same foraging areas is most likely due to *A. capensis* consuming a far greater number of crabs than other prey types, while *H. maculicollis* tends to consume more fish (Somers and Purves, 1996; Perrin and Carugati, 2000). However, coexistence is generally more likely to occur in heterogeneous environments presenting spatial variance for the species present (Hanski, 2008). Could this functioning dynamic between the two otter species be jeopardised in an instance in which the availability of a primary prey type, such as fish, is greatly reduced due to human disturbance? This is a question that arose during a recent study of otter ecology in Gauteng Province, South Africa (Chapter 2) when a small number of *H. maculicollis* spraint was found in the same area as *A. capensis* spraint. Diet analysis concluded that crab was the most consumed prey type for both otter species, with fish consumed the least. This raises concerns for the otters in Gauteng as the province is heavily developed with constant urban expansion posing a threat to surrounding natural environment and rivers flowing through urbanised areas.

Biodiversity tends to decrease as the level of urbanisation increases (McKinney, 2006), which may impact prey available to the two otters. The introduction of industrial, agricultural and domestic waste into rivers can greatly impact the assemblage of species present in rivers (Bernhardt *et al*., 2008). Fish and crab comprise the majority of both otter species' diets, which could be greatly affected by a decline in preferred prey species due to deteriorating water quality (see Mangadze, Bere and Mwedzi, 2016). This decline in preferred prey species in urban rivers could lead to increased competition between *H. maculicollis* and *A. capensis* which in turn could greatly affect their population numbers. In order to better understand the diet of *H. maculicollis* in an urban environment, I set out to determine their dietary composition in Gauteng Province, South Africa and compare our findings to studies involving the same species in relatively open and undisturbed environments, as well as to the diet of *A. capensis*. Previous dietary

studies have found that both species are reliant on crab and fish, but also display a certain amount of flexibility in prey choice (Somers and Purves, 1996; Perrin and Carugati, 2000).

Methodology

Eleven rivers in the north-eastern part of South Africa were chosen for this study. Two major cities are situated in Gauteng, Tshwane to the north and Johannesburg further south, with high levels of urbanisation evident throughout the province (GCRO, 2013). The chosen rivers flowed through both urban and peri-urban areas of Gauteng. Sampling sites were identified at 5 km intervals along the selected rivers (Figure 1). The study was conducted during the dry season (autumn and winter) between June 2012 and October 2014. A distance of 200 m along each riverbank was surveyed up and downstream from each 5-km site for signs and all spraints found were collected using re-sealable plastic bags, which were sealed and labelled with the GPS coordinates (using a hand-held Garmin eTrex Vista Cx GPS device) and the date. Samples were stored at -10°C in a refrigerated unit at the University of the Witwatersrand, Johannesburg until further analysis of the spraint was conducted. Prior to diet analysis, the spraint was swabbed to collect DNA for species identification (see Appendix B for detailed methods).

Following modified methods used by Perrin and Carugati (2000), each spraint sample was individually washed with water over a 1mm sieve to collect prey remains, which were placed on newspaper and air dried for 24 hours. The percentage of each prey type in each spraint was measured by placing a piece of graph paper underneath a petri dish containing the spraint. The total number of graph paper squares covered by specific prey remains was counted and the percentage of the prey contained in the spraint was calculated. Prey was identified visually to class level and where possible to order level using photographs of remains and museum specimens of animals and skeletons. The percentage of each prey type occurring in a single spraint was determined, followed by the calculation of percentage occurrence (PO) and relative occurrence (RO) for each prey type. Prey availability was based on data from the reference frequency of

occurrence of fish species in South Africa (FROC) (Kleynhans, Louw and Moolman, 2007), personal observation of crab holes in riverbanks and actual sightings of prey. Data were arcsine transformed prior to running statistical tests as the data did not follow a normal distribution. A general linear model (GLM) with a Fisher LSD post hoc test was used to determine any significant differences between prey items present in the samples. Comparisons between results from this study and previous studies were conducted using a two-tailed z-test. All statistical analyses were conducted using STATISTICA 10© (StatSoft, Inc. 2011), and the significance level was set at $p < 0.05$.

