



Small mammal community dynamics and associated trophic niche widths in a mining- impacted catchment.

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Declaration

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

A handwritten signature in dark ink, consisting of several loops and a long horizontal stroke, positioned above a horizontal line.

(Signature of Candidate)

Signed at Wits University on 14 November, 2013.

Abstract

The aim of this research was to contextualise, in terms of community structure, food web metrics and mercury contamination, small mammal communities living in the areas surrounding tailings storage facilities (TSFs) on a gold-mining property near Carletonville, South Africa. Tailings are liquid slurry containing waste material produced during the processing of ore. Pollution released into the environment by TSFs exposes wildlife to potential health hazards, such as elevated mercury concentrations. Therefore, this study focussed on describing the 1) small mammal population dynamics, 2) isotopic niche widths, and 3) mercury bioaccumulation in small mammal hair, to provide insight into the interactions between mining pollutants (indicated by mercury) and small mammal ecology and food webs.

During the study, six rodent species (order Rodentia, families Muridae and Nesomyidae) and two shrew species (order Soricomorpha, family Soricidae) were captured. Three Muridae (sub-family Murinae) species were: the striped mouse, *Rhabdomys dilectus* (n = 147); the multimammate mouse, *Mastomys sp.* (n = 77); and the rock rat (or veld rat), *Aethomys sp.* (n = 1). Two Muridae (sub-family Otomyinae) species were: the vlei rat, *Otomys auratus* (n = 14); and the Angoni vlei rat, *O. angoniensis* (n = 4). The Nesomyidae (sub-family Dendromurinae) species was Brant's climbing mouse, *Dendromus mesomelas* (n = 4). The two insectivorous shrew (family Soricidae) species captured were: the swamp musk shrew, *Crocidura mariquensis* (n = 46); and the forest shrew, *Myosorex varius* (n = 8).

This study confirmed that small mammal species diversity and abundance are influenced by the amount and quality of vegetation present and the cover it provides in the lower height classes (leaf litter and 0 – 30cm above ground). Disturbances to vegetation, such as through the occurrence of fire at the West Wits Mining Complex (WWMC), influenced small mammals both directly (likely through injury or death) and indirectly by removing resources such as cover and food. Although small mammal hair mercury concentrations did not exceed international regulatory standards, small mammal hair did reflect the general mercury abundance in their habitats.

Hair mercury concentrations also reflected trophic strategy, as insectivores had significantly higher hair mercury concentrations than omnivores and herbivores. Whilst not significant, there was a trend in omnivores toward higher hair mercury concentrations than herbivores as expected. Hence, bioaccumulation of mercury is evident in the terrestrial food web at WWMC, but not to the point where mercury would be considered a health hazard to wildlife or humans by international regulatory organisations as no clear measurable impact by chronic exposure to mercury pollution was found. The structure of the small mammal community at the WWMC, containing primary and secondary consumers, can therefore be used to gain more detailed insight into the interactions of food webs and environmental pollution in the mining environment.

Key words: small mammals, mercury, isotopes, trophic niche, mine pollution, bioaccumulation.

Disclaimer

The chapters contained in this dissertation have been formatted as standalone manuscripts for subsequent submission for publication purposes. Therefore, unavoidable repetition may occur between chapters.

Dedication

To my family

Acknowledgements

I would like to thank AngloGold Ashanti (LTD) for the opportunity to collect field data on their West Wits Mining Complex property; Isabel Weiersbye of the Ecological Engineering and Phytotechnology Programme (EEPP) for providing funding and insight; Julien Lusilao Makiese of the EEPP for the use of his elemental soil and water data, and for conducting the fur sample analysis; and Kwaku Acheampong, Sumeshni Pillay, and MacDonald Wanenge for their extensive help in the field.

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1. General Introduction

1.1 Background

The gold mining industry is one of the oldest and largest anthropogenic contributors to large-scale environmental transformation within South Africa. As the single largest producer of waste products in South Africa (DWAF 2001, van Eeden *et al.* 2009), gold mining has a significant impact on an estimated 6000km² of land on the Witwatersrand Basin alone (Weiersbye and Witkowski 2003). Tailings are liquid slurry containing waste material produced during the processing of ore. The environmental implications of tailings storage facilities (TSFs) include contamination of surface and groundwater by acid mine drainage (AMD), contamination of the water table by surface run-off from the TSFs, air and water pollution from wind erosion of dry tailings, possible collapse of TSFs releasing large amounts of slimes into the surrounding areas, physical and aesthetic modification to the landscape, and unfavourable soil conditions that prevent plant growth to stabilise the TSFs (Weiersbye *et al.* 2006, Oelofse *et al.* 2007).

During 2009, AngloGold Ashanti LTD (hereafter referred to as AGA or ‘the mine’) was extracting between three and ten grams of gold per tonne of ore extracted (AngloGold Financial 2009). To extract gold from ore, both metal- and acid-based compounds are used (Mendez and Maier 2008). Many of the metal-based compounds used contain numerous harmful metals and metalloids, such as mercury (Hg), lead (Pb) and chromium (Cr), at both lethal and sub-lethal concentrations (Boyd 2010). The release of such metals into the environment

poses as a significant health hazard to wild and domestic fauna and flora, including humans (Kumah 2006, Oelofse *et al.* 2007).

Multiple studies over the last few decades have recorded the ability of metallic contaminants to accumulate in mammalian tissues, such as liver, kidney and hair (Johnson *et al.* 1978, Roberts and Johnson 1978, Hunter *et al.* 1989, Beernaert *et al.* 2007, McLean *et al.* 2009). The resulting concentrations of some heavy metals (e.g. Hg, Pb and cadmium, Cd) within mammalian tissues have shown significant correlations with environmental (e.g. soil) metal concentrations (Sobańska 2005, D'Have *et al.* 2006, McLean *et al.* 2009).

The accumulation of contaminants in mammalian tissues provides a tool for ecologists to use in monitoring contaminated ecosystems. However, the analysis of internal organs from mammals is often not viable, as it requires destructive sampling resulting in the removal of individuals from the population and is still prone to error for some pollutants, such as highly volatile methylmercury which can be lost during the dissection process (Haines *et al.* 2010). As the hair root is constantly connected to the bloodstream and contains keratin with cysteine sulfhydryl (thiol) groups that have an affinity to bind with various metals, mammalian hair reflects the relative levels of metals circulating throughout the body (Beernaert *et al.* 2007, Magos and Clarkson 2008). Hair is metabolically inert so it is also able to preserve volatile metal forms such as methylmercury for longer than organ tissue during sample processing (Harada *et al.* 1999, Magos and Clarkson 2008, Haines *et al.* 2010).

Hair has been successfully used as a tissue proxy in measuring the exposure of multiple species to metallic and metalloid contaminants, from humans (Harada *et al.* 1999, Tian *et al.* 2011) to sled dogs, *Canis familiaris* (Dunlap *et al.* 2007), Pinnipeds (Mendvedev *et al.* 1997), many small mammals (Clark *et al.* 1989, Hunter *et al.* 1989, Hariono *et al.* 1993, D'Have *et al.* 2005, Sobańska 2005, Beernaert *et al.* 2007) and marsupials (Burger *et al.* 1994, McLean *et al.* 2009). In one of the earliest studies using wild mammal hair, Cumbie (1975) measured mercury concentrations in the hair of raccoons (*Procyon lotor*) and bobcats (*Lynx rufus*). Hair mercury concentrations were higher than the traditionally used skeletal muscle and liver tissue, but mercury concentrations in all tissue types were significantly ($p < 0.01$) correlated. Thus, although absolute mercury concentrations differ between tissue types, comparisons and trends are similar irrespective of whether hair, muscle or liver tissue is used. Cumbie (1975) also found that juvenile raccoons tended to have lower hair mercury concentrations than adults from the same area, thus mercury reflects age to some extent.

In a more recent study, Sobańska (2005) assessed and acknowledged the usefulness of wild boar (*Sus scrofa*) hair as a non-invasive indicator of mercury pollution. Like Cumbie (1975), Sobańska's (2005) results displayed a strong positive correlation between hair mercury concentration and liver and kidney mercury concentrations. However, unlike Cumbie (1975), Sobańska (2005) found that young animals between one and two years of age had the highest hair mercury concentration. Nakagawa (1995) found a similar relationship between hair mercury concentration and age in humans, with the highest mercury concentrations measured in the hair of young adults. Hair collection and analysis

therefore presents a non-invasive alternative method of monitoring the movement of contaminants within an ecosystem and its food chains.

The effects of mining pollution may also be reflected in food webs and their structure (Wren 1986, Chasar *et al.* 2009, Choy *et al.* 2010, Zhang *et al.* 2010). Developments into the field of stable light isotope ecology have opened new doors for ecologists studying ecosystems and food webs. Using mass spectrometry, ecologists are able to measure ratios of several stable isotopes (commonly C and N) from environmental samples, such as plant and animal tissues (Preston and Owens 1983, Brenna *et al.* 1997). Natural variations in stable isotope ratios in food sources are informative, as the isotopic composition of consumer tissues will frequently reflect their food sources in a semi-predictable manner (Hobson and Clark 1992, Hobson 1999). By measuring the C isotope ratios (expressed as $\delta^{13}\text{C}$) of consumers, ecologists can determine the relative contribution of different carbon pools to the consumers' diet (eg: C3 and C4 plant contribution to herbivore diets, Fry 2008).

Similarly, the ratio of ^{15}N to ^{14}N (relative to atmospheric N and expressed as $\delta^{15}\text{N}$) tends to become enriched in the heavier isotope with each increasing trophic level, resulting in consumer tissue $\delta^{15}\text{N}$ values being 2.5‰ to 5‰ higher than the $\delta^{15}\text{N}$ values of their diets (Peterson and Fry 1987, Ambrose 1991, Kelly 2000, Herrera *et al.* 2003, Bearhop *et al.* 2004, Campbell *et al.* 2005, Thompson *et al.* 2005, Crawford *et al.* 2008). Spatial variance at the base of the food web may also be exhibited by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Bearhop *et al.* 2004). This can be useful in a study such as the one performed by Cerling *et al.* (2006) in which stable isotopes were used to monitor human-elephant conflicts that included the raiding of C₄ crops by

elephants (*Loxodonta africana*). The study found that for most of the year elephants browsed mainly on C₃ leaves, but for a short interval at the beginning of the wet season they would raid C₄ crops intensively.

Much like with heavy metals, metabolically inert tissues such as hair preserve the stable isotope profile of an animals' diet at the time of synthesis (White 1993, Schwertl *et al.* 2003, West *et al.* 2004, Crawford *et al.* 2008, Fry 2008). The diet of the consumer will therefore be reflected in the stable isotope composition of their tissue for the period in which that tissue was synthesized (Hobson and Clark 1992, Bearhop *et al.* 2002). The length of time that the record has been preserved can be calculated by measuring the tissue turn-over rate for the tissue that has been sampled (Fry 2008). Various studies have documented the turn-over rate of different tissues across a number of species (eg: Tieszen *et al.* 1983, MacAvoy *et al.* 2006), but if the study requires highly accurate tissue specific turn-over rates a control experiment should be performed (Dalerum and Angerbjorn 2005, Miron *et al.* 2006, Crawford *et al.* 2008).

Ecologists can therefore use $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to gain some insight into the diet and trophic niche information of mammals, even during the elusive stages of an animals' life cycle when they are otherwise hard to find or are difficult to capture (Bearhop *et al.* 2004, Crawford *et al.* 2008). Like any ecological tool, stable isotopes have limitations. Bearhop *et al.* (2004) describe three main assumptions that must be met for stable isotope analysis, specifically as a measure of trophic niche width to be effective: prey species must differ isotopically by a measurable amount; the isotope signatures at the food-web base, and the diets of prey species must remain relatively invariant over time; and the tissue analysed must reflect the

period over which niche width is expressed. Once these assumptions have been met, stable isotope variance as a measure of niche width can be used as a means complimenting traditional or ‘aggregate’ variability measures of animal populations, such as species richness and biomass (Bearhop *et al.* 2004, Layman *et al.* 2007a).

1.2 Problem statement

AngloGold Ashanti LTD is a major gold producer world-wide and has seven deep-level operations and one surface operation in South Africa (AngloGold Financial 2009). According to the Department of Water Affairs (DWAf 2001) South African gold mining produced 221 million tonnes of waste per annum by 1997. Over 270 TSFs in the Witwatersrand basin, covering over 400km², produce vast amounts of air (dust), soil and water (surface and groundwater) pollution (AngloGold Ashanti 2004). These TSFs are interspersed by a patchwork of human settlements (towns, informal settlements etc.) and remnant habitats (fragmented grasslands, rehabilitation areas etc.). Therefore, organisms living within these areas are exposed to mine-related pollution. Mercury is of special concern as it can have severe health effects above certain concentrations (Boyd 2010).

Of the animals living within these areas, small mammals such as rodents and shrews live in close proximity to contaminated soil and utilise contaminated food and water sources making them useful for fast and effective assessment of the accumulation and biomagnification of mercury in the environment. Pereira *et*

al. (2006) propose five reasons relevant to this study on why analyses of small mammal hair make good indicators of environmental metal contamination: 1) many species have large geographic ranges/ distributions and can be found in both contaminated and uncontaminated habitats, 2) they are an important component of terrestrial ecosystems and form the middle of some food chains, 3) they are in contact with the soil throughout their lifetime, exposing them to contaminants via ingestion and dermal absorption, 4) they usually have small home ranges making them good site-specific indicators, and 5) they can be sampled invasively without major damage to the population or they can be sampled non-invasively with relative ease. Their potential as good site-specific indicators is increased by the fact that most small mammals have low dispersal rates, especially when body condition may be compromised such as in highly polluted environments (Krebs *et al.* 1973).

1.3 Aim

The aim of this research was to better understand small mammal communities living within contaminated areas in close proximity to TSFs. These communities were contextualised using three measures: ‘traditional’ aggregate measures of small mammal community variability (e.g. biomass and species richness); trophic niche width metrics of small mammals within the food web using stable isotope analysis; and the evaluation of levels of mercury contamination within the hair of small mammals. Study sites were located along a mining pollution gradient for comparison of mercury accumulation to aggregate variability measures and trophic niche width metrics. The study therefore provides

insight into the interactions between mining pollutants (indicated by mercury) and small mammal ecology and food webs by measuring whether mercury pollution affects small mammal species diversity and density, whether mercury pollution causes compression or expansion of isotopic niche widths, and whether mercury pollution poses a health risk to small mammals at the AngloGold Ashanti West Wits Mining Complex (WWMC). The chapters contained in this dissertation follow the three objectives below, but have been formatted as standalone manuscripts for subsequent submission for publication purposes. Therefore, unavoidable repetition may occur between chapters.

1.4 Objectives

Research question 1: What is the small mammal community composition in different areas of the WWMC?

The first objective was to describe small mammal community structure using ‘traditional’ aggregate measures of community variability. Small mammal populations were assessed using the Shannon-Wiener diversity and evenness indices, the minimum number of animals alive (MNA), trapping success, biomass and density. The percent aerial cover (PAC) provided to small mammals by vegetation was used to assess the relationship between small mammal community structure and vegetation cover (Bowers and Dooley 1993, Els and Kerley 1996, van Deventer and Nel 2006, Kok *et al.* 2012).

Hypothesis 1: Small mammal communities at the WWMC will vary according to the level of disturbance created by pollution bioavailability and site specific vegetation structure.

Research question 2: Does small mammal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ suggest fundamental differences in diet between/among the study sites that are attributable to heavy metal contamination?

The second objective is to compare small mammal communities within contaminated areas using stable isotope analysis bi-plots as a measure of community-wide variability that complements aggregate variability measures. Based on Elton's (1927) conception of the consumer trophic niche, community-wide metrics provide a means of comparing specific trophic structures for consumer species on spatial and temporal scale. This method is more detailed than aggregate variability measures as it employs continuous measures of trophic structure (Layman *et al.* 2007a).

Hypothesis 2: Small mammal community trophic niche width will shift (either increase in size as animals are forced to utilize a wider range of resources, or decrease in size as pollutants exclude the use of some resources) with variable heavy metal contamination, potential interspecific competition and seasonal variation in food resources.

Research question 3: How do levels of mercury concentrations in small mammal hair at the WWMC compare 1) between captured species 2) to the soil and vegetation mercury concentrations measured in the same area by Makiese (2012), and 3) to the target and intervention levels of international regulatory organisations?

The third objective is to: 1a) compare the concentrations of mercury in the hair of small mammals both within and between species and sites in the area around the WWMC; 1b) use C and N stable light isotope analysis to compare mercury concentration to relative isotopic trophic position to investigate bioaccumulation of mercury in small mammals living in mining environments; 2) compare small mammal hair mercury concentrations to the baseline environmental concentrations measured by Makiese (2012); and 3) compare small mammal hair mercury concentrations to target and intervention levels stipulated by international regulatory bodies.

Hypothesis 3: The soil, plants and animals found within the study sites will display both elevated mercury levels compared to target levels stipulated by international regulatory bodies, and bioaccumulation of mercury within the terrestrial food web.

**2. Population ecology of rodents and shrews on an AngloGold
Ashanti Ltd mining complex, South Africa.**

2.1 Introduction

Rodents are the most diverse order of mammals and are found throughout the world, with 80 species occurring in the southern African region alone (Apps 2000, Skinner and Chimimba 2005). Insectivorous shrews are not as diverse as rodents (only 16 species are found in Southern Africa), but share similar habitats, distributions and life traits with rodents (Apps 2000, Skinner and Chimimba 2005). Both rodents and insectivorous shrews will be referred to hereafter as ‘small mammals’.

Small mammals occur in a wide range of environments, with small mammal abundance determined by habitat productivity (Abramsky 1978), as well as factors such as species physiology, their nutritional needs and the animals successional roles (Taylor 1970, Bowers and Brown 1982, Price and Jenkins 1986, Kerley and Erasmus 1992, Ellison *et al.* 1993, Els and Kerley 1996, Kerr and Packer 1997, van Deventer and Nel 2006, Kok *et al.* 2012). Small mammal community structure and their associated habitat specialisation is often a result of the vegetation characteristics surrounding the community (Rosenzweig and Winakur 1969, Taylor 1970, Kerley 1992a, Els and Kerley 1996, Monadjem 1997a, Monadjem 1999, Cramer and Willig 2002, van Deventer and Nel 2006, Kryštufek *et al.* 2007, Kok *et al.* 2012).

Vegetation characteristics, specifically as food resources, apply a direct adaptive pressure on herbivorous and omnivorous small mammals (Martin 1998, Monadjem and Perrin 1997a) as most rodent species are omnivorous (Landry 1970, Kerley 1992b, Monadjem 1997b), and consume both seeds and vegetation

(Price and Jenkins 1986, Kam *et al.* 1997). Vegetation characteristics also influence insect diversity and abundance, applying an indirect adaptive pressure on insectivorous and omnivorous small mammals through their food resources (Scott *et al.* 2011).

Because of the relationship between the small mammals and vegetation communities, small mammals can be used as indicators of an ecosystem's health (Brown 1975, Korn 1987, Avenant 2011), a useful tool in conservation areas, or in transformed areas that have been identified for rehabilitation (Avenant 2000, Avenant 2011). Rehabilitating habitats display successional changes in vegetation characteristics over time (van Aarde *et al.* 1996). Similarly, the small mammal communities living in those habitats also display successional changes (van Aarde *et al.* 1996, Ferreira and van Aarde 1996, Ferreira and van Aarde 2000).

The successional changes in the small mammal community occur over both the long term, following vegetation community changes, and the short term, following seasonal changes in resource availability and quality (van Aarde *et al.* 1996, Ferreira and van Aarde 1996, Monadjem and Perrin 1998a, Ferreira and van Aarde 2000, Monadjem and Perrin 2003). With a high enough abundance for reliable statistical analysis, their ease of study, their community characteristics and important role in their habitats, small mammals can act as reliable indicators of successional states in transformed environments (Brown 1975, Monadjem 1997a, Monadjem 1999, van Deventer and Nel 2006).

However, habitats in active mining areas are prone to undergo multiple or frequent disturbances, preventing complete rehabilitation of vegetation and

succession in small mammal communities (van Aarde *et al.* 1996). Therefore, multiple factors such as small mammal responses to fire (outlined in Kerley and Erasmus 1992, Monadjem and Perrin 1997b), the tendency for habitats with intermediate levels of disturbance to have higher species diversity than habitats with very low or very high levels of disturbance (Paine and Vadas 1969), and mechanisms that allow ecologically similar small mammals to coexist (outlined in Monadjem and Perrin 2003, Kinahan and Pillay 2008) also contribute to the composition of small mammal communities.

South Africa's gold mining industry is considered the single largest producer of waste products and one of the largest contributors to environmental transformation in the country (DWAF 2001, Weiersbye and Witkowski 2003). The Witwatersrand Basin contains over 270 tailings storage facilities (TSFs), covering over 400km² (AngloGold Ashanti 2004), which comprise powdered processed rock containing high concentrations of harmful compounds such as forms of arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) (AngloGold Ashanti 2004; AngloGold EMP 2009). Seepage and runoff from TSFs contaminate groundwater, creating large acid mine drainage plumes around the TSFs, while wind carries and deposits vast amounts of air (dust) pollution (AngloGold Ashanti 2004, AngloGold EMP 2009). These TSFs are interspersed by a patchwork of human settlements (towns, informal settlements etc.) and remnant habitats (fragmented grasslands, rehabilitation areas etc.).

Vegetation growing within these affected areas absorbs water from acid mine drainage plumes, drawing the harmful contaminants out of the soils and into their tissues (Weiersbye and Witkowski 2003). This process forms the basis of the

concept of phytoremediation (Raskin *et al.* 1994). During its application, phytoremediation results in significant changes to vegetation species composition in the designated area. Through phytoremediation, polluted groundwater can affect the diversity and abundance of both vegetative and invertebrate food sources for small mammals, impacting herbivorous, omnivorous and insectivorous small mammal species found in these areas (Scott *et al.* 2011).

The success of large-scale phytoremediation projects relies on the constant monitoring and adaptation of project efforts (Oelofse *et al.* 2007). The need for monitoring programmes creates opportunities for research groups to perform research on multiple environmental interactions within areas that have been affected by mining.

The aim of this study was to better understand small mammal communities living within contaminated areas in close proximity to TSFs. These communities were contextualised using aggregate measures of small mammal community variability (e.g. species abundance, species biomass and species richness) through long-term data collection. Study sites were placed along a known mercury pollution gradient to reflect different levels of pollutant exposure (Makiese 2012). Natural habitat disturbance, such as fire and flooding, and anthropogenic habitat disturbance, such as mechanical disturbance (e.g. bulldozing) and cattle (*Bos primigenius*) grazing were included in the study as they affect the variability of small mammal communities. The combination of chemical and physical disturbances was used to discuss the relationship between small mammal community diversity and mine-related habitat disturbance. It was expected that small mammal communities at the AGA West Wits complex would

vary with site-specific vegetation structure and the effect of mine-related and physical habitat disturbance therein.

2.2 Materials and Methods

2.2.1 Study period

The study was conducted between November 2010 and November 2012 (hereafter referred to as the study period). Eight trapping sessions were undertaken at Sites A and B; November 2010, February 2011, April 2011, October 2011, December 2011, March 2012, April 2012 and October/November 2012. Four trapping sessions were undertaken at sites C and D; December 2011, March 2012, April 2012 and October/November 2012. These sampling periods are an attempt to cover a full seasonal cycle. The area undergoes a summer rainfall regime, with rains beginning in October/November and persisting until April (SAWS 2013). Maximum rainfall occurs between February and April (100 – 200mm; SAWS 2013). Rainfall decreases substantially from May to June, with little to no rainfall during July and August (0 – 50mm; SAWS 2013). A trapping session consisted of four consecutive nights at each site within the above mentioned months following the minimum limit for consecutive trapping nights determined by Avenant and Cavallini (2007).

2.2.2 Study sites

The study was conducted at four study locations (sites A to D) on an AGA property on South Africa's Gauteng Highveld region, referred to as the West Wits mine complex (WWMC, Figure 1) Varkenslaagte catchment, near Carletonville, Gauteng. The geology of the Witwatersrand Basin, upon which the sites were located, is characterised by volcanic and sedimentary rock types, usually covered with sandy soils (AngloGold Ashanti 2004). The sites were located along the

Varkenslaagte stream, which is known to carry elevated levels of pollution from the TSF to the north of Site A (Appendix A, Makiese 2012), with the stream running past the other three sites located further downstream.

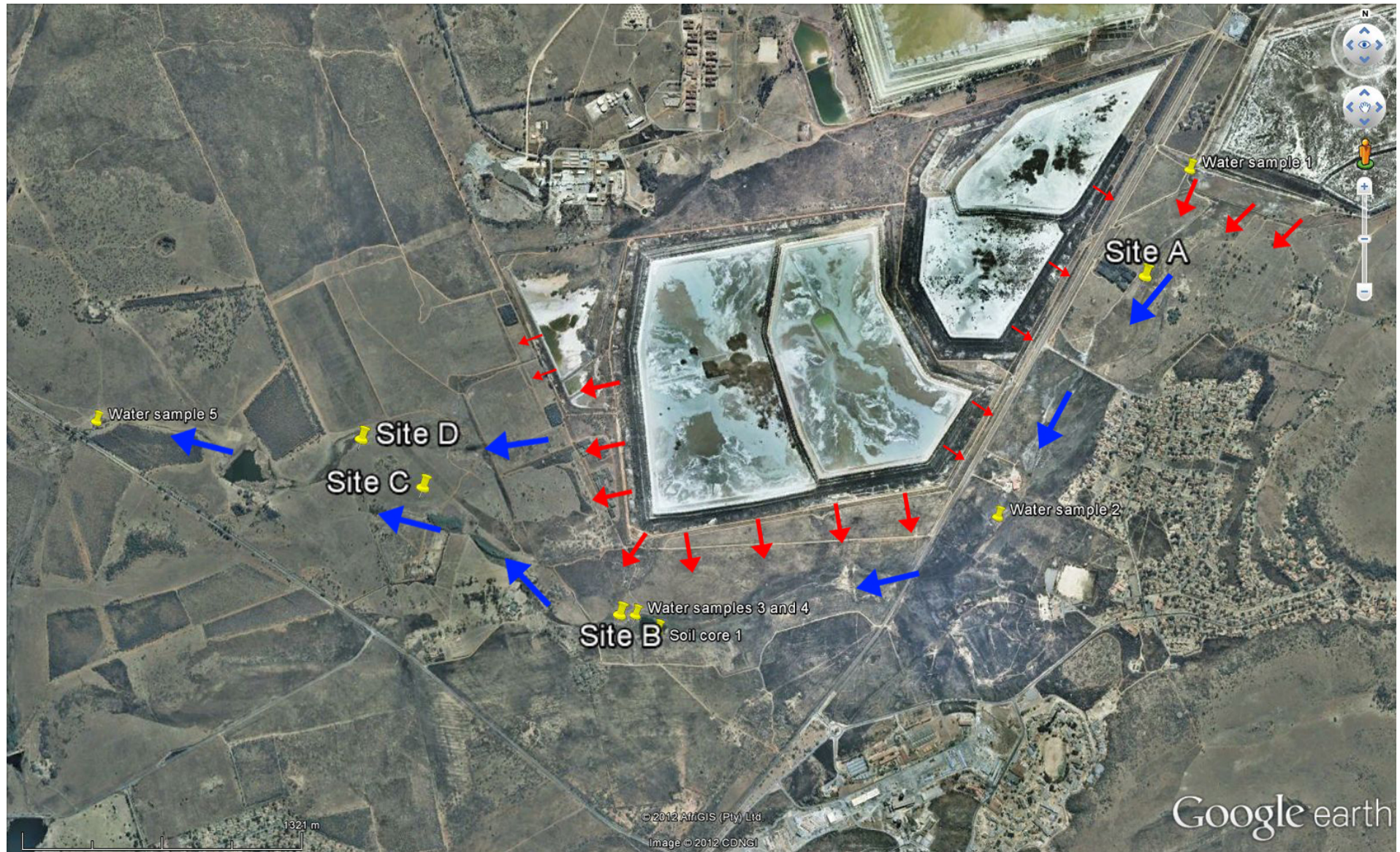


Figure 2.1: Satellite image (Property of Google) of the four study sites (Large yellow pins, A to D) located along the Varkenslaagte stream (blue arrows indicate direction of flow) at the AngloGold Ashanti West Wits Mine Complex, Gauteng. Red arrows indicate direction of contamination plumes from the adjacent TSFs. Small yellow pins indicate locations of water and soil samples taken for preliminary Hg and IHg analysis (Makiese 2012).

The two 'upstream' sites (A: S 26° 25.936', E 27° 22.183' and B: S 26° 26.820', E 27° 20.740') were relatively close to TSFs (~440m), while the two 'downstream' sites (C: S 26° 26.513', E 27° 20.176' and D: S 26° 26.395', E 27° 19.998') were further from TSFs (~1000m and ~1250m respectively). Sites C and D were added to the study in December 2011 to increase the disturbance variability amongst sites along a proposed pollution gradient (Appendix A). The relatively small distance between Sites C and D (~350m), however, increased the potential for small mammal movement between the sites.

A previous study (Makiese 2012) measured the concentrations of inorganic mercury (IHg (Hg^{2+})) and methylmercury (MHg) in stream water (Appendix A; 0.003 - 13.055 ng.L^{-1} IHg and 0.000 - 7.835 ng.L^{-1} MHg) and soils (Appendix B; 51.4 – 565.4 $\mu\text{g kg}^{-1}$ IHg and 0.0 – 11.3 $\mu\text{g kg}^{-1}$ MHg) at a number of locations along the length of the stream (Figure 2.1; Appendix C). Mercury is highly toxic and a major chemical pollutant produced by the mining process as it has a LD_{50} (Lethal Dose for 50% of tested rats and mice) in the range 25.9mg Hg/kg to 77.7mg Hg/kg in the form of mercuric chloride (Kostial *et al.* 1978, ATSDR 1999). The minimum risk level (MRL) for IHg is 2 $\mu\text{g/kg/day}$, whereas for MHg the MRL is 0.3 $\mu\text{g/kg/day}$ (ATSDR 1999). Hence, a rodent with an average weight of 100g would need to ingest only 0.2 μg of IHg and 0.03 μg of MHg per day to be at risk of mercury toxicity.

The total Hg concentration (IHg and MHg combined) was also measured in the common reed, *Phragmites australis* (37.8 $\mu\text{g/kg}$) within Site A, and bulrushes, *Typha spp.* (31.9 $\mu\text{g/kg}$) approximately 1300 metres downstream of Site A. Concentrations of IHg and MHg in the stream water increased from upstream

of Site A, downstream towards Site B, with the IHg concentration in the dam above Site B being much higher at 13.055ng/L within the dam water (Appendix A). Concentrations of water IHg and MHg decreased downstream of Site B with the lowest concentrations just downstream of Site D (Appendix A). Surface soil (0 – 5cm) samples taken within the reed beds at Site A ($344.2\mu\text{g}/\text{kg}^{-1}$), Site B ($628.4\mu\text{g}/\text{kg}^{-1}$), and Site D ($124.9\mu\text{g}/\text{kg}^{-1}$) were much higher than the surface soil samples taken within the Varkenslaagte stream ($38.9\mu\text{g}/\text{kg}^{-1}$ at Site A to $58.5\mu\text{g}/\text{kg}^{-1}$ just upstream of Site B).

Site A was located in a grassland and reed bed used for the phytoremediation of runoff from the northern TSF. The stream was lined with *P. australis* (Ca.) Trin. ex Steud., the common reed, as it is a robust C_3 wetland macrophyte capable of removing contaminants from soil and water (Ghassemzadeha *et al.* 2008, Bonanno and Lo Giudice 2010). Couch grass (*Cynodon dactylon* (L.) Pers.), weeping love grass (*Eragrostis curvula* (Schrader.) Nees.), and boer love grass (*E. chloromelas* Steud. Complex) were also common at Site A. The soil in Site A was observed to contain no rock, and high levels of clay and soil moisture.

