# THE EFFECT OF WASTEWATER WORKS ON FORAGING BEHAVIOUR AND METAL CONTENT OF *NEOROMICIA NANA* (FAMILY: VESPERTILIONIDAE)

by

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# ABSTRACT

Anthropogenic disturbance from urbanization has introduced a range of contaminants into freshwater ecosystems. Wastewater Treatment Works (WWTW) in particular, deposit effluent with high metal concentrations directly into rivers. These pollutants may affect river biota directly or through modifications to habitat and prey. Therefore, the impact of metal pollution through a food chain should be evident in high trophic level predators such as *Neoromicia nana*. *N. nana* is a small, insect-eating bat that occurs in forest and riparian habitats in Africa. Most importantly, it is an urban exploiter, i.e. a species that takes advantage of anthropogenic food and habitat resources. I investigated the foraging behaviour and metal content of *N. nana* at wastewater-polluted sites (WWTW sludge tanks and sites downstream of wastewater discharge into the rivers) and unpolluted sites (sites upstream of wastewater discharge) at three urban rivers in Durban, South Africa, during winter and summer. To assess water quality, I determined cadmium, copper, chromium, iron, nickel, zinc and lead concentrations using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). To investigate the foraging behaviour of *N. nana*, I quantified relative *N. nana* abundance, and feeding activity from recorded echolocation calls. Using ICP-OES, I quantified metal concentrations in three tissues (liver, kidney and muscle). My results show that concentrations of most metals were generally lowest upstream, intermediate at downstream sites and highest at the tanks. The relative abundance and feeding activity of *N. nana* were significantly higher at wastewater-polluted sites than at upstream sites, despite there being significantly more insect orders upstream. However, pollution-tolerant Chironomidae (Diptera), were significantly more abundant at wastewater-polluted sites. Indeed, at wastewater-polluted sites, Diptera represented the highest percentage of insects in the diet of *N. nana*. Essential metals (copper, zinc and iron) were detected in all tissue samples of *N. nana*. In contrast, the toxic metals cadmium, chromium and nickel were present in tissue of bats only at wastewater-polluted sites (except one upstream occurrence of cadmium). This suggests that these metals may accumulate in tissue through the ingestion of pollutant-exposed prey. Thus, metal pollution from WWTWs affects not only water quality of rivers, but also the diversity of resident aquatic insects and ultimately the ecology of *N. nana* populations, which may pose serious long-term health risks for these top predators.

# PREFACE

The experimental work described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, from January 2009 to December 2011, under the supervision of Dr. M. Corrie Schoeman and co-supervision of Dr. Dalene Vosloo and Dr. Robin L. Mackey.

This study represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

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I, Samantha Naidoo declare that

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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis:

Publication 1: **Foraging behaviour of the banana bat (*Neoromicia nana,* Family: Vespertilionidae*)* at rivers polluted by wastewater works.**

S. Naidoo, D. Vosloo, R.L. Mackey& M.C. Schoeman (in prep. for *Journal of Zoology*).

Author contributions: SN led the writing, and collected and analysed the data; SN and MCS conceived the ideas and experimental design; MCS, DV and RLM contributed to the writing. The manuscript will be submitted in December 2011.

Publication 2: **Metal content in kidney, liver and muscle tissue of the banana bat (*Neoromicia nana,* Family: Vespertilionidae*)* foraging at wastewater-polluted rivers.**

S. Naidoo, M.C. Schoeman, R.L. Mackey & D. Vosloo (in prep. for *Water SA*).

Author contributions: SN led the writing, and collected and analysed the data; SN DV, and MCS conceived the ideas and experimental design; SN and DV conducted the laboratory work; MCS, DV and RLM contributed to the writing. The manuscript will be submitted in early January 2012.

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# CHAPTER 1: INTRODUCTION
# THE EFFECT OF WASTEWATER POLLUTION ON A HIGH TROPHIC LEVEL PREDATOR AND ITS PREY AT RIVERS

## *1.1. Background*

The global expansion of cities is increasing rapidly, with large areas of natural land being transformed into urbanized landscapes (McKinney, 2006). It is projected that the majority of this urbanization will occur in coastal countries such as South Africa, where anthropogenic stress on the environment is already widespread (McKinney, 2006). To cater for the growing human populations, industrial development and services are increased. However, this development creates serious habitat alteration, which impacts on both the environment and its resident wildlife. The physical manipulation of the landscape by anthropogenic activities such as fragmentation has had predominantly negative impacts on the resident fauna and flora (Schmiegelow & Monkkonen, 2002).

In addition to the physical land-transformation, a chief anthropogenic disturbance to the urban environment is pollution. River pollution is currently a major problem, and there has been a recent influx of data highlighting the poor state of South African rivers in urban areas (eThekwini Municipality State of the Rivers Report, 2007). The rapid rate of urbanization in South Africa has resulted in the introduction of a range of contaminants into freshwater ecosystems (Gleick, 1998). Increased industrial development produces inorganic and organic pollutants, such as chemical runoff from textile factories and sewage effluent that are deposited directly into rivers (Sacks & Buckley, 1998).

These pollutants often have an adverse effect on biodiversity (Nedeau *et al*., 2003; Azrina *et al*., 2006; Vörösmarty *et al*., 2010). They may have a direct effect on organisms or may influence them through modifications to the habitat or prey. Within the previous century, the species diversity of aquatic invertebrates (Williams *et al*., 2003) and aquatic vertebrates (Reash & Berra, 1987) has decreased significantly in polluted rivers. For instance, the global diversity

of non-marine mollusks has been steadily decreasing, with 708 freshwater mollusc species included in the 2002 IUCN Red List of Threatened Species (Lydeard *et al*., 2004). Vertebrates such as the African bullfrog (*Pyxicephalus adspersus*), have shown a rapid decline in numbers in urban reserves in South Africa (Oberholster *et al*., 2008). This is due to developmental and behavioural abnormalities resulting from anthropogenic water pollution (Bridges & Semlitsch, 2000). Furthermore, industrial and pharmaceutical substances containing xenobiotic chemicals such as endocrine disrupting chemicals (EDCs) are found in specific pollution sources such as wastewater (Fossi *et al.,* 2002). These may have serious negative effects on the reproductive, endocrine and immune systems of pollutant exposed organisms (Fossi *et al.,* 2002).

Wastewater treatment works (WWTWs) provide an ideal model to investigate the toxic effect of river pollution on exposed organisms. They are ubiquitous in urban landscapes and are constantly in operation. Wastewater treatment works are an essential service, linked to urbanization and the concentration of human populations associated with it. They also have a precise point of effluent discharge into the river, allowing for a clear partitioning of sites receiving or not receiving wastewater. Furthermore, the type of pollution can be assessed from identifying wastewater constituents (Leland *et al*., 1974).

Wastewater effluent contains both industrial and domestic input including solids, pathogens and organic and inorganic pollutants (Gagnon & Saulnier, 2003). Various types of operational practices are employed by wastewater treatment plants to treat waste effluent. Conventional operational practices such as the screening and spraying of wastewater onto percolating filter beds have been largely replaced by more recently designed systems (Govender, 2002). The system most often used in South Africa is the process of activated sludge, because it is able to cater for larger populations and requires small land space (Govender, 2002). Domestic and industrial waste influent received by treatment plants employing this system, undergoes an aerobic biological process whereby wastewater is degraded using microbial communities (Lalbahadur, 2005). The wastewater is treated in aeration and settling tanks. It is then chlorinated to remove pathogenic organisms before being discharged into rivers (Jackson *et al*., 2002). However, the sludge produced in the tanks used for this process contains high levels of metals (Govender, 2002; Braum, 2004). These metals, including lead (Pb), cadmium (Cd), chromium (Cr) and nickel (Ni), are particularly toxic to living organisms when ingested in large quantities or over a long period of time

(Andres *et al*., 2000). The processes used to treat wastewater focus on the removal of solids, nutrients and pathogenic bacteria, often neglecting the treatment of metals (Gagnon & Saulnier, 2003).

In addition to the metals in the wastewater sludge, are large amounts of organic waste. High input from nutrients such as nitrogen (N) and phosphorous (P) compounds, promote eutrophication where elevated quantities of organic matter are produced (Yount & Crossman, 1970). Pollution-tolerant insects often thrive in eutrophied waters (Yount & Crossman, 1970). Consequently, pollution-tolerant insects such as the family of aquatic flies known as the chironomid midges, often occur at a high density at artificial tanks containing wastewater (Broza *et al*., 2003). The growth rate of chironomids is rapid, with a quick generation turnover, ensuring constant availability to the ecosystem (Menzie, 1981). Although chironomid larvae are found in sediment, the chironomid life cycle is dominated by the adult stage which is spent on and above the water surface (Ristola, 2000). Chironomid midges are capable of enduring polluted environments (Postma *et al.* 1995). Because they are strongly associated with polluted water, chironomids have long been used as indicators of poor water quality (Chutter, 1972). They are generally more abundant at sites located downstream of sewage discharge points than at sites located upstream of the effluent discharge into rivers (Abbott *et al*., 2009).

In the wastewater, particulate matter upon which the midges feed contains metal toxicants (Stuijfzand *et al*., 2000). Midges, amongst few other insect groups, are able to accumulate metals without being affected (Groenendijk *et al*., 1998). Calculated bioconcentration ratios indicate that metal pollutants bioaccumulate in even higher concentrations in the predators of the affected insects (Hsu *et al*., 2006). Unable to undergo metabolism, most metallic elements that are not excreted, become stored in the body (Fritsch *et al*., 2010). In turn, the physiology and anatomy of the organism may be negatively impacted (Walker, 1998). For instance, the great tit (*Parus major*) is an insectivorous predator, which has shown marked impairment in breeding success of populations in Finland as a result of metal exposure (Eeva *et al*., 2009). Decreased clutch size and hatching success of *P. major* populations were evident in a polluted area when compared to unexposed populations (Eeva *et al*., 2009). In addition to the well established negative effects of metal pollution observed in predatory bird species, metal

concentrations measured in small mammal species also indicate that metal exposure can have acute or chronic effects on mammalian health (Fritsch *et al*., 2010).

Bats are top mammalian predators of many ecosystems. In addition, bats are excellent indicators of habitat quality (Jones *et al*., 2009). This is because the life history characteristics of bats render them vulnerable to environmental changes (Kunz, 1982; Walsh & Harris, 1996). The long life span of bats is especially important because the accumulation of certain metals is specifically associated with age (Walker *et al*., 2002). In addition, the slow reproductive rate of bats allows for clear trends of population decline or increase to be elucidated (Jones *et al*., 2009). Rivers are important foraging habitats for numerous bat species (Racey *et al*., 1998; Warren *et al*., 2000). Insect-eating bats may be particularly vulnerable to water pollution because riparian vegetation and the emergent aquatic insects upon which bats feed are in direct contact with the polluted water (Walsh & Harris, 1996).

Kalcounis-Rueppell *et al*. (2007) compared the abundance and feeding rates of insectivorous bats at sites upstream and downstream of a sewage output in North Carolina, USA. The abundance and foraging activity of *Eptesicus fuscus* was lower downstream than upstream of the sewage discharge point. However, *Perimyotis subflavus,* a species that specializes in riparian habitats (Ford *et al*., 2005), was more abundant and fed more extensively at downstream sites (Kalcounis-Rueppell *et al*., 2007). Thus, poor water quality does not affect all bat species negatively. Some bat species may in fact, in the short term, benefit from the proliferation of prey insects in polluted water. Vaughan *et al*. (1996) also investigated differences in bat activity above and below sewage outputs and found that while certain species such as *Pipistrellus pipistrellus* were most active upstream of a pollution source; others such as *Myotis* species concentrated their feeding activity downstream of the pollution source. In addition, Racey *et al*. (1998) compared two rivers with differing water qualities and established that a river with inferior water quality could support bat activity and insect density as great as a healthy river. Most studies on the impacts of wastewater pollution on bats have been undertaken in Europe and the USA.

Studies in southern Africa have only recently begun to elucidate these effects (Naidoo *et al*., in press). Abundance and species richness of insectivorous bats was higher at a polluted river than at an unpolluted river in Durban, South Africa (Naidoo *et al*., in press). Furthermore, bat

species composition and phenotypic structure differed between the polluted and the unpolluted river (Schoeman & Waddington, in press). The majority (approximately 41%) of bats recorded at the polluted river was represented by *Neoromicia nana*. *N. nana* is generally known as the banana bat because it typically roosts in rolled-up banana leaves (LaVal & LaVal, 1977). It is a small (3 - 4 grams), insect-eating bat that commonly occurs in forest and riparian habitats throughout sub-Saharan Africa (Monadjem *et al*., 2010). Most importantly, *N. nana* is an urban exploiter; i.e. a species that takes advantage of food or habitat resources provided by humans (Jung & Kalko, 2011). The small size and the fact that it is an urban exploiter suggest that *N. nana* would exploit the increased availability of small chironomid midges at sewage-polluted sites. Furthermore, chironomid activity is at its peak during the early evening (Broza *et al*., 2003), which correlates with the foraging period of *N. nana*. The toxic effects of pollution should thus be evident in *N. nana*, making this species an ideal model predator to assess the impact of wastewater pollutants through a food chain.