Results

A fairly small number (eight) of *H. maculicollis* samples was found during the study. Crab comprised the majority of the diet as opposed to fish which was expected due to the piscivorous nature of *H. maculicollis* (Table 1). Insect, bird and mammal remains were also present in the spraint samples. Unlike in previous studies, no amphibian remains were found and vegetation was found in one of the peri-urban samples. There was no difference between the amount of crab taken in samples from this study compared to other studies, but a significant difference was seen when comparing the amount of fish taken, with far less fish remains detected in the Gauteng samples (Table 2). A comparison of prey remains comprising the diets of *H. maculicollis* and *A. capensis* indicated that there was no significant difference in the percentage of any of the prey items (Table 3).

Table 1 Diet comparison of the spotted-necked otter (*Hydrictis maculicollis*) across two areas of differing levels of urbanisation in Gauteng, South Africa. (X = number of samples with prey type present; PO = percentage of occurrence of prey type; RO = relative occurrence of prey type).

	Peri-urban area (n = 6)			Urban area (n = 2)		
Prey	X	PO (%)	RO (%)	X	PO (%)	RO (%)
Crab	4	66.67	36.36	2	100	40
Insect	2	33.33	18.18	2	100	40
Bird	2	33.33	18.18	0	0	0
Mammal	1	16.67	9.09	1	50	20
Fish	1	16.67	9.09	0	0	0
Vegetation	1	16.67	9.09	0	0	0

Table 2 Comparing the current study percentage occurrence (PO) and relative occurrence (RO) of prey types to PO and RO measured in previous studies of *H. maculicollis* diet in areas with lower human impact. A = current study; B = Perrin and Carugati (2000); C = Somers and Purves (1996); α = 0.05. Significance difference of $p < 0.05$ (*), and significance difference of $p < 0.001$ (**).

Prey type (%)	A (n = 8)	B (n = 516)	C (n = 79)
Amphibian	0/0	43*/25*	13.9/8
Bird	25/12.5	2**/1**	2.5**/1.5*
Crab	75/37.5	43/25	65.8/38
Fish	12.5/6.3	64*/37*	81**/46.7*
Insect	50/25	18*/11	3.8**/2.2**
Mammal	25/12.5	1**/0.4**	1.3**/0.7**

Table 3 Percentage of occurrence (PO) and relative occurrence (RO) of prey types from *H. maculicollis* and *A. capensis* spraint samples found along the banks of sampled rivers.

Prey type (%)	*H. maculicollis* (n = 8)	*A. capensis* (n = 171)
Amphibian	0/0	0/0
Bird	25/12.5	27.49/13.95
Crab	75/37.5	88.3/44.81
Fish	12.5/6.3	20.47/10.39
Insect	50/25	26.32/13.35
Mammal	25/12.5	22.22/11.28

Discussion

Anthropogenic disturbances to natural environments have led to the decrease in native species population sizes, which has a cascade effect on associated species in the food web (Madsen and Prang, 2001). Variation in prey availability, due to seasonal or habitat change, requires adaptation in the trophic niche of predators to accommodate for the lower presence of certain prey. Results from this study indicate a broader trophic niche being utilised by *Hydrictis maculicollis* along rivers impacted by human development in Gauteng Province, South Africa. The otters are consuming a higher number of what has previously been considered supplementary prey types (e.g. birds, mammals, insects), over fish which is considered its preferred food source (Somers and Purves, 1996; Perrin and Carugati, 2000). The high presence of uncharacteristic prey making up the diet of *H. maculicollis* raises some concerns regarding prey species population numbers, as a reduction in fish numbers could be causing *H. maculicollis* to rely more on other prey types, possibly leading to inter- and intra-specific competition over resources (Hanski, 2008). Data from the River Ecostatus Monitoring Programme – REMP) indicate that fish frequencies are decreasing and lower than expected in rivers surveyed for this study, most likely affected by the levels of river modification, water pollution and siltation present in the rivers along which *H.*

maculicollis spraint was found (Singh and Todd, 2015). Comparing the diets of *H. maculicollis* and *A. maculicollis* yielded no differences between the percentage of each prey type taken. Previous studies (Somers and Purves, 1996; Perrin and Carugati, 2000) focusing on the coexistence of these two otter species have shown *H. maculicollis* eating more fish than *A. capensis*, which could be the factor allowing both species to cohabit the same stretches of a river without conflict over resources. If no difference is being observed between the prey consumed along the rivers in Gauteng, competition may become more prominent. This shift in preference may result in additional interspecific conflicts if both otter species depend on crabs as their main source of food in urban areas (see Caro and Stoner, 2003). Competition between the two species may be further elevated by human disturbance in the area, leading to a decrease in suitable habitat for resources specific to the otters (Hanski, 2008).