Site B was situated approximately 3kms downstream of Site A, below a dam on the Varkenslaagte stream. The stream in this site was lined with multiple wetland plant species, the most prominent of which were bulrushes (*Typha spp.*) and sedges (*Schoenoplectus spp.*), with some grasses (e.g. *Cynodon spp.* and *E. curvula*) and some *P. australis* prior to the October 2011 sampling period. Observed soil clay and soil moisture were similar to Site A, but Site B soils also

contained rocks left over from dam construction and exposed bedrock further away from the stream.

Site C was located approximately 1km downstream of Site B, and was further from the Varkenslaagte stream relative to the other three sites (approximately 50m from the bank as opposed to 10m or less in the other three sites). As such, Site C did not support any wetland plant species. The site prominently supported bankrupt bush (*Seriphium plumosum*, formerly *Stoebe vulgaris*) and multiple grass species (including *Cynodon spp.*, *Eragrostis spp.*, and *Aristida spp.*). The soil was observed as sandy, dry and rockier than the other three sites.

Site D was located approximately 350m downstream of Site C, and was in close proximity to a wetland formed between the convergence of the Varkenslaagte stream and an overflow channel/stream from a nearby tailings effluent pond. Site D supported bulrushes (*Typha spp.*) and the common reed (*P. australis*) distributed along the northern edge, but was mostly vegetated with a similar mixture of grasses (including *Cynodon spp.*, *Eragrostis spp.*, and *Aristida spp.*) and bankrupt bush (*S. plumosum*) to Site C. The soil of Site D was observed as sandy and contained fewer, but larger rocks and more soil moisture than Site C.

Sites were exposed to a number of natural and anthropogenic disturbances during the study period. Site A was burned prior to the October 2011 and October/November 2012 sampling period. Localised flooding of Site A during heavy rains occurred in the wet period (February 2011 and March 2012). A minor spill from the northern TSF resulted in tailings entering Site A ,with the resulting

clean-up process for the TSF spillage razing the areas directly adjacent to Site A, completely removing all vegetation from two sides of the site in early 2012. Disturbances at Site B included localised flooding during heavy rains in the wet period (February 2011 and March 2012), fire damage prior to the October 2011 and October/November 2012 sampling periods and consistent, heavy grazing by cattle across the entire study period.

Site C underwent no known and recorded disturbances during the study period as it presented low grazing potential for cattle, and did not undergo any type of flooding as the Site's slightly higher geographical elevation and distance from the stream prevented flood waters from reaching it. Disturbances at Site D included some minor localised flooding during heavy rains in the wet period (February 2011 and March 2012) and consistent, light to medium grazing by cattle across the study period.

The four study sites were therefore grouped into three classes of disturbance based on disturbance severity. Site B was classified as highly disturbed based on the high levels background mercury pollution, localised flooding, fire damage and continuous heavy cattle-grazing. Site A was classified as intermediately disturbed as it underwent localised flooding, intermediate levels of mercury pollution and fire damage. Site D underwent low levels of disturbance, based on continuous light cattle grazing, minor localised flooding and had the lowest level of mercury pollution. Site C can be classified as having low to no disturbance as it did not undergo any of the physical disturbances seen in the other three study sites. The level of mercury pollution in Site C was unconfirmed, but it

was expected that mercury pollution will be lower than the other sites as Site C was located over fifty metres from the stream.

2.2.3 Small mammal captures

Fifty live traps were set for small mammals (Enviro-care MRT1 box traps, ± 10 cm x 7.5 cm x 25 cm), deployed in a 5x10 grid formation with 10 to 12 metres between traps (total area $\sim 500\text{m}^2$). The traps were baited with a mixture of peanut butter and rolled oats. Traps were covered with plant material to protect potential captures from environmental extremes and were checked at dawn and dusk of every day. All trapped small mammals were identified according to genus and species (according to Apps 2000 and Skinner and Chimimba 2005), weighed, sexed and reproductive status noted where possible. Captured animals were given a unique number by fur clipping (removing a small patch of their belly fur with scissors, a method less stressful on the animals than toe clipping (Korn 1987)) so that recaptures within the sampling week could be recorded. Animals were then released at the point of capture.

Captures were undertaken under the certification from the University of the Witwatersrand Ethics Committee to trap, handle and collect hair samples from captured small mammals (Certificate number 2011/45/2A). Permission was also granted by the Gauteng Provincial Government to do research within the province (Certificate CPF6 No. 0151).

2.2.4 Vegetation

The structure of the vegetation at each site for each month was described using an ocular Percent Aerial Cover (PAC) estimate, similar to the methods used by Bowers and Dooley (1993), Els and Kerley (1996) and van Deventer and Nel (2006). The PAC estimate for each site was determined by quantifying the amount of cover that the vegetation within a 1m² quadrat provided to small mammals. The PAC estimate was made at four different height classes: leaf litter (LL) on the ground, ground level to 30cm (0 – 30cm) above ground level, 30cm to 1m (30 – 100cm) above ground level, and higher than 1m (>100cm) above ground level. PAC was estimated at every fifth trap within the live trap grids, giving 15 PAC values per site. The prominent vegetation species present in each site were identified (Van Oudtshoorn 1999, van Ginkel *et al.* 2011).

2.2.5 Data analysis

Data was prepared using Office Excel 2010 (Microsoft Corporation 2010) before running statistics in R 2.15.2 (R Development Core Team 2010). Small mammal populations were assessed using Simpson's index of diversity (Simpson 1949) and the Shannon index of diversity (Shannon 1948), calculated using the BioDiversity Pro software and base log¹⁰ (McAleece *et al.* 1997), and checked using manual calculation (Krebs 1998, formulae in Appendix D). The Simpson index of diversity is more sensitive to changes in the more common species in the community sample, whilst the Shannon index is more sensitive to changes in rare species in the sample community (Krebs 1998). The reciprocal of Simpson's index (1/D) was also calculated as it provides an estimate of the number of

equally common species needed in a community to generate the observed heterogeneity of the sampled community (Williams 1964, MacArthur 1972).

The site specific minimum number alive (MNA) and species specific MNA were calculated as a representative of small mammal populations within the study sites. The MNA is defined in this study as the number of individuals caught at least once within the sampling periods (Krebs 1966). Trapping success, small mammal biomass (captured mass [g] per hectare), site density (abundance per hectare), and species specific density were also calculated. A rarefaction curve was calculated for each site across the sampling period as an indication of how species diversity would be affected by increasing the number of randomly captured individuals within each site (Gotelli and Colwell 2001).

The normality of the data was checked using Shapiro-Wilk's W Test (p-value range $<2.2e^{-16} - 0.0035$) (Shapiro and Wilk 1965). Subsequently, non-parametric Kruskal-Wallis one-way analysis of variance tests (Kruskal and Allen 1952) were used to test for changes in PAC estimates over time within each site. A Multiple Comparison test (with $p = 0.05$) was used to determine which sets were significant based on the Kruskal-Wallis results (Siegel and Castellan 1988) (performed using the "pgirmess" package by Giraudoux (2012) in R 2.15.2 (R Development Core Team 2010)).

Spearman's rank correlation coefficients (Spearman 1904) were calculated to test for any significant correlations between the small mammal community variability measures and the PAC of the vegetation. The Spearman's rank correlation test was used as it is a non-parametric test, giving a better

representation of the non-normally distributed data. The coefficient was used to test for correlations between: small mammal Simpson's index and PAC over time within each site, small mammal density (n/ha) and PAC over time within each site, and species specific MNA between species over time within each site (e.g. changes in *Rhabdomys dilectus* numbers over time vs. changes in *Mastomys sp.* numbers over time within Site B).

A Detrended Correspondence Analysis (DCA; Hill and Gauch 1980) was performed on the PAC at each trap in which a capture was made. It provides insight into which PAC height class affected the species-specific MNA over time within each site. The DCA copes better than the Spearman's rank correlation test for this type of species abundance data, as the DCA is less affected by rare species and the delayed effect of disturbance events, such as fires or flooding on small mammal populations (Quinn and Keough 2002).

2.3 Results

2.3.1 Overall small mammal abundance

Over the entire study period (eight sampling occasions), a total of 4120 trap nights were recorded. Trapping success averaged 11.9% (0.5% to 40.5%, Table 2.1) across all four sites within the study period, with Site D in the April 2012 sampling occasion having the highest capture success of 40.5%. The total number of unique individuals captured across the entire study period was 262, with 221 recaptures made (483 total captures made). The highest overall density was recorded at Site A during the April 2011 sampling period (127 animals/ha), while the lowest overall density was recorded at Site C during the December 2011 sampling period and Sites B and D during the October – November 2012 sampling period (2 animals/ha, Table 2.1). Site A had the highest average overall density (54 animals/ha, SD = 42.38), as well as a consistently high species diversity and species richness (Table 2.1). Site C had the lowest average density of the sites (9 animals /ha, SD = 6.63), and had the lowest species diversity of all the sites (Table 2.1), as only one species, the striped mouse (*R. dilectus*) was caught there throughout the study period.

Across the entire study period, six rodent species (order Rodentia, families Muridae and Nesomyidae) and two shrew species (order Soricomorpha, family Soricidae) were captured within the study sites (Table 2.2). Three Muridae (sub-family Murinae) species were: the striped mouse, *R. dilectus* (n = 147, recently split from *R. pumilio*; Rambau *et al.* 2003, Pillay *et al.* 2006, Castiglia *et al.* 2012); the multimammate mouse, *Mastomys* sp. (n = 77; *M. coucha* and

Table 2.1: Small mammal community abundance and diversity variables for each study site across the study period. Site C has no Evenness (E) values as the Shannon index (H') is zero as only a single species was present at the site. Species richness represents the number of species caught per site per month. Density and biomass represent overall values per site per month (i.e. cumulative values). Formulae for the Shannon index (H'), Evenness (E) and the Simpson's index (D) appear in Appendix D.

	November 2010		February 2011		April 2011		October 2011		December 2011				March 2012				April 2012				October - November 2012			
	A	B	A	B	A	B	A	B	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Trap nights	150	150	120	120	120	120	120	120	200	200	200	200	200	200	200	200	100	200	200	200	200	200	200	200
Trapping success (%)	18.0	3.3	14.2	7.5	20.0	8.3	4.2	9.2	7.0	13.0	2.5	16.5	18.5	6.5	4.5	20.5	26.0	21.0	10.5	40.5	5.5	0.5	6.0	1.0
No. Individuals caught	13	3	14	7	19	6	4	6	10	12	1	21	19	8	4	23	17	23	9	32	4	1	4	1
Total captures (incl. Recaps)	27	5	17	9	24	10	5	11	11	26	5	33	37	13	9	41	26	42	21	81	11	1	12	2
Density (n/ha)	86.7	20.0	93.3	46.7	126.7	40.0	26.7	40.0	20.0	24.0	2.0	42.0	38.0	16.0	8.0	46.0	34.0	46.0	18.0	64.0	8.0	2.0	8.0	2.0
Biomass (g/ha)	4026.7	753.3	1886.7	2613.3	3460.0	1566.7	560.0	3126.7	600.0	1004.0	64.0	1836.0	898.0	648.0	228.0	1452.0	1868.0	1320.0	454.0	1828.0	262.0	132.0	462.0	74.0
Species richness	3	2	3	3	4	2	3	3	5	2	1	3	5	2	1	2	6	3	1	3	2	1	1	1
Shannon index (H')	0.47	0.28	0.33	0.35	0.42	0.20	0.45	0.44	0.64	0.20	0.00	0.40	0.49	0.24	0.00	0.20	0.69	0.38	0.00	0.16	0.24	0.00	0.00	0.00
Evenness (J)	0.98	0.92	0.69	0.73	0.70	0.65	0.95	0.92	0.82	0.65	-	0.83	0.70	0.81	-	0.67	0.89	0.80	-	0.34	0.81	-	-	-
Simpson's index (D)	0.35	0.56	0.56	0.55	0.50	0.72	0.38	0.39	0.26	0.72	1.00	0.45	0.41	0.63	1.00	0.71	0.23	0.47	1.00	0.83	0.63	1.00	1.00	1.00
Simpson's Reciprocal (1/D)	2.86	1.80	1.78	1.81	1.99	1.38	2.67	2.57	3.85	1.38	1.00	2.24	2.42	1.60	1.00	1.40	4.31	2.12	1.00	1.21	1.60	1.00	1.00	1.00

M. natalensis, here referred to as *Mastomys sp.* as genetic analysis is required to distinguish between the two species); and the rock rat (or veld rat), *Aethomys sp.* (n = 1, *A. chrysophilus* and *A. ineptus*, here referred to as *Aethomys sp.* as genetic analysis is required to distinguish between the two species). Two Muridae (sub-family Otomyinae) species were: the vlei rat, *Otomys auratus* (n = 14, recently split from *O. irroratus*; Taylor *et al.* 2009, Engelbrecht *et al.* 2011); and the Angoni vlei rat, *O. angoniensis* (n = 4). The Nesomyidae (sub-family Dendromurinae) species was Brant's climbing mouse, *Dendromus mesomelas* (n = 4). The two insectivorous shrew (family Soricidae) species captured were: the swamp musk shrew, *Crocidura mariquensis* (n = 46); and the forest shrew, *Myosorex varius* (n = 8).

The striped mouse, *R. dilectus*, was the only species to be found at all four sites during the study period. *Rhabdomys dilectus* was also the only truly diurnal species captured during the study, as the other captured species are all mainly nocturnal, with occasional short periods of activity during the early morning or late afternoon (Bronner and Meester 1988, Brooks 1972, Choate 1972, Davis 1972, Kingdon 1997, Apps 2000, Skinner and Chimimba 2005).

The small mammal species can also be grouped according to trophic strategy. The four rodent species with captured weights less than 70g, *R. dilectus*, *Mastomys sp.*, *D. mesomelas* and *Aethomys sp.* are generally considered omnivores (Brooks 1972, Choate 1972, Curtis and Perrin 1979, Perrin 1980, Perrin and Curtis 1980, De Graaff 1981, Rowe-Rowe 1986, Willan 1992, Kingdon 1997, Monadjem 1997b, Monadjem and Perrin 1997b, Monadjem and Perrin 1998b, Apps 2000, Baxter and Hansson 2001, Skinner and Chimimba

2005, Kryštufek et al. 2007, Mulungu et al. 2011). The two rodents with captured weights greater than 70g, *O. auratus* and *O. angoniensis* are considered as specialist herbivores (Davis 1972, Curtis and Perrin 1979, Perrin 1980, Perrin and Curtis 1980, De Graaff 1981, Bronner and Meester 1988, Willan 1992, Monadjem 1997b, Kingdon 1997, Apps 2000, Baxter and Hansson 2001, Skinner and Chimimba 2005). The two shrew species, *C. mariquensis* and *M. varius* are, by definition, insectivorous (Yates 1984, Churchfield 1990, Kingdon 1997, Monadjem 1997b, Apps 2000, Skinner and Chimimba 2005).

The maximum species richness was at Site A (6 species captured) during the April 2012 sampling period. Across the entire sampling period, Site A had the highest cumulative number of species captured (7 species). Despite having a lower cumulative number of species captured (6 species) Site B achieved its maximum earlier than Site A during October 2011. Site D increased by one species for each of the sampling periods (5 cumulative species), while Site C remained constant with one species as mentioned above. The rarefaction curve (Figure 2.2) for the sampling period indicates that as the number of randomly captured individuals increases, Site A would yield the highest species diversity. The species diversity of Site B would increase quickly with low captures, but would then reach its maximum species diversity relatively quickly as well. Whilst the species diversity of Site D would not increase as quickly as that of Site B, the species diversity would reach a higher maximum as more random individuals within the site are caught. As only one species was captured in Site C, the number of individuals caught would not affect species diversity.

[illegible]

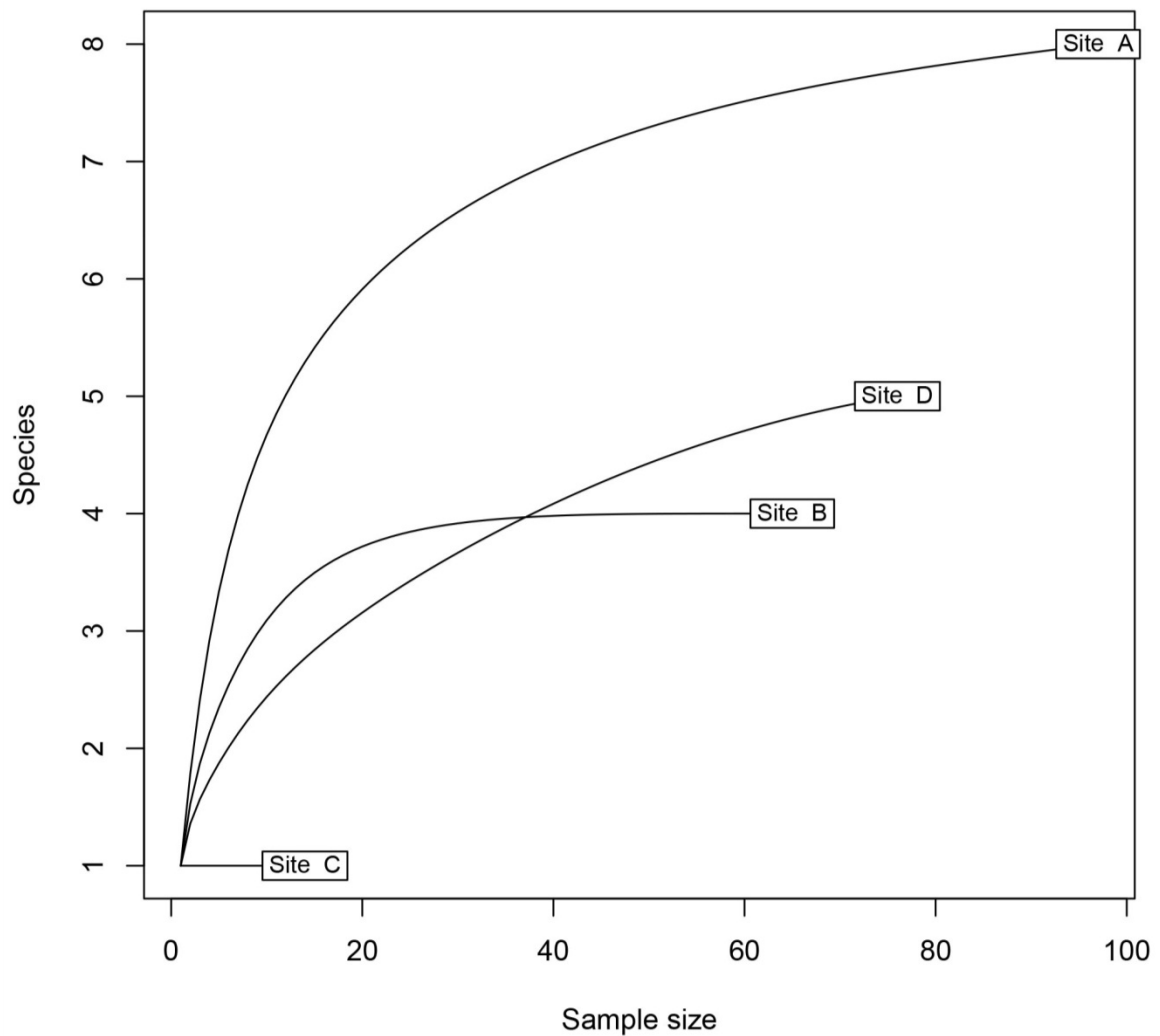


Figure 2.2: Rarefaction curve of small mammal species within each study site across the entire sampling period. Site A had the greatest projected species richness (7), followed by Site B (6) and Site D (5). Only a single species was captured at Site C. Sites A and B were sampled eight times (November 2010 to October/November 2012), whilst Sites C and D were only sampled four times (December 2011 to October/November 2012).

2.3.2 *Seasonal trends in small mammal abundance*

The density of small mammals at Site A decreased (from 126.67 animals/ha in April 2011 to 26.67 animals/ha in October 2011, Table 2.1) after a fire that consumed the majority of the vegetation at Site A. The overall density at Site B decreased from October 2011 to December 2011, following a fire prior to the October 2011 sampling period. The density of both Site A and B showed a decrease over the next two sampling periods after the fires, before starting to increase again (Table 2.1). However, Site A's density decreased again in the April 2012 sampling period as adjacent areas to Site A were bulldozed between the March and April 2012 sampling periods. The density in Site A decreased further during the winter of 2012, from 34 animals/ha in April 2012 to 8 animals per hectare in October-November 2012 (Table 2.1), by which time the bulldozed area adjacent to the site had increased. The density of Site B decreased over the winter of 2012, from 46 animals/ha in April 2012 to only 2 animals/ha in October-November 2012 (Table 2.1), as a fire during the dry season consumed the majority of the vegetation in Site B (refer to Figure 2.6 for Site B's vegetation PAC). The density of Site C increased from December 2011 (2 animals/ha) when it was instated until April 2012 (18 animals/ha), after which it decreased during the dry period to October-November 2012 (8 animals/ha; Table 2.1). The density of Site D showed a similar pattern of increasing from December 2011 (42 animals/ha) to April 2012 (64 animals/ha), before undergoing the largest relative decrease in density over the dry period to October-November 2012 to only 2 animals/ha (Table 2.1).

In terms of species-specific MNA, *C. mariquensis* was most common at Site A, while *Mastomys sp.* was most common at Site B and *R. dilectus* was most common at Sites C and D (Table 2.2). The MNA of *R. dilectus* varied the most during the study period, ranging from no animals caught (February 2011, Table 2.2) to 87 animals caught within a single sampling occasion across all four sites (April 2012, Table 2.2). It is interesting to note that both *R. dilectus* and *Mastomys sp.* were not captured at Site A until October 2011, by which time the capture rate of *C. mariquensis* was decreasing (Figure 2.3). There is evidence at Site A that *Mastomys sp.* have a negative correlation with the density of *C. mariquensis* ($r_s = -0.7828$, $S = 149.7505$, $p = 0.0216$), and *O. auratus* have a negative correlation with the density of *D. mesomelas* ($r_s = -0.7078$, $S = 143.4551$, $p = 0.0495$).

Both *R. dilectus* and *Mastomys sp.* continued to increase in Site A over the next two sampling periods, until *Mastomys sp.* again decreased between March and April 2012 (Figure 2.3), the same time during which the adjacent area was cleared by bulldozers. As *R. dilectus* captures at Site D increased between December 2011 and April 2012 to the highest recorded site- and species-specific MNA value of 29 individuals, *Mastomys sp.* captures decreased until no members of the species were caught in April 2012 (Figure 2.4). However, Site D was only sampled during the last four sampling occasions which could account for the weak statistical evidence of a negative correlation between *R. dilectus* and *Mastomys sp.* ($r_s = -0.1054$, $S = 11.0541$, $p = 0.8946$).

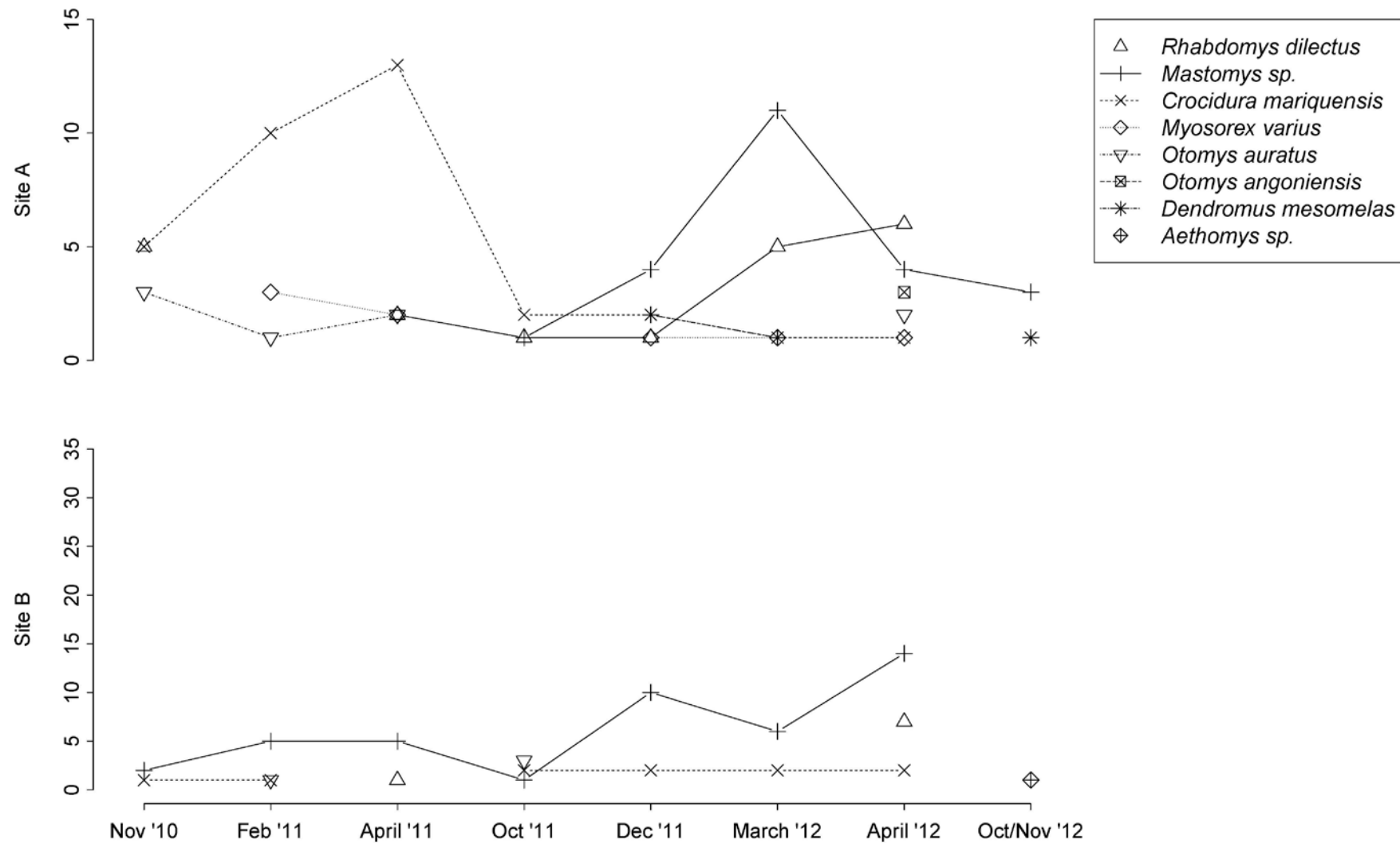


Figure 2.3: Species specific minimum number alive (MNA) values for Sites A and B across the study period. Same symbols without connecting lines between them indicate a period of no captures between periods with captures. No symbol for a specific month indicates that no individuals of that species were caught during that month at the relevant site.

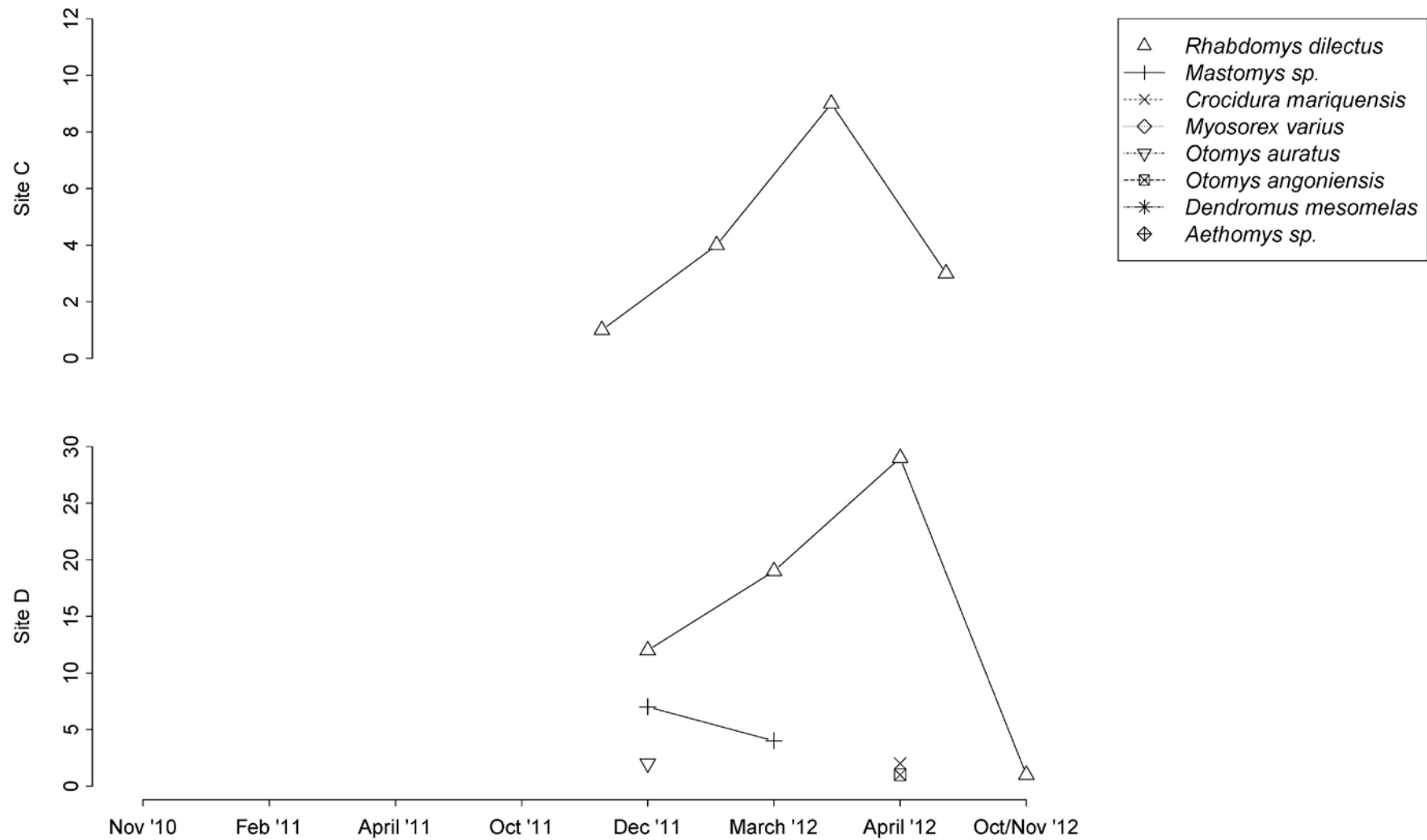


Figure 2.4: Species specific minimum number alive (MNA) values for Sites C and D across the study period. Sites C and D were only added to the study in December 2011. Same symbols without connecting lines between them indicate a period of no captures between periods with captures. No symbol for a specific month indicates that no individuals of that species were caught during that month at the relevant site.

2.3.3 Vegetation

The percent aerial cover (PAC) at Site A showed a decrease from February 2011 to October 2011 in the 0 – 30cm and 30 – 100cm height classes (Kruskal-Wallis $\chi^2 = 74.7763$, $df = 6$, $p = 4.268e^{-14}$ and $\chi^2 = 42.5653$, $df = 6$, $p = 1.422e^{-07}$ respectively), following a fire at the site two weeks before the estimates were made (Figure 2.5). The PAC for the vegetation >100cm showed a large decrease in variation between November 2010 and February 2011 (Figure 2.5), but remained consistently low throughout the study. The PAC of Site A increased from October 2011 to December 2011, with only minor changes occurring in March and April 2012. However, the PAC in the 0 – 30cm class was much lower during October-November 2012 than in April 2012 (Kruskal-Wallis $\chi^2 = 74.7763$, $df = 6$, $p = 4.268e^{-14}$). The land clearance by bulldozers only affected the areas adjacent to Site A, and no vegetation impact is evident in the PAC values. The variation in PAC values for Site A during April 2012 is lower than the months preceding it (Figure 2.5), as the site is characterised by a low plant species diversity comprising *P. australis* and the *E. curvula* and *E. chloromelas* complex.

The PAC at Site B shows a decrease from February 2011 in the 30 – 100cm height class (Kruskal-Wallis $\chi^2 = 30.3239$, $df = 6$, $p = 3.411e^{-05}$) in the October 2011 sampling period following the 2011 dry period and the fire in the week prior to sampling (Figure 2.6). Although the vegetation in Site B showed similar patterns of recovery to Site A over the months following the fire prior to the October 2011 sampling period (Figure 2.6), the variability at Site B remained higher than Site A. Despite this, the species variability within Site B was lower

after the fire than before, indicating that some plant species (e.g. *P. australis* and *E. curvula*) may not have had time to recover before the end of the study period (Figure 2.6).