## *1.2. Outline of thesis*

The main purpose of this study is to compare foraging behaviour and the metal content in tissues of *Neoromicia nana* at sites polluted and unpolluted by wastewater effluent. I investigate biotic and abiotic components of the ecosystem that are closely associated with bat activity and are also affected by wastewater pollution. In obtaining an overall representation of the risk of wastewater impact on *N. nana*, I ask the following questions and test the following predictions:

1. Do concentrations of wastewater-associated metals (copper, chromium, iron, nickel, zinc, lead and cadmium) differ between water samples collected at sites upstream of the point of wastewater effluent discharge into the rivers, at sludge tanks of WWTWs and at sites downstream of the point of wastewater effluent discharge into rivers (Chapter 2)? This provides a measure of water quality at the sites where foraging behaviour of *N. nana* was investigated.

2. How does wastewater pollution affect the foraging ecology of *N. nana* populations (Chapter 3)? I predicted that the relative abundance and feeding activity of *N. nana* would be higher at wastewater-polluted sites (sludge tank and downstream sites) than at sites situated upstream of the wastewater pollution, as a result of the high abundance of the pollution-tolerant chironomid midges specifically associated with wastewater and eutrophication (Marques *et al*., 1999). If so, I further predicted that there would be a significant correlation between the abundance of pollution-tolerant insects at wastewater-polluted sites and in the diet of *N. nana* at the sites.

3. What is the risk associated with foraging at wastewater-polluted rivers for *N. nana* (Chapter 4)? To investigate this, I quantified concentrations of the wastewater-associated metals (see Chapter 2) in the kidney, liver and muscle tissue of *N. nana* at sites unpolluted and polluted by wastewater. I predicted that metal concentrations in *N. nana* tissue would correlate with the metal concentrations in the water at the sites.

Finally, in Chapter 5 I synthesize the conclusions of the previous chapters, specifically within the framework of the main aims of the research. Factors contributing to differences in the foraging behaviour of *N. nana* at sites polluted and unpolluted by wastewater effluent, and the potential health and population effects in pollutant exposed *N. nana* are discussed. Implications of wastewater pollution for bat communities and river ecosystems are explored. To conclude, recommendations for future studies are made.

# CHAPTER 2:
# METAL CONTENT AS AN INDICATION OF WATER QUALITY IN RIVERS POLLUTED BY WASTEWATER EFFLUENT

## *2.1. Summary*

Wastewater effluent, which contains pollutants from domestic and industrial waste, is treated at WWTWs and then directly deposited at a discharge point into the river. Metals are not easily degraded and thus remain in the treated and discharged effluent, becoming toxic at elevated concentrations. Organisms, including insects upon which *N. nana* feeds, are exposed to the metals in the water, and in turn may cause elevated metal concentrations in these animalivorous bats. To obtain a measure of water quality and later evaluate possible health risks for *N. nana*, I used Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) to determine cadmium, copper, chromium, iron, nickel, zinc and lead concentrations at sites upstream of the point of wastewater effluent discharge into the rivers, at sludge tanks within the WWTWs, and at sites downstream of the point of wastewater effluent discharge into each of three rivers (Mdloti River, Little Amanzimtoti River, Umbilo River) selected in Durban, South Africa.

Concentrations of all metals tested at the Umbilo and Mdloti Rivers (Cu, Cr, Fe, Ni, Zn and Pb), and four of the six metals tested at the Little Amanzimtoti River (Cr, Ni, Zn and Pb), were highest at the tank sites. Nearly 80% of the ANOVAs performed were significant, with post-hoc tests showing differences between sites, for all metals except Ni and Pb. Fe was the metal with the highest content at all rivers, and Pb/ Cr were the lowest. Metal concentrations at the downstream sites were generally higher than at upstream sites, although a few of the metals were higher upstream. This may be the result of illegal waste discharge into the rivers, and other external factors such as use by residents of informal settlements. The point source pollution of WWTWs is evidently the most significant contributor of contaminants into the rivers. Furthermore, most of the metals are in excess of the Target Water Quality Range (TWQR) set for the aquatic ecosystem. Thus, predators such as *N. nana,* foraging at the tanks, may be at a higher health risk than those foraging at other points along rivers.

## *2.2. Introduction*

Although rivers comprise a small quantity of the biosphere's stored water, they serve an invaluable function in the preservation of life on earth (Allan & Flecker, 1993). Rivers provide physical, geological, chemical, hydrological and biological functions. For example, the flowing water of rivers is a key component to the water cycle and to the global movement of nutrients and minerals. In addition, river ecosystems support unique and diverse biotic communities and in turn affect the surrounding terrestrial life (Pompeu & Alves, 2005). While rivers are vital to the functioning of the natural world, they also provide important services such as agriculture, recreation, waste removal and renewable energy to mankind. Anthropogenic activities, however, have resulted in modifications which often cause major degradation to the functions of rivers (Paul & Meyer, 2001).

Wastewater pollution in particular is a major cause of river degradation. Industrial, business, drainage and domestic waste sent to wastewater treatment works (WWTWs) contain solids, pathogens and organic and inorganic pollutants (Gagnon & Saulnier, 2003). After treatment, the effluent is deposited at a discharge point into the river, ultimately going to marine outfalls. Organic material in the effluent is considerably reduced by chemical processes during treatment. However, metals are not biologically degradable (Moeletsi *et al*., 2004), and different metals may be toxic at varying concentrations (Newman & Unger, 2003). The term „toxic' can be defined as natural or synthetic chemical substances that have adverse effects on living organisms (DWAF, 1996). There are more than twenty metals classified as toxic, many of which contribute significantly to the pollution of river water (Nomanbhay & Palanisamy, 2005). Waste effluent from industries such as tanneries, electronics, electroplating, petrochemical, textile mills, paint/ alloy/ plastic manufacturers; and iron producers are some of the main sources of metals sent to WWTWs (DWAF, 1996; Chuah *et al*., 2005).

The most toxic metals found in WWTW effluent are lead (Pb) and cadmium (Cd) (Kazemipour *et al*., 2008). These metals are known as non-essential elements, because they are not required for biological functioning. Cd has strong carcinogenic properties, and Pb may induce neurological dysfunction with chronic exposure (Newman & Unger, 2003). Due to their high toxicity, the concentrations of these metals should be low in river water according

to the standards stipulated by the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996). However, an evaluation of a South African river (the Umtata River in the Eastern Cape) found that both Cd and Pb concentrations exceeded permissible amounts by ±100 fold (Fatoki *et al*., 2002). In freshwater, both Pb and Cd often exist as free ions and are therefore readily assimilated by aquatic organisms (DWAF, 1996).

Chromium (Cr) and nickel (Ni) are also non-essential metals which occur in wastewater. Cr, like other metals, occurs in several forms, each with a different degree of toxicity (DWAF, 1996). One broad effect of Cr is that it may temporarily reduce growth in young fish (DWAF, 1996), whereas high concentrations of Ni are both toxic and carcinogenic (Newman & Unger, 2003). The other metals most commonly occurring in wastewater, including copper (Cu), zinc (Zn) and iron (Fe), are essential to the biological functioning of organisms. However, they are potentially hazardous in highly elevated concentrations (Moeletsi *et al*., 2004), causing for example, acute or chronic physiological effects such as neurotoxicity (Du & Wang, 2009) and decreased reproductive success (Eeva *et al*., 2009) in exposed organisms.

Metals released into rivers can exist in solution as free cations (Newman & Unger, 2003), become adsorbed onto the sediment and/or by plants in the water (Wang *et al*., 2003), or get taken up by aquatic and semi-aquatic insects and other organisms living in or near the water (Rainbow, 2002).Ultimately these metal particles may end up in the bodies of higher predators (Hsu *et al*., 2006). For example, metal induced damage in the organs of aquatic predators such as fish often correspond to a high metal content in the surrounding water (Cerqueira & Fernandes, 2002; Grosell *et al*., 2003; van Heerden *et al*., 2004; van Heerden *et al*., 2006). Terrestrial predators such as animalivorous bats can also be affected by metal pollution in rivers by, for example, drinking water from rivers, and feeding on large quantities of river-dwelling insects (Walker *et al*., 2007). It is thus through the food chain that metal concentrations magnify from river water to predators (Vickerman & Trumble, 2003).

The aim of this chapter was to measure the water quality of three urban rivers in Durban, South Africa that are used by *Neoromicia nana*, by quantifying metals (cadmium, copper, chromium, iron, nickel, zinc, and lead) commonly found in wastewater, at sites upstream of the point of wastewater effluent discharge, at the sludge tanks of WWTWs, and at sites

downstream of the point of wastewater effluent discharge. I predicted that metal concentrations would be lowest at upstream sites and highest at sludge tanks.

## *2.3 Methods*

### *2.3.1. Study area*

The study was conducted in the urban landscape of Durban, South Africa (S29°58'; E30°57'). There are approximately 32 WWTWs which operate within the Durban Metropolitan (CEROI, 1999). These WWTWs collectively receive over 400 million litres of domestic and industrial waste per day (CEROI, 1999). The discharge volume of the waste effluent to rivers is high, amounting to an average of 220 million litres per day (eThekwini Municipality State of the Rivers Report, 2007). Three rivers which receive effluent from WWTWs that use the activated sludge tank system were selected: the Mdloti River (DWAF, 2009), the Little Amanzimtoti River (Naidoo *et al*., 2002) and the Umbilo River (Lacko *et al*., 1999) (Fig. 1). The WWTWs included the Verulam Wastewater Works (S29º38.38; E31º03.49) situated on the Mdloti River, the Kingsburgh Wastewater Works (S30º04.29; E30º51.26) situated on the Little Amanzimtoti River and the Umbilo Wastewater Works (S29º50.44; E30º53.31) situated on the Umbilo River. Domestic and industrial waste influent received by these treatment plants undergoes an aerobic biological process whereby wastewater is degraded using microbial communities (Lalbahadur, 2005). The wastewater is treated in aeration and settling tanks. It is then chlorinated to remove pathogenic organisms before being discharged into the rivers (Jackson *et al*., 2002).

The Verulam Wastewater Works is located in the northern region of Durban, the Kingsburgh Wastewater Works in the southern region, and the Umbilo Wastewater Works centrally (Fig. 1). Upstream and downstream sites at each river had similar abiotic and biotic features including water flow rate, width between banks (range 3 - 9 m), water surface clutter (Biscardi *et al*., 2007), and riparian vegetation (visual assessment). The distance between sites and the distance from outflow point were also similar among the rivers.

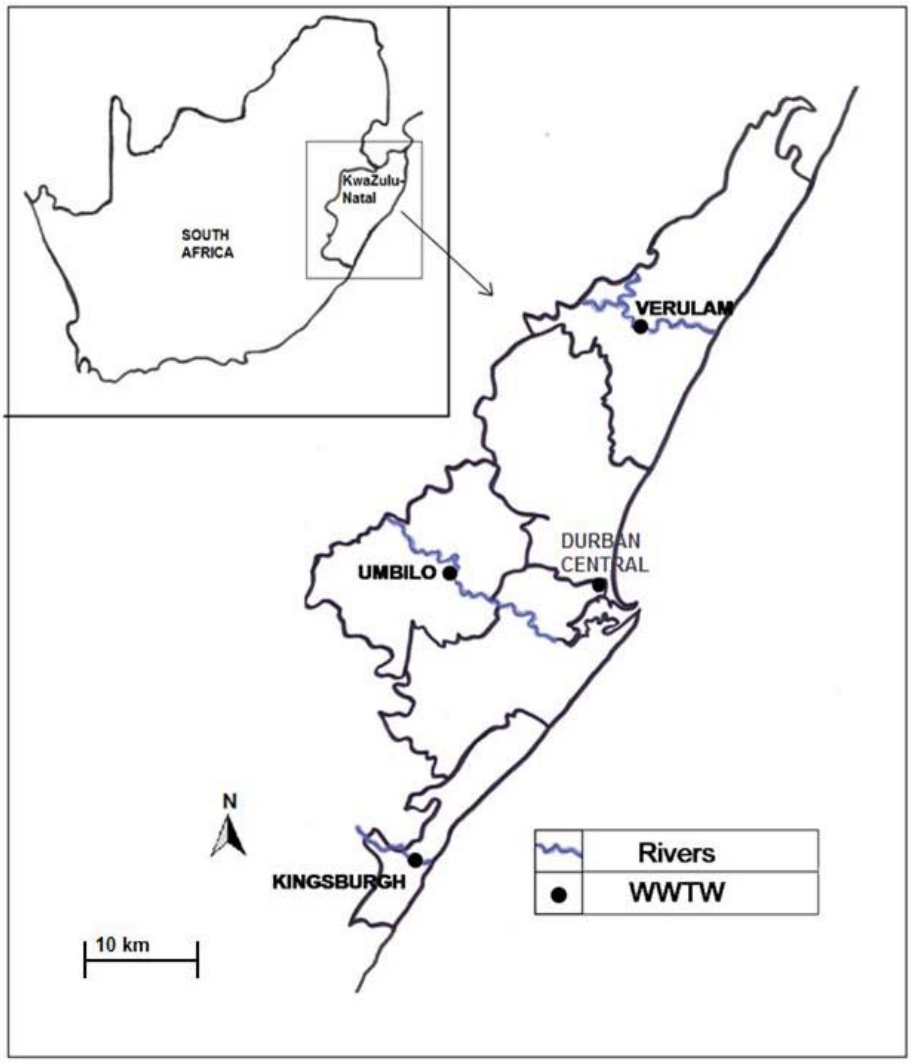


Fig. 1. Map of the study area in Durban, South Africa showing the location of the Verulam, Kingsburgh and Umbilo Wastewater Works on the Mdloti, Little Amanzimtoti and Umbilo Rivers respectively.