The constant threat of increasing degradation of riparian habitat in urban areas could lead to fish stock depletion, impacting on the predators reliant on fish as a food source. Several of the rivers surveyed are exposed to pollutants, waste disposal and mine drainage contamination, due to the close proximity to urban, industrial and mining areas (DWAF, 2003*a*; 2003*b*). These impactors may be affecting the fish populations in surveyed rivers, as well as other sensitive fauna (see Mangadze *et al*., 2016). Avoidance of these unfavourable conditions may be forcing fish to migrate further downstream, possibly resulting in a shift of *H. maculicollis* distribution to more suitable habitat with more abundant prey species, to reduce a possible conflict with *A. capensis* over the limited food supply.

In conclusion, it would appear that there is a possibility of unexpected prey consumption by *Hydrictis maculicollis* in Gauteng which could lead to resource conflict with *Aonyx capensis*. Further research is needed to confirm this as only eight spraint sample were found from *H. maculicollis* during a larger study on otter ecology in Gauteng. Both otter species are demonstrating foraging flexibility which may be enabling the otters to survive in an environment with reduced populations of "conventional" prey shown to be chosen in previous studies.

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APPENDIX B

DIFFERENTIATION OF TWO SOUTH AFRICAN OTTER SPECIES (*AONYX CAPENSIS* AND *LUTRA MACULICOLLIS*) FROM SPRAINT BASED ON PARTIAL *CYTB* PRIMER SETS

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Short communication

Differentiation of two South African otter species (*Aonyx capensis* and *Lutra maculicollis*) from spraint based on partial *CytB* primer sets

M. Thabang Madisha [a,b,*], Damian Ponsonby [c], Ute Schwaibold [c], Antoinette Kotzé [a,d], Raymond Jansen [b], Helene Brettschneider [a], Desiré L. Dalton [a,d]

[a] National Zoological Gardens of South Africa, P.O. Box 754, Pretoria, 0001, South Africa

[b] Department of Environmental, Water and Earth Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa

[c] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa

[d] Genetics Department, University of the Free State, PO Box 339, Bloemfontein 9300, South Africa

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ABSTRACT

Accurate species identification based on visual cues can be challenging due to morphological similarities and the cryptic nature of certain species. Thus a more conclusive method of identification is required, namely DNA barcoding. This is the case regarding two South African otter species, Cape Clawless otter (*Aonyx capensis)* and the spotted necked otter (*Lutra maculicollis*). Due to the cryptic nature of these animals faecal samples, known as spraints, are the easiest way of confirming the presence of the animal in an area. In this study, we compared results obtained for universal and partial *CytB* primer sets on collected spraint and tissue control samples. Universal *CytB* primers revealed a low percentage of amplified otter species from faecal samples (species specific amplification success of 10.9%) whereas, the partial *CytB* primer set resulted in successful amplification of 45 out of 55 (82%) samples. We were thus able to positively differentiate between the two otter species using the partial *CytB* primer set developed in this study. The ability to accurately identify species using partial DNA will be beneficial in understanding numerous aspects of the behaviour and ecological importance of animals in their environment.

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1. Introduction

Accurate species identification based on visual cues can often be difficult due to cryptic morphological features of species. In addition, morphological identification keys often rely on a certain life stage or gender of an animal to be used correctly (Hebert et al., 2003; Bonito, 2009; Chaves et al., 2012). In the case of two cryptic, co-occurring South African otter species, namely the Cape Clawless otter (*Aonyx capensis)* and the spotted necked otter (*Lutra maculicollis*), faeces (spraint) is used as a means of determining presence, space use and diet (Arden-Clarke, 1986; Van Niekerk et al., 1998; Perrin and Carugati, 2000; Angelici et al., 2005). However, due to similar diets the spraint can most often be visually similar, making it difficult to accurately identify the species occurring in a given area. In addition, erosion of spraint through exposure to environmental

* Corresponding author at: National Zoological Gardens of South Africa, P.O. Box 754, Pretoria, 0001, South Africa. Tel.: +27 12 339 2802.
E-mail address: thabang@nzg.ac.za (M.T. Madisha).

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