The vegetation structures at Sites C and D were very similar to each other within each height class (post-hoc multiple comparison test for Kruskal-Wallis $\chi^2 = 286.8585$, $df = 7$, $p < 2.2e^{-16}$). The PAC at Site C did not change over the four months of the Site's inclusion in the study period for any height class (Figure 2.7). However, the PAC in Site D made a significant increase from March 2012 to April 2012 in the 30 – 100cm class (Kruskal-Wallis $\chi^2 = 12.3566$, $df = 3$, $p = 6.256^{-3}$). The majority of the PAC at both sites was contributed by *S. plumosum* and a number of short grass species, and fell into the 0 – 30cm height class. Very few species (e.g. *S. plumosum*) at sites C and D grew tall enough to provide meaningful cover for small mammals in the 30 – 100cm and >100cm height classes (Figures 2.7 and 2.8).

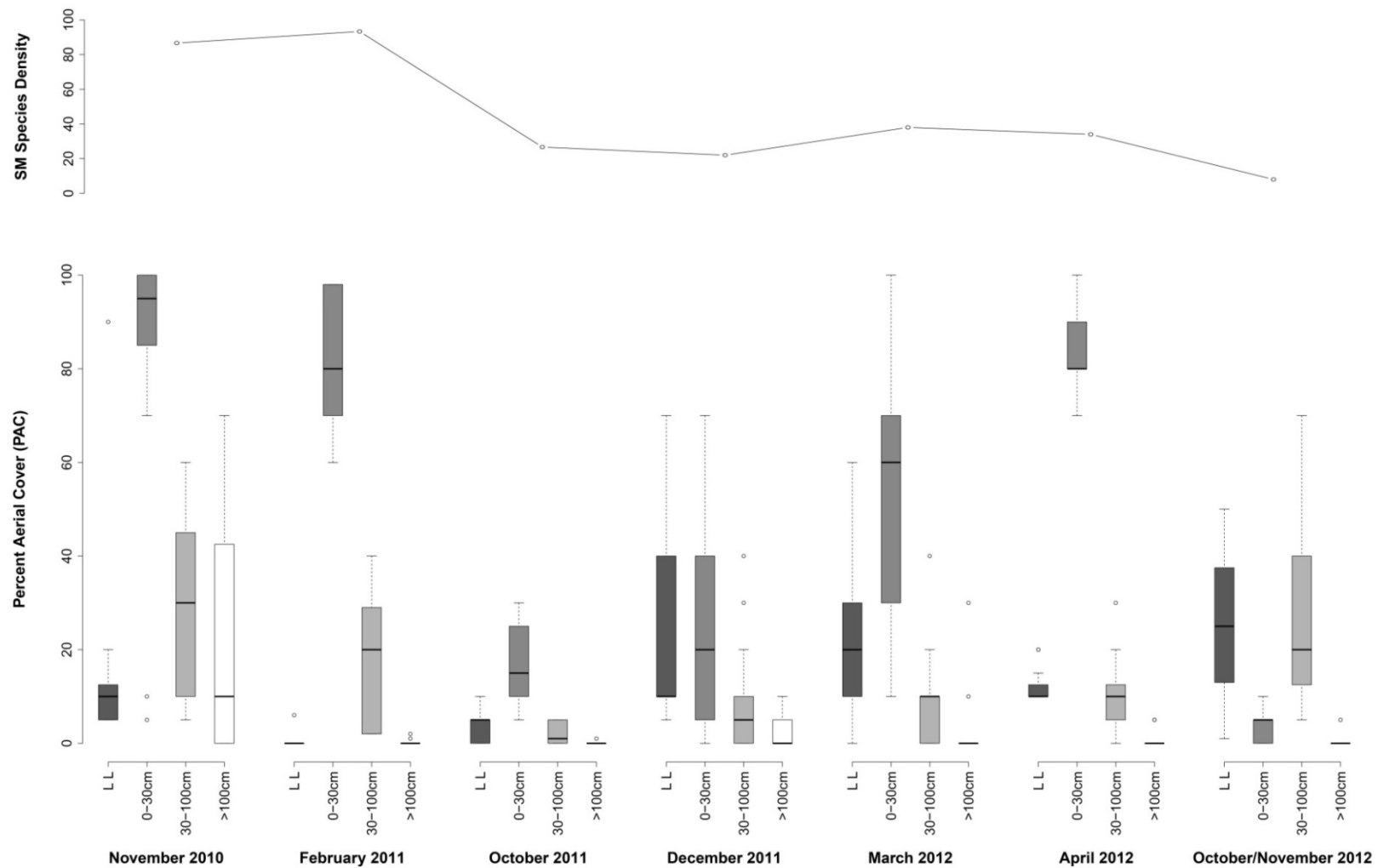


Figure 2.5: Boxplot of Site A vegetation PAC estimates at the four height classes for each month (April 2010 excluded). Open dots represent outliers to the mean. Line graph displays the total density (n/ha) of the Site A small mammal community for each month. No significant correlations were found between PAC and the total density (Spearman's $p > 0.05$). Fires occurred prior to the October 2011 and 2012 sampling periods. Leaf litter (LL) = dark gray; 0 – 30cm = medium gray; 30 – 100cm = light gray; >100cm = no fill.

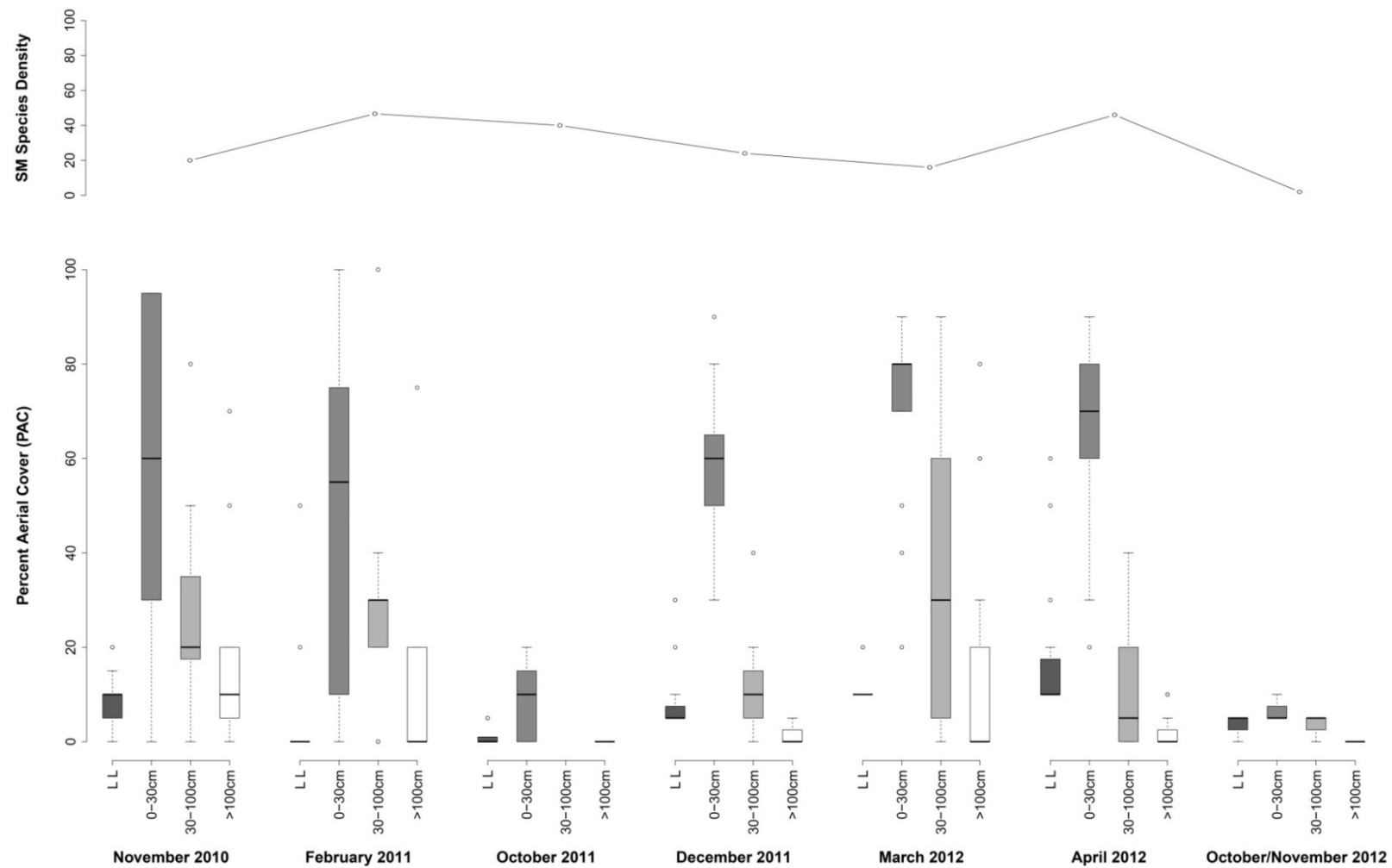


Figure 2.6: Boxplot of Site B vegetation PAC estimates at the four height classes for each month (April 2010 excluded). Open dots represent outliers to the mean. Line graph displays the total density (n/ha) of the Site B small mammal community for each month. No significant correlations were found between PAC and the total density (Spearman's $p > 0.05$). Fires occurred prior to the October 2011 and 2012 sampling periods. Leaf litter (LL) = dark gray; 0 – 30cm = medium gray; 30 – 100cm = light gray; >100cm = no fill.

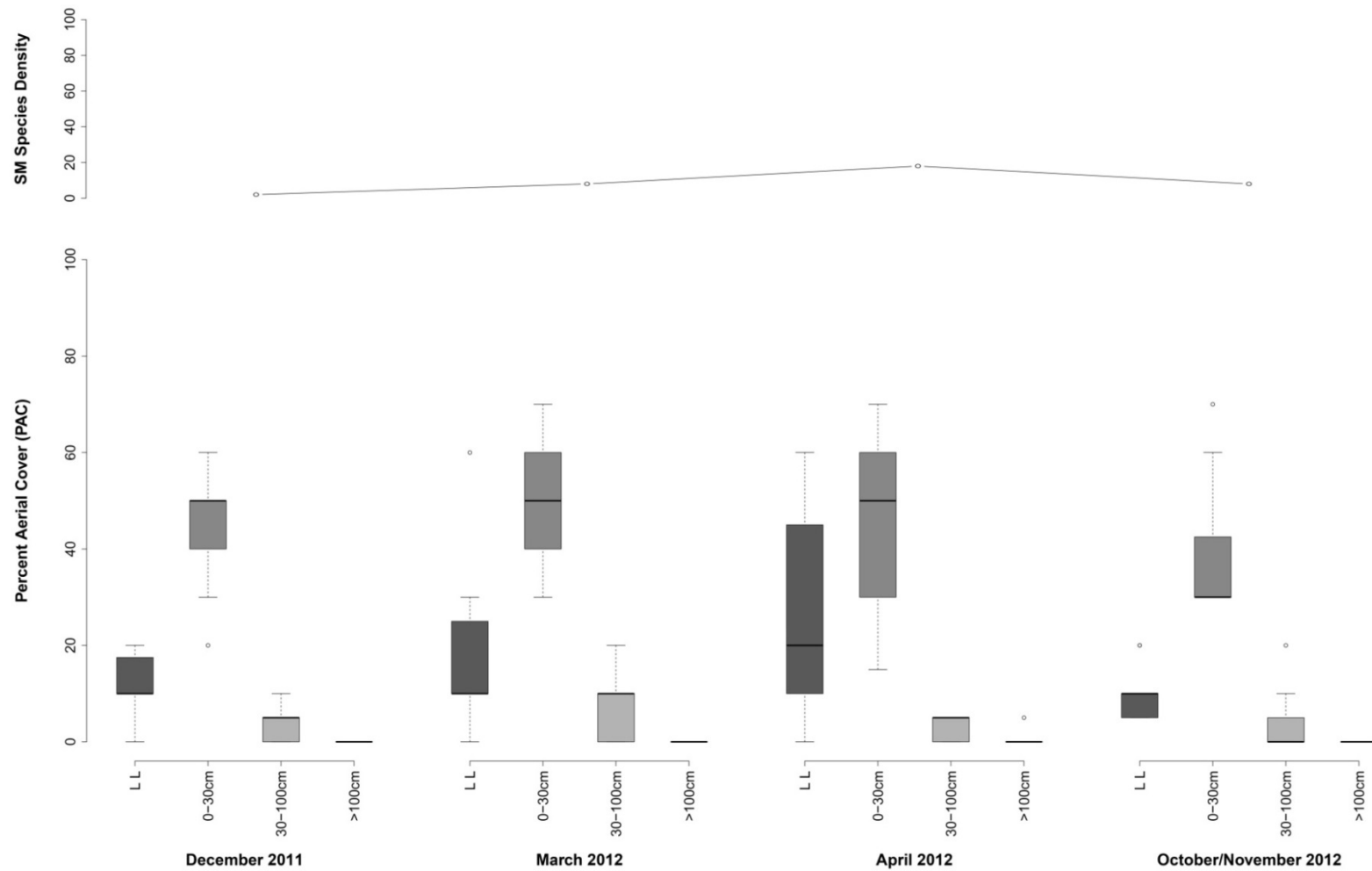


Figure 2.7: Boxplot of Site C vegetation PAC estimates at the four height classes for each month (since the addition of Site C in December 2010). Open dots represent outliers to the mean. Line graph displays the total density (n/ha) of the Site C small mammal community for each month. No significant correlations were found between PAC and the total density (Spearman's $p > 0.05$). Leaf litter (LL) = dark gray; 0 – 30cm = medium gray; 30 – 100cm = light gray; >100cm = no fill.

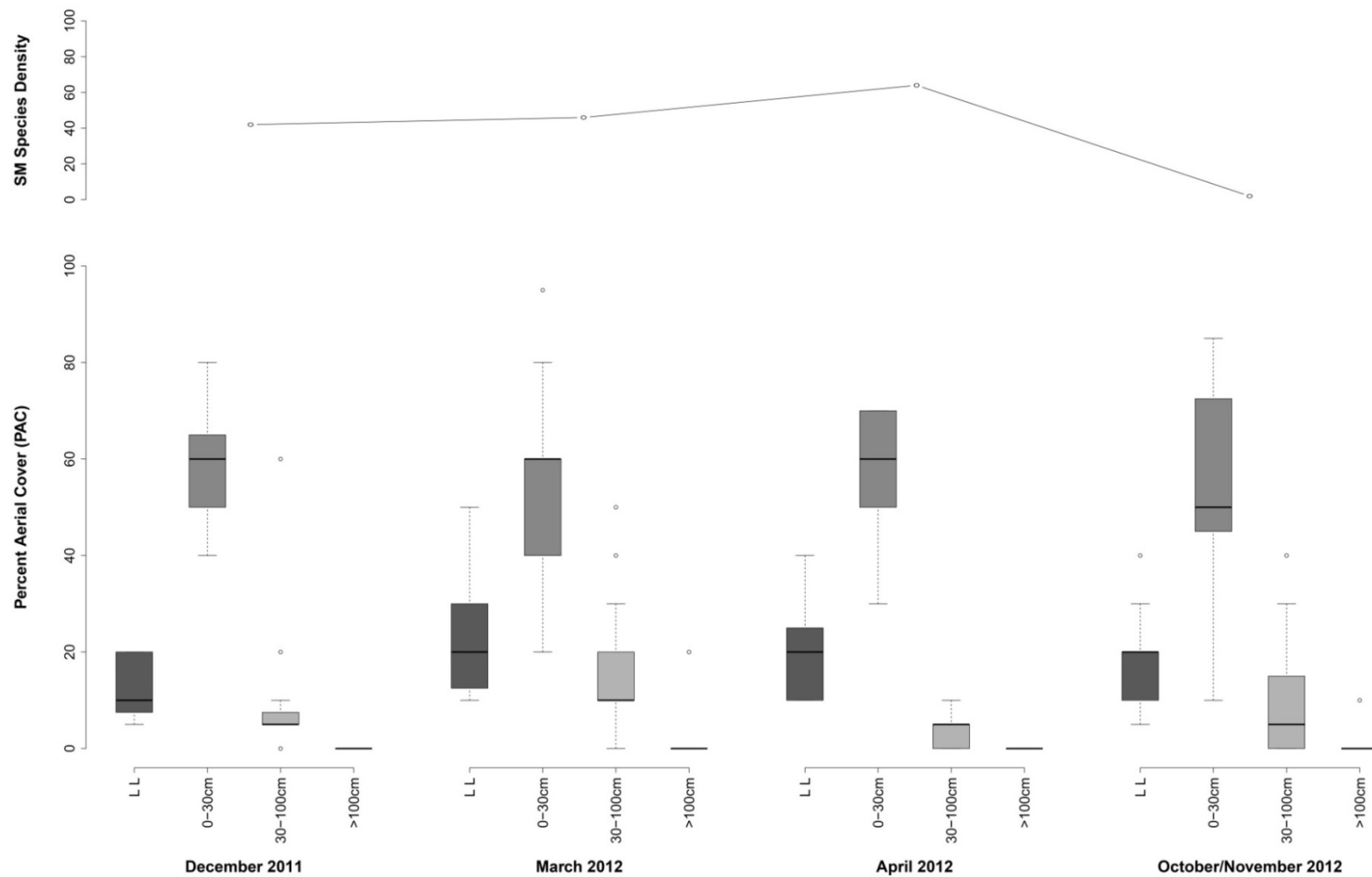


Figure 2.8: Boxplot of Site D vegetation PAC estimates at the four height classes for each month (since the addition of Site D in December 2010). Open dots represent outliers to the mean. Line graph displays the total density (n/ha) of the Site D small mammal community for each month. No significant correlations were found between PAC and the total density (Spearman's $p > 0.05$). Leaf litter (LL) = dark gray; 0 – 30cm = medium gray; 30 – 100cm = light gray; >100cm = no fill.

2.3.4 Vegetation and small mammal interactions

PAC was not correlated with small mammal Simpson's Diversity Index (r_s range -0.8000 – 0.2108, S range 7.8918 – 91.3167, p -value range 0.1289 to 1.000) or small mammal density (r_s range -0.4000 – 0.8165, S range 1.8350 – 59.0551, p -value range 0.1289 – 1.000). However, whilst not statistically significant, the small mammal densities do appear to follow the changes in vegetation that occurred at Site A and Site B (Figures 2.5 and 2.6). A weak correlation ($-0.7 < r_s < 0.7$) is evident in both the 0 – 30cm and 30 – 100cm height classes in Site A ($r_s = 0.6429$, $S = 20.6833$, $p = 0.1389$ and $r_s = 0.6307$, $S = 20.6833$, $p = 0.1289$ respectively). The Spearman's rank correlation test was unable to discern any correlations between PAC and species-specific MNA in Sites B, C and D.

The detrended correspondence analysis (DCA, Figure 2.9) was used to illustrate the effects of each height class on the species-specific MNA within each site across the study period. The further a point falls from the origin (0,0), the larger the influence of the height classes that extend in the similar direction as the point. Hence, within Site A the 30 – 100cm height class had the lowest effect on the species-specific MNA, with only *C. mariquensis* numbers in December 2011 (Site A point 2, Figure 2.9) appearing to be influenced the most by that class. Most notably in Site A, *D. mesomelas* was strongly influenced by the 0 – 30cm height class in October-November 2012 (Site A point 8, Figure 2.9). This characteristic was shared in Site B by both *Mastomys sp.* and *O. auratus* in October 2011 (Site B points 27 and 31 respectively, Figure 2.9), and to not quite

the same extent by *R. dilectus* in Site D during October-November 2012 (Site D point 45, Figure 2.9).

The abundance of *C. mariquensis* displayed the most influence by the higher height classes (30 – 100cm and >100cm) of any species throughout the study during March 2012 (Site B point 25, Figure 2.9), the time during which PAC in Site B for the 30 – 100cm and >100cm classes was highest (Figure 2.6). Inferences as to the influence of vegetation height class on *Aethomys sp.* cannot be made, as only a single individual was captured. However, the position on the DCA graph of the *Aethomys sp.* individual during October-November 2012 (Site B point 22, Figure 2.9) suggests that it is influenced most by the 30 – 100cm height class.

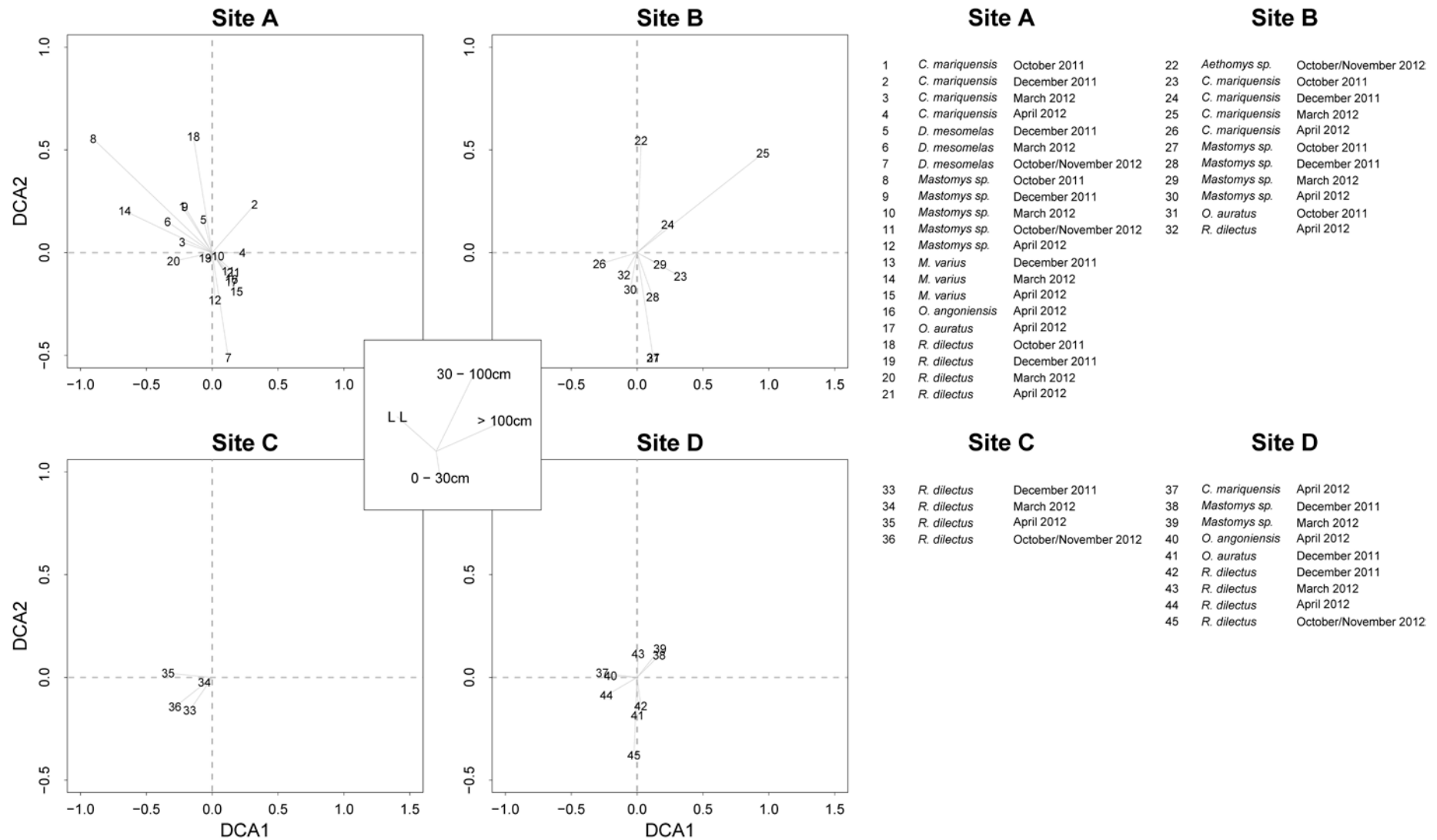


Figure 2.9: Detrended correspondence analysis (DCA) between species-specific MNA and the PAC at each trap in which a capture was made for each month and Site. The direction in which each number is drawn along the first two axes from the DCA corresponds to the direction of the height classes in the central block. Distance from the origin indicates the relative influence of each height class on the species-specific MNA. Axes are in standard deviation (SD) units. Vegetation height classes appear in the centre square (LL = leaf litter, 0 – 30cm, 30 – 100cm, >100cm).

2.4 Discussion

Small mammal species diversity and density is dependent on vegetation structure and the cover it provides, especially in the lower vegetation height class. In general, the vegetation PAC and small mammal species abundance were closely related, with the small mammal species responding to changes in vegetation structure. Whilst the Spearman's rank correlation tests did not return significant relationships between PAC and small mammal abundance, the DCA illustrates that the majority of the species-specific MNA values appear to be most influenced by the lower height classes (LL and 0 – 30cm), which is in concordance with other work (Rosenzweig and Winkaur 1969, Kerley 1992a, Els and Kerley 1996, Monadjem 1999, van Deventer and Nel 2006, Kok *et al.* 2012).

The dependence of small mammals on the lower vegetation height classes for protection and food leaves them more susceptible to the effects of fire (Rowe-Rowe and Lowry 1982, Breytenbach 1987, Kerley and Erasmus 1992, Monadjem and Perrin 1998a, Attuquayefio and Wuver 2003, Lindenmayer *et al.* 2008). Fire can either affect small mammals by directly causing death or injury, or indirectly by removing vegetation which provides food and protection from predation (Bendell 1974, Kern 1981, Kerley and Erasmus 1992, Monadjem and Perrin 1997a, Monadjem and Perrin 1998a, Lindenmayer *et al.* 2008, Banks *et al.* 2011). To escape fire, small mammals can either leave the area or take shelter in fire-free microhabitats such as burrows (Bendell 1974, Frost 1984, Bowland and Perrin 1988, Bronner 1992, Attuquayefio and Wuver 2003, Banks *et al.* 2011). Species that do not burrow, such as *Otomys* species, *D. mesomelas*, *C. mariquensis* and *M.*

varius therefore rely on mobility to escape fires, making them more vulnerable (Breytenbach 1987, Bowland and Perrin 1988, Simons 1991, Monadjem and Perrin 1998a, Attuquayefio and Wuver 2003, Banks *et al.* 2011).

Diurnal species, such as *R. dilectus* which rely on low-lying vegetation for protection from predation leave burnt areas within days of a fire (Breytenbach 1987). Nocturnal species are more able to survive in areas with low cover after a fire, and hence maintain their presence in the burnt area (Willan and Bigalke 1981, Frost 1984, Monadjem and Perrin 1998a, Attuquayefio and Wuver 2003). This survival is increased if the nocturnal species is able to exploit a wide range of food sources, such as the omnivorous *Mastomys sp.*, *D. mesomelas* and to some extent *Aethomys sp.*, but decreases for those species that are either more specialist feeders or prefer dense vegetation, such as the *Otomys* species, *C. mariquensis* and *M. varius* (Willan and Bigalke 1981, Frost 1984, Monadjem and Perrin 1998a).

Such responses to fire disturbances can be seen in this study. In both Site A and B, *Mastomys sp.* abundance increased following the disturbance created by the September/October 2011 fires, while *R. dilectus* abundance in these sites was slower to increase. However, as the vegetation in Site A recovered, with only low levels of localised flooding disturbance along its edges in the wet season, *R. dilectus* abundance increased by above that of *Mastomys sp.* which began to decline by April 2012. A fire removed Site A's vegetation again during September/October 2012, after which no *R. dilectus* were captured in the site, whilst *Mastomys sp.* was captured again, albeit in low numbers during the October-November 2012 sampling period.

In Site B which underwent consistent intermediate disturbance by grazing cattle and the associated trampling of vegetation (Bowland and Perrin 1989, Monadjem 1999, Goheen *et al.* 2004, Hoffmann and Zeller 2005, Muck and Zeller 2006), *Mastomys sp.* numbers persisted throughout the study and continued to increase until the fire in September/October 2012. However, after the fire, the only species captured in Site B was *Aethomys sp.*, and then only a single animal was captured so no inferences can be made with regards to its response to disturbances on a mining property. In Site B, *R. dilectus* did not appear to be able to increase in abundance, possibly as a result of the consistent disturbance by grazing cattle, the two fires in September/October 2011 and 2012, and the intense localised flooding, restricting the growth of *R. dilectus* numbers.

The reverse pattern was seen in Sites C and D where there were no apparent changes in vegetation structure. Site C had no evidence to infer physical disturbance during the study period, and contained no *Mastomys sp.*. Site D underwent consistent, low level disturbance from grazing cattle on the northern border of the site. Across the majority of the site however, disturbance was low enough to allow *R. dilectus* abundance to double between December 2011 and April 2012. During this time, *Mastomys sp.* abundance decreased to the point where no *Mastomys sp.* were caught in April 2012, the same time that *R. dilectus* abundance reached its recorded maximum. However, after the winter dry period of 2012, the small mammal abundance in Site D decreased drastically, with *R. dilectus* density falling from 120 animals/ha to only 2 animals/ha. However, wide fluctuations of *R. dilectus* numbers have been observed before, as the breeding season increases numbers greatly before a high winter mortality rate decreases

population numbers in the following months (Brooks 1974, David and Jarvis 1985, Perrin *et al.* 2001, Schradin and Pillay 2005a and b).

The specific reason for this decrease in small mammal abundance is unknown, as the vegetation structure had not changed, no fires had burnt the site and there was no visible increase in the level of cattle grazing. One possible scenario is the presence of heightened levels chemical pollutants, such as organic and inorganic Hg in the water, soil and vegetation of the site. In most cases, heavy metal pollution causes genetic damage, and as such there are few morphological differences between small mammals in polluted and non-polluted areas, unless the pollution levels are extremely high causing acute physiological responses (Sanchez-Chardi and Nadal 2007). Such a scenario will be investigated further in the following chapters of this dissertation.

Perhaps the most surprising of the studies outcomes was the presence of *D. mesomelas* in Site A. Because of its discontinuous distribution and classification as a specialist species (Apps 2000, Skinner and Chimimba 2005), *D. mesomelas* is considered a rare species that would not usually be found in what is considered a highly impacted mining area. Monadjem (1999) found *D. mesomelas* to be restricted to tall, thick grasslands and as such are negatively affected by overgrazing and other activities that modify the grassland structure. However, *D. mesomelas* was present in very low numbers, and was only found in December 2011, March 2012 and October-November 2012, during the wet season when vegetation PAC in the LL and 0 – 30cm height classes was relatively high. The DCA confirms that the lower vegetation height classes contribute the most to the presence of *D. mesomelas*.

Another species with an apparent sensitivity to disturbance is the insectivorous *C. mariquensis*. Prior to the September/October 2011 fire in Site A, *C. mariquensis* had the highest densities of the small mammals trapped. However, after the fire in September/October 2011, *C. mariquensis* densities underwent a severe decline, and did not increase again during the study period. The *C. mariquensis* densities in Site B are unfortunately not comparable to those of Site A, as the species density remained consistently low (only one to two individuals capture per trapping session). Despite the recovery of vegetation in Sites A and B following the September/October 2011 fires, the *C. mariquensis* numbers did not increase in both sites in the months following the fire. A single occurrence of *C. mariquensis* was recorded at Site D during April 2012, but by the October/November 2012 sampling period, the species was no longer captured at any of the study sites.

The other shrew species, *M. varius* had a similar response to the fire disturbance in Site A, with no captures in October 2011 after the September/October 2011 fire, and very low densities in the following three sample periods. No captures were made of *M. varius* after the September/October 2012 fire. The Vlei Rat, *O. auratus* was not captured in Site A after the September/October 2011 fire, apart from two individuals captured in April 2012. As a strictly herbivorous species, *O. auratus* depends on grasses and newly sprouting *P. australis* shoots, along with their rhizomes and roots, as a food resource (Davis 1972, Bronner and Meester 1988), much of which was burnt by the fires in September/October 2011 and 2012. *O. angoniensis* was only recorded in April 2012 and as such it does not display any patterns.