### *2.3.2. Collection and preparation of samples*

Water samples were collected in 500 ml plastic collection bottles at sites upstream of the point of wastewater effluent discharge, at the sludge tanks of WWTWs, and at sites downstream of

the point of wastewater effluent discharge. At each site, water was collected at sunset to correspond with the feeding emergence times of bats, on consecutive days in January 2010, between the summer and winter bat/ insect sampling periods (Chapter 3). Three replicates were taken at each of the nine sites, resulting in a total of 27 samples. To minimize the possibility of contamination and eradicate bacterial growth, the 500 ml plastic bottles were soaked overnight in a 10% acid wash (distilled water and $HNO_3$), and then washed in distilled water. They were then prepared for sample collection with 2 ml 65% concentrated nitric acid (Jackson *et al*., 2007). At each site, water was collected just below the surface. After allowing for overnight nitric acid digestion, the samples were filtered through Advantec GA - 55 (47 mm) glass fiber filter membranes on a filtration pump, to remove particulates. Ten ml of the filtered water was refrigerated until analysis for metal content.

### *2.3.3. Inductively Coupled Plasma Optical Emission Spectrometry* (*ICP-OES*)

Metal content was determined using ICP-OES. This method includes the following components: a sample introduction system, ICP torch, high frequency generator, transfer optics, and spectrometer and computer interface (Halday, 2007). A metal solution of 5 mg/L concentration was prepared using cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), nickel (Ni), zinc (Zn) and lead (Pb). This was further diluted with double distilled water to produce calibration standards of the following concentrations: 1 mg/L; 0.5 mg/L; 0.25 mg/L; 0.1 mg/L; 0.05 mg/L and 0.01 mg/L. The accuracy of the calibration standards were tested using the ‚automated analysis control' function on WinLab32 ICP Continuous software (Perkin Elmer, USA). Baseline calibration curves were set for each metal (Table 1) to create a reference wavelength at which metal content is determined in each sample. An ICP-OES (Perkin Elmer, Optima 5300 DV) was then used to measure the content of each metal in the samples.

Although mercury (Hg) is also a toxic metal which is of major concern as a pollutant in rivers (Moeletsi *et al*., 2004), it was not included in the suite of metals (Cd, Cu, Cr, Fe, Ni, Zn and Pb) tested at the rivers in this study. This is because Hg is a hydride and detection therefore

requires specialized equipment, i.e. a cold vapor system, which is not commonly available (Henry & Miles, 2001).

Table 1. Baseline wavelengths (nm) selected for individual elements. Samples were analysed for copper (Cu), chromium (Cr), iron (Fe), nickel (Ni), zinc (Zn) and lead (Pb). Cadmium was excluded from the analysis because concentrations were below the detection limit of ICP-OES.

| **Element** | **Baseline wavelength (nm)** |
|---|---|
| Cu | 327.393 |
| Cr | 267.716 |
| Fe | 238.204 |
| Ni | 231.604 |
| Zn | 206.200 |
| Pb | 220.353 |
| Cd | Excluded (Values below detection limit) |

### *2.3.4. Target water quality range (TWQR) index*

South African Water Quality Guidelines (SAWQG) specify target concentrations of metals for different water uses. For aquatic ecosystems, the Target Water Quality Range (TWQR) is not a water quality criterion, but rather a management objective (DWAF, 1996). In the current study a metal concentration index (I) was calculated as (I) = measured water metal concentration/ TWQR (Table 3). Where (I) $\leq$ 1, the metal content measured at the site complies with the TWQR. Where (I) > 1, the metal content measured at the site exceeds the TWQR.

### *2.3.5. Statistical analysis*

One-way ANOVAs were used to compare differences between upstream, tank and downstream sites for each of the metals at the rivers. Assumptions of normality and equality of variance for the metal content data were tested using a 1-sample Kolmogorov-Smirnov Test

and a Levene's Test, respectively. The assumptions were satisfied for all metals at each of the three rivers. Tukey HSD post-hoc tests were performed on significant ANOVAs. All results were reported as means ±standard deviation.

## *2.4. Results*

### *2.4.1. Inductively Coupled Plasma Optical Emission Spectrometry* (*ICP-OES*)

The concentrations of Cr, Cu, Fe, Ni and Zn were significantly different between upstream, tank and downstream sites at the Umbilo River (all $P < 0.05$, Table 2, Fig. 2). There were significant differences between upstream and tank, tank and downstream, and upstream and downstream sites for all metals except Cu and Fe (Table 2). There were no significant differences between the downstream and tank sites for Cu, and between the downstream and upstream sites for Fe. Although there was no significant difference between the sites for Pb, the concentration of this metal was higher at the tanks than at the upstream or downstream sites. The concentrations of the rest of the metals were generally lowest at the upstream site, intermediate at the downstream site and highest at the tank site (Fig. 2). The metals occurred in the following order of descending concentration at the Umbilo River: Fe > Zn > Cr > Ni > Cu > Pb.

At the Little Amanzimtoti River; Cr, Cu, Fe, Zn and Pb (i.e. all metals except Ni) concentrations were significantly different between upstream, tank and downstream sites (all $P < 0.05$, Table 2, Fig. 3). With the exception of Cu and Fe, there were significant differences between upstream and tank, and tank and downstream sites for all metals (Table 2). The concentration of Ni at the Little Amanzimtoti River was higher at the tanks than at the upstream or downstream sites, albeit not statistically significant. The concentrations of the other metals were similar at upstream and downstream sites and highest at the tank sites (Fig. 3), with the exception of Cu and Fe, which had the highest concentrations at the upstream site. The metals at the Little Amanzimtoti River occurred in the following order of descending concentration: Fe > Zn > Cu > Ni > Pb > Cr.

There was a significant difference between upstream, tank and downstream sites at the Mdloti River for Fe, Zn, Cu and Cr (all $P < 0.05$, Table 2, Fig. 4). Tukey HSD post-hoc tests revealed that there were no significant differences between the upstream and downstream sites, except for Fe. However, there were numerous significant differences between the tanks and upstream sites, and the tanks and downstream sites, with metals having the highest concentration at the tank site (Fig. 4). Cr, Cu, Ni and Fe were higher upstream than downstream. The metals occurred in the following order of descending concentration at the Mdloti River: Fe > Zn > Cu > Ni > Cr > Pb.

(a)

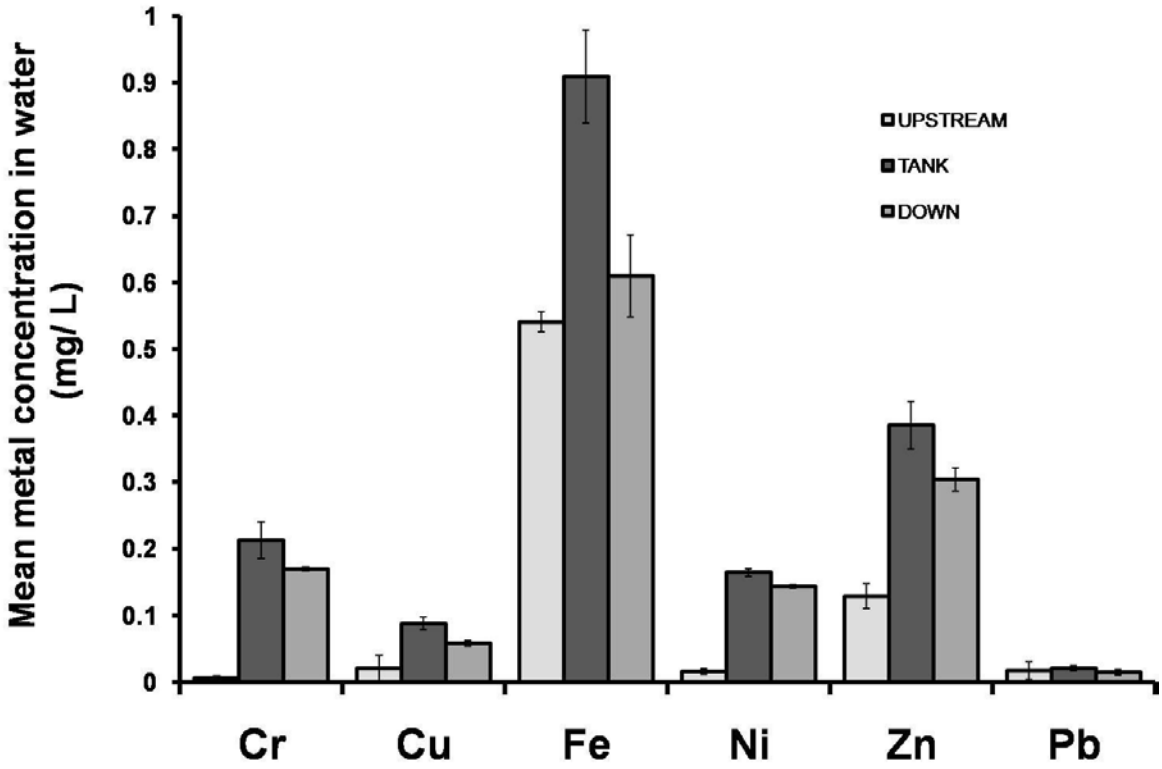


Fig. 2. Means (±std error) concentrations (mg/L) of chromium (Cr), copper (Cu), iron (Fe), nickel (Ni) and lead (Pb) at upstream, tank and downstream sites at the Umbilo River.

(b)

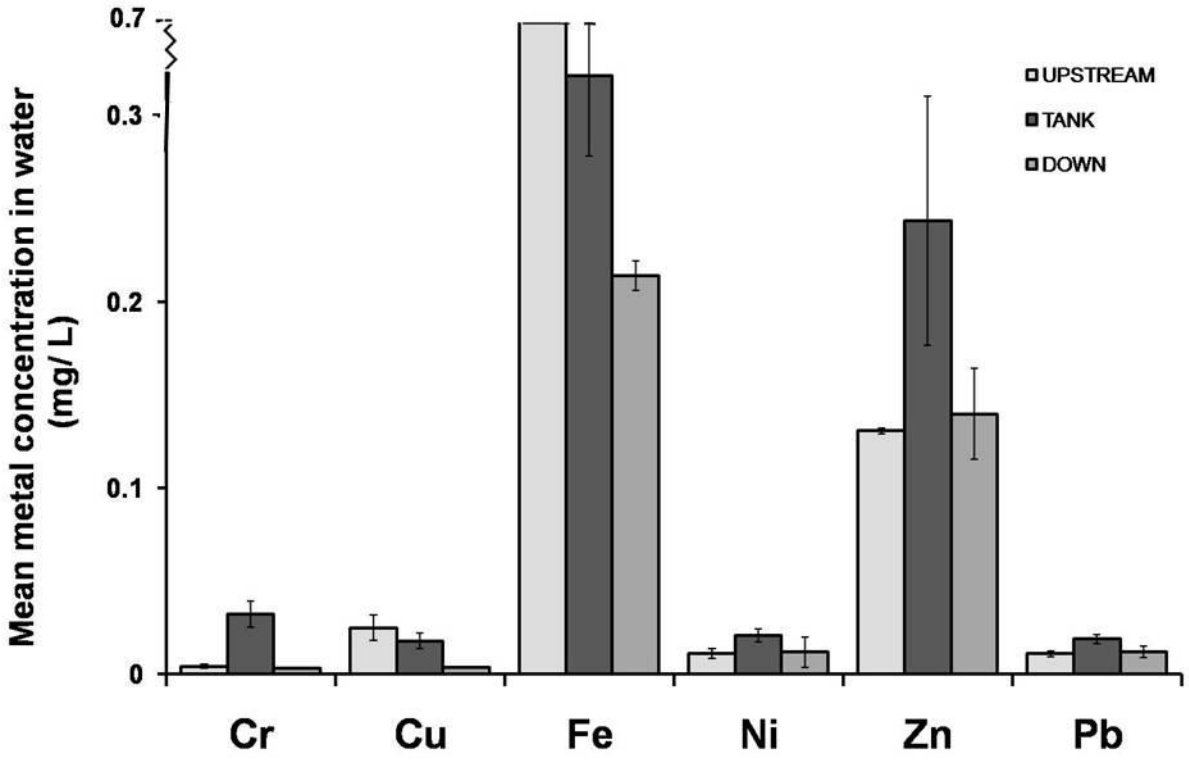


Fig. 3. Means (±std error) concentrations (mg/L) of chromium (Cr), copper (Cu), iron (Fe), nickel (Ni) and lead (Pb) at upstream, tank and downstream sites at the Little Amanzimtoti River.