It is generally supported that a habitat with intermediate levels of disturbance will maintain higher small mammal species diversity (Paine and Vadas 1969, Ferreira and van Aarde 2000, Kryštufek *et al.* 2007, Lindenmayer *et al.* 2008) than a habitat with low or no levels of disturbance (e.g. van Deventer and Nel 2006). Areas with little or no disturbance provide a habitat for more competitive species to dominate, causing less competitive, pioneering species to decrease in abundance (Ferreira and van Aarde 1996, Avenant and Cavallini 2007). Based on the vegetation PAC over the four sampling sites, it seems that the theory that intermediate levels of disturbance do maintain higher species diversity, as Sites A and B had higher levels of disturbance and small mammal species diversity than Sites C and D.

Mastomys sp. is confirmed as a generalist pioneer species in a number of studies, and as such is known to occur in disturbed areas before decreasing in abundance as the vegetation rehabilitates and other, more competitive or specialist small mammal species begin to increase in abundance (e.g. Green *et al.* 1980, Leirs *et al.* 1994, Ferreira and van Aarde 1996, Monadjem and Perrin 1997b, Monadjem and Perrin 1998b, Monadjem 1999, Ferreira and van Aarde 2000, Venturi *et al.* 2004, Odhiambo *et al.* 2008, Avenant and Cavallini 2007, Avenant 2011). Both *R. dilectus* and *Mastomys sp.* occupy similar positions in the food web, and utilise similar diet niches separated only by temporal activity, exploiting insets, seeds and plant material (Veenstra 1958, Curtis and Perrin 1979, Green *et al.* 1980, Monadjem and Perrin 1998b, Venturi *et al.* 2004, Schradin 2005). As such the changes in the respective species abundance could be a reflection of resource competition driving species successions in the study sites as the sites

recover from disturbances. It is possible that the low level of disturbance created by the clearing of vegetation next to the site, coupled with the increased numbers of competitive omnivorous *R. dilectus* and *Mastomys sp.* was enough to force the omnivorous *D. mesomelas* out of the study site.

Kinahan and Pillay (2008) mention several mechanisms that can lead to ecologically similar small mammals coexisting, including spatial segregation (Heske *et al.* 1994), temporal segregation (as with *Mastomys sp.* and *R. dilectus*) (Jones *et al.* 2001), different behaviours (Price *et al.* 2000), social tolerance (Perri and Randall 1999) and dietary partitioning (Kotler and Brown 1999, Price and Heinz 1984). However, in areas with frequent or severe disturbances, such as may be found in a mining area, limited resources restrict these mechanisms, maintaining a level of interspecific competition (Yarnell *et al.* 2007). Repetitive disturbances such as those in Sites A and B can limit resources to the point where mechanisms such as resource partitioning are no longer viable. In the two sites without major disturbance, Sites C and D, the apparent species succession continued until the pioneer species, *Mastomys sp.* was no longer captured.

Clearly an area such as a mining property like the West Wits mine complex is subject to numerous factors that influence small mammal community composition. Disturbances in the form of chemical waste, such as metal pollutants (refer to Appendix A of this chapter and Chapter 4 of this thesis); mechanical transformation, such as bulldozing areas in an attempt to clean up tailings spills; cattle (grazing and trampling); and wildfires and flooding on mining properties can all be regarded as mine-related disturbances that can be controlled through environmental management. However, the prioritisation of practices within the

mining industry often results in detrimental environmental transformation (for example, the prioritisation of extracting gold from ore results in chemical pollutants released into the environment) (Oelofse *et al.* 2007). Hence, it is important for environmental managers in mining areas to have a good understanding of the modified ecosystems with which they work, in order to maintain ecological integrity and biodiversity.

This study provides some working insight into the interactions between small mammal communities and the environment in which small mammals occur on the West Wits mine complex. As the small mammal communities fluctuate in response to disturbances, there is evidence that ecosystems on mine properties adhere to some extent to the intermediate disturbance hypothesis. Using this knowledge, environmental managers can assess an area based on its level of disturbance and estimate the general composition of small mammal communities within the area. However, the variability of the environments found on mine properties can result in the presence of unexpected species and as a consequence small mammal community composition should not be inferred without field data to provide a more accurate description. Once confirmed, the small mammal community can provide a proxy that can give insight into the impact of a cryptic disturbance factor that is difficult or expensive to measure, such as chemical pollutants.

3. Using stable light isotope analysis to compare small mammal isotopic niche widths in a mercury-polluted environment.

3.1 Introduction

The concept of the ecological niche, developed by Hutchinson (1978) describes an n -dimensional hypervolume, with environmental (scenopoetic) and trophic (bionomic) components of niche space as the axes. In recent years, ecologists have begun to use the concept of ‘isotopic niche space’ (Newsome *et al.* 2007) as a proxy for describing ecological niche space (Bolnick *et al.* 2003, Bearhop *et al.* 2004). The use of multivariate bi-plots (typically using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in illustrating the isotopic values of animal tissue can be used to represent the delta-space or isotopic niche of the animal (Newsome *et al.* 2007). The stepwise increase in nitrogen stable light isotope ratio ($\delta^{15}\text{N}$) with trophic level is analogous to Hutchinson’s (1978) bionomic axes (Minagawa and Wada 1984, Wada *et al.* 1987, Fry 1988). The carbon stable light isotope ratio ($\delta^{13}\text{C}$) can be used to describe the primary carbon source, reflecting both the bionomic and scenopoetic axes (Craig 1953, Chisholm *et al.* 1982).

However, it is important to remember that whilst they are comparable, the ecological niche and the isotopic niche are not synonymous and should not be confused (Newsome *et al.* 2007, Jackson *et al.* 2011). As Newsome *et al.* (2007) state, “the isotopic niche is a potentially powerful way to investigate ecological niches”. To this end, ecologists have been developing new statistical tools for comparing the isotopic variation of organisms at varying levels, such as both the community and individual level (Bearhop *et al.* 2004, Layman *et al.* 2007a, 2007b, Turner *et al.* 2010, Hammerschlag-Peyer *et al.* 2011, Jackson *et al.* 2011).

Layman *et al.* (2007a) proposed a set of methods to quantitatively characterise aspects of trophic structure at the community level. One such method was the use of convex hull areas in $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ space to represent trophic diversity. Jackson *et al.* (2011) expanded on the methods proposed by Layman *et al.* (2007a), adding the use of Bayesian standard ellipse areas as a more robust means of dealing with small sample sizes. The area of the convex hull is sensitive to sample size, as the area tends to increase as sample size increases, leaving it susceptible to ‘outliers’ (Layman *et al.* 2007a, Hoeinghaus and Zeug 2008, Jackson *et al.* 2011). The standard ellipse area used by Jackson *et al.* (2011) deals with this issue by utilising Bayesian inference techniques, resulting in a ‘core area’ of approximately 40% of the values in the isotopic niche regardless of sample size (Batschelet 1981). However, the convex hulls used by Layman *et al.* (2007a) better emphasize the role of the individual in the overall dispersion of the community’s isotopic niche space (Jackson *et al.* 2011).

Conspecific individuals within a population have traditionally been assumed to be ecologically equivalent (Bolnick *et al.* 2003, Svanbäck and Bolnick 2007). The width of a population’s isotopic niche space depends on the individuals living within the community (Bearhop *et al.* 2004). A population with a large isotopic niche space could either contain generalist individuals which exploit a wide range of food resources (Type A generalisation), or specialist individuals that each focus on specific, but isotopically different food resource (Type B generalisation) (Van Valen 1965, Grant *et al.* 1976, Bearhop *et al.* 2004). However, alongside the refinement of stable light isotope methodology, a large number of studies have shown that ‘generalist’ species are in fact comprised of

relatively specialised individuals (Type B generalisation) (Table 1 in Bolnick *et al.* 2003). This resource use specialisation, or ‘individual specialisation’, occurs in populations containing heterogeneous individuals which only exploit a subset of the population’s overall resource base (Bolnick *et al.* 2003). Individual specialisation facilitates factors that influence a population, such as its stability, fitness and the level intraspecific competition (Schoener 1986, Bolnick *et al.* 2003).

In the face of interspecific competition, classic theory (e.g. Hutchinson 1978, Van Valen 1965) suggests that a species experiencing more interspecific competition will have a smaller realised niche than a species experiencing less interspecific competition. As interspecific competition increases, resource use overlap between sympatric species will eventually result in competition exclusion (Pianka 1974). Additionally, elevated mercury pollution reduces foraging ability (Weis and Khan 1990, Grippo and Heath 2003). Hence, the isotopic niche space of a species should decrease as interspecific competition and mercury pollution increases (Weis and Khan 1990, Grippo and Heath 2003, Jackson *et al.* 2012). Alternatively, Svanbäck and Bolnick (2007) argue that it is also possible for the isotopic niche space of a species to increase as interspecific competition increases, as individuals are forced into exploiting a wider range of food resources in order to meet energy requirements. However, Jackson *et al.* (2012) found that interspecific competition resulted in a decrease or displacement of the less-competitive species’ isotopic niche width in a field study on two sympatric introduced benthic omnivores, carp (*Cyprinus carpio*) and Louisiana swamp crayfish (*Procambarus clarkii*) in Lake Naivasha, Kenya.

In this chapter, the position of each species in the binomial delta-space was calculated and assessed as a description of the relative trophic position of species or individuals within a community. This method is more detailed than the aggregate variability measures used in Chapter 2 as it employs continuous measures of trophic structure (Layman *et al.* 2007a). Based on Elton's (1927) conception of the consumer trophic niche, community-wide metrics provide a means of comparing specific trophic structures for consumer species on a spatial and temporal scale, giving insight into fundamental differences in small mammal diet potentially attributable to mercury pollution both within and between the study sites. Seasonal variation in food resources (such as declining seed and insect abundance in the early dry season) was expected to cause a shift in small mammal delta-space position towards more constant food sources such as foliage. Additionally, it was expected that small mammal community trophic niche width would shift with variable mercury contamination and interspecific competition: either a) isotopic niche width would decrease with increasing mercury pollution and interspecific competition as they exclude access to certain food resources (Jackson *et al.* 2012); or b) isotopic niche width would increase with increasing mercury pollution and interspecific competition as animals are forced to exploit a wider range of resources (Svanbäck and Bolnick 2007) .

3.2 Materials and Methods

The following materials and methods for the study period, study sites and small mammal capture are the key points taken from the more detailed descriptions found in the Material and Methods section of Chapter 2: Population Dynamics.

3.2.1 *Study period*

The study was conducted between November 2010 and November 2012 (hereafter referred to as the study period). Eight trapping sessions were undertaken at Sites A and B; November 2010, February 2011, April 2011, October 2011, December 2011, March 2012, April 2012 and October/November 2012. Four trapping session were undertaken at sites C and D; December 2011, March 2012, April 2012 and October/November 2012. These sampling periods were an attempt to cover a full seasonal cycle.

3.2.2 *Study sites*

The study was conducted at four study locations (sites A to D) on an AGA property on South Africa's Gauteng Highveld region, referred to as the West Wits mine complex (WWMC, Figure 3.1) Varkenslaagte catchment, near Carletonville, Gauteng. These sites encompassed a range of Hg (and other mining) contamination with the two 'upstream' sites (A: S 26° 25.936', E 27° 22.183' and B: S 26° 26.820', E 27° 20.740') were relatively close to TSFs (~440m), while the

two 'downstream' sites (C: S 26° 26.513', E 27° 20.176' and D: S 26° 26.395', E 27° 19.998') were further from TSFs (~1000m and ~1250m respectively). The relatively small distance between Sites C and D (~350m) carried some potential for small mammal movement between the sites.

A previous study (Makiese 2012) measured the concentrations of inorganic mercury (IHg (Hg^{2+})) and methylmercury (MHg) in stream water (Appendix A) and soils (Appendix B) at a number of locations along the length of the stream (Figure 1; Appendix C). Surface soil (0 – 5cm, Appendix B) samples taken within the reed beds at Site A ($344.2\mu\text{g.kg}^{-1}$), Site B ($628.4\mu\text{g.kg}^{-1}$), and Site D ($124.9\mu\text{g.kg}^{-1}$) were much higher than the surface soil samples taken within the Varkenslaagte stream ($38.9\mu\text{g.kg}^{-1}$ at Site A to $58.5\mu\text{g.kg}^{-1}$ just upstream of Site B). The Dutch Target and Intervention Values (SRC 2009) (published by the Government of the Netherlands and also known as the 'New Dutch List') specifies that the target value for soil mercury concentration is 0.30mg.kg^{-1} , with a soil mercury concentration of 5.15mg.kg^{-1} requiring further investigation and a soil mercury concentration above 10mg.kg^{-1} requiring immediate intervention.

Concentrations of IHg and MHg measured in the stream water (Makiese 2012) increased from upstream of Site A, downstream towards Site B, with the IHg concentration in the dam above Site B being much higher at 13.055ng/L within the dam water (Appendix A). Concentrations of water IHg and MHg decreased downstream of Site B with the lowest concentrations just downstream of Site D (Appendix A). The total mercury concentration (IHg and MHg combined) was also measured by Makiese (2012) in the common reed,

Phragmites australis (37.8µg/kg) within Site A, and bulrushes, *Typha spp.* (31.9µg/kg) approximately 1300 metres downstream of Site A.

Mercury is highly toxic and a major chemical pollutant produced by the mining process as it has a LD₅₀ (Lethal Dose for 50% of tested rats and mice) in the range 25.9mg Hg/kg to 77.7mg Hg/kg in the form of mercuric chloride (Kostial *et al.* 1978, ATSDR 1999). The minimum risk level (MRL) for IHg consumption is 2µg/kg/day, whereas for MHg the MRL is 0.3µg/kg/day (ATSDR 1999). Hence, a rodent with an average weight of 100g would need to ingest only 0.2µg of IHg and 0.03µg of MHg per day to be at risk of mercury toxicity.

Site A was located in a grassland and reed bed used for the phytoremediation of runoff from the northern TSF. The stream was lined with *P. australis* (Ca.) Trin. ex Steud., the common reed, as it is a robust C₃ wetland macrophyte capable of removing heavy metals from soil and water (Ghassemzadeha *et al.* 2008, Bonanno and Lo Giudice 2010). Also common at Site A are couch grass (*Cynodon dactylon* (L.) Pers.), weeping love grass (*Eragrostis curvula* (Schrud.) Nees.), and boer love grass (*E. chloromelas* Steud. Complex).

Site B was situated approximately 3kms downstream of Site A, below a dam on the Varkenslaagte Stream. The stream in this site was lined with multiple wetland plant species, the most prominent of which were bulrushes (*Typha spp.*) and sedges (*Schoenoplectus spp.*), with some grasses (e.g. *Cynodon spp.* and *E. curvula*) and some *P. australis* prior to the October 2011 trapping session. Site C was located approximately 1km downstream of Site B, and was further from the

Varkenslaagte Stream relative to the other three sites (approximately 50m from the bank as opposed to 10m or less in the other three sites). As such, Site C did not support any wetland plant species. The site prominently supported bankrupt bush (*Seriphium plumosum*, formerly *Stoebe vulgaris*) and multiple grass species (including *Cynodon spp.*, *Eragrostis spp.*, and *Aristida spp.*).

Site D was located approximately 350m downstream of Site C, and was in close proximity to a wetland formed between the convergence of the Varkenslaagte Stream and an overflow channel/stream from a nearby tailings effluent pond. Site D supported bulrushes (*Typha spp.*) and the common reed (*P. australis*) distributed along the northern edge, but was mostly vegetated with a similar mixture of grasses (including *Cynodon spp.*, *Eragrostis spp.*, and *Aristida spp.*) and bankrupt bush (*S. plumosum*) to Site C.

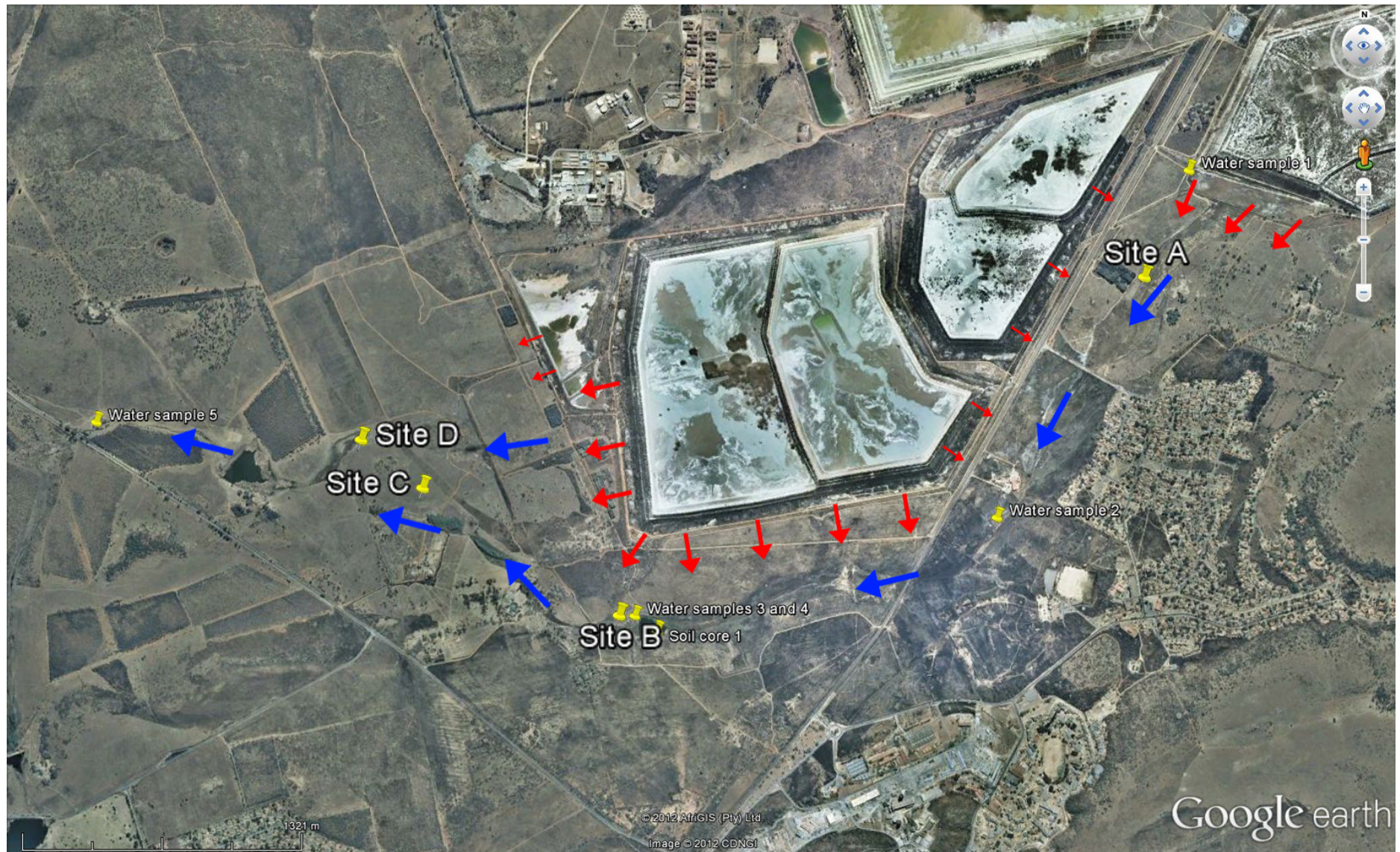


Figure 3.1: Satellite image (property of Google) of the four study sites (large yellow pins, A to D) located along the Varkenslaagte Stream (blue arrows indicate direction of flow) at the AngloGold Ashanti West Wits Mine Complex, Gauteng. Red arrows indicate direction of contamination plumes from the adjacent TSFs. Small yellow pins indicate locations of water and soil samples taken for preliminary Hg and IHg analysis (Makiese 2012).

3.2.3 *Small mammal captures*

A trapping session consisted of trap deployment over four consecutive nights in each of the four study sites. Four consecutive nights is the minimum limit determined by Avenant and Cavallini (2007). Fifty live traps were set for small mammals (Enviro-care MRT1 box traps, ± 10 cm x 7.5 cm x 25 cm), deployed in a 5x10 grid formation with 10 to 12 metres between traps (total area $\sim 500\text{m}^2$). All trapped small mammals were identified according to genus and species (according to Apps 2000 and Skinner and Chimimba 2005). Captures were undertaken under the certification from the University of the Witwatersrand Ethics Committee to trap, handle and collect hair samples from captured small mammals (Certificate number 2011/45/2A). Permission was also granted by the Gauteng Provincial Government to do research within the province (Certificate CPF6 No. 0151).

3.2.4 Stable isotope samples

Small mammal hair samples (0.45 – 0.65mg) were analysed for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable light isotopes using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of Cape Town (Preston and Owens 1983, Brenna *et al.* 1997). All isotope samples were prepared according to the guidelines stipulated by the Stable Isotope Facility, University of California Davis

(<http://stableisotopefacility.ucdavis.edu/13cand15nsamplepreparation.html>).

3.2.5 Data analysis

The relatively new isotopic niche width metrics developed by Layman *et al.* (2007a) and Jackson *et al.* (2011) were used for exploratory analyses of community wide trophic niches (detailed below). Data were prepared using Office Excel 2010 (Microsoft Corporation 2010) before running statistics in R 2.15.1 (R Development Core Team 2010). Isotope ratios were expressed as per mil (parts-per-thousand over a reference material, ‰) using a standard reference material in δ -notation as described in Fry (2008). The analyses described below were performed on three levels of small mammal community: overall metrics where data were combined across the entire study (space and time) for each species, spatial metrics where data were compared both within and between each site, and temporal metrics where data were compared across time within each site. As the spatial effect on small mammal isotopic niche width was an important factor, the temporal metrics for each month were calculated within each site without

combining data across sites within each month, as the spatial metrics did with time. Only species containing three or more individuals were included in the community analyses.

Five community-wide metrics that describe important aspects of trophic structure were calculated based on the methods described by Layman *et al.* (2007a): $\delta^{15}\text{N}$ range (NR), $\delta^{13}\text{C}$ range (CR), mean distance to centroid (CD), mean nearest neighbour distance (MNND), and standard deviation of nearest neighbour distance (SDNND). The first three metrics (NR, CR and CD) are community-wide measures of trophic diversity (involving all species within an area) and can be used to estimate isotopic niche width. The last two metrics (MNND and SDNND) are representations of relative distance between species in a trophic structure and can be used to describe trophic redundancy (Layman *et al.* 2007a). These five metrics will hereafter be collectively referred to as ‘Layman metrics’. These metrics are calculated using mean species specific isotopic values, calculated by averaging the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic values of all individuals within each species (Layman *et al.* 2007a).

Stable light isotope Bayesian ellipses (hereafter referred to as SIBER metrics) were calculated as bi-plots for C and N as a representation of ‘niche space’, following the methods described by Jackson *et al.* (2011). The SIBER metrics were calculated using the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of multiple individuals of each species within the small mammal community (performed using the “siar” package by Parnell [2010] in R 2.15.1 [R Development Core Team 2010]). The SIBER metrics use Bayesian inference to calculate the standard ellipse area for a species (reported in permil as it incorporates $\delta^{15}\text{N}$ as the y-axis and $\delta^{13}\text{C}$ as the x-

axis), with a correction for the small sample size of rare species included in the calculations. Hence, the corrected standard ellipse area will hereafter be referred to as the SEAc. The overlap between SEAc's was calculated as an indication of isotopic niche redundancy, and is reported as the percentage of the total SEAc of each variable that falls within the overlapping area.

SIBER metrics were chosen for the comparison of small mammal isotopic niche widths over the total area of a Convex Hull method described by Layman *et al.* (2007a) as they are theoretically unbiased with respect to sample size, incorporate uncertainty in the sampled data and error arising from the sampling process, and allow for robust statistical comparison between communities despite differences in sample size (Jackson *et al.* 2011).

In this chapter, I compared isotopic niche widths between small mammal species and their relative exposure to mercury pollution, but did not attempt to infer potential food resources being utilised by the animals. Hence, I did not make assumptions about the sources of the respective isotopes. I also did not infer trophic level based on nitrogen as again, the food sources have not been measured, and neither have the pathways of construction, recombination and sorting of the isotopes during digestion, metabolism and tissue synthesis (Martinez del Rio and Wolf 2005). Food source isotope values have been taken from the literature (Appendix E) and are only used as a reference for where the food sources might fall on the binomial delta-space. They are not used in any calculations and may not represent actual values at the study sites. *Aethomys sp.* was not included in the calculations as only a single individual was captured and sampled.

3.3 Results

3.3.1 Small mammal captures

A full description of the small mammal capture results can be found in Chapter 2: Population Dynamics. Over the entire study period (eight sampling occasions), a total of 4120 trap nights were recorded. Trapping success averaged 11.9% (0.5% to 40.5%) across all four sites within the study period, with Site D in the April 2012 trapping session having the highest capture success of 40.5%. The total number of unique individuals captured across the entire study period was 262, with 221 recaptures made (483 total captures made).

Across the entire study period, six rodent species (order Rodentia, families Muridae and Nesomyidae) and two shrew species (order Soricomorpha, family Soricidae) were captured within the study sites (Table 3.1). Three Muridae (sub-family Murinae) species were: the striped mouse, *Rhabdomys dilectus* (n = 147); the multimammate mouse, *Mastomys* sp. (n = 77); and the rock rat (or veld rat), *Aethomys* sp. (n = 1). Two Muridae (sub-family Otomyinae) species were: the vlei rat, *Otomys auratus* (n = 14); and the Angoni vlei rat, *O. angoniensis* (n = 4). The Nesomyidae (sub-family Dendromurinae) species was Brant's climbing mouse, *Dendromus mesomelas* (n = 4). The two insectivorous shrew (family Soricidae) species captured were: the swamp musk shrew, *Crocidura mariquensis* (n = 46); and the forest shrew, *Myosorex varius* (n = 8). The striped mouse, *R. dilectus*, was the only species found at all four sites during the study period. *R. dilectus* was also the only truly diurnal species captured during the study, as the other captured species are all mainly nocturnal, with occasional short periods of activity during

the early morning or late afternoon (Bronner and Meester 1988, Brooks 1972, Choate 1972, Davis 1972, Kingdon 1997, Apps 2000, Skinner and Chimimba 2005).

The small mammal species can also be grouped according to trophic strategy. *R. dilectus*, *Mastomys* sp., *Aethomys* sp. and *D. mesomelas* are generally classed as omnivores (Brooks 1972, Choate 1972, Curtis and Perrin 1979, Perrin 1980, Perrin and Curtis 1980, De Graaff 1981, Rowe-Rowe 1986, Willan 1992, Kingdon 1997, Monadjem 1997b, Monadjem and Perrin 1997b, Monadjem and Perrin 1998b, Apps 2000, Baxter and Hansson 2001, Skinner and Chimimba 2005, Kryštufek *et al.* 2007, Mulungu *et al.* 2011). *Otomys auratus* and *O. angoniensis* are classed as specialist herbivores (Davis 1972, Curtis and Perrin 1979, Perrin 1980, Perrin and Curtis 1980, De Graaff 1981, Bronner and Meester 1988, Willan 1992, Monadjem 1997b, Kingdon 1997, Apps 2000, Baxter and Hansson 2001, Skinner and Chimimba 2005). The two shrew species are, by definition, insectivorous (Yates 1984, Churchfield 1990, Kingdon 1997, Monadjem 1997b, Apps 2000, Skinner and Chimimba 2005).

3.3.2 Overall metrics

The small mammal community had an overall nitrogen range (NR) of 5.60‰, carbon range (CR) of 3.63‰, total area (TA) of 10.06‰, distance to centroid (CD) of 1.70‰, and mean nearest neighbour distance (MNND) and standard deviation of nearest neighbour distance (SDNND) of 1.39‰. The overall small mammal community metrics were calculated using the mean isotope composition of each species (hence the small ranges), whilst species metrics were calculated using the isotopic compositions of individuals within the species. The insectivorous shrew, *C. mariquensis* occupied the highest overall position on the $\delta^{15}\text{N}$ scale (Figure 3.2) with the second largest NR (11.96‰), whilst the other insectivorous shrew species, *M. varius* had the lowest NR and CR (3.43‰ and 2.55‰ respectively). The omnivorous *Mastomys sp.* and *R. dilectus* displayed large NRs (11.79‰ and 12.27‰ respectively), as well as the largest CRs (11.41‰ and 13.20‰ respectively, Table 3.1).

Based on the SIBER metrics (Table 3.1), the seven small mammal species were divided into three loose groups of SEAc values. The three largest SEAc values were spaced well apart within delta-space, with *O. angoniensis* having the largest SEAc (57.95‰), *O. auratus* the second largest (40.99‰), and *Mastomys sp.* the third (33.26‰). The mid-group of SEAc values were spaced much closer relative to each other within delta-space. The SEAc of *C. mariquensis* (26.46‰) was similar to that of *R. dilectus* (24.27‰) and *D. mesomelas* (23.49‰). The final group only contained *M. varius* with the smallest SEAc (10.30‰). Despite their similar NR and CR, *Mastomys sp.* had a larger SEAc than *R. dilectus*. The CD for each species tended to increase in the same order as the SEAc, with the exception

of *O. auratus*. The shrew, *M. varius*, with the smallest SEAc, had the smallest CD (1.98‰). Despite having a medium NR and CR, *O. angoniensis* had the highest SEAc (57.95‰), CD (4.63‰), MNND (2.71‰) and SDNND (4.82‰) a possible reflection of the influence of differences in individual diet.

The largest overlaps in isotopic niche were among the species with large, centrally distributed SEAc values, whilst the species with relatively small SEAc values distributed towards the edge of the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ranges had smaller SEAc overlaps, as would be expected. The largest overlap of SEAc was between *O. auratus* and *Mastomys sp.* ($Oau = 72.45\%$, $Ms = 89.64\%$), two species with large SEAc. The second and third largest overlaps were between *O. auratus* and *O. angoniensis* ($Oau = 70.58\%$, $Oa = 49.93\%$) and *O. angoniensis* and *Mastomys sp.* ($Oa = 49.57\%$, $Ms = 86.70\%$). The overlap between *D. mesomelas* and *C. mariquensis* was the smallest overlap between two species ($Dm = 5.74\%$, $Cm = 5.10\%$). The overlap between *R. dilectus* and *Mastomys sp.* comprised nearly half of *R. dilectus*' SEAc ($Rd = 43.73\%$, $Ms = 32.03\%$). The largest overlap for *C. mariquensis* occurred with *O. auratus* ($Cm = 35.59\%$, $Oau = 22.97\%$).

Table 3.1: Overall metrics describing each small mammal species captured, composited across the study. Numbers of individuals sampled per species appear in parentheses. SEAc = sample size corrected standard ellipse area; NR = $\delta^{15}\text{N}$ range; CR = $\delta^{13}\text{C}$ range; CD = distance to centroid; MNND = mean nearest neighbour distance; SDNND = standard deviation of nearest neighbour distance.