(c)

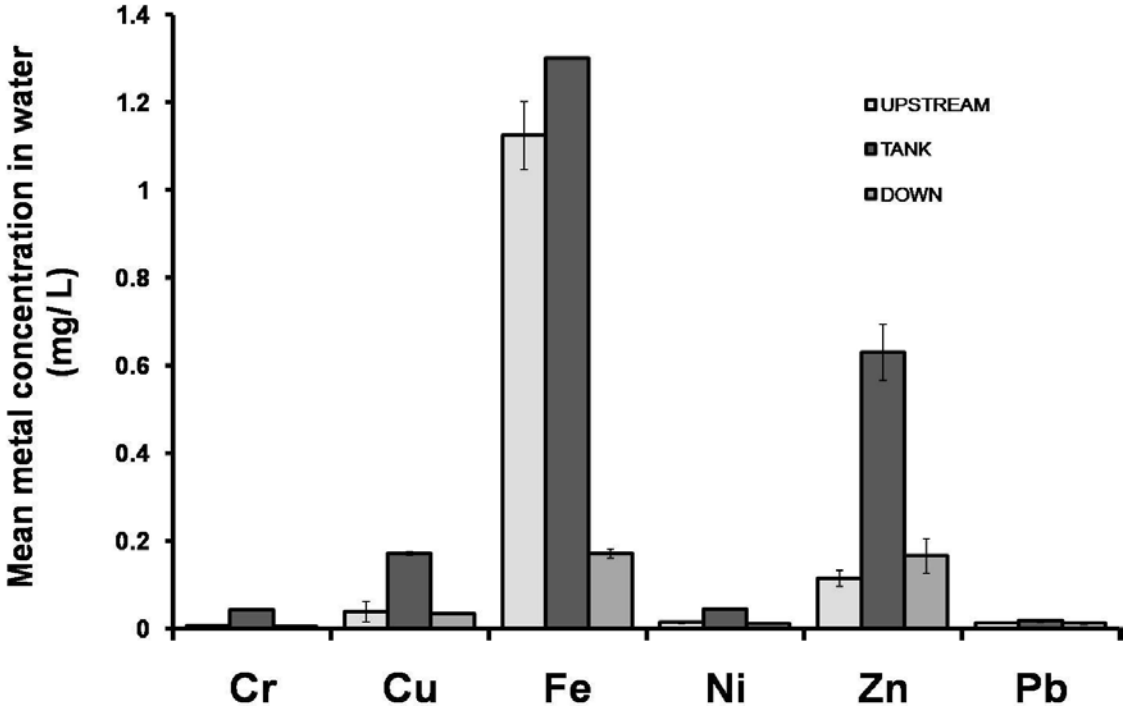


Fig. 4. Means (±std error) concentrations (mg/L) of chromium (Cr), copper (Cu), iron (Fe), nickel (Ni) and lead (Pb) at upstream, tank and downstream sites at the Mdloti River.

Table 2. Results of one-way ANOVAs (n = 9 per metal) and significant Tukey HSD post- hoc tests between upstream (U), tank (T) and downstream (D) sites, for metals tested at the Umbilo, Little Amanzimtoti and Mdloti Rivers. (* indicates significance at the $P < 0.05$ level)

| Metals | One-Way ANOVA | Significant differences based on Tukey post-hoc tests |
|---|---|---|
| UMBILO | | |
| Cr | $F = 136.8$; $*P < 0.0005$ | D < T; D > U; U < T |
| Cu | $F = 20.7$; $*P = 0.002$ | D > U; U < T |
| Fe | $F = 20.6$;$*P = 0.002$ | D < T; U < T |
| Ni | $F = 948.3$; $*P < 0.0005$ | D < T; D > U; U < T |
| Zn | $F = 79.2$; $*P < 0.0005$ | D < T; D > U; U < T |
| Pb | $F = 0.37$; $P = 0.708$ | - |
| LITTLE AMANZIMTOTI | | |
| Cr | $F= 48.8$; $*P < 0.0005$ | D < T; U < T |
| Cu | $F = 16.9$; $*P = 0.003$ | D < T; D < U |
| Fe | $F = 72.4$; $*P < 0.0005$ | D < U; U > T |
| Ni | $F = 3.1$; $P = 0.121$ | - |
| Zn | $F = 6.9$; $*P = 0.028$ | D < T; U < T |
| Pb | $F = 9.5$; $*P = 0.014$ | D < T; U < T |
| MDLOTI | | |
| Cr | $F = 1669.9$; $*P < 0.0005$ | D < T; U < T |
| Cu | $F = 100.8$; $*P < 0.0005$ | D < T; U < T |
| Fe | $F = 545.7$; $*P < 0.0005$ | D < T; D < U; U < T |
| Ni | $F = 99.1$; $*P < 0.0005$ | D < T; U < T |
| Zn | $F = 120.3$; $*P < 0.0005$ | D < T; U < T |
| Pb | $F = 3.5$; $P > 0.05$ | - |

*2.4.2. Target water quality range (TWQR) index*

For Cr, Cu, Zn and Pb (that have available TWQR values), I > 1 for all except Cr, at the three rivers, i.e. this indicates that the concentrations of the metals at the sites exceeded the TWQR stipulated. Compliance with TWQR was generally closer at upstream sites, whereas the metal concentration index at all tank sites were much higher than the TWQR.

Table 3. TWQR and concentration index (I) for Cr, Cu, Zn and Pb concentrations of upstream (U), tank (T) and downstream (D) sites at the Umbilo, Little Amanzimtoti and Mdloti rivers. If (I) ≤ 1, metal content at site complies with TWQR; If (I) > 1, metal content at site exceeds TWQR (indicated in bold).

| Metal | TWQR (mg/L) | UMBILO (I) | | | LITTLE AMAN. (I) | | | MDLOTI (I) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | U | T | D | U | T | D | U | T | D |
| Cr | 0.95 | **30.38** | **24.19** | 0.57 | **4.57** | 0.43 | 0.62 | 6.00 | 0.38 | 0.95 |
| Cu | **68.89** | **292.22** | **192.22** | **82.22** | **58.89** | **11.11** | **127.78** | **571.11** | **108.89** | **68.89** |
| Fe | - | - | - | - | - | - | - | - | - | - |
| Ni | - | - | - | - | - | - | - | - | - | - |
| Zn | **64.33** | **192.67** | **152.00** | **65.33** | **121.67** | **69.83** | **57.00** | **314.50** | **82.67** | **64.33** |
| Pb | **85.00** | **101.67** | **71.67** | **53.33** | **93.33** | **58.33** | **56.67** | **86.67** | **56.67** | **85.00** |

- indicates that TWQR has not yet been set.

## *2.5. Discussion*

In this chapter I compared the concentrations of individual metals (copper, lead, zinc, nickel, chromium and iron), at sites upstream of the point of wastewater effluent discharge into the rivers, at sludge tanks and at sites downstream of the point of wastewater effluent discharge at three rivers. I predicted that metal concentrations would be lowest at upstream sites and

highest at tank sites, based on industrial input of metals into WWTW effluent. My results were partially consistent with this prediction: the concentrations of all metals tested at the Umbilo and Mdloti Rivers (Cr, Cu, Fe, Ni, Zn and Pb), and four of the six metals tested at the Little Amanzimtoti River (Cr, Ni, Zn and Pb), were highest at the tank sites. Tukey HSD post-hoc tests showed significant differences between the upstream and tank sites for fourteen of the fifteen significant ANOVAs obtained. Similarly, metal content measured in industrial effluent at the Potsdam WWTW in Cape Town, South Africa, was higher than the metal content in the treated effluent for discharge into the river (Halday, 2007). In another study, which determined the occurrence of metals (including the six tested in the current study), along a Turkish river receiving wastewater effluent, metal concentrations were also higher at the WWTW than downstream metal concentrations (Ustun, 2009).

Cr, Cu, Fe, Ni, Zn and Pb are frequently produced from industrial activities (Chipasa, 2003; Gagnon *et al*., 2009). Among the metals tested, Fe and Zn concentrations were highest at the three rivers. This is a general trend for metals in wastewater, and has been reported in other studies (Chipasa, 2003; Karvelas *et al*., 2003). Although Fe is deposited in habitats naturally from weathering processes (DWAF, 1996), it is anthropogenic sources that contribute to the excess concentrations of Fe in rivers (Gerzau *et al*., 2003). Fe is used in the chlor-alkali, household chemical, fungicide, and petro-chemical industries (DWAF, 1996). Fe is an essential metal for living organisms, and has a limited bio-availability (fraction of the total metal concentration that may be taken up by an organism) (Van Leeuwen *et al*., 2005). Nevertheless, at extremely elevated concentrations Fe is toxic to organisms. Zn, which is also an essential metal, is used widely in industry. Industrial sources of Zn include the galvanizing of metal and the manufacturing of dyes, pigments (paints and cosmetics), pharmaceuticals, fertilizers and insecticides (DWAF, 1996). Although Zn is essential for biological functioning, it can also be toxic to aquatic organisms (DWAF, 1996).

In general, Fe and Zn are the most abundant metals in wastewater, followed by Cu and Ni (Karvelas *et al*., 2003). This was found at the Mdloti River and the Little Amanzimtoti River. At the Umbilo River, however, Cr concentrations were higher than Cu, suggesting an additional source of Cr at the Umbilo WWTW. Copper is a metal which occurs naturally from weathering processes, but is also extensively used in iron and steel producing industries, and as a component of algicides, fungicides and pesticides (DWAF, 1996). Despite its natural

occurrence, Cu may become toxic when in excess concentrations (Gaetke & Chow, 2003), and is thus regarded as potentially hazardous by the United States Environmental Protection Agency (USEPA) (DWAF, 1996). Ni is used in welding, electroplating and electroforming. In addition, Ni is used to manufacture products such as nickel-cadmium batteries, electronic equipment, tools, machinery, armaments, jewellery and appliances (Denkhaus & Salnikow, 2002).

Metals generally found in the lowest concentrations in wastewater are Pb, Cr and Cd, respectively (Karvelas *et al*., 2003). Cd concentrations were below the detection limit and thus excluded from the analyses. Pb concentrations were the lowest of all metals tested at the rivers except at the Little Amanzimtoti River, where Cu concentration was lowest. Pb is produced from the mining, smelting and refining of lead and other metals (Hoffman *et al*., 2001). Despite the low concentrations at which Pb was measured, it was still much higher than the TWQR. Pb is a non-essential metal and can therefore have detrimental effects on organisms even at the lowest concentrations (Hoffman *et al*., 2001). The TWQR is thus particularly low for Pb due to this high toxicity to life (DWAF, 1996). The TWQR is merely a guideline for the management of freshwater ecosystems (DWAF, 1996). This may play a strong role in the influence of WWTWs to not adhere strictly to the metal concentrations stipulated in the South African Water Quality Guidelines. More than 80% of all sites exceeded TWQRs, with just Cr at upstream sites and two downstream sites in compliance. The fact that the concentrations of metals at downstream sites were still above TWQR concentrations suggests that the removal of metals from the wastewater effluent was not efficient.

I found evidence for the prediction that metal concentrations at the upstream sites would be lowest. However, Pb at the Umbilo River, Cr at the Little Amanzimtoti River, and Cr, Cu, Fe and Ni at the Mdloti River had upstream concentrations that were higher than those at the downstream sites, but lower than at the tank sites. Furthermore, at the Little Amanzimtoti River, upstream Cu and Fe concentrations were much higher than those at both downstream and tank sites. It is possible that these metal concentrations were lower downstream because conditions there were more suitable for metal particles to settle into the sediment (Rondeau *et al.*, 2000). Higher concentrations of nutrient input from WWTW effluent results in the presence of particulate matter in the water. This biomass often binds easily to free ions of metal in the water, causing them to sink and settle in sediment (Sauve *et al*., 1998). This

process of complex formation, along with other physico-chemical processes, may contribute to the lower metal concentrations observed in surface water at these downstream sites. Future studies should quantify metal concentrations in the sediment at the sites.