Species	SEAc	NR	CR	CD	MNND	SDNND
<i>O. auratus</i> (9)	40.99	9.01	11.04	4.21	2.24	0.91
<i>O. angoniensis</i> (4)	57.95	10.47	9.59	5.25	4.32	5.14
<i>M. varius</i> (3)	10.30	3.43	2.55	1.75	2.47	0.17
<i>C. mariquensis</i> (16)	26.46	11.96	8.63	3.85	1.32	1.25
<i>D. mesomelas</i> (4)	23.49	4.44	7.28	3.15	3.06	1.20
<i>R. dilectus</i> (87)	24.27	12.27	13.20	3.53	0.62	0.61
<i>Mastomys sp.</i> (63)	33.13	11.80	11.41	4.26	0.68	0.53

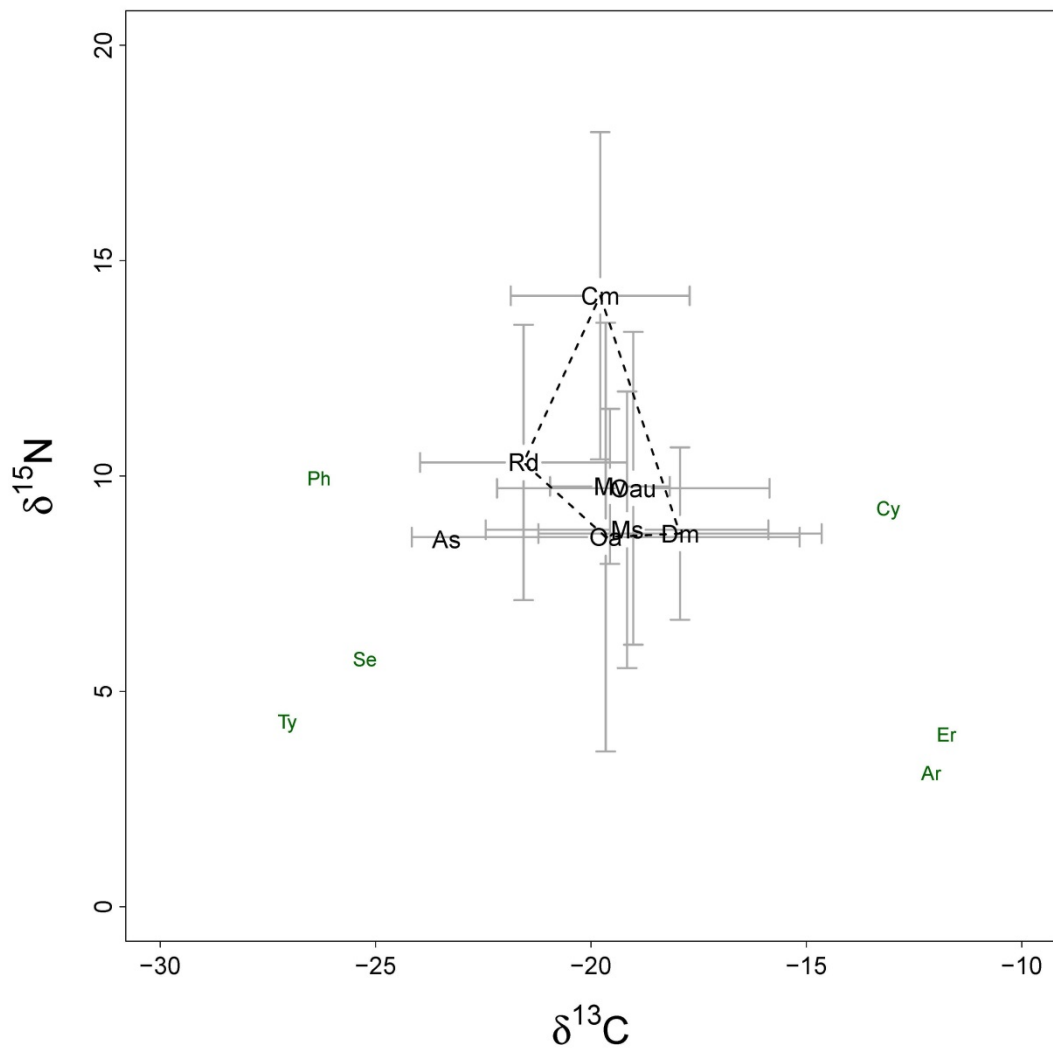


Figure 3.2: Overall community stable isotope bi-plots describing each small mammal species captured, composited across the study. Each point with error bars represents the mean value of 3 – 87 individuals of that species. *Aethomys sp.* (point Ac) has no error bars as only a single sample was collected during the study. The convex hull used to calculate the total area (TA) of the community's isotopic niche width is represented by the dotted line. Vegetation values from literature included as an indication of general vegetation position in isotopic niche space (from Appendix E). Small mammal abbreviations: Ms = *Mastomys sp.*; Rd = *R. dilectus*; Oau = *O. auratus*; Oa = *O. angoniensis*; Cm = *C. mariquensis*; Dm = *D. mesomelas*; Mv = *M. varius*; As = *Aethomys sp.*. Vegetation abbreviations: Ph = *P. australis*; Se = sedges; Ty = *Typha spp.*; Cy = *Cynadon spp.*; Er = *Eragrostis spp.*; Ar = *Aristida spp.*

3.3.3 Spatial metrics – within site comparisons

The NR of the small mammal community at Site A (5.46‰) was higher than Sites B and C (4.81‰, Table 3.2), possibly a reflection of a wider variety of nitrogen sources at Site A. However, the CR for Site A (3.80‰) and Site D (3.86‰) were both higher than that of Site B (1.87‰, Table 3.3). Hull TA (3.35‰, Figure 3.3) and CD (1.75‰) were smallest at Site B. However, variation within individual species at Site B (e.g. species-specific NR, CR, CD, and MNND) was relatively high compared to the other sites (Table 3.3). Site A had the smallest MNND (1.39‰) and SDNND (0.38‰) as more species were captured at Site A (7 species) than in Sites B and D (4 species). As only a single species was captured at Site C, *R. dilectus*, it could not be included in the site-specific community calculations.

Table 3.2: Site-specific community metrics, composited across time. Numbers of individuals sampled per site appear in parentheses. [Hg] = Reed bed surface soil mercury concentration in $\mu\text{g.kg}^{-1}$ (ppb); Spp. = number of species sampled with $n > 2$; NR = $\delta^{15}\text{N}$ range; CR = $\delta^{13}\text{C}$ range; TA = total area of hull; CD = distance to centroid; MNND = mean nearest neighbour distance; SDNND = standard deviation of nearest neighbour distance.

Site	[Hg]	Spp.	NR	CR	TA	CD	MNND	SDNND
A (58)	344.2	7	5.46	3.80	11.31	2.19	1.39	0.38
B (42)	628.4	4	4.81	1.87	3.35	1.75	1.65	1.48
D (63)	124.9	4	4.81	3.86	8.52	2.33	2.44	1.64

Within Site A (Table 3.3), the omnivorous *D. mesomelas* had the greatest SEAc (23.49‰), CD (3.15‰) and MNND (3.06‰), but also had a low sample size ($n = 4$ individuals). *Mastomys sp.* had the largest CR (10.42‰), whilst *R. dilectus* had the largest NR (7.52‰). The CD for the majority of Site A's species was similar (within 2 – 3‰), with only *C. mariquensis* (1.75‰) and *D. mesomelas* (3.15‰) falling outside the range (Table 3.4). *Mastomys sp.* had the lowest MNND (0.79‰), and a relatively low SDNND (0.79‰), as it had the largest sample size ($n = 23$). The herbivorous *O. angoniensis* had both the smallest SEAc (4.15‰) and NR (1.11‰), much lower than that of *O. auratus* (SEAc = 15.20‰ and NR = 6.68‰). However, the difference between the CR of *O. angoniensis* (4.71‰) and *O. auratus* (6.31‰) was not as large (Table 3.3).

Within Site B, sample size restricted analysis to two omnivorous and one insectivorous species (Table 3.3). The omnivorous *Mastomys sp.* and *R. dilectus* had very similar SEAc values (39.53‰ and 39.52‰ respectively) despite their differences in sample size ($n = 29$ and $n = 6$ individuals respectively). However, *R. dilectus* had the smallest NR (9.61‰) and largest CR (13.20‰), a relatively large difference compared to the difference between the NR and CR of *Mastomys sp.* (11.65‰ and 10.70‰ respectively). The SEAc (17.68‰), CR (4.38‰) and CD (2.69‰) of the insectivorous *C. mariquensis* in Site B was less than half of that of the two omnivorous species. As only *R. dilectus* was found at Site C, it could not be compared to other species within the site. However, the *R. dilectus* at Site C had a much smaller SEAc (10.02‰) and CR (4.06‰) than the *R. dilectus* populations at the other three sites (Table 3.3).

Within Site D, sample size restricted analysis to *Mastomys sp.* and *R. dilectus* (Table 3.4). The SEAc of *Mastomys sp.* (40.10‰) at Site D was more than double that of *R. dilectus* (18.32‰). Although *Mastomys sp.* and *R. dilectus* had very similar CR values (9.19‰ and 9.00‰ respectively), *Mastomys sp.* had a larger NR (11.86‰) than that of *R. dilectus* (9.98‰). As the sample size of *R. dilectus* ($n = 52$) was much higher than that of *Mastomys sp.* ($n = 11$), the CD, MNND and SDNND of *R. dilectus* were all lower than those of *Mastomys sp.* (Table 3.3).

The smallest overlap between species' SEAc values at Site A was between *Mastomys sp.* and *C. mariquensis* ($Ms = 3.12\%$ and $Cm = 2.85\%$), followed by *R. dilectus* and *C. mariquensis* ($Rd = 4.28\%$ and $Cm = 4.10\%$). The largest overlap at Site A was between *Mastomys sp.* and *R. dilectus* ($Ms = 60.20\%$ and $Rd = 57.43\%$). The same was seen at Site B, with the largest overlap between *Mastomys sp.* and *R. dilectus* (55.79% and 55.80%), a small overlap between *R. dilectus* and *C. mariquensis* ($Rd = 2.24\%$ and $Cm = 5.01\%$), and no measurable overlap between *Mastomys sp.* and *C. mariquensis*. Site D only had a single overlap, that between *Mastomys sp.* and *R. dilectus* (17.40% and 38.09%).

Table 3.3: Site-specific metrics describing each small mammal species captured with $n > 3$, composited across time. Numbers of individuals sampled per species appear in parentheses. SEAc = sample size corrected standard ellipse area; NR = $\delta^{15}\text{N}$ range; CR = $\delta^{13}\text{C}$ range; CD = distance to centroid; MNND = mean nearest neighbour distance; SDNND = standard deviation of nearest neighbour distance.

Site	Species	SEAc	NR	CR	CD	MNND	SDNND
A	<i>Mastomys sp.</i> (23)	14.12	6.23	10.42	2.66	0.79	0.79
	<i>R. dilectus</i> (13)	14.81	7.52	7.32	2.59	1.12	1.02
	<i>O. auratus</i> (5)	15.20	6.68	6.31	2.99	2.40	2.18
	<i>O. angoniensis</i> (3)	4.15	1.11	4.71	2.07	1.91	2.19
	<i>C. mariquensis</i> (7)	15.44	7.38	4.47	2.90	1.17	0.61
	<i>M. varius</i> (3)	10.30	3.43	2.55	1.75	2.47	0.17
	<i>D. mesomelas</i> (4)	23.49	4.44	7.28	3.15	3.06	1.20
B	<i>Mastomys sp.</i> (29)	39.53	11.65	10.70	4.67	1.04	0.66
	<i>R. dilectus</i> (6)	39.52	9.61	13.20	5.15	2.59	1.16
	<i>C. mariquensis</i> (7)	17.68	10.71	4.38	2.69	2.28	1.71
C	<i>R. dilectus</i> (16)	10.02	9.29	4.06	2.55	0.93	0.63
D	<i>Mastomys sp.</i> (11)	40.10	9.98	9.19	4.38	2.32	1.20
	<i>R. dilectus</i> (52)	18.32	11.86	9.00	3.07	0.65	0.48

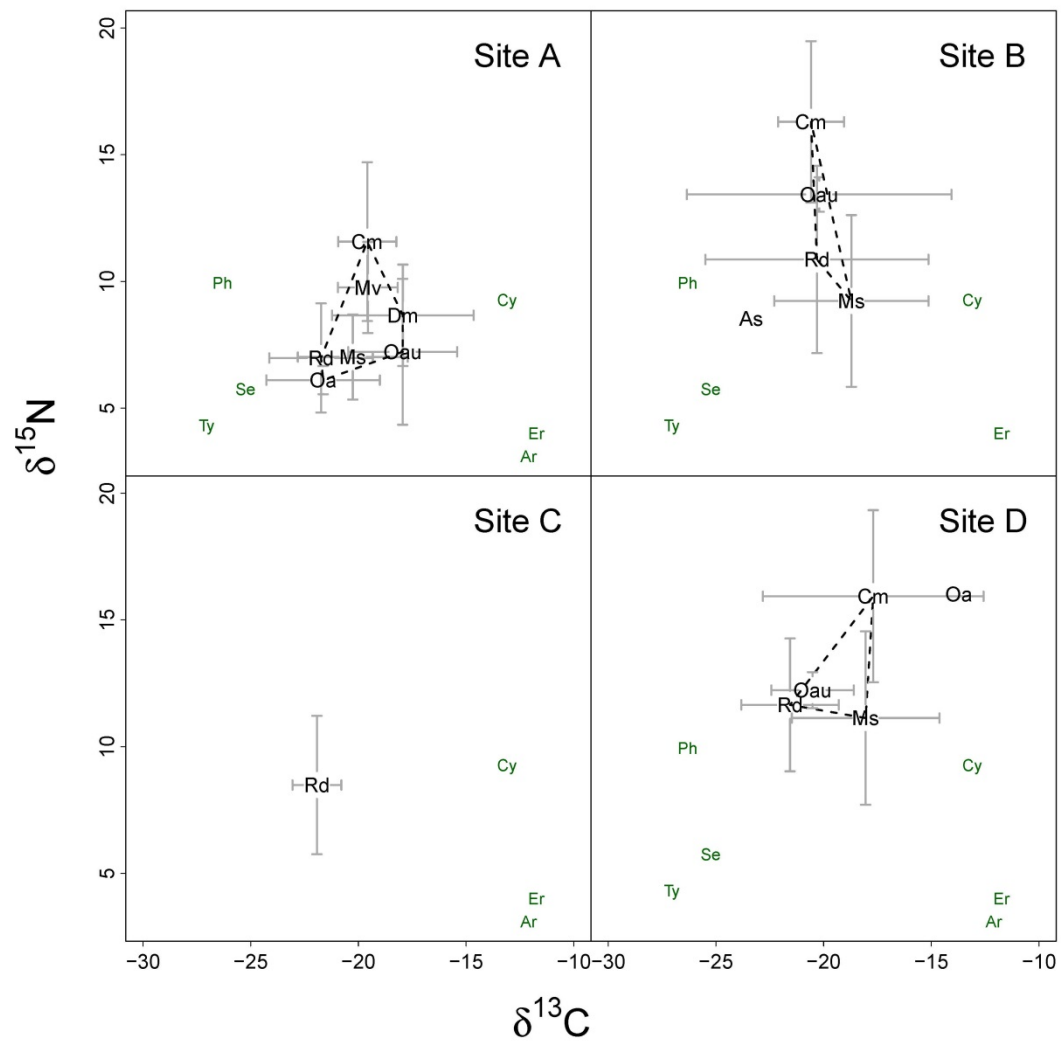


Figure 3.3: Site-specific community stable isotope bi-plots describing each small mammal species captured, composited across time. Each point with error bars represents the mean value of individuals of that species. Points with no error bars represent species with $n < 2$, excluding them from analysis. The convex hull used to calculate the total area (TA) of the community's isotopic niche width is represented by the dotted line. Vegetation values from literature included as an indication of general vegetation position in isotopic niche space (from Appendix E). Small mammal abbreviations: Ms = *Mastomys* sp.; Rd = *R. dilectus*; Oau = *O. auratus*; Oa = *O. angoniensis*; Cm = *C. mariquensis*; Dm = *D. mesomelas*; Mv = *M. varius*; As = *Aethomys* sp.. Vegetation abbreviations: Ph = *P. australis*; Se = sedges; Ty = *Typha* spp.; Cy = *Cynadon* spp.; Er = *Eragrostis* spp.; Ar = *Aristida* spp.

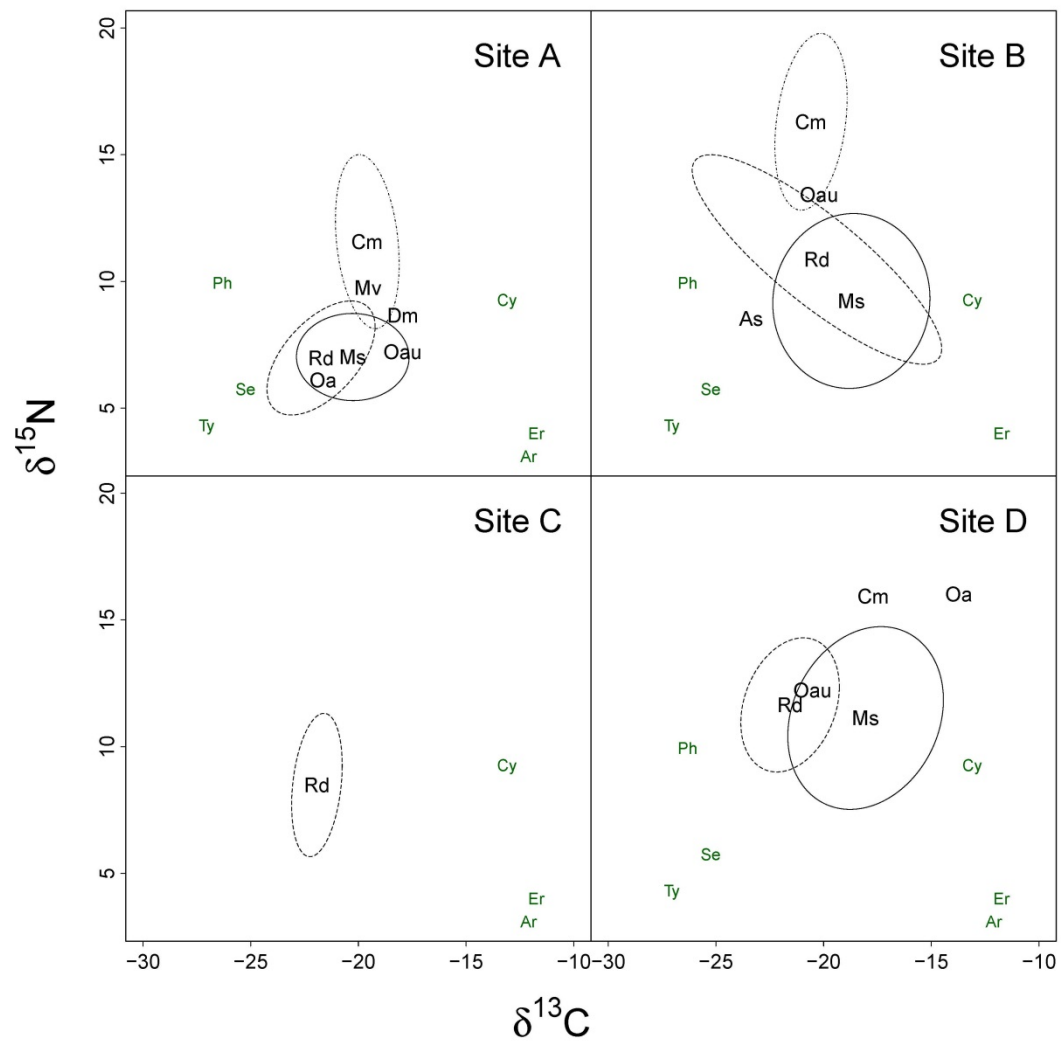


Figure 3.4: Site-specific community stable isotope bi-plots describing each small mammal species captured, composited across time. Ellipses represent the SEAC for species with $n > 3$ in more than one site, i.e.: Ms = solid ellipse; Rd = dashed ellipse; Cm = dotted ellipse. Vegetation values from literature included as an indication of general vegetation position in isotopic niche space (from Appendix E). Small mammal abbreviations: Ms = *Mastomys* sp.; Rd = *R. dilectus*; Oau = *O. auratus*; Oa = *O. angoniensis*; Cm = *C. mariquensis*; Dm = *D. mesomelas*; Mv = *M. varius*; As = *Aethomys* sp.. Vegetation abbreviations: Ph = *P. australis*; Se = sedges; Ty = *Typha* spp.; Cy = *Cynodon* spp.; Er = *Eragrostis* spp.; Ar = *Aristida* spp.

3.3.4 Spatial metrics – between site comparisons

Sample size again restricted analysis to *Mastomys sp.*, *R. dilectus* and *C. mariquensis* (Table 3.3, Figures 3.4 and 3.5). The NR and CR for *Mastomys sp.* were both largest in Site B (11.65‰ and 10.65‰ respectively), where disturbance impact was highest, but interestingly, Site D had a marginally larger SEAc (40.10‰) than in Site B. Both SEAc and NR of *Mastomys sp.* at Site D were larger than those at Site A, where disturbance impact was higher (Table 3.3). Unlike *Mastomys sp.*, the SEAc of *R. dilectus* and *C. mariquensis* were largest in Site B. However, *R. dilectus* SEAc was also smaller in Site A than Site D despite their levels of disturbance. Hence, Site B had the largest relative variation in species-specific diet, suggesting a larger diet breadth in individuals where disturbance impact was highest.

The overlap of SEAc for *Mastomys sp.* (Figure 3.5) was smallest between Site A (17.04%) and Site D (6.00%), and largest between Site B (65.55%) and Site D (64.62%). Similarly, the overlap of SEAc for *R. dilectus* (Figure 3.5) was smallest between Sites A and D where no measurable overlap was found, and largest between Site B (34.39%) and Site D (74.18%). The overlap of the *R. dilectus* SEAc for Site C was largest with Site A (C = 54.67% and A = 37.01%), followed by Site D (C = 35.58% and D = 19.47%) and Site B (C = 24.82% and B = 6.29%). The overlap between Sites A and B (Figure 3.5) was lowest for *R. dilectus* (A = 6.17% and B = 2.31%), followed by *C. mariquensis* (A = 13.71% and B = 11.97%) and *Mastomys sp.* (A = 69.11% and B = 24.7%).

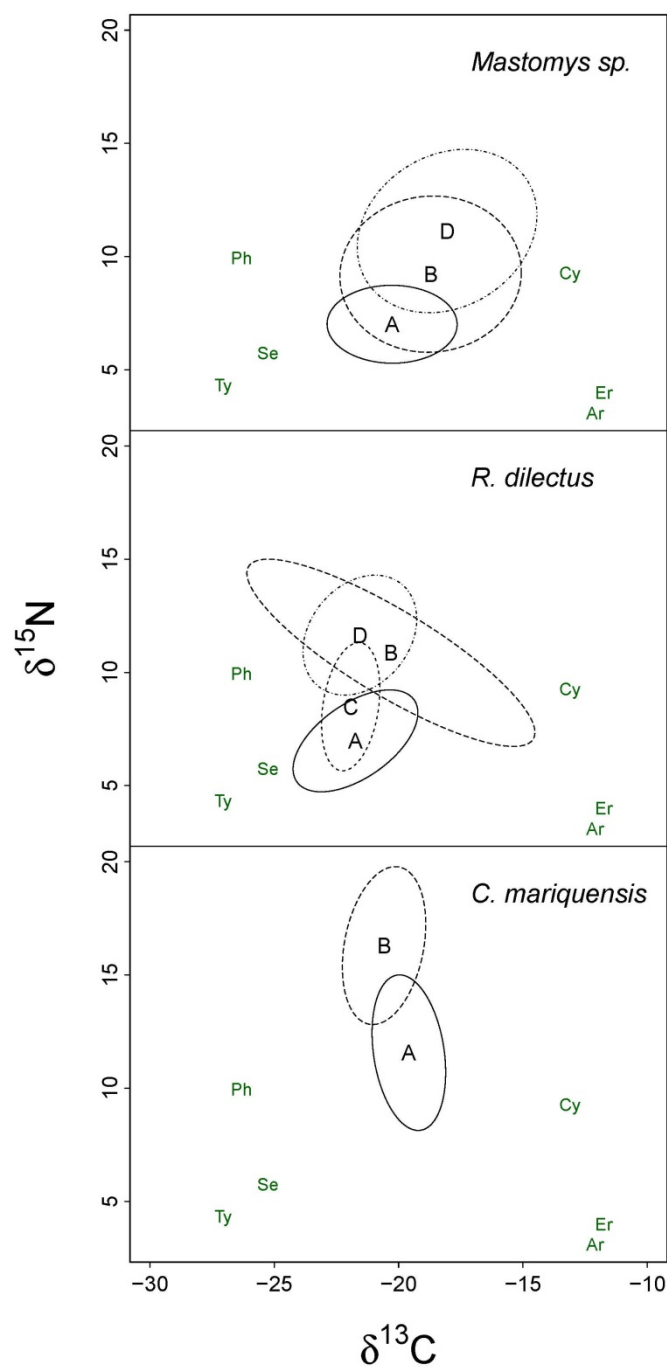


Figure 3.5: Stable isotope bi-plots describing the site-specific SIAR ellipses of *Mastomys sp.*, *R. dilectus* and *C. mariquensis*. Ellipses represent the SEAc for species with $n > 3$ in more than one site, i.e.: Site A = solid ellipse; Site B = dashed ellipse; Site C = dotted ellipse; Site D = dot-dash ellipse. Vegetation values from literature included as an indication of general vegetation position in isotopic niche space (from Appendix E). Vegetation abbreviations: Ph = *P. australis*; Se = sedges; Ty = *Typha spp.*; Cy = *Cynadon spp.*; Er = *Eragrostis spp.*; Ar = *Aristida spp.*

3.3.5 Temporal metrics

Sample size limited analyses to *Mastomys sp.* and *R. dilectus* post December 2011 (Table 3.4, Figures 3.6 and 3.7). The tissue turn-over rate of fur on the lower back of captive *Mastomys sp.* and *R. dilectus* was approximately three months. Therefore, the measured isotopic composition of fur samples represents an average isotopic composition of the food sources utilised by the individual over the three months prior to sampling (Tieszen *et al.* 1983, Fry 2008).

Within Site A, the SEAc of *Mastomys sp.* in April 2012 did not overlap with any other months' SEAc (Figure 3.6). All of the metrics calculated for *Mastomys sp.* at Site A in April 2012 were the smallest for the species across the entire study (Table 3.4) as the individuals sampled in April 2012 had very similar isotopic compositions, possibly as a result of decreased plant diversity. The overlap between the SEAc of the other three months remained low (range 0.17 – 33.74%). Within Site B, the overlap of SEAc between December 2011 and the months following was very low (Figure 3.6), a possible indication of a change in isotopic trophic dynamics caused by the TSF spill in January 2012. The greatest overlap between two months for *Mastomys sp.* occurred in Site D, between December 2011 and March 2012 (Dec = 42.52% and Mar = 82.96%), suggesting minimal changes in individual diets during that time.

The *R. dilectus* individuals sampled in Site A had lower calculated metrics in March 2012 than in April 2012 (Table 3.4). The SEAc of April 2012 in Site A almost completely overlaps that of March 2012 (Mar = 93.79% and Apr = 17.12%, Figure 3.7), as the SEAc of April 2012 is more than five times larger than that of March 2012 (Table 3.4), the opposite of what was displayed by

Mastomys sp. Whilst Site B only produced an ellipse in April 2012 (Figure 3.7), the SEAc was large relative to other sites (Table 3.4). The NR and CR of the April 2012 *R. dilectus* population at Site B were also relatively high (9.61‰ and 9.90‰ respectively) compared to the average *R. dilectus* NR and CR across all sites (6.93‰ and 5.55‰ respectively).

The SEAc of *R. dilectus* in Site C displayed a large increase from March 2012 (3.54‰) to April 2012 (10.75‰) and October/November 2012 (15.91‰). Despite the increase in SEAc and CR (Table 3.4), the trophic niches did not move much in the binomial delta-space (Figure 3.7); resulting in relatively large overlaps between all months (range 13.80 – 83.44%). Interestingly, whilst SEAc and CR increased continuously in time in Site C, the NR decreased from March 2012 to October/November 2012 (Table 3.4).

The SEAc of Site D almost doubled from December 2011 (11.47‰) to March 2012 (21.42‰), as March 2012 had the largest NR and CR (Table 3.4). Site D's SEAc decreased from March 2012 to April 2012 (17.17‰) along with NR and CR (Table 3.4), despite the number of captured individuals increasing far more from March to April 2012, than from December 2011 to March 2012. March 2012 and April 2012 had the largest overlap of all of the *R. dilectus* populations (Mar = 69.25% and Apr = 86.42%) and were closely positioned to each other within the binomial delta-space suggesting little change in diet during this period. Both *Mastomys sp.* and *R. dilectus* were situated lower on the $\delta^{15}\text{N}$ scale in December 2011 compared to March and April 2012 across all four sites.

Table 3.4: Metrics describing *Mastomys sp.* and *R. dilectus* populations within each site across time, where $n > 3$. Numbers of individuals sampled per species appear in parentheses. Spp. = Species; SEAc = sample size corrected standard ellipse area; NR = $\delta^{15}\text{N}$ range; CR = $\delta^{13}\text{C}$ range; CD = distance to centroid; MNND = mean nearest neighbour distance; SDNND = standard deviation of nearest neighbour distance.

Spp.	Site	Month	SEAc	NR	CR	CD	MNND	SDNND
<i>Mastomys sp.</i>	A	Dec '11 (4)	3.26	3.17	3.47	1.95	0.89	0.42
		Mar '12 (11)	10.66	4.01	5.45	2.27	1.05	0.89
		Apr '12 (4)	0.95	0.75	1.35	0.57	0.59	0.29
		Oct/Nov '12 (3)	12.46	2.76	10.42	4.75	3.98	5.71
	B	Dec '11 (10)	15.25	8.62	4.53	2.55	1.03	0.67
		Mar '12 (4)	28.90	2.75	10.70	4.70	2.85	1.11
		Apr '12 (14)	37.18	11.36	9.06	4.28	1.52	1.33
	D	Dec '11 (7)	55.86	9.98	9.19	4.97	3.51	0.67
		Mar '12 (4)	28.63	4.86	6.89	3.06	3.59	1.09
<i>R. dilectus</i>	A	Mar '12 (4)	3.63	1.31	2.98	1.04	1.12	0.62
		Apr '12 (6)	19.86	6.46	6.24	2.97	2.33	2.31
	B	Apr '12 (5)	20.37	9.61	9.90	4.22	2.90	2.32
	C	Mar '12 (4)	3.54	6.93	0.69	2.39	2.12	0.84
		Apr '12 (7)	10.75	6.88	2.96	2.45	1.14	1.09
		Oct/Nov '12 (4)	15.91	4.92	4.06	2.46	2.96	0.35
	D	Dec '11 (11)	11.47	7.62	6.03	2.34	1.31	1.13
		Mar '12 (15)	21.42	10.93	9.00	2.81	1.38	1.70
		Apr '12 (25)	17.17	7.75	8.09	2.94	0.68	0.34

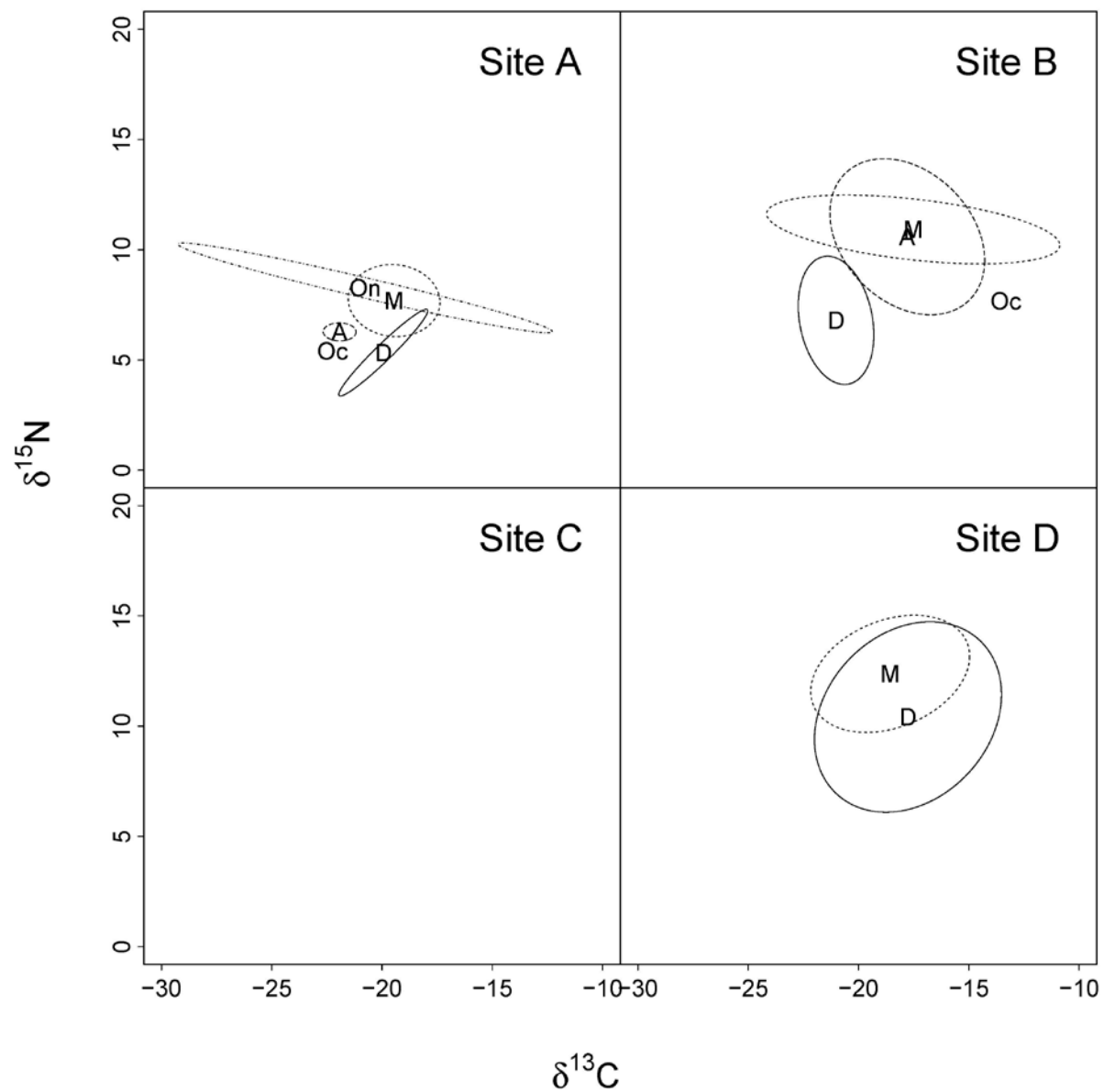


Figure 3.6: Stable isotope bi-plots describing the SIAR ellipses of *Mastomys sp.* through time in Sites A to D. No *Mastomys sp.* were captured in Site C. Points represent the centroid of ellipses (if present) or samples with $n < 3$. Ellipses represent the SEAc for species with $n > 3$ in more than one month, i.e.: Oc = October 2011 (no ellipse); D = December 2011 (solid ellipse); M = March 2012 (dotted ellipse); A = April 2012 (dashed ellipse); On = October/November 2012 (dot-dash ellipse).

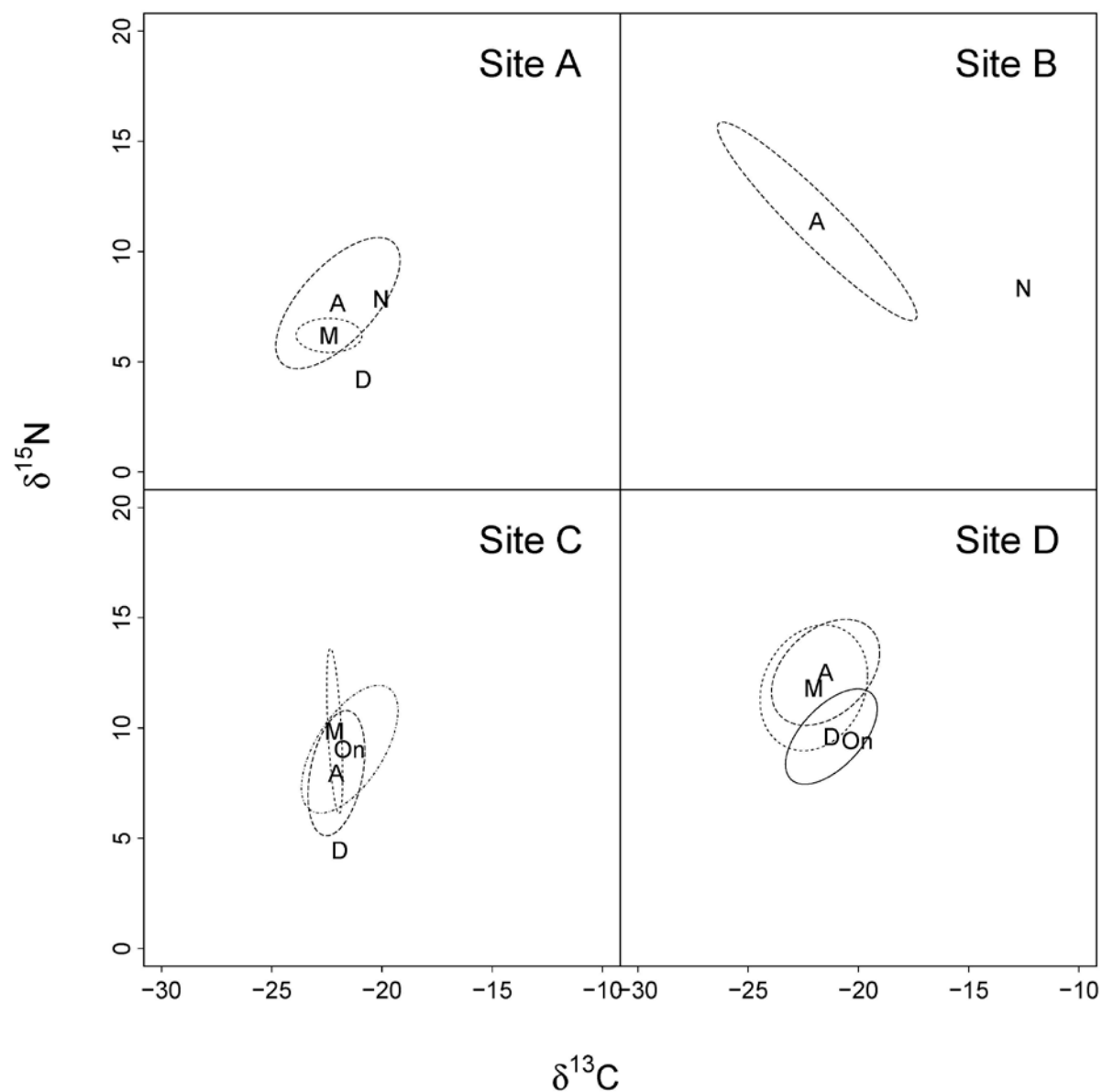


Figure 3.7: Stable isotope bi-plots describing the SIAR ellipses of *R. dilectus* through time in Sites A to D. Points represent the centroid of ellipses (if present) or samples with $n < 3$. Ellipses represent the SEAc for species with $n > 3$ in more than one month, i.e.: N = November 2010 (no ellipse); Oc = October 2011 (no ellipse); D = December 2011 (solid ellipse); M = March 2012 (dotted ellipse); A = April 2012 (dashed ellipse); On = October/November 2012 (dot-dash ellipse).

3.4 Discussion

3.4.1. What do isotope analyses suggest about specific trophic structures of consumer species on spatial and temporal scales?

a) By species

The insectivorous shrew, *C. mariquensis* displayed enriched $\delta^{15}\text{N}$ relative to all other small mammal species captured across all four study sites (Figure 3.2, 3.3 and 3.4). This enrichment, linked with the large $\delta^{15}\text{N}$ range and smaller $\delta^{13}\text{C}$ range reflects the species' reliance on insects as a food source (Kingdon 1997, Apps 2000, Skinner and Chimimba 2005). Interestingly, the second insectivorous shrew species, *M. varius* did not appear as enriched in ^{15}N at a general community level (Figure 3.2). However, at Site A, the only site at which *M. varius* was captured, *M. varius* also displayed enriched $\delta^{15}\text{N}$ relative to the other species (Figures 3.3 and 3.4).

The two herbivorous species, *O. angoniensis* and *O. auratus* had similar positions in delta-space as the four omnivorous species at a community level (Figure 3.2). However, the high variability of the positions in delta-space and the $\delta^{15}\text{N}$ ranges of *O. angoniensis* and *O. auratus* suggests a larger variety of nitrogen sources than would be expected of specialist herbivores that do not prey on insects (Davis 1972, Curtis and Perrin 1979, Kingdon 1997, Apps 2000, Skinner and Chimimba 2005).

The omnivorous *R. dilectus* and *Aethomys* sp. (of which only a single individual was captured) were relatively ^{13}C depleted; positioning them closer to

C3 plants than to C4 plants. The aggregate variability measures used in Chapter 2 suggested a succession whereby resource competition resulted in *R. dilectus* numbers increasing above those of *Mastomys sp.* once vegetation had recovered from a disturbance. However, isotope analyses suggest that resource competition between the two species was lower than expected, based on isotopic niche overlap and *R. dilectus* being relatively ^{13}C depleted compared to *Mastomys sp.*. The final omnivorous species, *D. mesomelas* displayed slight ^{13}C and ^{15}N enrichment relative to both *R. dilectus* and *Mastomys sp.* at Site A, the only site where *D. mesomelas* was caught. The differences in their positions in delta-space, coupled with individual variation within each species (illustrated by CR and NR) suggest that some level of resource partitioning allows coexistence between small mammal species (Kinahan and Pillay 2008).

b) Spatial scale

The differences between species' isotopic niche widths are more evident at the spatial scale, suggesting that site-specific food resources influence small mammal diet. At a site-specific community level, the smallest total area of the convex hull was at Site B, where small mammal hair and reed bed soil mercury concentrations were highest (discussed further in Chapter 4). The small total area and distance to centroid indicates that species were grouped relatively closely in delta-space at Site B, in this case because of the relatively low $\delta^{13}\text{C}$ range and species diversity of the Site B community. However, at a species level, variation (Figures 3.3 to 3.5) was highest at Site B as individuals in each species exploited a wider range of potential food sources than their counterparts at the other sites.

Lower $\delta^{15}\text{N}$ at Site A in *Mastomys sp.* and *R. dilectus* suggested a lower reliance on *P. australis* and seeds than at other study sites. *P. australis* has a relatively high leaf $\delta^{15}\text{N}$ (range 9.59 – 10.79‰, Wainright *et al.* 2000), whilst the seeds of *Eragrostis spp.* and *Aristida spp.* have enriched $\delta^{15}\text{N}$ relative to their leaves and stems (*Eragrostis spp.* ~1.5‰ enrichment, *Aristida spp.* ~0.5‰ enrichment, Codron *et al.* 2005a, 2005b).

Only the isotopic composition of *Mastomys sp.* clearly reflected the relative abundance of C4 plants from Site A through to Site D (Figure 3.5). The shift of *Mastomys sp.* towards C4 seeds as the plant abundance increased supports findings that *Mastomys sp.* tends towards granivory where available (Veenstra 1958, Meester and Hallett 1970, Isaäcson 1975, Oguge 1995, Monadjem and Perrin 1998b, Kinahan and Pillay 2008, Odhiambo *et al.* 2008, Mulungu *et al.* 2011). The lack of variation in the position of *R. dilectus* on the $\delta^{13}\text{C}$ scale across sites suggests that the changing abundance of C3 and C4 plants did not influence their feeding preferences. *R. dilectus* is a widespread generalist, displaying a large variation in its dietary preferences, consuming grain, foliage, flowers and even bark (Hechter-Schulz 1962, Perrin 1980, Rowe-Rowe 1986, Baxter and Hansson 2001, Schradin and Pillay 2005a, Kryštufek *et al.* 2007).

c) Temporal scale

Seasonal variation of food resources may lead to changes in the exposure of small mammals to mercury pollution over time (Wren 1986, Zhang *et al.* 2010). The relatively low abundance of insects and seeds during the winter months was expected to decrease both $\delta^{15}\text{N}$ and mercury concentrations – evident

in the isotopic composition of small mammals captured in October/November and December. There was, however, only minor evidence of seasonal variation in the isotopic niche width of both *Mastomys sp.* and *R. dilectus* across the four study sites (Figures 3.6 and 3.7). *Mastomys sp.* and *R. dilectus* had consistently lower $\delta^{15}\text{N}$ during the dry season (evident in the December 2011 trapping period) relative to the wet season.

Mastomys sp. and *R. dilectus* isotopic niche widths within each site fluctuated in size by at least 50% over the study period, whilst the actual position of the species in delta-space showed less variation. However, the fluctuations in isotopic niche width did not appear to follow a discernible trend, apart from having lower $\delta^{15}\text{N}$ following the dry season. The isotopic niche width of *Mastomys sp.* in Site A during April 2012 was the smallest of any niche space calculated, and did not overlap with the niche space of any other *Mastomys sp.* community from other months. The extreme decline in isotopic niche width from March 2012 (10.66‰) to April 2012 (0.95‰), along with the very small suite of Layman metrics indicates that the *Mastomys sp.* within Site A shifted from exploiting isotopically different food resources to isotopically similar resources. This likely reflects a decreased plant diversity within Site A in March to April 2012 (see Chapter 2 of this thesis), with an increased abundance of *Cynodon spp.* covering most of the area, with the remainder covered by some sparse *Typha spp.* and *P. australis*.

3.4.2 Are fundamental differences in small mammal diet (described by the isotope analysis) attributable to mercury pollution?

As mercury is a harmful pollutant (ATSDR 1999), it was expected that exposure to it would negatively influence the isotopic niche width of small mammals. However, mercury is just one of a group of heavy metal contaminants produced by the mining industry that may have potentially toxic effects on wildlife. The ability of generalist feeders, such as *R. dilectus* to exploit a wide range of food resources may aid in mitigating the health hazards of mercury and its bioaccumulation.

Specialist feeders, especially those with a high trophic position that are unable to exploit a wide range of food resources, such as *C. mariquensis* are more susceptible to the negative effects of mercury contamination and its bioaccumulation. If this is the case, the variation in isotopic trophic niche widths would be expected to increase or decrease for insectivores to a greater extent than omnivores at sites with higher mercury concentrations. Unfortunately, small sample size limited analysis of herbivorous species to Site A, so their isotopic niche widths could not be compared across sites (and hence across differing levels of mercury contamination).

Isotopic variation in small mammal species was lower at Site A (medium Hg, low plant diversity) than both Site B (high Hg, high plant diversity) and Site D (low Hg, high plant diversity). However, Site A had the highest small mammal species diversity and density, whilst Sites B and D had fewer species (Chapter 2). As such, it is not possible to determine whether the decrease in isotopic variability in Site A relative to Sites B and D was caused by mercury pollution. However, it

does appear that plant diversity influences small mammal isotopic variability, either directly as a food source (for herbivores and omnivores, Abramsky 1978) or indirectly through insects (for insectivores, Jones *et al.* 2011).

3.4.3 Summary

Stable light isotope analyses can provide greater insights into community structure and variability than those found using traditional aggregate measures. Based on aggregate measures of variability, such as species abundance and richness, a greater level of interspecific competition is evident (See Chapter 2) than when the isotopic niche widths of species are compared. Therefore, the resource and temporal partitioning displayed by *Mastomys sp.* and *R. dilectus* is enough to allow the coexistence of the two species within the study sites. The apparent competition exclusion of *Mastomys sp.* by *R. dilectus* seen in Chapter 2 is evidently less influenced by resource competition than expected. Comparison of isotopic niche widths of the small mammals at these study sites support the finding that vegetation, both as structural cover and as a food resource is a major driver of small mammal community structure (Abramsky 1978, Korn 1987, Ferreira and Van Aarde 1996, Monadjem 1997b, Greenwood *et al.* 2002, Monadjem and Perrin 2002, Mahlaba and Perrin 2003, van Deventer and Nel 2006, Avenant and Cavallini 2007, Pocock and Jennings 2008, Jones *et al.* 2011).

There was little evidence of direct influence of mercury pollution on the isotopic niche width of small mammals in the mining area. However, mercury pollution may have an indirect effect on small mammals as it can affect food

resources, for example by influencing the amount of nitrogen available to plants (Sage and Pearcy 1987). Animals with a broader isotopic niche width, and hence a broader range of potential food resources that they can utilise, such as *Mastomys* sp. and *R. dilectus*, may be more robust to mercury pollution. Specialist species with a smaller range of available food resources and a higher trophic position, such as *C. mariquensis* (Apps 2000, Skinner and Chimimba 2005) will be more negatively affected, should mercury pollution remove a potential food resource or bioaccumulation increase above safe levels.

**4. Mercury bioaccumulation in small mammal hair on an
AngloGold Ashanti Ltd mining complex, South Africa.**

4.1 Introduction

The gold mining industry is the largest producer of waste products in South Africa (DWAF 2001, van Eeden *et al.* 2009). During the extraction process, compounds containing potentially harmful heavy metals, such as mercury (Hg) are used to remove gold from ore (Tarras-Wahlberg *et al.* 2001, Mendez and Maier 2008, Boyd 2010). Waste products containing remnants of these compounds can therefore become a health hazard if released into the environment in high enough concentrations (Kumah 2006, Oelofse *et al.* 2007).

Mercury can be found in the environment in three broad forms; metallic or elemental mercury, inorganic mercury, and organic mercury (ATSDR 1999). Mercury, especially in the form of methylmercury has the potential to bioaccumulate within the food web (EPA 1984, Wren 1986, WHO 1990, Lewis and Furness 1991, ATSDR 1999, Sánchez-Chardi *et al.* 2007, Haines *et al.* 2010, Zhang *et al.* 2010). At high enough concentrations, mercury can have serious detrimental health effects, such as Minamata disease, genetic damage and even death (WHO 1990, 1991, ATSDR 1999, Harada *et al.* 1999). Methylmercury is soluble and mobile, contributing to its greater level of accumulation in biological tissue than inorganic mercury compounds (Riisgård and Hansen 1990). Concentrations of methylmercury in aquatic vertebrate top-predators have been found to be 10,000 to 100,000 times higher than the concentration of the ambient water in which they live (EPA 1979, 1984, WHO 1990, 1991).

Whilst levels of bioaccumulation of mercury in terrestrial ecosystems have been found to be lower than in the more complex aquatic ecosystems (Lindqvist

1991, Zhang *et al.* 2010), there is still the potential for harm (ATSDR 1999). Research on edible mushroom species (of the *Pleurotus*, *Lepiota*, *Lepista*, *Amanita*, *Boletus* and *Phaxillus* genera) by Bressa *et al.* (1988) and Kalac *et al.* (1991) concluded that bioaccumulation of mercury in mushrooms was significant enough to create a potential health risk for humans consuming the mushrooms. However, research (Mortimer 1985, Lindqvist *et al.* 1991, Granato *et al.* 1995) has shown that higher plants incorporate less mercury in their above-ground tissues than their roots, and that roots tend to have mercury concentrations comparable to that of the surrounding soil.

Aquatic macrophytes are known to accumulate methylmercury in their shoots, whilst inorganic mercury tends towards the older parts of the plant and roots where it is excreted (Dolar *et al.* 1971, Czuba and Mortimer 1980, Mortimer 1985, Baldantoni *et al.* 2004, Batty and Younger 2004, Bragato *et al.* 2006, Bonanno and Lo Giudice 2010). The majority of mercury accumulated in aquatic macrophytes therefore occurs in the below ground organs of the plant (Jana 1988, Bonanno and Lo Giudice 2010). However, the linear relationship between heavy metal concentrations in sediment and water, and the different organs of *P. australis* has led to its widespread use as a 'biomonitor' species used in phytoremediation (Bonanno and Lo Giudice 2010).

Methylmercury also has a strong affinity to bind with organic matter (Blume and Brummer 1991, Lee and Iverfeldt 1991). Hence, invertebrates, such as earthworms (Lumbricina) bioaccumulate mercury through consumption of contaminated organic matter and through direct contact with contaminated soil (Cocking *et al.* 1994). Predation on these invertebrates by insectivorous and

omnivorous vertebrates could pass the contamination on to the next trophic level (Cocking *et al.* 1994, Zhang *et al.* 2010). It therefore follows that the biomagnification of mercury results in a higher concentration of mercury in the tissues of carnivores, followed by omnivores and herbivores (Wren 1986).

Wild mammals can act as good indicators of heavy metal pollution (Chyla 1998, Chyla *et al.* 2000) as they are relatively long-lived and accumulate pollutants over multiple trophic levels over a relatively long time (Sobańska 2005). Their physiological similarities with humans can provide an early warning system when environmental toxicity levels become dangerously high (Su *et al.* 2008). Hair has been used as a sample tissue to measure mercury in a wide range of mammals, including humans (Doi and Ui 1975, Harada *et al.* 1977, 1999, Ikingura and Akagi 1996, Olivero-Verbel *et al.* 2011, Tian *et al.* 2011), dogs, *Canis lupus familiaris* (Hansen and Danscher 1995, Dunlap *et al.* 2007), wild boar, *Sus scrofa* (Sobańska 2005), sea lions, *Eumetopias jubatus* (Beckman *et al.* 2002), seals, *Arctocephalus gazella* (Sun *et al.* 2006), bobcats, *Lynx rufus*, and raccoons, *Procyon lotor* (Cumbie 1975), river otters, *Lontra canadensis* (Ben-David *et al.* 2001, Klenavic *et al.* 2008), opossum, *Didelphis virginiana* (Burger *et al.* 1994), caribou, *Rangifer tarandus* (Duffy *et al.* 2005) and muskrats, *Ondatra zibethicus* (Stevens *et al.* 1997).

As destructive methods of sampling animal tissues for mercury contamination result in individuals being removed from populations, there has been an increasing interest to use non-invasive sampling methods that prevent the need to remove whole individuals (Fossi and Leonzio 1994, Sobańska 2005). The use of hair as a means of measuring mercury load in animals has been practiced

for decades (e.g. Nelson *et al.* 1971, Nord *et al.* 1973, Huckabee *et al.* 1972, Cumbie 1975). The constant connection between the hair root and the bloodstream results in the incorporation of mercury into the hair follicle in proportion to the concentration of mercury in the blood (EPA 1996, ATSDR 1999, Beernaert *et al.* 2007, Magos and Clarkson 2008). As hair is metabolically inert, it is able to preserve volatile methylmercury for longer than organ tissue (Karz 1998, WHO 1990, Harada *et al.* 1999, Klenavic *et al.* 2008, Magos and Clarkson 2008, Haines *et al.* 2010).

Few studies have combined mercury analysis with stable light isotope analysis, despite the potential insights into food web ecology that stable light isotope analysis can provide, and the fact that hair samples can be used for both analyses. As nitrogen stable light isotope ratios ($\delta^{15}\text{N}$) increase in a step-wise fashion along with trophic level, they can be used as a measure to describe the relative trophic position of organisms (Minagawa and Wada 1984, Wada *et al.* 1987, Fry 1988). In their study of a terrestrial food web adjacent to an Arctic seabird colony, Choy *et al.* (2010) used stable light isotope analysis to determine whether mercury levels were enriched by deposition of guano by seabirds. Chasar *et al.* (2009) used stable light isotopes to measure the efficiency of trophic enrichment of methylmercury in stream ecosystems. Through the use of nitrogen isotopes, Chasar *et al.* (2009) were able to show that methylmercury concentrations increased significantly with increasing trophic position, but that top-predator methylmercury concentration was influenced more by the availability of methylmercury at the base of the food web than by their relative trophic position at the top of the food web.

With the aid of stable light isotope analysis, bioaccumulation of mercury in areas surrounding South Africa's gold mines can be monitored for potentially dangerous levels of mercury pollution. Makiese (2012) performed a study on mercury speciation in the area surrounding AngloGold Ashanti's West Wits mining complex (WWMC), providing baseline water, soil and vegetation mercury concentrations. International regulatory organisations, such as the United States' Agency for Toxic Substances and Disease Registry (ATSDR), the Government of the Netherlands' Dutch Environmental Standards, and the World Health Organisation (WHO) have stipulated target and intervention levels for mercury in the environment. Therefore, the aim of this chapter was to: 1) compare the concentrations of mercury in the hair of small mammals in the area around the WWMC; 2) use carbon and nitrogen stable light isotope analysis to compare mercury concentration to relative isotopic trophic position to investigate bioaccumulation of mercury in small mammals living in mining environments; 3) compare small mammal hair mercury concentrations to the baseline environmental concentrations measured by Makiese (2012); and 4) compare small mammal hair mercury concentrations to target and intervention levels stipulated by international regulatory bodies. It is expected that the WWMC study sites will display both elevated mercury levels, and bioaccumulation of mercury within the terrestrial food web.

4.2 Materials and Methods

The following materials and methods for the study period, study sites and small mammal capture are the key points taken from the more detailed descriptions found in the Material and Methods section of Chapter 2: Population Dynamics.

4.2.1 Study period

The study was conducted between November 2010 and November 2012 (hereafter referred to as the study period). Samples for mercury analysis were collected in October and December 2011, and March, April and October/November 2012. These sampling periods were selected in an attempt to cover a full seasonal cycle.

4.2.2 Study sites

The study was conducted at four study locations (sites A to D) on an AGA property on South Africa's Gauteng Highveld region, referred to as the West Wits mine complex (WWMC, Figure 3.1) Varkenslaagte catchment, near Carletonville, Gauteng. The two 'upstream' sites (A: S 26° 25.936', E 27° 22.183' and B: S 26° 26.820', E 27° 20.740') were relatively close to TSFs (~440m), while the two 'downstream' sites (C: S 26° 26.513', E 27° 20.176' and D: S 26° 26.395', E 27° 19.998') were further from TSFs (~1000m and ~1250m respectively). The relatively small distance between Sites C and D (~350m) carried some potential for small mammal movement between the sites.

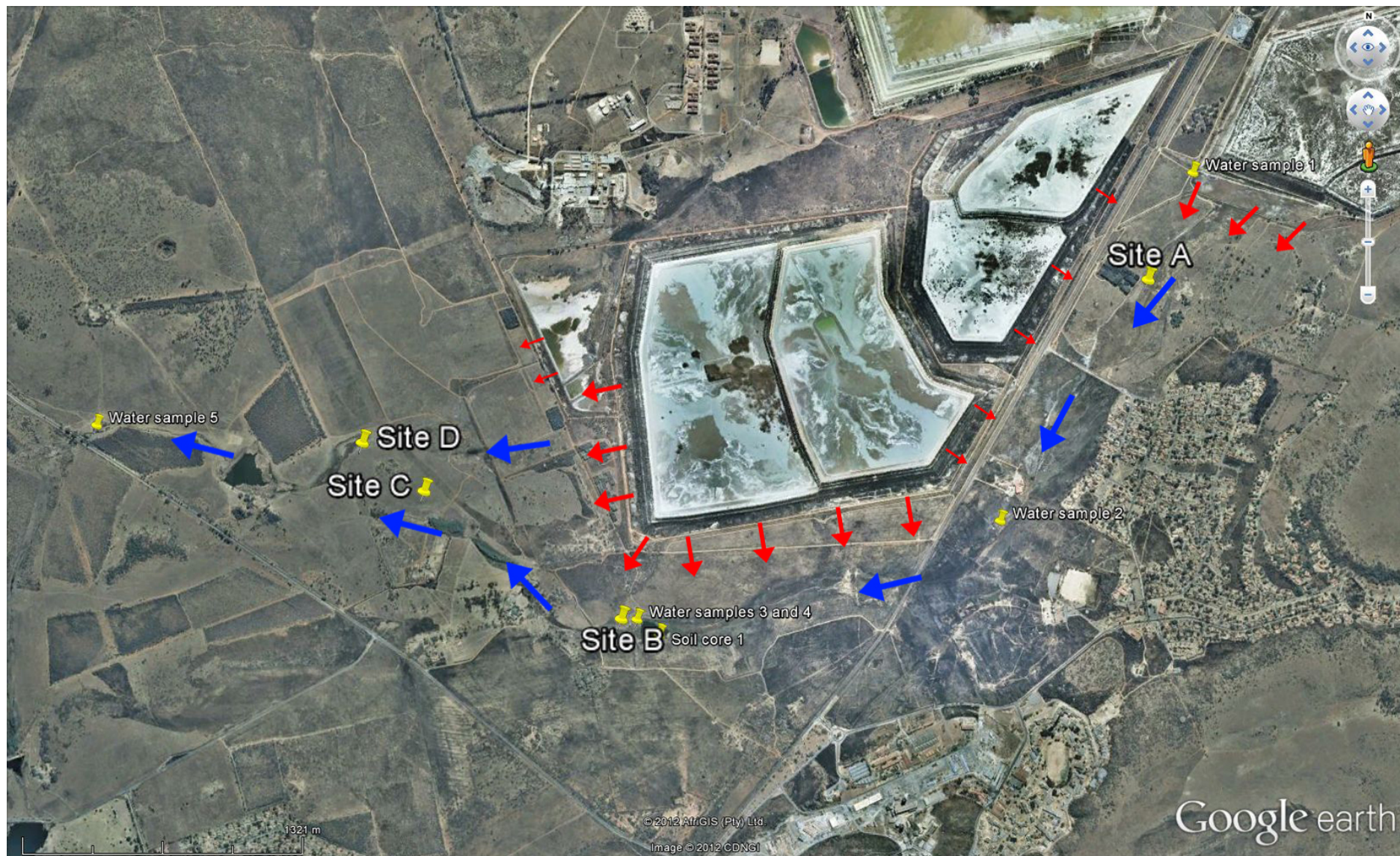


Figure 4.1: Satellite image (property of Google) of the four study sites (large yellow pins, A to D) located along the Varkenslaagte Stream (blue arrows indicate direction of flow) at the AngloGold Ashanti West Wits Mine Complex, Gauteng. Red arrows indicate direction of contamination plumes from the adjacent TSFs. Small yellow pins indicate locations of water and soil samples taken for preliminary Hg and IHg analysis (Makiese 2012).

Site A was located in a grassland and reed bed used for the phytoremediation of runoff from the northern TSF. The stream was lined with *Phragmites australis* (Ca.) Trin. ex Steud., the common reed, as it is a robust C₃ wetland macrophyte capable of removing heavy metals from soil and water (Ghassemzadeha *et al.* 2008, Bonanno and Lo Giudice 2010). Also common at Site A are couch grass (*Cynodon dactylon* (L.) Pers.), weeping love grass (*Eragrostis curvula* (Schrud.) Nees.), and boer love grass (*E. chloromelas* Steud. Complex).

Site B was situated approximately 3kms downstream of Site A, below a dam on the Varkenslaagte Stream. The stream in this site was lined with multiple wetland plant species, the most prominent of which were bulrushes (*Typha spp.*) and sedges (*Schoenoplectus spp.*), with some grasses (e.g. *Cynodon spp.* and *E. curvula*) and some *P. australis* prior to the October 2011 trapping session. Site C was located approximately 1km downstream of Site B, and was further from the Varkenslaagte Stream relative to the other three sites (approximately 50m from the bank as opposed to 10m or less in the other three sites). As such, Site C did not support any wetland plant species. The site prominently supported bankrupt bush (*Seriphium plumosum*, formerly *Stoebe vulgaris*) and multiple grass species (including *Cynodon spp.*, *Eragrostis spp.*, and *Aristida spp.*).

Site D was located approximately 350m downstream of Site C, and was in close proximity to a wetland formed between the convergence of the Varkenslaagte Stream and an overflow channel/stream from a nearby tailings effluent pond. Site D supported bulrushes (*Typha spp.*) and the common reed (*P. australis*) distributed along the northern edge, but was mostly vegetated with a

similar mixture of grasses (including *Cynodon spp.*, *Eragrostis spp.*, and *Aristida spp.*) and bankrupt bush (*S. plumosum*) to Site C.

4.2.3 *Small mammal captures*

A trapping session consisted of trap deployment over four consecutive nights in each of the four study sites. Four consecutive nights is the minimum limit determined by Avenant and Cavallini (2007). Fifty live traps were set for small mammals (Enviro-care MRT1 box traps, ± 10 cm x 7.5 cm x 25 cm), deployed in a 5x10 grid formation with 10 to 12 metres between traps (total area $\sim 500\text{m}^2$). All trapped small mammals were identified according to genus and species (according to Apps 2000 and Skinner and Chimimba 2005). Captures were undertaken under the certification from the University of the Witwatersrand Ethics Committee to trap, handle and collect hair samples from captured small mammals (Certificate number 2011/45/2A). Small mammal research was carried out under a Gauteng Provincial Government Permit, Certificate CPF6 No. 0151.

4.2.4 *Mercury samples*

Small mammal hair samples collected in the field were stored below 4°C as soon as possible after collection. They were then washed in a three-stage process, using deionised water to remove contaminants from the surface of the hair according to the methods described by Sobańska (2005). Following washing, the samples were freeze-dried for at least 48 hours before being weighed (0.0010

– 0.1035mg) using an analytical balance with a precision of 10^{-4} g (Precisa 180A, Switzerland) at the Department of Chemistry, University of the Witwatersrand. Dry samples underwent acid digestion in an open-microwave oven (CEM, USA) at 70W for four minutes using a 25% tetramethylammonium hydroxide (TMAH, Sigma Aldrich) solution as TMAH was found to allow complete alkaline hydrolysis of protein, lipids and sugars (Tseng *et al.* 1997). Digested samples were diluted with deionized water and stored below 4°C until they were analysed using an inductively coupled plasma mass spectrometry (ICP-MS) instrument (SPECTROMASS 2000, Germany). For a more detailed description of the sample preparation and analysis process, see Makiese (2012).