Moreover, there are pollution sources other than WWTWs which may have contributed to these increased metal concentrations at upstream sites. Besides storm water runoff, there are many commercial and industrial sources of metal pollutants that are unknown because of illegal dumping activities (Moeletsi *et al*., 2004). For example, it was noted in the State of the Rivers Report that tent hiring companies were washing tents on rocks at upstream sites of the Little Amanzimtoti River (eThekwini Municipality State of the Rivers Report, 2007) using cleaning detergents manufactured with Fe (DWAF, 1996). The other notable source of Fe from cleaning detergents is most likely from washing activities at the river by members of nearby informal settlements. The unusually high Cu concentration at this upstream site may be due to nearby piping systems which release Cu during erosion. This enters into storm water runoff flowing into rivers (DWAF, 1996).

In summary, upstream sites had lower metal concentrations, and thus lower risk of metal toxicity than WWTW tanks and downstream sites. This shows that the point source pollution of WWTWs was the most significant contributor of metal contaminants into the rivers. This means that animals such as *Neoromicia nana* foraging near the WWTW tanks were probably exposed to these metals, resulting in a higher risk of accumulated concentrations of metals in the tissues and organs of their bodies (Chapter 4). Future work should investigate the interactions of metals with physical characteristics of the environment. For instance, pH affects the availability and toxicity of metals in the aquatic environment (eThekwini Municipality State of the Rivers Report, 2007). Nonetheless, pH readings taken at various points along the three rivers indicated that the pH levels were relatively neutral (ranging from 6 to 8). Future studies should also quantify concentrations of organic pollutants and synthetic compounds at the rivers to assess the general health and quality of the aquatic systems.

# CHAPTER 3:
# FORAGING ECOLOGY OF THE BANANA BAT (*NEOROMICIA NANA*, FAMILY: VESPERTILIONIDAE*)* AT RIVERS POLLUTED BY WASTEWATER WORKS

## *3.1. Summary*

Wastewater pollution decreases ecosystem quality. Negative effects on a species may transfer up the food chain and impact other trophic levels. To understand how WWTWs affect *N. nana* populations that forage along wastewater-polluted rivers, the activity and foraging behaviour of *N. nana* was evaluated at upstream, tank and downstream sites situated along the Umbilo, Little Amanzimtoti and Mdloti Rivers (summer 2010 and winter 2009). Relative bat abundance (represented by an activity index, AI) was significantly higher at wastewater-polluted sites (tank and downstream sites) than at upstream sites at all rivers, regardless of season. The Umbilo and Little Amanzimtoti Rivers had the highest relative bat abundance at downstream sites, while at the Mdloti River it was highest at the tank. Similarly, feeding activity (number of feeding buzzes) was significantly higher at wastewater-polluted sites than upstream, but did not differ significantly between tank and downstream sites. Total insect abundance was significantly highest at downstream sites. The number of insect orders was significantly higher at upstream and downstream sites, than at tanks. The most abundant insect order at wastewater-polluted sites at all rivers was Diptera, of which > 80% were comprised of pollution-tolerant Chironomidae (non-biting midges). The abundance of midges was significantly lower at upstream sites than at wastewater-polluted sites. Dietary analysis revealed that the insect order constituting the highest proportion in the diet of *N. nana* at wastewater-polluted sites was indeed Diptera. The proportion of insect orders in the diet of *N. nana* captured at most tank and downstream sites was significantly correlated to the proportion of insect orders captured at the site. This suggests that the bats feed opportunistically at the sites, exploiting swarms of midges associated with wastewater. It can thus be concluded that *N. nana* activity along rivers within the urban landscape is concentrated at WWTWs and sites downstream of effluent discharge into rivers. Thus, wastewater pollution along rivers affects the composition of insect communities and, in turn, influences the activity and foraging behaviour of *N. nana* populations within the landscape.

## *3.2. Introduction*

Studies of the negative impacts of metal pollutants on vertebrates have focused predominantly on birds because they are heavily dependent on both insect and fish prey (Rattner, 2009). Research into the effects of river metal pollutants on small mammal predators has thus far, focused on voles, mice and shrews (Wijnhoven *et al*., 2007; Fritsch *et al*., 2010). These small mammals generally exhibit short life expectancies and high reproduction rates (Shore & Rattner, 2001). In contrast, bats have long life spans, slow reproductive rates and long population recovery periods (Jones *et al*., 2009). Bats have thus been advocated as viable bioindicator species for demonstrating environmental change (Jones *et al*., 2009).

Although biodiversity is generally negatively affected by pollution, the impact of river pollution on bats is not clear, with several studies showing less negative effects on bat populations (Vaughan *et al*., 1996; Racey *et al*., 1998; Park & Cristinacce, 2006; Kalcounis-Rueppell *et al*., 2007; Abbot *et al*., 2009). For example, in North Carolina, USA, certain insectivorous bats (eg. *Perimyotis subflavus,* which is a species that specializes in riparian habitats (Ford *et al*., 2005)), were found to be more abundant at sites downstream of a sewage output (Kalcounis-Rueppell *et al*., 2007). Similarly, Vaughan *et al*. (1996) found that while certain species were more active upstream of a sewage output, *Myotis* species concentrated their feeding activity downstream of the pollution source. Sewage polluted water promotes eutrophication, which in turn affects the composition of aquatic insect populations (Lawrence & Gresens, 2004). Pollution-tolerant insects such as chironomid midges increase in abundance, altering the insect prey base available to animalivorous bats (Racey *et al*., 1998).

The majority of studies that investigated the impacts of wastewater-polluted rivers on bats have been limited to Europe and the USA. Little is known about the impact of wastewater pollution on bats in other regions, particularly Africa. In Africa, *Neoromicia nana* is an animalivorous bat species that has been observed to forage in riparian habitat (Rautenbach *et al*., 1996, Monadjem & Reside, 2008). Approximately 41% of the bats recorded at a polluted river in Durban, South Africa, were *N. nana* (Naidoo *et al*., in press). *N. nana* is a particularly small bat (3 - 4 grams; Monadjem *et al*. 2010) and would therefore exploit the increased availability of small pollution-tolerant chironomid midges at wastewater-polluted sites. The toxic effects of pollution should

thus be evident in this animal, making *N. nana* an ideal model predator to investigate the impact of wastewater pollutants through a food chain.

The aim of this chapter was to evaluate the influence of wastewater pollution on the foraging ecology of *N. nana* populations at sites polluted and unpolluted by wastewater effluent along rivers. I predicted that the relative abundance and feeding activity of *N. nana* would be higher at wastewater-polluted sites (sludge tank and downstream sites) than at sites situated upstream of the wastewater pollution, as a result of the high abundance of the pollution-tolerant chironomid midges specifically associated with wastewater and eutrophication (Marques *et al*., 1999). I further predicted that there would be a significant correlation between the abundance of pollution-tolerant insects at wastewater-polluted sites and in the diet of *N. nana* at the sites.

## *3.3 Methods*

### *3.3.1. N. nana sampling and identification*

*N. nana* was sampled during winter (June/July 2009) and summer (March/April 2010), for three nights at each of the three sites per river (Chapter 2), for each season. At each river, one site was situated upstream of the point of effluent discharge into the river, one at the sludge tanks in the wastewater treatment works and one site downstream of the point of wastewater effluent discharge (Chapter 2). I captured *N. nana* at each site with mist nets which were set across the rivers and next to the sludge tanks, from 18h30 and 18h00 until 22h00 and 21h00 in summer and winter, respectively. Nets were checked every 10 to 15 minutes.

Bats were sexed and identified to species using a taxonomic key (Monadjem *et al*., 2010). Bat species other than *N. nana* were released where they were caught. *N. nana* were held individually in cotton bags until the next morning to collect faecal pellets. I measured forearm length (to nearest 0.01 mm) with calipers, and body mass with a Pesola scale (to nearest 0.5 g). The presence of cartilaginous epiphyseal plates was used to determine age (juvenile or adult) (Anthony, 1988).

In addition, I passively monitored the relative abundance and feeding activity of *N. nana* by recording bat echolocation calls at each site from 18h30 and 18h00 until 22h30 and 21h00 in summer and winter, respectively. Echolocation calls were recorded using an Avisoft Ultrasound 116 Bat Detector (Avisoft Bioacoustics, Berlin, Germany) connected to a laptop computer (Hewlett Packard Pavilion 6210 notebook). At upstream and downstream sites, the recording equipment was set up alongside the river with the microphone positioned at a 45° angle to record bats flying directly above the river. At tank sites, the recording equipment was set up a few metres from the sludge tank, with the microphone pointing toward the tank.

Batsound Pro-Sound Analysis software (version 3.31b, Pettersson Elektronik AB, Upsala, Sweden) was used to analyze the recorded echolocation calls. A sampling rate of 500 000 Hz (16 bits, mono) with a threshold of 16 was used. The dominant harmonic, i.e. peak frequency, and the bandwidth from each recorded bat pass was examined in a power spectrum (size 1024) (Schoeman & Jacobs, 2008) (Fig. 1a), and duration of the call was noted from the oscillogram. *N. nana* calls were identified by comparing peak echolocation frequency and duration of call with reference calls (Monadjem *et al*., 2010).

### *3.3.2. N. nana relative abundance/ activity index (AI)*

I quantified the relative abundance of *N. nana* with an acoustic activity index (AI) (Miller, 2001). I defined a bat pass as a series of echolocation calls made by one individual (Saunders & Barclay, 1992). If *N. nana* passes occurred within a 1 min interval, *N. nana* was said to be present in that count (Miller, 2001). AI per night for each site was calculated as:

$$\mathrm{AI} = \sum_{1}^{n} \mathrm{P} \ (\mathrm{x}\ 100)$$

where P = sum of presence counts and n = number of 1 min intervals for the sampling night.

### *3.3.3. N. nana feeding activity*

I quantified the feeding activity of *N. nana* at each site as the number of feeding buzzes (Fenton *et al*., 1977). Feeding buzzes (Fig. 1b) consist of high pulse-repetition rates of echolocation pulses emitted by animalivorous bats as they capture prey (Griffin *et al*., 1960). All feeding buzzes recorded per night were counted.

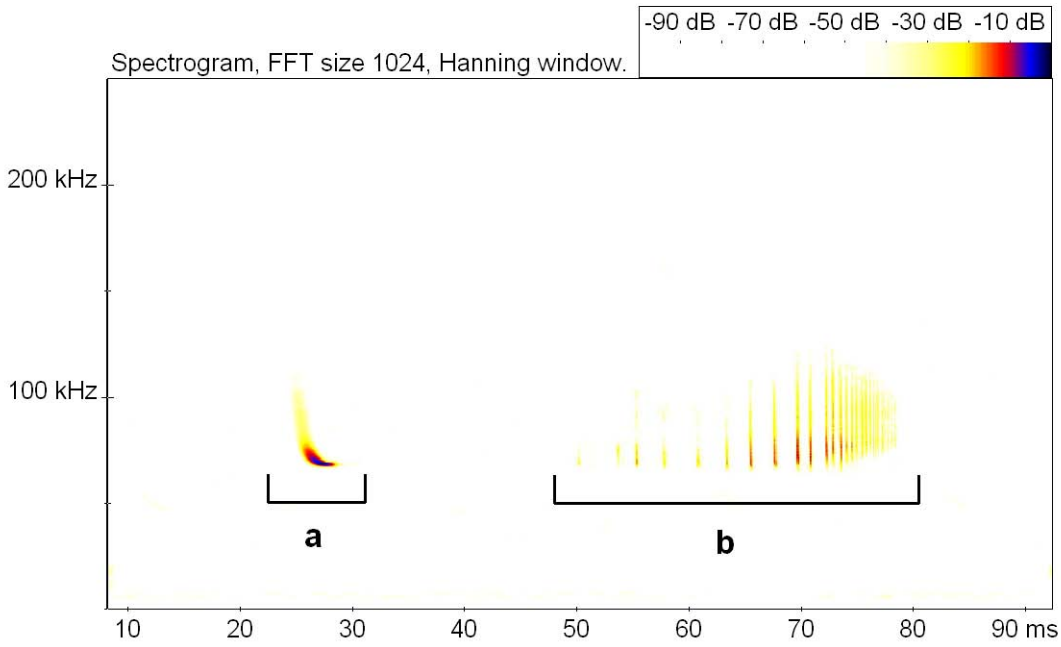


Fig. 1. (a) Echolocation call (Mean peak frequency ±SD: 68.6 ±2.0 kHz (n = 10)) and (b) feeding buzz of *Neoromicia nana*

### *3.3.4. Insect diversity*

At each site I captured nocturnal insects with a 22 W black-light bucket trap (Black, 1974) for the same time period that mist nets were set. Black-light traps effectively sample Diptera, Lepidoptera and Coleoptera (Nabli *et al*., 1999), which are the insect orders most frequently recorded in the diets of South African insectivorous bats (Aldridge & Rautenbach, 1987; Schoeman & Jacobs, 2011). The black-light trap was positioned along sludge tanks or above

water level near the river (~1m above water, 1 - 3 m from the edge of the river), and at least 50 m away from the mist nets to prevent the light from affecting bat activity. The collected insects were placed in jars and stored in a freezer. In addition, I captured insects by sweep netting (20 sweeps) along the edge of the river or tank, every hour from the start to the end of the sampling period per night. All insects collected from the sweeps and the light trap were pooled and identified to order using a taxonomic text (Scholtz & Holm 1985). Diptera were further analysed to obtain the abundance of chironomid midges (Family: Chironomidae). At least one individual from each collected order was mounted on a slide and used as a reference library for dietary analyses (see below).

### *3.3.5. N. nana dietary analysis*

Five faecal pellets from each bat (Whitaker *et al*., 1996) and a minimum of 20 pellets in total (when possible) from *N. nana* bats captured at the upstream, tank and downstream site at each river (Whitaker *et al*., 1999) were collected for dietary analyses. Faecal samples were individually teased apart in 70% alcohol. Remnants of insect exoskeletons were identified to order with the aid of a classification key (Scholtz & Holm, 1985) and a reference collection of insects trapped at each site. The percentage of the total pellet volume comprising each order present was visually estimated following Whitaker (1988), where the percent insect order per bat was calculated from the average percent insect order per pellet.

### *3.3.6. Statistical analysis*

Three-way ANOVAs were used to compare differences in *N. nana* relative abundance, *N. nana* feeding activity, total insect abundance, insect order richness and midge abundance among sites (upstream, tank and downstream) and rivers (Umbilo, Little Amanzimtoti, Mdloti), and between seasons (summer and winter). Assumptions of normality and equality of variance for the data were tested using a 1-sample Kolmogorov-Smirnov Test and a Levene's Test, respectively. If assumptions were not satisfied, the data were rank transformed and non-parametric tests were run. Tukey HSD post-hoc tests were performed on significant ANOVAs.

The dietary composition of *N. nana* bats captured during winter and summer at each site was compared with the insect abundances at each of the rivers using a Pearson's correlation, or a Spearman's rank correlation if assumptions were not satisfied. All analyses were performed with SPSS 19.0, using alpha of 0.05.

## *3.4. Results*

### *3.4.1. N. nana relative abundance and feeding activity*

*N. nana* emits low duty-cycle, frequency modulated echolocation calls (LD-FM). The peak echolocation frequency of *N. nana* was 68.6 ±2.0 kHz, with a bandwidth of 14.1 ±3.7 kHz and a call duration of 4.6 ±0.8 ms (means ±SD; n = 10).

There was a significant difference in the relative abundance of *N. nana* among upstream, tank and downstream sites ($F = 84.424$; $df = 2$; $P < 0.0005$). Tukey HSD post-hoc tests showed that *N. nana* relative abundance was significantly higher at downstream than at tank and upstream sites ($P < 0.0005$). The lowest relative abundance was at the upstream sites (Fig. 2; Table 1). There was no difference in *N. nana* relative abundance among rivers, however there was an interaction effect between river and site ($F = 6.783$; $df = 4$; $P < 0.0005$). The significant interaction indicates that the difference among sites was not consistent among rivers. There were no significant seasonal differences in *N. nana* relative abundance.

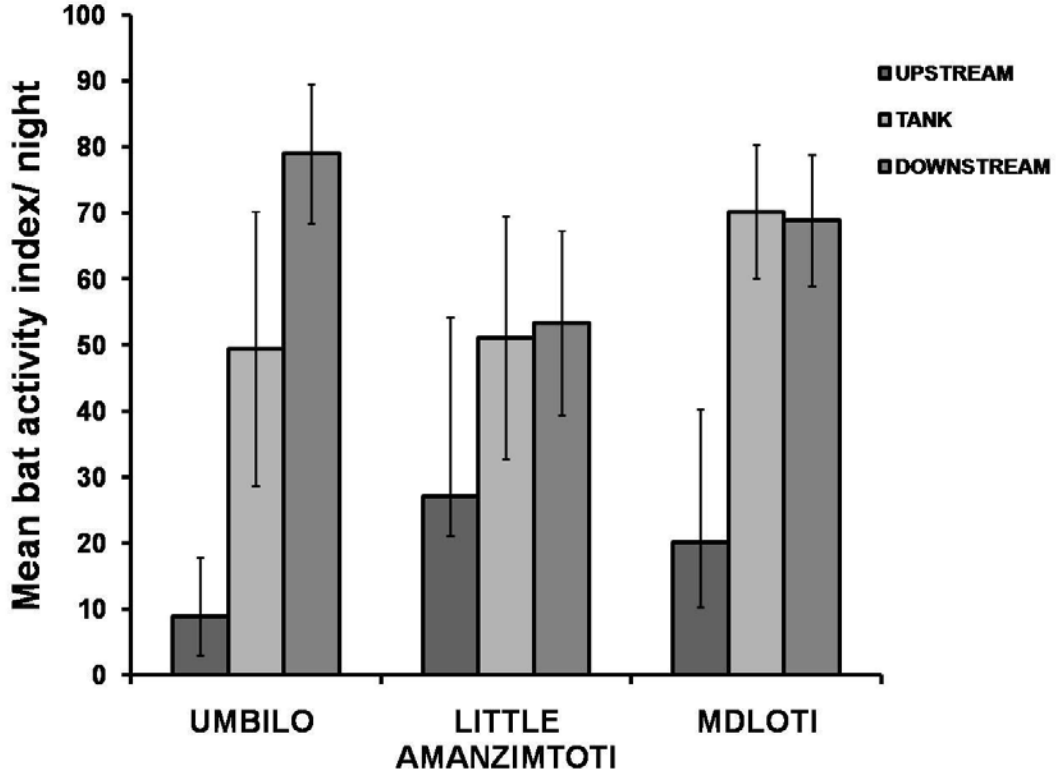


Fig. 2. Mean (bars: ±SD) AI/night at upstream, tank and downstream at the Umbilo, Little Amanzimtoti and Mdloti Rivers.

Similar to relative abundance, feeding activity was significantly higher at wastewater-polluted sites than at upstream sites ($F = 10.315$; $df = 2$; $P < 0.0005$) (Fig. 3; Table 2), but did not differ significantly between tank and downstream sites. There were no significant seasonal differences in *N. nana* relative abundance or feeding activity. Feeding activity differed significantly between rivers, with the Little Amanzimtoti River having a significantly lower number of feeding buzzes than the Umbilo and Mdloti rivers ($F = 9.438$; $df = 2$; $P < 0.0005$).

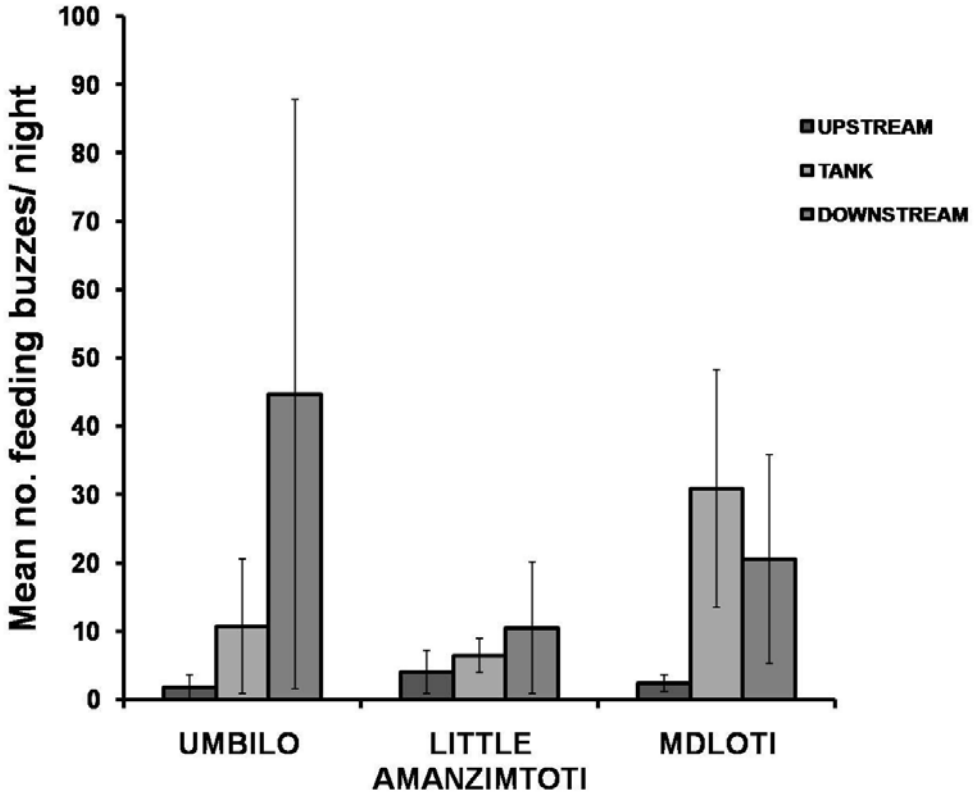


Fig. 3. Mean (bars: ±SD) number of feeding buzzes/night at upstream, tank and downstream sites at the Umbilo, Little Amanzimtoti and Mdloti Rivers.

### *3.4.2. Insect abundance and richness of orders*

The total number of insects captured during both sampling seasons was 3742 at the Umbilo River, 3209 at the Little Amanzimtoti River and 3513 at the Mdloti River. There was a significant difference in insect abundance among the upstream, tank and downstream sites ($F$ = 4.818; $df$ = 2; $P$ = 0.015) (Table 1). Furthermore, Tukey HSD post-hoc tests showed that downstream sites had a significantly higher insect abundance than tank sites ($P$ = 0.014). There was a significantly larger number of insects captured during summer than in winter ($F$ = 313.867; $df$ = 1; $P$ < 0.0005). In addition, there were significant interaction effects between site and season ($F$ = 8.462; $df$ = 2; $P$ = 0.001), river and season ($F$ = 4.038; $df$ = 2; $P$ = 0.028), site and river ($F$ = 16.911; $df$ = 4; $P$ < 0.0005), and season, site and river ($F$ = 18.062; $df$ = 4; $P$ < 0.0005).

The number of insect orders also differed significantly among sites, with Tukey HSD post-hoc tests showing a significantly higher order richness at upstream and downstream sites than at tank sites ($F = 10.627$; $df = 2$; $P < 0.001$). A significantly higher number of insect orders was encountered during summer than in winter ($F = 64.234$; $df = 1$; $P < 0.001$). At all three rivers, the most prevalent order at the upstream sites was Coleoptera (45.8 - 69.8%) and the most prevalent order at wastewater-polluted sites was Diptera (41.2 - 73.1%) (except for the Umbilo downstream site, which varied) (Table 1). The Neuroptera, Dermaptera and Mantodea orders were rare (i.e. ≤ 2%) and were pooled into one category classified as "other". Trichoptera and Ephemeroptera were more abundant at upstream sites than at wastewater-polluted sites (Table 1).

More than 80% of Diptera at the tank and downstream sites comprised chironomid midges (Family: Chironomidae). The mean abundance of midges/night was significantly lower at upstream sites than at wastewater-polluted sites ($F = 156.086$; $df = 2$; $P < 0.0005$) (Fig. 4; Table 2). In addition, midge abundance was significantly lower at downstream sites than at the tank sites (Tukey HSD post-hoc tests, all $P < 0.0005$) (Fig. 4; Table 2). Midge abundance also differed significantly between seasons and among rivers. There was a significantly higher midge abundance in summer than in winter ($F = 143.973$; $df = 1$; $P < 0.0005$), at the Little Amanzimtoti and Mdloti rivers than at the Umbilo River ($F = 10.372$; $df = 2$; $P < 0.0005$). There were significant interaction effects between site and season ($F = 36.468$; $df = 2$; $P < 0.0005$), and between site and river ($F = 10.540$; $df = 4$; $P < 0.0005$). Thus, significant differences obtained for site were not constant for both seasons and for all rivers.