4.2.5 Stable isotope samples

Small mammal hair samples (0.45 – 0.65mg) were analysed for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable light isotopes using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of Cape Town (Preston and Owens 1983, Brenna *et al.* 1997). All isotope samples were prepared according to the guidelines stipulated by the Stable Isotope Facility, University of California Davis

(<http://stableisotopefacility.ucdavis.edu/13cand15nsamplepreparation.html>).

4.2.6 Data analysis

Data were prepared using Office Excel 2010 (Microsoft Corporation 2010) before running statistics in R 2.15.1 (R Development Core Team 2010). Mercury concentrations were calculated as $\mu\text{g.kg}^{-1}$ (ppb). Isotope ratios were expressed as per mil (parts-per-thousand over a reference material, ‰) using a standard reference material in δ -notation (i.e. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) as described in Fry (2008). Of the 50 samples analysed for mercury, 45 underwent stable light isotope analysis. An alpha-level of 0.05 was used for all statistical tests. The data was tested for normality using a Shapiro-Wilks W test (p-value range 4.897e^{-11} - 0.0032) (Shapiro and Wilk 1965).

As such, the non-parametric Kruskal-Wallis rank sum test (Kruskal and Allen 1952) was used to test for differences in the distribution of parameters between multiple groups (e.g. differences in Hg concentration between insectivores, omnivores and herbivores). A Multiple Comparison test was used to determine which sets were significant based on the Kruskal-Wallis results (Siegel and Castellan 1988) (performed using the “pgirmess” package by Giraudoux (2012) in R 2.15.2 (R Development Core Team 2010)). A Wilcoxon rank sum test was used in cases where only two groups were present (e.g. differences between *Rhabdomys dilectus* Hg concentration in Sites C and D).

Spearman’s rank correlation coefficients (Spearman 1904) were calculated to test for any significant correlations between the small mammal mercury concentrations and body weight, as well as mercury concentrations and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. The Spearman’s rank correlation test was used as it is a non-parametric test, giving a better representation of the non-normally distributed data.

The standard ellipse areas corrected for small sample size (SEAc, Jackson *et al.* 2011) were calculated for small mammal individuals that were also sampled for mercury concentration (i.e. a subsample of the individuals analysed in Chapter 3). Average species and community (based on site) mercury concentrations were compared to their respective SEAc values.

4.3 Results

4.3.1 Small mammal mercury concentrations

Samples for mercury analysis ($N = 50$) were taken from eight small mammal species captured during the study. Each species was classified as an insectivore, omnivore or herbivore according to literature and stable isotope analysis, in Chapters 2 and 3 of this thesis. Two insectivorous shrew species, *Crocidura mariquensis* ($n = 8$) and *Myosorex varius* ($n = 1$) were sampled. Most small mammal species captured were omnivorous; *Mastomys sp.* ($n = 27$), *R. dilectus* ($n = 6$), *Dendromus mesomelas* ($n = 4$), and *Aethomys sp.* ($n = 1$). Samples were collected from two captured herbivorous small mammal species, *Otomys auratus* ($n = 2$) and *O. angoniensis* ($n = 1$). Species-specific mercury concentrations are summarised in Table 4.1.

A shrew species, *C. mariquensis* had the highest median mercury concentration ($1320.42 \mu\text{g.kg}^{-1}$), but only the second highest individual mercury concentration (an individual of unknown sex with a mercury concentration of $4030.36 \mu\text{g.kg}^{-1}$). Interestingly, the single sample from the other insectivorous shrew, *M. varius* recorded the lowest mercury concentration ($12.92 \mu\text{g.kg}^{-1}$). The highest individual mercury concentration was recorded in a male *Mastomys sp.* ($5141.77 \mu\text{g.kg}^{-1}$). Median hair mercury concentrations are a better representation than mean mercury concentrations across treatments as a number of individuals with high mercury concentrations strongly influenced means and errors (Figure 4.2).

Table 4.1: Species specific hair mercury concentrations ($\mu\text{g.kg}^{-1}$) pooled across sites. Data appears in descending order of median hair mercury concentrations as outliers resulted in skewed average hair mercury concentrations. Numbers of individuals sampled per species appear in parentheses after species names.

Species	Min	Max	Median	Average	STD Deviation
<i>C. mariquensis</i> (8)	334.92	4030.36	1320.42	1574.72	1129.25
<i>D. mesomelas</i> (4)	12.23	2814.95	172.27	792.93	1351.58
<i>Mastomys sp.</i> (27)	47.07	5141.77	162.95	396.79	967.20
<i>R. dilectus</i> (6)	20.49	683.04	89.56	219.50	266.01
<i>O. angoniensis</i> (1)	81.46	81.46	81.46	81.46	N/A
<i>O. auratus</i> (2)	58.64	59.35	59.00	59.00	0.50
<i>Aethomys sp.</i> (1)	36.81	36.81	36.81	36.81	N/A
<i>M. varius</i> (1)	12.92	12.92	12.92	12.92	N/A

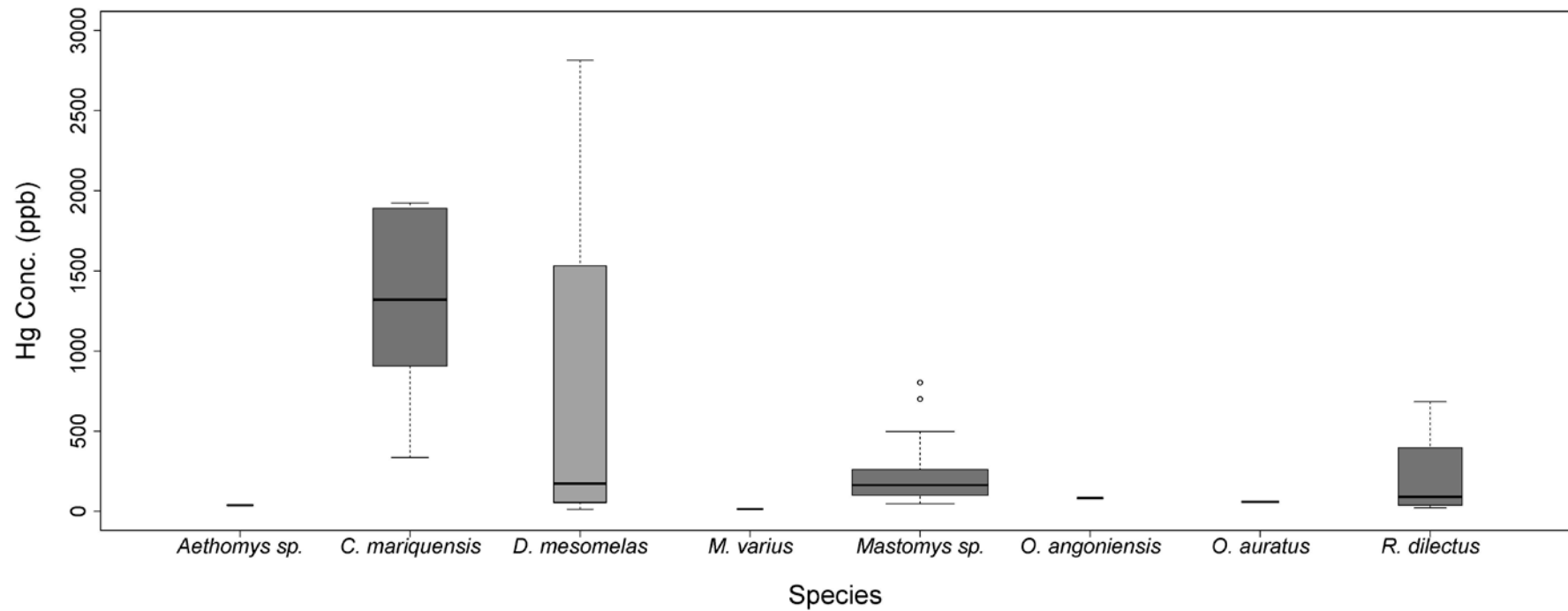


Figure 4.2: Boxplot of species specific hair mercury concentrations ($\mu\text{g.kg}^{-1}$ or ppb). Box widths are proportional to the square-roots of the number of samples collected for each species ($\sqrt{n}$). *Aethomys sp.*, *M. varius*, *O. angoniensis* and *O. auratus* appear only as horizontal lines because of their small sample sizes. Open circles represent points that lie beyond the ends of the whiskers. Points above 3000ppb: *C. mariquensis* = 4030.36ppb; *Mastomys sp.* = 5141.77ppb.

Of the eight captured small mammal species, only two species had significantly different mercury concentrations, *Mastomys sp.* and *C. mariquensis* (Kruskal-Wallis $\chi^2 = 21.511$, $df = 7$, $p = 0.003$), confirmed by the post-hoc multiple comparison test. Apart from *C. mariquensis*, the hair mercury concentration of *Mastomys sp.* was statistically similar to the other six species. The hair mercury concentration of *C. mariquensis* was marginally higher than *O. auratus* (multiple comparison test at $p = 0.05$, observed difference = 35.125 versus critical difference = 35.999) and *R. dilectus* (multiple comparison test at $p = 0.05$, observed difference = 23.958 versus critical difference = 24.592).

Four of the eight species were caught in sufficient numbers to test for correlation between mercury concentration and body weight; *C. mariquensis*, *D. mesomelas*, *Mastomys sp.*, and *R. dilectus*. However, no correlations were found between species' mercury concentrations and body weights. The difference in mercury concentration between male and female small mammals was not statistically significant at both a community and species level. The concentration of mercury in small mammal hair did not change over time at the community level. The only species with a large enough sample size to test for temporal changes of mercury concentration was *Mastomys sp.*, for which there were no significant differences.

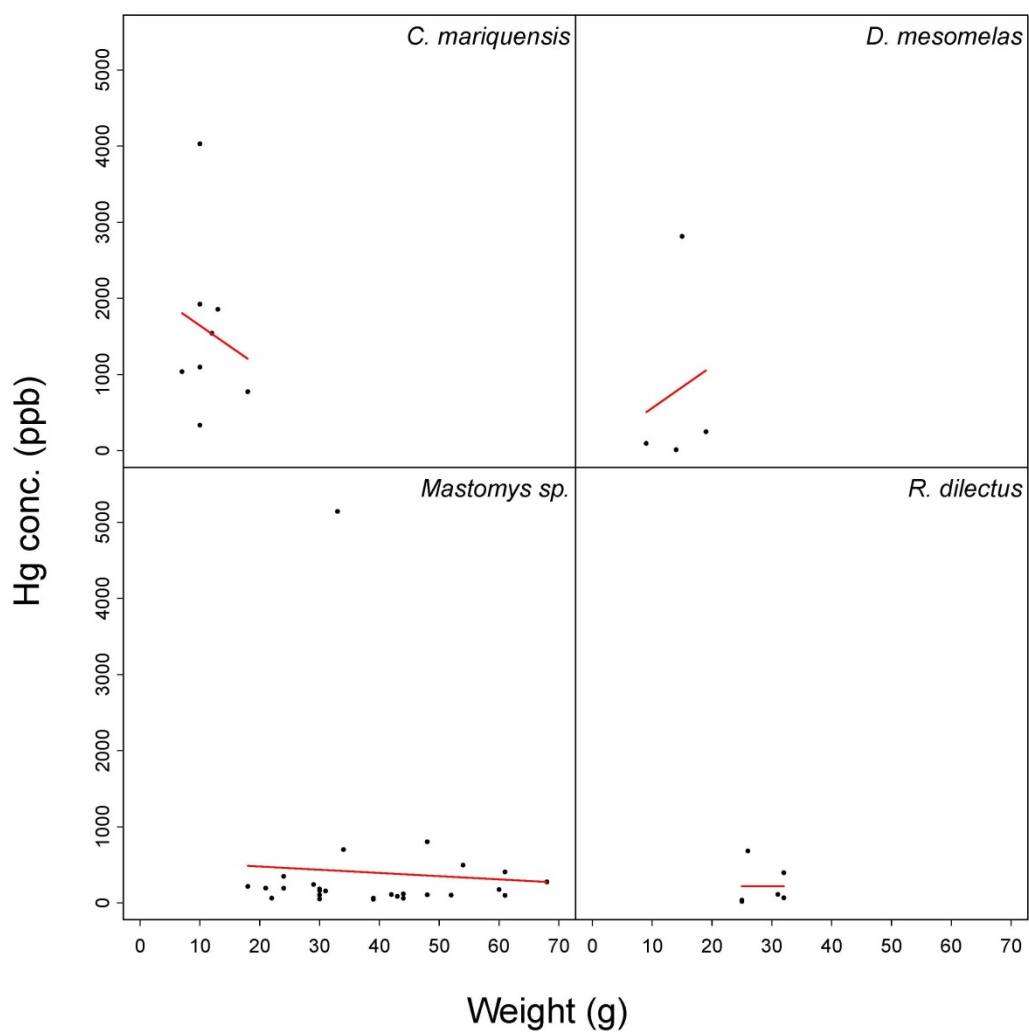


Figure 4.3: Small mammal body weight (g) versus hair mercury concentration ($\mu\text{g.kg}^{-1}$ or ppb). Solid lines represent simple linear regressions. No significant correlations were found at $p = 0.05$ (r_s range -0.025 – 0.600, S range 4.000 – 3276.000, p range 0.140 – 1.000).

It was also possible to test for differences between sites for *Mastomys sp.*. The mercury concentrations of individuals in Site A were significantly lower than those in Site B (Kruskal-Wallis $\chi^2 = 9.372$, $df = 2$, $p = 0.009$). The hair mercury concentrations of *Mastomys sp.* individuals in Site B were marginally higher than those in Site D (multiple comparison test at $p = 0.05$, observed difference = 11.667 versus critical difference = 11.819). A Wilcoxon rank sum test was used to test for differences between Sites C and D for *R. dilectus*, but showed that there was no difference (Wilcoxon $W = 8$, $p = 0.381$).

Interestingly, only Sites B and D showed a significant difference in mercury concentration at a community level (Kruskal-Wallis $\chi^2 = 9.705$, $df = 3$, $p = 0.021$). Site C had the highest median hair mercury concentration ($539.94\mu\text{g.kg}^{-1}$), but was based on only two hair samples from a single species, *R. dilectus* (Figure 4.4). Site B had the second highest median hair mercury concentration ($305.71\mu\text{g.kg}^{-1}$), followed by Site A ($98.83\mu\text{g.kg}^{-1}$) and Site D ($67.14\mu\text{g.kg}^{-1}$).

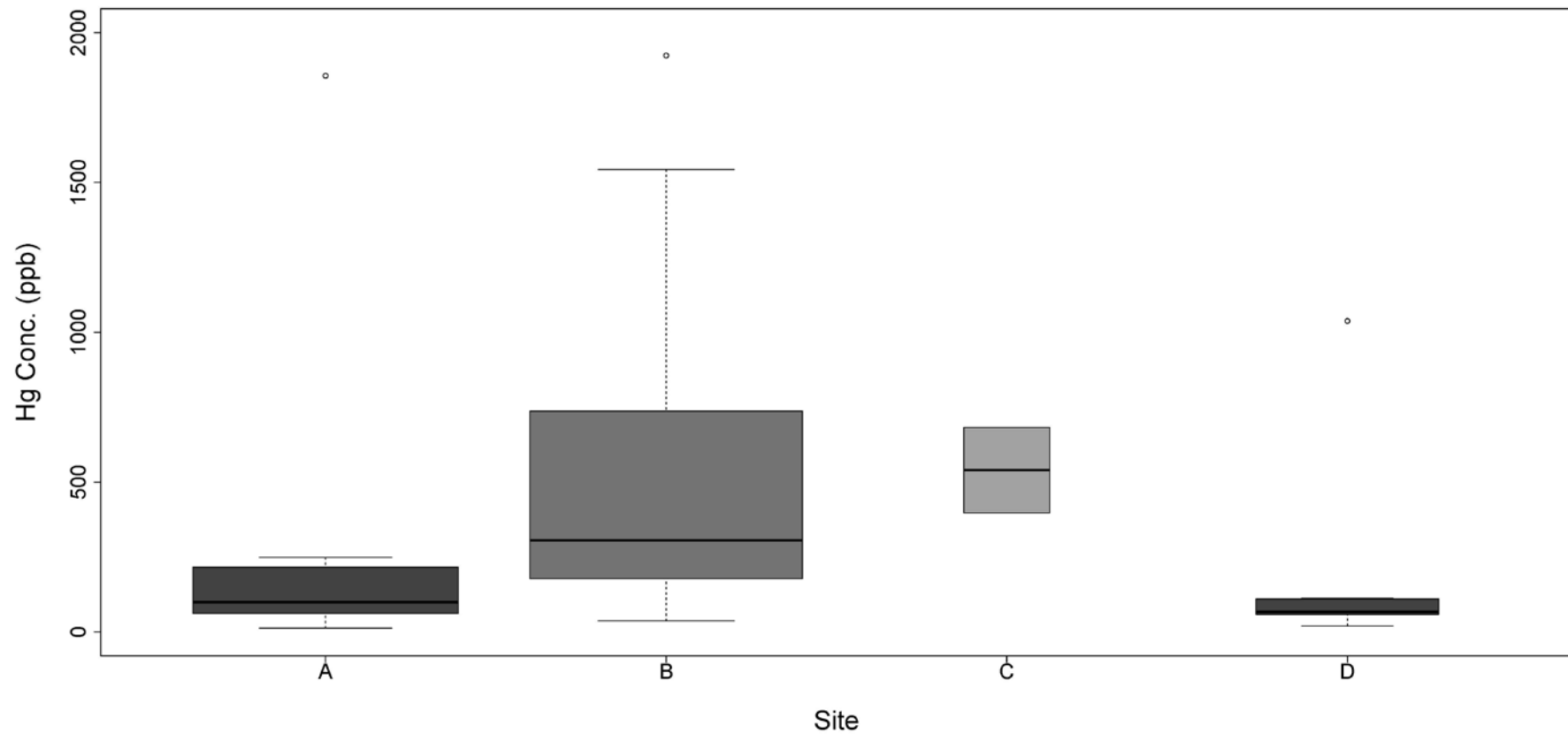


Figure 4.4: Boxplot of site specific hair mercury concentrations ($\mu\text{g.kg}^{-1}$ or ppb). Box widths are proportional to the square-roots of the number of samples collected for each species ($\sqrt{n}$). Site C does not have upper or lower whiskers as $n = 2$. Site B contained the highest reed-bed soil mercury concentration (628.4ppb), followed by Site A (344.2ppb) and Site D (124.9ppb). Open circles represent points that lie beyond the ends of the whiskers. Points above 2000ppb: Site A = 5141.77ppb, 4030.36ppb, 2814.95ppb.

4.3.2 Isotopic trophic niches and mercury concentration

Due to sample size constraints, only *C. mariquensis*, *D. mesomelas*, *Mastomys sp.*, and *R. dilectus* were used in the isotopic trophic niche analyses. There were no significant correlations found between species' mercury concentration and $\delta^{15}\text{N}$ (r_s range -0.800 – 0.446, S range 18.000 – 122.000, p range 0.134 – 0.958) for *C. mariquensis*, *D. mesomelas*, and *R. dilectus* (Figure 4.5). The positive correlation, albeit slight, between *Mastomys sp.* mercury concentration and $\delta^{15}\text{N}$ was the only statistically significant correlation ($r_s = 0.446$, S = 1816, p = 0.021). None of the four species displayed a significant correlation between mercury concentration and $\delta^{13}\text{C}$ (r_s range -0.021 – 0.800, S range 14.000 – 3344.000, p range 0.242 – 0.919).

The standard ellipse areas (SEAc) were calculated for all four species (Table 4.2, Figure 4.6). The largest was *C. mariquensis* (38.31‰), followed by *Mastomys sp.* (33.33‰), *R. dilectus* (25.20‰), and *D. mesomelas* (23.49‰). *C. mariquensis* also had the highest average $\delta^{15}\text{N}$ (14.40‰) and median hair mercury concentration (1320.42 $\mu\text{g.kg}^{-1}$). Interestingly, *D. mesomelas* had the second highest median hair mercury concentration, followed by *Mastomys sp.* and *R. dilectus* (Table 4.2). The second highest average $\delta^{15}\text{N}$ was of *R. dilectus*, followed by *D. mesomelas* and *Mastomys sp.* (Table 4.2).

Despite the overall lack of correlation between small mammal mercury concentration and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, mercury concentration differed based on the published trophic strategies of the small mammals (Figure 4.7). Insectivorous small mammals (*C. mariquensis* and *M. varius*, median = 1097.69 $\mu\text{g.kg}^{-1}$) had

significantly higher mercury concentrations than both the omnivorous (*Mastomys* sp., *R. dilectus*, *D. mesomelas*, and *Aethomys* sp., median = 137.64 $\mu\text{g.kg}^{-1}$) and the herbivorous (*O. angoniensis* and *O. auratus*, median = 59.35 $\mu\text{g.kg}^{-1}$) small mammal species (Kruskal-Wallis $\chi^2 = 11.682$, df = 2, p-value = 0.003). Omnivorous and herbivorous small mammals had similar mercury concentrations, as expected.

Table 4.2: Standard ellipse area (SEAc,‰), average $\delta^{15}\text{N}$ (‰), average $\delta^{13}\text{C}$ (‰), and median hair mercury concentration ($\mu\text{g.kg}^{-1}$) of the four small mammal species with large enough sample sizes (appear in parentheses).

Species	Median Hg	SEAc	Avg. $\delta^{15}\text{N}$	Avg. $\delta^{13}\text{C}$
<i>C. mariquensis</i> (8)	1320.42	38.31	14.40	-19.21
<i>D. mesomelas</i> (4)	172.27	23.49	8.66	-17.94
<i>Mastomys</i> sp. (27)	162.95	33.33	8.52	-19.58
<i>R. dilectus</i> (6)	89.56	25.20	9.97	-21.22

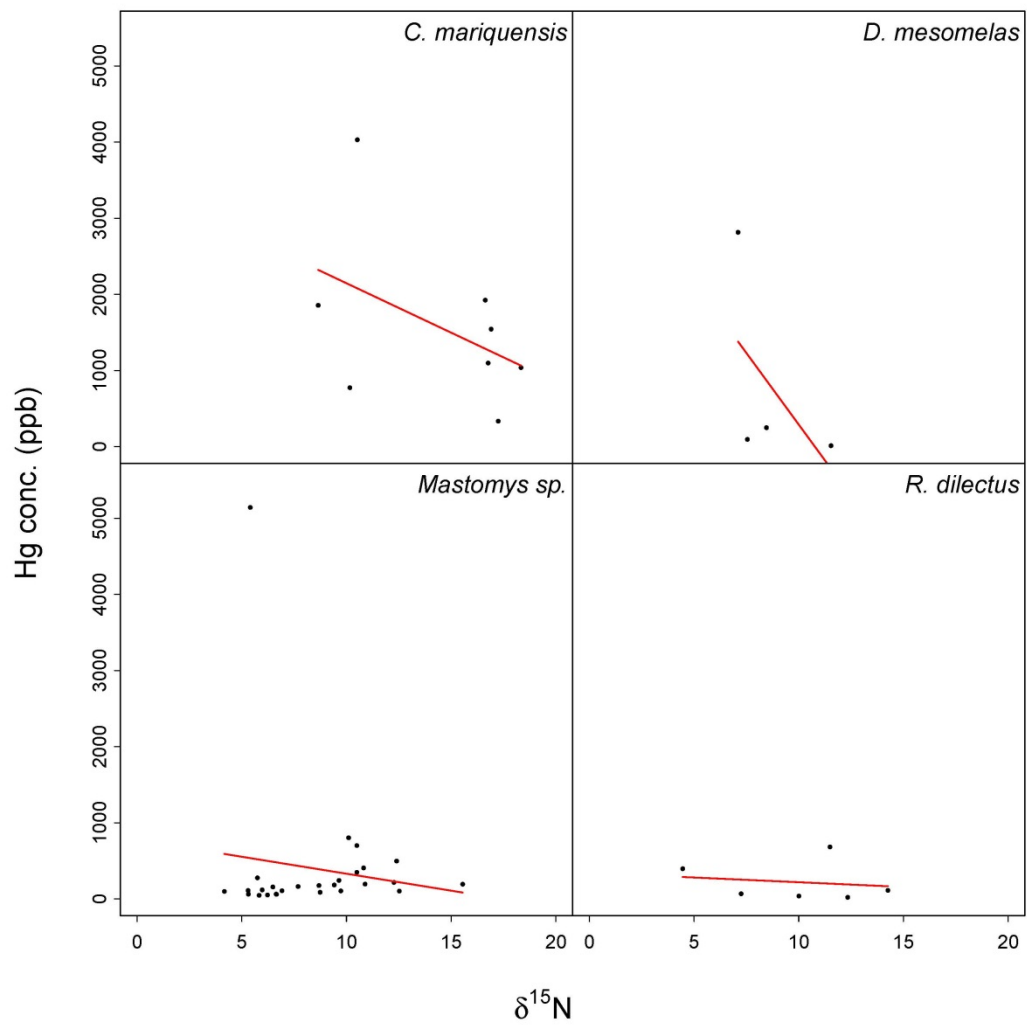


Figure 4.5: Small mammal $\delta^{15}\text{N}$ (‰) versus hair mercury concentration ($\mu\text{g.kg}^{-1}$ or ppb). Solid lines represent simple linear regressions. No significant correlations were found at $p = 0.05$ (r_s range $-0.800 - 0.446$, S range $18.000 - 122.000$, p range $0.134 - 0.958$) for *C. mariquensis*, *D. mesomelas*, and *R. dilectus*. A positive correlation between *Mastomys sp.* mercury concentration and $\delta^{15}\text{N}$ ($r_s = 0.446$, $S = 1816$, $p = 0.021$) was evident.

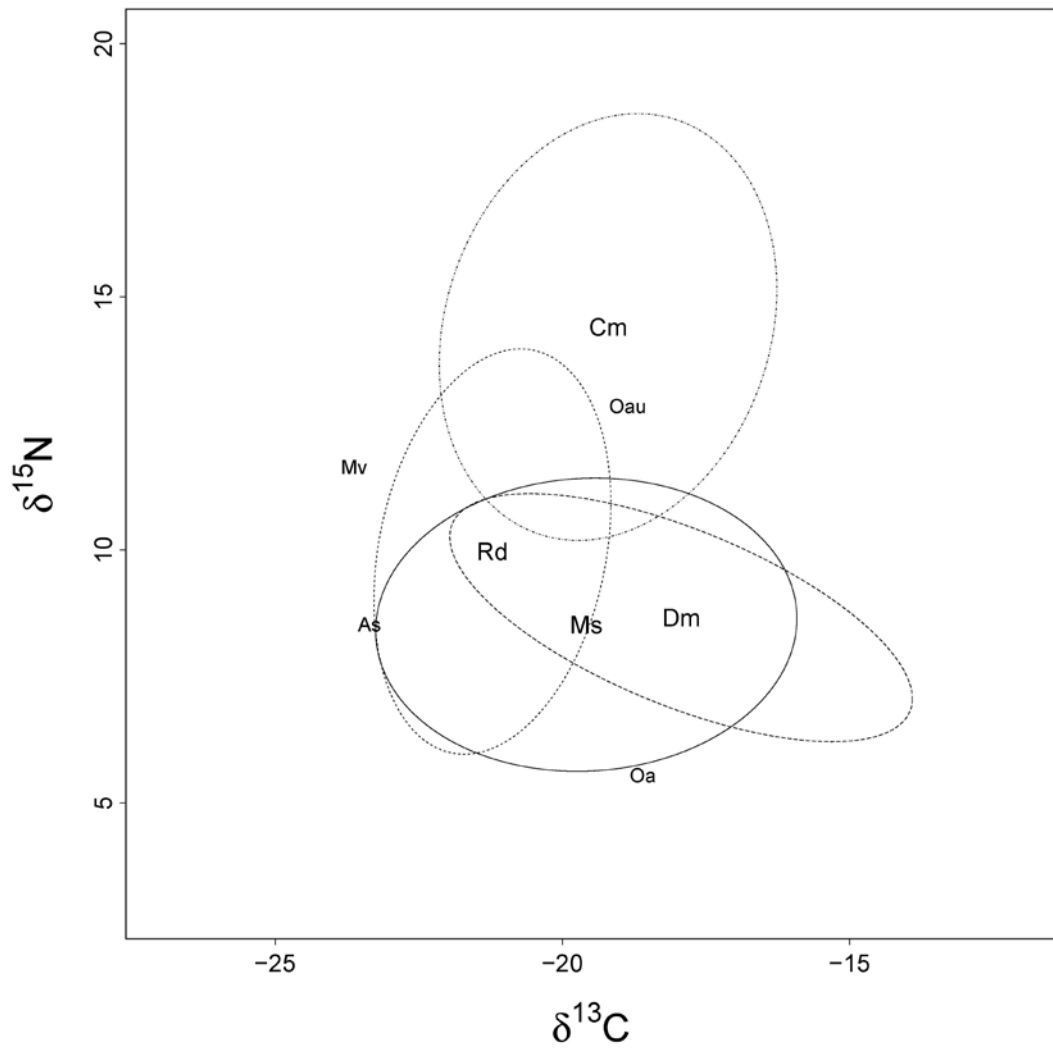


Figure 4.6: Species-specific community stable isotope bi-plots (‰) describing each small mammal species captured, pooled across the study sites. Ellipses represent the SEAc for species with $n > 3$ in more than one site, i.e.: Ms = solid ellipse; Rd = dotted ellipse; Cm = dot-dash ellipse, Dm = dashed ellipse. Species with $n < 3$ included as single points, i.e.: Oau, Oa, As, and Mv. Small mammal abbreviations: Ms = *Mastomys sp.*; Rd = *R. dilectus*; Oau = *O. auratus*; Oa = *O. angoniensis*; Cm = *C. mariquensis*; Dm = *D. mesomelas*; Mv = *M. varius*; As = *Aethomys sp.*.

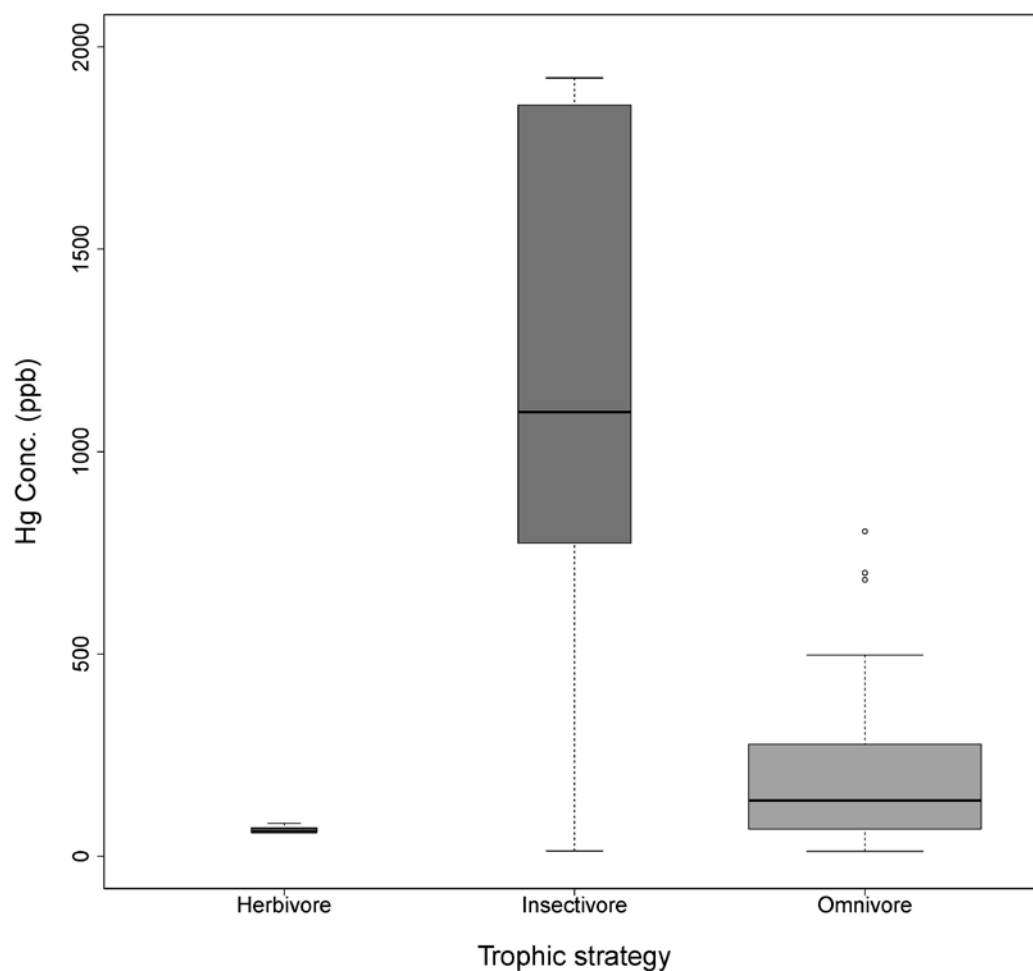


Figure 4.7: Boxplot of hair mercury concentrations ($\mu\text{g.kg}^{-1}$ or ppb) based on small mammal trophic strategy. Herbivore variation is low as $n = 3$. Box widths are proportional to the square-roots of the number of samples collected for each species ($\sqrt{n}$). Open circles represent points that lie beyond the ends of the whiskers. Points above 2000ppb: Insectivores = 4030.36ppb; Omnivores = 5141.77ppb, 2814.95 ppb.