Table 1. Means (±SD) percent abundance (per night) of the insect orders Coleoptera (Col), Diptera (Dip), Lepidoptera (Lep), Hemiptera (Hem), Hymenoptera (Hym), Trichoptera (Trich) and Ephemeroptera (Ephem) captured at upstream (U), tank (T) and downstream (D) sites at the Umbilo, Little Amanzimtoti and Mdloti Rivers during winter (2009) and summer (2010).

| | | | % Insect Order | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **River** | **Site** | **Season** | **Col** | **Dipt** | **Lep** | **Hem** | **Hym** | **Trich** | **Ephem** | **Other** |
| Umbilo | U | S | 69.8 ±33.8 | 9.5 ±10.1 | 1.6 ±1.0 | 6.4 ±3.6 | 11.3 ±2.1 | 0.5 ±0.3 | 0.7 ±0.6 | 0.1 ±0.3 |
| | | W | 45.8 ±50.8 | 12.4 ±7.1 | 2.9 ±3.6 | 34.4 ±31.9 | 1.2 ±2.0 | 2.2 ±1.0 | 1.2 ±0.1 | 0.0 |
| | T | S | 26.5 ±3.7 | 44.0 ±12.1 | 4.9 ±3.1 | 19.1 ±5.8 | 4.5 ±1.5 | 0.0 | 0.0 | 1.1 ±1.8 |
| | | W | 19.9 ±24.8 | 73.1 ±17.3 | 2.3 ±1.6 | 4.1 ±5.7 | 0.0 | 0.0 | 0.0 | 0.6 ±0.8 |
| | D | S | 44.1 ±7.9 | 16.1 ±1.4 | 1.3 ±0.4 | 35.6 ±5.7 | 0.8 ±0.2 | 0.1 ±0.1 | 0.0 | 1.9 ±1.7 |
| | | W | 27.6 ±3.9 | 58.6 ±6.8 | 3.4 ±1.0 | 9.0 ±4.8 | 1.4 ±1.9 | 0.0 | 0.0 | 0.0 |
| Little Amanzimtoti | U | S | 57.7 ±14.5 | 10.9 ±7.8 | 6.0 ±1.2 | 12.4 ±6.0 | 8.6 ±5.2 | 2.1 ±1.1 | 0.7 ±1.0 | 1.6 ±0.7 |
| | | W | 46.0 | 19.0 | 4.8 | 12.7 | 14.3 | 3.2 | 0.0 | 0.0 |
| | T | S | 29.4 ±14.2 | 52.8 ±7.5 | 2.0 ±0.8 | 11.9 ±4.1 | 3.2 ±1.7 | 0.1 ±0.4 | 0.3 ±0.2 | 0.3 ±0.2 |
| | | W | 39.2 ±10.3 | 43.2 ±7.9 | 2.8 ±0.7 | 14.0 ±5.4 | 0.8 ±1.4 | 0.0 | 0.0 | 0.0 |
| | D | S | 20.1 ±12.9 | 47.2 ±4.5 | 5.1 ±2.0 | 12.0 ±2.4 | 14.8 ±4.6 | 0.4 ±0.5 | 0.0 | 0.4 ±0.4 |
| | | W | 21.0 ±6.0 | 52.1 ±16.4 | 3.8 ±1.0 | 12.2 ±1.2 | 10.1 ±4.0 | 0.2 ±2.2 | 0.0 | 0.6 ±0.5 |

Table 1 Continued

| River | Site | Season | % Insect Order | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Col** | **Dipt** | **Lep** | **Hem** | **Hym** | **Trich** | **Ephem** | **Other** |
| Mdloti | U | S | 48.1 ±4.0 | 4.1 ±3.0 | 6.9 ±2.4 | 31.8 ±9.9 | 1.4 ±0.7 | 6.1 ±1.2 | 0.5 ±0.5 | 1.1 ±0.5 |
| | | W | 46.9 ±6.2 | 11.5 ±3.7 | 11.5 ±3.7 | 28.3 ±5.0 | 0.9 ±1.2 | 0.9 ±0.4 | 0.0 | 0.0 |
| | T | S | 31.9 ±6.6 | 55.8 ±3.7 | 2.5 ±1.4 | 6.7 ±3.4 | 1.5 ±0.7 | 0.2 ±0.7 | 0.0 | 1.5 ±1.5 |
| | | W | 37.2 ±7.7 | 55.8 ±7.5 | 2.1 ±0.5 | 3.9 ±1.3 | 1.0 ±0.5 | 0.0 | 0.0 | 0.0 |
| | D | S | 28.1 ±7.2 | 41.2 ±5.7 | 10.5 ±2.9 | 9.2 ±4.0 | 7.3 ±4.5 | 1.0 ±0 | 0.0 | 2.6 ±1.6 |
| | | W | 17.8 ±8.9 | 65.5 ±19.7 | 7.4 ±2.5 | 7.8 ±5.2 | 1.5 ±1.5 | 0.0 | 0.0 | 0.0 |

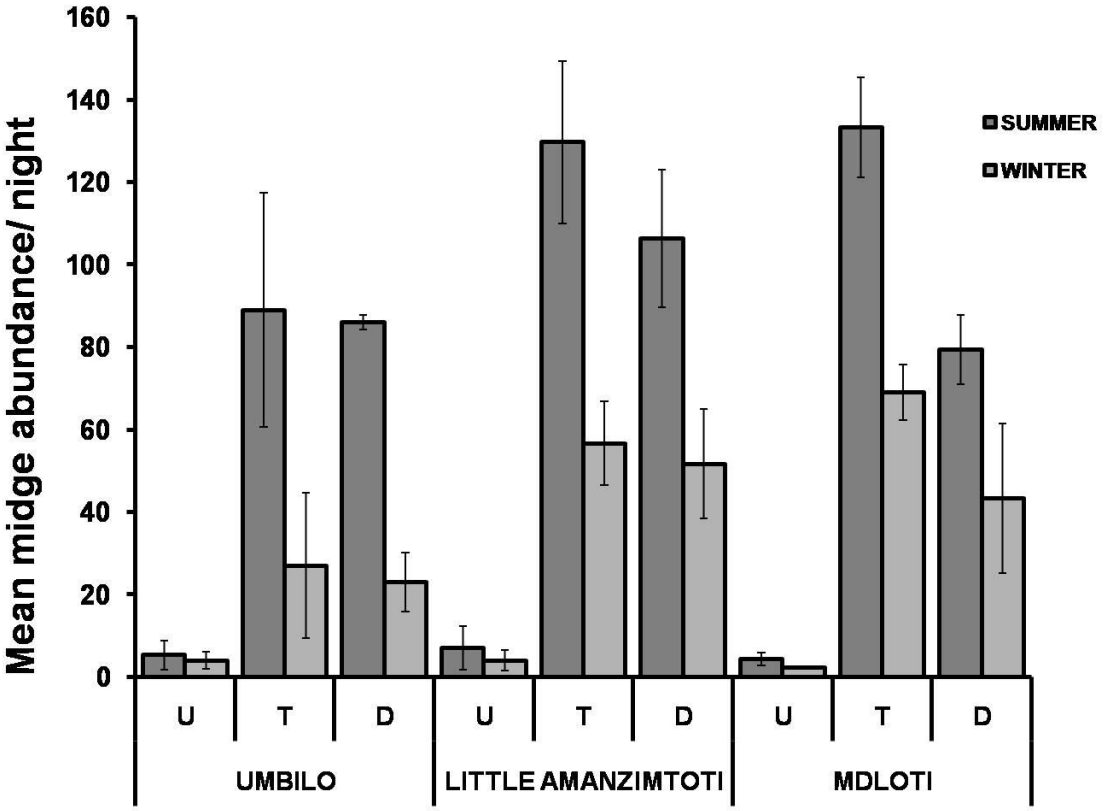


Fig. 4. Mean (bars: ±SD) midge abundance/night at upstream (U), tank (T) and downstream (D) sites at the Umbilo, Little Amanzimtoti and Mdloti Rivers during winter (2009) and summer (2010).

### *3.4.3. N. nana diet*

At least 20 faecal pellets were analyzed for *N. nana* captured at the upstream, tank and downstream sites except at the Umbilo upstream site where no faecal pellets were collected from the single *N. nana* captured. The proportions of insect orders in the diet of *N. nana* captured at tank sites were significantly correlated to the proportions of insect orders captured at the respective tank site at Umbilo ($r = 0.900$, n = 6; $P = 0.014$) and Mdloti ($r = 0.964$, n = 6; $P = 0.002$) in summer, and Little Amanzimtoti in summer ($r = 0.978$, n= 6; $P = 0.001$) and winter ($r = 0.979$, n= 6; $P = 0.001$) (Table 3). There was a significant correlation between the proportions of insect orders in the diet and those captured at the upstream site at Mdloti River, for both summer and winter (summer: $r = 0$.

931, n = 6; $P = 0.007$/ winter: $r = 0.884$, n = 6; $P = 0.019$). There were also significant correlations between the insects in the diet and those captured at downstream sites at Mdloti in both seasons (summer: $r = 0.974$, n = 6; $P = 0.001$/ winter: $r = 0.987$, n = 6; $P = 0.001$), Umbilo in winter ($r = 0.974$, n = 6; $P = 0.001$) and Little Amanzimtoti in summer ($r = 0.966$, n = 6; $P = 0.002$). At all tank and downstream sites, the insect order constituting the highest proportion (or second highest by ≤ 1%) in the diet of *N. nana* was Diptera (Table 3).

Table 2. Summary of lowest (1), intermediate (2) and highest (3) *N. nana* relative abundance, *N. nana* feeding activity and midge abundance at upstream, tank and downstream sites at the Umbilo, Little Amanzimtoti and Mdloti Rivers during both sampling seasons.

| River | Site | *N. nana* relative abundance | *N. nana* feeding activity | Midge abundance |
|---|---|---|---|---|
| Umbilo | Upstream | 1 | 1 | 1 |
| | Tank | 2 | 2 | 3 |
| | Downstream | 3 | 3 | 2 |
| Little Amanzimtoti | Upstream | 1 | 1 | 1 |
| | Tank | 2 | 2 | 3 |
| | Downstream | 3 | 3 | 2 |
| Mdloti | Upstream | 1 | 1 | 1 |
| | Tank | 3 | 3 | 3 |
| | Downstream | 2 | 2 | 2 |

Table 3. Mean (±SD) percent volume of the insect orders Coleoptera (Col), Diptera (Dip), Hymenoptera (Hym), Hemiptera (Hem), Lepidoptera (Lep) and Trichoptera (Trich) in the diet of *N. nana* captured at upstream (U), tank (T) and downstream (D) sites at the Umbilo, Little Amanzimtoti and Mdloti Rivers during winter (2009) and summer (2010).

| | | | | **Insect Order (% volume diet composition)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **River** | **Site** | **Season** | **No. of pellets** | **Col** | **Dipt** | **Lep** | **Hem** | **Hym** | **Trich** |
| Umbilo | T | S | 15 | 14.0 ±7.6 | 61.0 ±5.3 | 9.3 ±3.8 | 11.7 ±10 | 2.7 ±2.3 | 1.3 ±2.3 |
| | | W | 10 | 35.5 ±17.7 | 35.0 ±21.2 | 8.0 ±4.2 | 8.5 ±0.7 | 13.0 ±1.4 | 0 |
| | D | S | 5 | 37.0 ±2.7 | 46.0 ±8.2 | 9.0 ±5.5 | 6.0 ±8.2 | 2.0 ±2.7 | 0 |
| | | W | 15 | 21.7 ±24.3 | 50.0 ±25.7 | 12.7 ±10 | 8.0 ±9.2 | 7.7 ±6.8 | 0 |
| Little Amanzimtoti | U | S | 5 | 53.0 ±2.7 | 4.0± 5.5 | 20.0 ±0 | 10.0 ±6.1 | 13.0 ±6.7 | 0 |
| | | W | 10 | 24.0 ±9.9 | 28.0 ±7.1 | 20.5 ±12 | 14.0 ±7.1 | 9.5 ±3.5 | 4.0 ±5.7 |
| | T | S | 20 | 35.3 ±12.7 | 46.8 ±10.3 | 6.0 ±1.8 | 8.5 ±7.2 | 3.5 ±4.7 | 0 |
| | | W | 15 | 40.0 ±29.9 | 39.0 ±28 | 9.33 ±4 | 9.0 ±4.4 | 2.7 ±3.1 | 0 |
| | D | S | 5 | 12.0 ±2.7 | 60.0 ±12.2 | 7.0 ±2.7 | 7.0 ±6.7 | 14.0 ±13.4 | 0 |
| | | W | 20 | 32.3 ±8.5 | 13.0 ±10.1 | 27.5 ±5.8 | 25.8 ±12.7 | 1.5 ±3 | 0 |

Table 3 Continued

| River | Site | Season | No. of pellets | Insect Order (% volume diet composition) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Col | Dipt | Lep | Hem | Hym | Trich |
| Mdloti | U | S | 10 | 41.5 ±23.3 | 2.0 ±2.8 | 7.0 ±7.1 | 32.5 ±0.7 | 15.0 ±15.6 | 2.0 ±2.8 |
| | T | S | 20 | 22.3 ±15.4 | 52.8 ±33.3 | 7.3 ±4.9 | 15.8 ±14.9 | 2.0 ±4 | 0 |
| | | W | 40 | 11.9 ±11.2 | 57.5 ±15.4 | 3.8 ±2.9 | 23.9 ±11.7 | 3.0 ±3.3 | 0 |
| | D | S | 20 | 23.8 ±6.9 | 45.8 ±10.4 | 7.8 ±2.1 | 13.3 ±4.6 | 9.5 ±7.5 | 0 |
| | | W | 43 | 22.4 ±15.4 | 61.3 ±16.7 | 1.5 ±1.6 | 14.4 ±8 | 0 | 0.33 ±1 |

## *3.5. Discussion*

In accordance with my prediction, the relative abundance and feeding activity of *N. nana* were highest at wastewater-polluted sites and lowest at sites located upstream of the wastewater pollution at all three rivers. My results are consistent with previous published studies that showed high abundances and feeding rates of focal bat species below sewage discharge points (Vaughan *et al*., 1996; Racey *et al*., 1998; Kalcounis-Rueppell *et al*., 2007; Abbot *et al*., 2009). For instance, *Pipistrellus pygmaeus* foraging along rivers in Ireland were significantly more active at sites located downstream of a sewage outfall than at sites located upstream of the outfall (Abbot *et al*., 2009). Vaughan *et al*. (1996) surveyed bat activity upstream and downstream of WWTWs, and concluded that treatment works may be important foraging areas for particular insectivorous bats such as *Myotis daubentonii*. These bat activity and foraging patterns are associated with increased insect productivity as a result of eutrophication and in Europe, the increase in abundance of *Myotis daubentonii* has been directly attributed to the increased incidence of eutrophic streams across the mainland (Kokurewicz, 1995).