4.3.3 Baseline environmental mercury concentrations

A previous study (Makiese 2012) measured the concentrations of inorganic mercury (IHg (Hg^{2+})) and methylmercury (MHg) in soils (Table 4.3) at a number of locations along the length of the stream (Figure 4.1; Appendix C). Surface soil (0 – 5cm) mercury concentrations measured within the Varkenslaagte stream ranged from $38.9\mu\text{g.kg}^{-1}$ at Site A to $58.5\mu\text{g.kg}^{-1}$ just upstream of Site B (Table 4.3). The total mercury concentration (IHg and MHg combined) was also measured for the common reed, *P. australis* ($37.8\mu\text{g.kg}^{-1}$) within Site A, and bulrushes, *Typha spp.* ($31.9\mu\text{g.kg}^{-1}$) approximately 1300 metres downstream of Site A. Surface soil samples taken within the reed beds at Site A ($344.2\mu\text{g.kg}^{-1}$), Site B ($628.4\mu\text{g.kg}^{-1}$), and Site D ($124.9\mu\text{g.kg}^{-1}$) were much higher than the surface soil samples taken within the Varkenslaagte stream (Table 4.3).

Concentrations of IHg and MHg in the stream water increased from upstream of Site A, downstream towards Site B, with the IHg concentration in the dam above Site B being much higher at 13.055ng/L within the dam water (Appendix A). Concentrations of water IHg and MHg decreased downstream of Site B with the lowest concentrations just downstream of Site D (Appendix A).

Table 4.3: Mercury concentrations ($\mu\text{g.kg}^{-1}$ or ppb) in soil cores collected by Makiese (2012) from within the Varkenslaagte stream and within the reed-beds at the study area. All samples $n = 3$. Depths of samples (cm) appear in parentheses next to original sample codes. bdl = below detection limit, nd = not determined.

Description	Original sample code	Hg _{TOT}	IHg	MHg	%MHg
Core from V. stream at Site A	16K(0-5)	38.9 ± 2.1	nd	nd	-
Core from reed-bed at Site A	16L(0-5)	344.2 ± 13.1	331.8 ± 5.0	1.9 ± 0.5	0.6
Core from V. stream upstream of Site B	17E(0-2)	58.5 ± 3.0	nd	nd	-
Core from reed-bed at Site B	19F(0-2)	628.4 ± 7.6	505.2 ± 1.4	8.8 ± 0.5	1.7
Core from reed-bed at Site D	23(0-2)	124.9 ± 6.5	119.6 ± 4.7	bdl	0

4.3.4 International Regulatory Standards

The majority of studies on the toxic effects of mercury have been performed on animals, with the intention of extrapolating the data to humans (WHO 2003) leading to regulatory values developed for human risk and exposure to mercury. The minimum risk level (MRL) for the ingestion of IHg is 2µg/kg/day, whereas for MHg the MRL is 0.3µg/kg/day (ATSDR 1999, IPCS 2003). It is generally accepted that Minamata disease begins to display symptoms in humans at 50ppm mercury in human hair (Doi and Ui 1975, Harada *et al.* 1977), approximately 50 times higher than the median mercury concentration in shrew hair measured here.

Mercury has a LD₅₀ (Lethal Dose for 50% of tested rats and mice) in the range 25.9mg Hg/kg to 77.7mg Hg/kg in the form of inorganic mercuric chloride (Kostial *et al.* 1978, ATSDR 1999). It is more difficult to assess the toxicity of organic forms of mercury, such as methylmercury, as the toxic effects are strain- and sex-specific in mice (ATSDR 1999). Yasutake *et al.* (1991) reported that a single oral dose of 16mg Hg/kg methylmercuric chloride killed 60% of male mice, but no females. Only at doses of 40mg Hg/kg did female mortality increase above 60%. Mitsumori *et al.* (1981) reported that dietary exposure of methylmercuric chloride at 3.1mg Hg/kg/day over 26 weeks resulted in increased mortality in both male and female mice. Chronic dietary exposure over 104 weeks to methylmercuric chloride increased male mortality at 0.69mg Hg/kg/day, but did not increase female mortality at up to 0.6mg Hg/kg/day (Mitsumori *et al.* 1990).

The Dutch Target and Intervention Values (SRC 2009) (published by the Government of the Netherlands and also known as the 'New Dutch List') specifies that the target value for soil mercury concentration is 0.30mg.kg^{-1} , with a soil mercury concentration of 5.15mg.kg^{-1} requiring further investigation and a soil mercury concentration above 10mg.kg^{-1} requiring immediate intervention. The soil intervention value was later modified in the Circular for Soil Remediation Target and Intervention Values (SRC 2009) to specify a concentration of 36mg.kg^{-1} for inorganic mercury and 4mg.kg^{-1} for organic mercury, such as methylmercury. The Circular for Soil Remediation Target and Intervention Values (SRC 2009) also states that the maximum permissible risk for ingestion by humans ($\text{MPR}_{\text{human}}$) is $0.1\mu\text{g/kg/day}$ organic mercury, and $2.0\mu\text{g/kg/day}$ inorganic mercury. The target value given for shallow ground water is $0.05\mu\text{g.l}^{-1}$, whilst the deep ground water target value is $0.01\mu\text{g.l}^{-1}$, and the intervention value for both is $0.3\mu\text{g.l}^{-1}$. The guidelines for drinking water published by WHO (2004) sets the upper limit at $1\mu\text{g.l}^{-1}$ for total mercury, approximately a hundred times higher than the maximum water mercury concentration measured in the Varkenslaagte stream by Makiese (2012).

4.4 Discussion

4.4.1. Hair mercury concentrations: general comparisons

All eight captured species displayed large variation in hair mercury concentration. However, individual variation in mercury concentration is not unexpected as the utilisation of different food sources exposes animals to different levels of mercury pollution (Martin and Coughtrey 1982, Peterle 1996, Evans *et al.* 1998, Sobańska 2005). The insectivorous shrew, *C. mariquensis* displayed the highest hair mercury concentrations as a species, nearly double that of *D. mesomelas* with the second highest hair concentrations. Unfortunately, only a single individual from the second insectivorous shrew species, *M. varius* could be sampled for mercury analysis. Based on its trophic strategy we would expect *M. varius* to display higher levels of hair mercury concentration.

Like a number of other studies (Wren 1986, Burger *et al.* 1994, Sobańska 2005, Haines *et al.* 2010), we did not find a correlation between animal sex and mercury concentration. Female animals had a slightly higher but not statistically significant different mercury concentration than males, a pattern seen in previous studies but one that has not been extensively studied. It is possible that sex-specific resource partitioning contributes to the differences in mercury concentration (Ashraf *et al.* 1995).

Although hair mercury concentration was expected to increase with body weight (as a proxy for age) (Brooks 1972), there was no relationship found between body weight and mercury concentration. Three of the species analysed, *C. mariquensis*, *D. mesomelas*, and *R. dilectus* displayed relatively little variation

in body weight, but large variation in mercury concentration. The fourth species, *Mastomys sp.*, displayed a larger range of body weights, but individuals all had relatively low mercury concentrations (below 1ppm). It is therefore unlikely that mercury concentration has any adverse effects on the body weight of small mammals living within the study area. Therefore, mercury levels appear low enough to not present a serious health hazard to the small mammals captured here. However, neither the neurological effects of mercury, nor the effects of other heavy metal pollutants generally found alongside mercury were investigated.

4.4.2. Hair mercury concentration and relative isotopic trophic niches

Most plants do not incorporate a large amount of mercury into their growing tissues (ATSDR 1999, Baldantoni *et al.* 2004, Batty and Younger 2004, Bragato *et al.* 2006, Bonanno and Lo Giudice 2010). Some plants, such as aquatic macrophytes like *P. australis* are known to accumulate mercury and other pollutants in their shoots (ATSDR 1999), increasing the risk of exposure to herbivores and omnivores that feed on them. However, vegetation is still a relatively small source of mercury to herbivores and omnivores when compared to the potential source of mercury that invertebrates present to insectivores (Wren 1986). Both *P. australis* and *Typha spp.* sampled at the study area had relatively low mercury concentrations ($37.8\mu\text{g.kg}^{-1}$ and $31.9\mu\text{g.kg}^{-1}$, Makiese 2012) when compared to the potential concentrations found in insects and invertebrates such as grasshoppers (e.g. *Locusta migratoria*) and earthworms (up to $1000\mu\text{g.kg}^{-1}$, Martin and Coughtrey 1982, Peterle 1996, Zhang *et al.* 2010).

Mercury concentration in the hair of insectivorous shrews was significantly higher than that of omnivorous and herbivorous small mammals. The hair mercury concentration of omnivores and herbivores was relatively similar, as was their position in isotopic delta-space (refer to Figure 4.6). *C. mariquensis* was positioned the highest on the $\delta^{15}\text{N}$ scale, as is expected of an insectivore, but interestingly, the *M. varius* individual is positioned lower down the scale, amongst the omnivores and herbivores. Despite being second highest on the $\delta^{15}\text{N}$ scale – suggesting a greater dietary reliance on insects or other high trophic level items, *R. dilectus* had a much lower hair mercury concentration than *C. mariquensis*, and had lower hair mercury than both *D. mesomelas* and *Mastomys sp.*.

Some individuals of both *Mastomys sp.* and *D. mesomelas* had exceptionally high hair mercury concentrations at Site A, possibly as a result of one of the disturbances events that took place at the site, such as the tailings spill in January 2012. The individual with the highest recorded hair mercury concentration was a *Mastomys sp.* captured at Site A in March 2012, two months after the tailings spill potentially released large amounts of pollutants into the site. The variability displayed in the $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and mercury concentrations of each species implies that variation in individual diets influences the amount of mercury accumulated by small mammals. Hence, individuals of the same species can be exposed to different levels of mercury pollution based on their feeding behaviour.

4.4.3. Hair and baseline environmental mercury concentrations

Concentrations of mercury in stream water, submerged soil, and reed-bed soil at the study sites followed the same general pattern, with Site B having the highest concentrations, followed by Site A and then Site D, as seen in the small mammal hair mercury concentrations. Baseline environmental mercury pollution data were not available for Site C. While the two animals trapped at Site C had the highest hair mercury concentrations (Figure 4.4), lack of environmental data and the small sample sizes limited the site's inclusion in the analyses. The median hair mercury concentrations can therefore be said to follow on from the known baseline environmental mercury concentrations, with Site B the highest, followed by Site A and Site D.

The reed-bed surface soil concentrations are important as it is there that the animals come into direct contact with the contaminated soil, and where food resources such as *P. australis* are found. Artificial wetlands, such as those at the study sites, are used in phytoremediation as they are considered as 'sinks' for heavy metals (Hart 1982), where waterborne heavy metals are transferred to the sediment through the processes of adsorption to organic matter and sedimentation (Zwolsman *et al.* 1993). As mercury is strongly sorbed to humic material (Blume and Brummer 1991), requiring chemical or biological reduction and bioconversion to desorb it (Andersson 1979, EPA 1979), it has almost negligible leaching rates, with the majority of mercury remain in the top 15cm of soil (Granato *et al.* 1995).

Bonanno and Lo Giudice (2010) found that root mercury concentrations in *P. australis* tended to be higher than that of the surrounding soils, but that there is

low mobility of mercury between below-ground organs and above-ground organs, with roots containing approximately five times the mercury concentration of stems. Unfortunately, baseline vegetation mercury concentrations were measured in above-ground stems and leaves, and not below ground organs (Makiese 2012). Hence, the mercury concentrations of the *P. australis* and *Typha spp.* growing within the reed beds were much lower than the surface-soil mercury concentrations. The median hair mercury concentrations for each site were higher than the vegetation baseline mercury concentrations as small mammals accumulated mercury gained from ingesting vegetation in their hair. Hair mercury concentrations were lower than their respective reed-bed surface soil mercury concentrations because mercury tends to remain in surface soils, and the low mobility between below-ground and above-ground plant organs, on which small mammals feed.

4.4.4. International Regulatory Standards

Hair mercury concentrations in all small mammals captured here were lower than the WHO (1990) advisory maximum tolerable level for human of 6ppm. The advisory maximum tolerable level is based on the amount of mercury that would accumulate in hair if mercury intake were maintained at the minimum risk level set by the ATSDR (1999), calculated based on rat and mice mortality rates in the laboratory. Most individuals captured here had hair mercury concentrations well below 1ppm, with only a few *C. mariguensis*, *D. mesomelas*, and *Mastomys sp.* individuals above 1ppm.

Human safety standards tend to err on the side of caution, as illustrated by the advisory maximum tolerable level of mercury in hair (6ppm) being nearly a tenth of the level at which the symptoms of Minamata disease begin to manifest (50ppm hair mercury concentration). The maximum tolerable level of mercury in hair appears higher than would be expected, as it is calculated using a hair: blood ratio of 250 (Phelps *et al.* 1980, Suzuki *et al.* 1992, ATSDR 1999). Therefore, the advisory maximum tolerable level of mercury in hair (6ppm) is equivalent to 0.024ppm mercury in blood.

Chronic exposure of small mammals to mercury in the environment is of the greatest concern, as acute mercury poisoning is unlikely except in the case of extreme point pollution, such as a break in a TSF wall. However, when the low mercury concentrations in *P. australis* and *Typha spp.* is considered along with the low hair mercury concentrations measured in small mammals, the data suggests a rate of exposure below the dangerous thresholds described by Mitsumori *et al.* (1990). Despite this, the presence of mercury could indicate the presence of other heavy metal pollutants, which could present potential health risks (Bonanno and Lo Giudice 2010). Also, as primary and secondary consumers that bioaccumulate mercury, the small mammals themselves could contribute to elevated mercury concentrations in predators.

The environmental baseline mercury concentrations measured by Makiese (2012) were similar to the target values specified by the Dutch Standards (SRC 2009). Whilst the reed-bed surface soil concentrations at Site A and B were both slightly elevated compared to the target value of $300\mu\text{g.kg}^{-1}$, they were not nearly as high as the new intervention levels set at $4000\mu\text{g.kg}^{-1}$ in 2009 (SRC 2009). The

water mercury concentrations in the Varkenslaagte stream were also lower than the Dutch Standard target value of $0.05\mu\text{g.kg}^{-1}$ for shallow surface water (SRC 2009).

4.4.5 Conclusion:

From this preliminary analysis, I found that as insectivorous shrews hold a higher trophic position than the other small species at the study sites and display elevated mercury concentrations, reflected in both their $\delta^{15}\text{N}$ and hair mercury concentrations. Although hair mercury concentrations did not exceed WHO (1990, 1991) guidelines, the small mammals measured here generally reflected the mercury abundance in their specific habitats (i.e. soil, plant and hair mercury concentrations were all high at Site B, whilst at Site A all concentrations were intermediate and at Site D all concentrations were low). Hair mercury concentration also reflected trophic strategy, with insectivores consistently higher in mercury than omnivores and herbivores. Thus, mercury in these animals is indicative of their environments and trophic positions, and can potentially be used as biological indicators of mercury accumulation in the food chain in polluted environments.

5. Synthesis and Conclusion

The aim of this research was to contextualise, in terms of community structure, food web metrics and mercury contamination, small mammal communities living in the areas surrounding tailings storage facilities (TSFs) on a gold-mining property near Carletonville, South Africa. Pollution released into the environment by TSFs exposes wildlife to potential health hazards, such as elevated mercury concentrations (ATSDR 1999, AngloGold Ashanti 2004, Boyd 2010). Therefore, this study focussed on describing the 1) small mammal population dynamics, 2) isotopic niche widths, and 3) mercury bioaccumulation in small mammal hair, to provide insight into the interactions between mining pollutants (indicated by mercury) and small mammal ecology and food webs.

5.1 *Small mammal population dynamics*

The first objective of this study was to describe the small mammal community structure and composition in different parts of the West Wits Mining Complex (WWMC) using traditional aggregate measures of variability (e.g. the Shannon-Wiener diversity and evenness indices). It was expected that these measures would vary in small mammal communities according to the level of disturbance created by pollution bioavailability and site-specific vegetation structure. Disturbances that altered vegetation structure were included in this first part of the study as it was expected that such disturbances would also alter small mammal community structure because of the relationship between small mammal

community structure and the surrounding vegetation (Paine and Vadas 1969, Brown 1975, Korn 1987, Kerley and Erasmus 1992, Avenant 2011, Kok *et al.* 2012).

This study confirmed that small mammal species diversity and abundance are influenced by the amount and quality of vegetation present and the cover it provides in the lower height classes (leaf litter and 0 – 30cm above ground) (Rosenzweig and Winakur 1969; Kerley 1992a; Els and Kerley 1996; Monadjem 1999; van Deventer and Nel 2006, Kok *et al.* 2012). Disturbances to vegetation, such as through the occurrence of fire at the WWMC, influenced small mammals both directly (likely through injury or death) and indirectly by removing resources such as cover and food.

Disturbances at Sites A and B modified small mammal community structure, and in Site A, *Mastomys sp.* numbers increased following fires, but were later surpassed in abundance by *Rhabdomys dilectus* once the vegetation had recovered and attained a certain level of cover. However, in Site B, *Mastomys sp.* abundance persisted whilst *R. dilectus* abundance did not increase, possibly as a result of continuous disturbance from cattle grazing and trampling. In Site C and D where disturbance was lower, *R. dilectus* were captured in the highest abundance. Interestingly, a number of species thought to be sensitive to disturbances such as fire and pollution were captured during the study. The presence of one such species, *Crocidura mariquensis*, is important, as it creates the potential to develop a monitoring programme for the bioaccumulation of harmful contaminants within the WWMC.

The fluctuation of the small mammal community indicates that it is, at least in part, subject to the intermediate disturbance hypothesis (Paine and Vadas 1969, Ferreira and van Aarde 2000). Numerous factors influence the ecosystems in which small mammals occur, and as such it is important for environmental managers to understand the modified ecosystems in which they work in order to maintain ecological integrity and biodiversity (Bowland and Perrin 1988 and 1989, Avenant and Cavallini 2007, Bonanno and Lo Giudice 2010). Studying small mammal communities not only provides insight for environmental managers about the obvious disturbances such as fire and grazing, but can also provide insight into more cryptic disturbances such as the effect of harmful contaminants on the food web. The small mammal communities at the WWMC can therefore be used as indicators of the ecosystem's health during its rehabilitation (Brown 1975, Korn 1987, Avenant 2000, Avenant 2011).

5.2 Isotopic niche widths

The second objective was to use stable light isotope analyses to describe the relative position of small mammals within isotopic delta-space as a means of investigating whether mine-produced mercury pollution affects small mammal isotopic niche widths. Seasonal variation of food resources, such as seed and insect abundance, was expected to cause a shift in small mammal delta-space position towards more constant food sources (e.g. foliage). It was also expected that isotopic niche width would either a) decrease with increasing mercury concentration and interspecific competition (Jackson *et al.* 2012), or b) increase

with increasing mercury concentration and interspecific competition (Svanbäck and Bolnick 2007).

Stable light isotope analysis confirmed that the insectivorous shrew, *C. mariquensis* holds a higher relative isotopic trophic position than the omnivorous and herbivorous species captured. All four species captured in Site B displayed elevated $\delta^{15}\text{N}$ compared to the same species at Sites A and D, apart from *Mastomys sp.* which displayed elevated $\delta^{15}\text{N}$ in Site D. The isotopic composition of *Mastomys sp.* across sites reflected the abundance of C3 grasses, supporting findings that the species is granivorous where possible (Veenstra 1958, Monadjem and Perrin 1998b) - both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ became enriched as C3 seeding grass abundance increased (seeds of C3 grasses are generally 0.5 – 1.5‰ enriched in ^{15}N relative to their foliage; Codron *et al.* 2005a, 2005b). Isotopic variation in *R. dilectus* did not appear to reflect the abundance of any plant types, supporting findings that the species is a widespread generalist (Hechter-Schulz 1962, Perrin 1980, Schradin and Pillay 2005a). The differences of the position of *Mastomys sp.* and *R. dilectus* in delta-space suggested that interspecific competition between the two species was smaller than assumed using the aggregate variability measures used in Chapter 2.

In Site B, where soil and small mammal hair mercury concentrations were highest, species had broader $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ranges than their counterparts in other sites, but as a community, small mammals in Site B displayed relatively similar average $\delta^{13}\text{C}$ values. *R. dilectus* in Site B showed the greatest trophic variability, reflected in both the SIAR and Layman metrics calculated. However, the lack in any discernible patterns between mercury concentration, small mammal species

diversity and density, and isotopic variation provides little evidence of direct influence by mercury pollution on small mammals at the WWMC. Instead, it is likely that site-specific vegetation has a greater effect on small mammal isotopic composition than the range of mercury pollution examined here and interspecific competition.

Mercury pollution, however, may have an indirect effect on small mammal isotopic niche width by affecting food sources, such as plants by limiting plant access to micronutrients like nitrogen (Sage and Pearcy 1987). As different food resources have different mercury concentrations, animals with a broader range of potential food resources will be exposed to a wider range of mercury concentrations (both high and low [omnivores]). Animals with a narrow range of potential food resources will be exposed to a smaller range of mercury concentrations (either high [insectivores] or low [herbivores]). As the shrew, *C. mariquensis* holds the highest trophic position of the small mammals captured, and is fairly common, it is a good indicator species of bioaccumulation within the food web.

5.3 Mercury bioaccumulation in small mammal hair

The final objective of the study was to compare mercury concentrations measured in the hair of small mammals a) between captured species, b) to the environmental concentrations measured by Makiese (2012), and c) to the target and intervention levels stipulated by international regulatory organisations. The comparison of mercury concentrations in small mammal hair between species

entailed direct comparison between species, and comparisons between species within the context of their relative isotopic trophic positions. It was expected that mercury concentrations in both the environment and small mammal hair would be elevated above international target levels, and that bioaccumulation of mercury in the terrestrial food web would be evident.

Individual variation in hair mercury concentration was large, likely as a result of the utilisation of different food resources by individuals, exposing small mammals to different levels of mercury pollution (Martin and Coughtrey 1982, Peterle 1996, Evans *et al.* 1998, Sobańska 2005). The insectivorous shrew, *C. mariquensis* had the highest species specific hair mercury concentrations. As such, insectivorous small mammal hair mercury concentrations were significantly higher than those of omnivorous and herbivorous small mammals. The highest recorded individual hair mercury concentration was measured in a *Mastomys sp.* individual, but hair mercury concentrations did not exceed the advisory maximum tolerable level for mercury in hair stipulated by WHO (1990, 1991).

Small mammal hair mercury concentrations followed the site-specific patterns of soil mercury concentrations. Site B contained the most elevated soil and hair mercury, followed by Site A and Site D. Soil mercury concentrations were generally lower than the Dutch Standard target values (SRC 2009), except in the reed-beds at Site A and B where mercury concentrations were higher. However, mercury concentrations were still much lower than the Dutch Standards intervention levels (SRC 2009), at which point direct human intervention is required. Water mercury concentrations were nearly a thousand times lower than the WHO (2004) standard for drinking water mercury concentrations.

Although small mammal hair mercury concentrations did not exceed international regulatory standards, small mammal hair did reflect the general mercury abundance in their habitats. Hair mercury concentrations also reflected trophic strategy, as insectivores had significantly higher hair mercury concentrations than omnivores and herbivores. Whilst not significant, there was a trend in omnivores toward higher hair mercury concentrations than herbivores as expected. Hence, bioaccumulation of mercury is evident in the terrestrial food web at WWMC, but not to the point where mercury would be considered a health hazard to wildlife or humans by international regulatory organisations.

5.4 Overall conclusions and implications

This study indicated that small mammal communities living in the mining-polluted environment surrounding the Varkenslaagte stream are influenced by disturbances to vegetation and associated factors, but no clear measurable impact by chronic exposure to mercury pollution was found. Mercury concentrations were lower than expected given the visible degree of contamination, and do not seem to show a marked health risk to small mammals at the current levels. However, there is the risk that a large enough release of pollution into the environment, such as through the release of tailings from a storage facility could increase mercury concentrations to the point of there being a risk of acute mercury poisoning. The accumulation of mercury in the environment over time could also increase the potential of chronic mercury exposure.

Whilst the study would have benefitted from the inclusion of such factors as control study sites, the unique structure of the highly impacted and modified Varkenslaagte catchment makes it difficult to find sufficiently comparable non-polluted control sites. The inclusion of non-polluted control sites would have increased the number of confounding factors when trying to compare polluted versus non-polluted sites. The aim of the study was to compare mercury levels in the catchment and its small mammals to international regulatory levels, and to test for bioaccumulation within the food webs of which small mammals play an important role.

The variable level of bioaccumulation of pollutants in the food web makes it difficult to predict pollution levels in different animals without taking samples (Shore and Douben 1994), making the direct measurement of environmental pollution levels alone insufficient when assessing the bioavailable fraction of pollutants (Newman 1998, Beernaert *et al.* 2007). The structure of the small mammal community at the WWMC, containing primary and secondary consumers, can therefore be used to gain more detailed insight into the interactions of food webs and environmental pollution in the mining environment.

As discussed by Krebs *et al.* (1973) and Pereira *et al.* (2006), small mammals, as used in this study, make good indicators of environmental pollution. Most importantly for a study of this kind, 1) small mammals are relatively easy and inexpensive to capture and sample non-invasively, 2) carefully homogenised hair samples can be used for a wide suite of analyses including elemental (such as Hg or other heavy metals) and stable light isotope analysis, and 3) as primary and

secondary consumers, small mammals span multiple trophic positions allowing researchers to measure the potential bioaccumulation of contaminants.

A well-designed and efficient environmental pollution monitoring programme should therefore include the use of small mammals as biological indicators, using both population dynamics and food web metrics, to compliment the environmental information about the investigated problem. In future, a study of this kind should include more detailed environmental information about specific food resources utilised by small mammals, such as the isotopic composition and pollution levels in specific plant and insect prey species, so that sources of contaminant exposure can be accurately identified and the bioaccumulation factors calculated. The measurement of environmental contaminants at the same time as biological sampling would aid in discerning the effect of contaminants on small mammal population dynamics. The use of small mammals as biological indicators would also benefit from detailed research into the effect that mining pollution has on small mammal behaviour, feeding ability and resource selection.

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Appendices:

Appendix A: Concentration of inorganic mercury (IHg) and methylmercury (MHg) in West Wits Mine Complex waters.

Sample	T (°C)	pH	Eh (mV)	Ec (mS cm ⁻¹)	IHg (ng L ⁻¹)	%RSD	MHg (ng L ⁻¹)	%RSD	%MHg
1(15A)	19.8	5.92	387.7	3.82	0.004	6.5	0.292	25.8	98.5
2 (17A)	20.4	6.67	482.9	3.88	0.330	2.0	7.835	6.3	96.0
3 (19A)	20.0	7.51	452	3.64	13.055	2.9	6.547	4.8	33.4
4 (20A)	20.0	7.74	445	3.55	0.195	2.4	3.436	6.6	94.6
24B	20.3	8.95	415	2.03	0.099	6.6	0.007	32.2	6.7
5 (25B)	19.3	8.97	384	2.02	0.003	9.4	bdl	-	0

bdl = below detectable limit

Appendix B: Concentration of inorganic mercury (IHg) and methylmercury (MHg) in West Wits Mine Complex sediments and tailings. Depth in cm in parenthesis following sample code.

Sample code (n =3)	Hg _{TOT} (µg kg ⁻¹)	IHg (µg kg ⁻¹)	%RSD	MHg (µg kg ⁻¹)	%RSD	%MHg
8	267.0 ± 20.7	290.9 ± 3.6	1.3	1.9 ± 0.2	8.7	0.6
16G(0-2)	49.3 ± 1.9	nd	-	nd	-	-
16K(0-5)	38.9 ± 2.1	nd	-	nd	-	-
16L(0-5)	344.2 ± 13.1	331.8 ± 5.0	1.5	1.9 ± 0.5	26.2	0.6
16L(5-10)	nd	205.0 ± 0.8	0.4	bdl	-	0.0
16L(10-15)	nd	57.8 ± 1.0	1.7	bdl	-	0.0
16L(15-20)	nd	55.3 ± 3.2	5.7	bdl	-	0.0
16L(20-25)	nd	274.7 ± 9.6	3.5	bdl	-	0.0
16L(25-30)	nd	192.4 ± 2.3	1.2	bdl	-	0.0
17E(0-2)	58.5 ± 3.0	nd	-	nd	-	-
19F(0-2)	628.4 ± 7.6	505.2 ± 1.4	2.3	8.8 ± 0.5	5.8	1.7
19F(2-4)	nd	420.4 ± 2.0	0.5	4.7 ± 0.1	2.1	1.1
19F(4-6)	nd	336.9 ± 16.7	4.9	8.1 ± 2.1	26.6	2.3
19F(6-10)	nd	565.4 ± 15.0	2.6	11.3 ± 1.0	8.7	2.0
19fF(10-15)	nd	76.0 ± 1.8	2.4	bdl	-	0.0
19F(15-20)	nd	51.4 ± 2.4	4.7	3.0 ± 0.0	0.4	5.4
23(0-2)	124.9 ± 6.5	119.6 ± 4.7	3.9	bdl	-	0.0

bdl: below detection limit

nd: not determined

Appendix C: West Wits Mine Complex water and soil sampling details (collected winter 2009) as they appear on Figure 1.

Sample ID	Description	GPS
8	Soil core from Old North TSF	S26°25.638' E027°22.337'
15A	Water sample from Vaarkenslaagte canal (Top of canal below TSF)	S26°25.725' E027°22.376'
16G	Core from Old Reed Bed	S26°25.706' E027°22.380'
16K	Core from V. canal	
16L	Core from Reeds near V. canal	
16Y	<i>P. australis</i> from V. canal	
17A	Water samples below Buldens Warehouse	S26°26.492' E027°21.847'
17C	<i>Typha. spp</i> below Buldens Warehouse	S26°26.492' E027°21.847'
17E	Soil core below Buldens Warehouse	S26°26.492' E027°21.847'
19A	Water from Dam	S26°26.790' E027°20.822'
19F	Soil core	S26°26.798' E027°20.872'
20A	Water samples downstream from dam	S26°26.801' E027°20.787'
23	Soil core	S26°26.846' E027°20.855'
24B	Water samples	S26°26.832' E027°19.190'
25B	Stream before flows under Welverdened Rd	S26°26.373' E027°19.216'

Appendix D: Formulae used in the calculation of the Simpson Diversity Index (Equations 1) and the Shannon-Wiener Diversity Index (Equation 2) and Evenness index (Equation 3). Equations from Krebs 1998.

$$D = \sum_{i=1}^S \frac{n_i(n_i - 1)}{N(N - 1)}$$

Equation 1

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

Equation 2

$$E = \frac{H'}{H_{\max}}$$

Equation 3