Of the wastewater-polluted sites at the Umbilo and Little Amanzimtoti rivers, the relative abundance and feeding activity of *N. nana* was higher at downstream sites than at tank sites. The differences in relative abundance and feeding activity between wastewater-polluted sites, were however, not significantly different. The higher relative abundance and feeding activity of *N. nana* may be attributed to the total insect abundance, which was highest at downstream sites. There were significant correlations between the insects in the diet and those captured at the downstream sites on the Umbilo River in winter, the Mdloti River in summer and winter, and the Little Amanzimtoti downstream site in summer. Thus, *N. nana* at the downstream sites opportunistically feed on the available insect prey.

The most prevalent insect order at the wastewater-polluted sites was Diptera. Furthermore, more than 80% of Diptera at wastewater-polluted sites comprised chironomid midges, with the highest abundance of chironomid midges at the tank sites. The tank sites, however, had the lowest insect order richness. This suggests that fewer orders were thriving under the physical and chemical conditions at the tank sites compared to upstream and downstream sites. One reason may be the high metal content at the tank sites (see Chapter 2). The insect orders Ephemeroptera and

Trichoptera, which are pollution-sensitive invertebrate taxa and indicative of „cleaner' water (Dinakaran & Anbalagan, 2007), were most abundant at upstream sites. Pollution-sensitive insect taxa are susceptible to the toxic effects of metals. For instance, mayfly larvae often die before emergence when exposed to metals (Hatakeyama, 1989).

However, pollution-tolerant insect taxa such as chironomid midges can tolerate high levels of metals (Postma *et al.,* 1995). Consequently, chironomid midges often occur at high densities near wastewater (Broza *et al*., 2003). Chironomid midges at metal-contaminated sites contain particularly high concentrations of metals in the midgut tissue (Krantzberg & Stokes, 1990). This is a result of the ability of midges to accumulate metals without being adversely affected, as illustrated by the occurrence of metal-adapted genetic strains of chironomid midges at sites downstream of pollution (Groenendijk *et al*., 1998). Although chironomid midge larvae captured at sites polluted with industrial discharge have shown morphological deformities such as head capsule and mouthpart asymmetry, there were no observed adverse effects on their survival and growth (Al-Shami *et al*., 2010). Thus, the high abundances of chironomid midges at the wastewater-polluted sites may be due to the insects' ability to tolerate and store metal pollutants.

At the tank sites for two of the rivers (Umbilo and Mdloti), there were significant correlations between the insects in the diet and those at the site in summer. The dietary analysis also revealed that at both tank and downstream sites, the insect order comprising the largest proportion in *N. nana's* diet was Diptera. Thus, the bats were exploiting the large swarms of chironomid midges present at the tank sites during this season. A similar pattern was found in studies of European *Myotis* species, which often fed opportunistically on swarms of insects, specifically chironomid midges, which were abundant near sewage outputs (Vaughan, 1980; Vaughan *et al*., 1996).

Contrary to these patterns, the *N. nana* diet at upstream sites generally comprised Coleoptera and Lepidoptera. There was a significant correlation between the proportions of insect orders in the diet and those captured at the upstream site, at the Mdloti River. This suggests that the bats were feeding opportunistically on the insects available at this upstream site. Conversely, at the upstream site of the Little Amanzimtoti River, bats were found to forage selectively on Lepidoptera. In addition to the large component of Coleoptera in the diet, the diet of these bats contained a large amount of Lepidoptera, even though Lepidoptera were not as abundant as other

insect orders, including Diptera, at the site. Thus, even when Diptera was available, *N. nana* selected other orders at upstream sites.

An important conclusion arising from this chapter is that within the urban landscape, *N. nana* activity is concentrated at wastewater-polluted sites along rivers. The diet of the bats indicates that they are exploiting the high abundance of chironomid midges occurring at the tanks and downstream sites. Because pollution-tolerant chironomid midges are able to store metal pollutants without being negatively affected, the opportunistic feeding of *N. nana*, at wastewater-polluted sites puts this bat species at risk of being exposed to the high metal levels at those sites.

# CHAPTER 4:
# THE RISK OF FORAGING AT WASTEWATER-POLLUTED RIVERS: METAL LEVELS IN *N. NANA*

## *4.1. Summary*

Metal pollutants may directly or indirectly affect the health of wild mammals. In terrestrial organisms, metals are most often taken up through the drinking of polluted water and the ingestion of contaminated prey. *N. nana* investigated in this study may be exposed to wastewater metals through their prey. To determine whether the metal pollutants detected in the water at upstream and wastewater-polluted (tank and downstream) sites accumulate in *N. nana* that forage there, I quantified the concentration of metals (Cu, Cr, Fe, Ni, Zn, Pb and Cd) in the liver, kidneys and pectoral muscle of individuals captured at the sites. Pb was below detection levels. Essential metals (Cu, Zn and Fe) were detected in the muscle, kidney and liver of *N. nana* captured at all sites. In contrast, Cd, Cr and Ni were detected in tissue collected from only wastewater-polluted sites (except one occurrence of Cd at an upstream site) for all rivers. Cr and Ni were present in the kidney and muscle of bats only at wastewater-polluted sites. Cd, however, was present in all tissue types. This is of particular concern as Cd is a highly toxic metal and is known to have a high tendency to accumulate in tissue over time. I further investigated whether the concentrations of metals measured in liver, kidney and muscle of *N. nana* were related to concentrations in the water at upstream and wastewater-polluted sites. There was a positive significant relationship between tissue metal and water metal concentrations for only kidney samples. This relation between metal concentration at the sites and in the bat kidney tissue suggests that there is potential for transfer of metals through the food chain. More specifically, important toxic metals, Cd, Cr and Ni, may be accumulating in organs and may therefore pose negative long-term health effects for *N. nana*. This chapter thus provides an indication of potential risk for *N. nana* foraging at wastewater-polluted rivers.

## *4.2. Introduction*

Numerous studies conducted in the past few decades have documented the severe negative impact of anthropogenic pollutants on the health of wildlife (for review see Rattner, 2009). Pollutants, including metals, may directly or indirectly affect the health of individual organisms, resulting in impaired physiology (Sanchez-Chardi *et al*., 2009), reproduction (Eeva *et al*., 2009) and behaviour (Bridges & Semlitsch, 2000) or, in severe cases, mortality (Hoenerhoff & Williams, 2004). In terrestrial organisms, metals are most often taken up through the drinking of polluted water and the ingestion of contaminated prey (Brueske & Barret, 1991).

Metal pollution is known to have harmful impacts on predatory bird species (Rattner, 2009). For example, significant impairments in the breeding performance of passerine birds, such as the tree swallow (*Tachycineta bicolor*), have been observed in metal polluted areas (Brasso & Cristol, 2008). Studies have attempted to elucidate the effects of metal pollution on small mammals (voles, mice and shrews) that feed in metal contaminated habitats (Metcheva *et al*., 2003; Hamers *et al*., 2006; Wijnhoven *et al*., 2007).

In an analysis of metal loadings of the herbivorous snow vole (*Chionomys nivalis*), there was a significant correlation between metal content in the food source and in vole liver (Metcheva *et al*., 2003). Bioaccumulation of metals is, however, characteristically more prominent in carnivorous small mammals than in herbivorous small mammals (Alleva *et al*., 2006; Hamers *et al*., 2006). In a risk assessment of metals for herbivorous and carnivorous small mammals, metal concentrations were higher in the kidneys of the insectivorous shrew, *S. araneus,* than in the herbivorous bank vole, *C. glareolus* (Hamers *et al*., 2006). *S. araneus* also had the highest metal concentration in a study of seven small mammal species representing various feeding guilds (Wijnhoven *et al*., 2007). Thus, animalivorous bats such as *N. nana*, which feed extensively on insect prey, are at a high risk for metal bioaccumulation in the body.

Metals accumulate at different concentrations in different organs (Johnson *et al*., 1978). For instance, the accumulation of toxic metals, such as Cd, is most evident in the kidneys (Johnson *et al*., 1978). The accumulation of metals in different tissue types is influenced by factors such as calcium, phosphorus and vitamin D levels (Sobel *et al*., 1940). Although metals may be detected

in various body tissues, they most often occur at higher concentrations in the target organs associated with detoxification, i.e. the kidney and liver (Hunter & Johnson, 1982). Metal residues may additionally accumulate in muscle tissue (Hunter & Johnson, 1982).

The lethal effects of large contaminant doses on bats have been well documented (Clark *et al*., 1978). However, sub-lethal effects, such as metal-induced damage in target organs, are usually undetected in bats (Clark & Shore, 2001). To date, little research (approximately 30% of all bat contaminant studies) has been conducted on metal pollutants in bats (Clark & Shore, 2001). In addition, there have been no studies that have quantified metal concentrations in bat species in Africa, or evaluated metal concentrations in animalivorous bats foraging at wastewater-polluted rivers.

In Chapter 3, it was established that *N. nana* concentrated their feeding activity at wastewater-polluted sites (tank and downstream sites) compared to sites located upstream of wastewater pollution along the Umbilo, Little Amanzimtoti and Mdloti rivers in Durban. Furthermore, their diet at these wastewater-polluted sites consisted of predominantly Dipteran prey (specifically chironomid midges), which are in direct contact with the metal-polluted water at the sites. Thus, *N. nana* may be exposed to wastewater metals through their prey. In Chapter 2, it was established that metal concentrations in the water at upstream sites were low compared to those measured in the water at wastewater-polluted sites. The aim of this chapter was to, therefore, determine whether the metal pollutants detected in water samples from sites upstream of and at wastewater-polluted sites are also found in individuals of *N. nana* that forage there. I quantified the concentration of seven metals (Cu, Cr, Fe, Ni, Zn, Pb and Cd) in the liver, kidneys and pectoral muscle of *N. nana* captured at upstream and wastewater-polluted sites and tested whether the concentration of metals in the tissues of *N. nana* were correlated with the metal concentrations in the water at the site. I predicted that metal concentrations would be higher in tissues of *N. nana* foraging at tank and downstream sites than at those foraging at sites upstream of wastewater pollution.

## *4.3. Methods*

### *4.3.1. Preparation of samples*

Twenty six *N. nana* were collected from upstream (n = 3), tank (n = 15), and downstream (n = 8) sites along the Umbilo, Little Amanzimtoti and Mdloti rivers during the summer sampling period (March/April 2010) (see Chapter 3 for further details). Ethical approval was obtained for the capture of *N. nana* for dissection. The bats were euthanized and dissected, using autoclaved instruments, for the collection of liver, kidney and pectoral muscle samples. Newly sterilised tools were used for each individual, to prevent metal contamination between samples. Only adults were used for the analysis, to control for differences in metal content between juveniles and adults. The removed tissue samples were kept at -80 °C until preparation for metal analysis. Samples were dried at 60 °C for two days in an oven and then weighed to obtain the dry mass of the sample. Using a ratio of 45.5 ml $HNO_3$: 1 g tissue, the dried tissue samples were digested overnight in 65% concentrated nitric acid. To remove particulate matter, the digested samples were diluted with distilled water (1: 2) and filtered through syringe filters with a diameter of 25 mm and pore size of 0.45 µm (PALL, Acrodisc). The liquid filtrate was kept for analysis. To test recovery rates, the same procedure used to prepare *N. nana* tissue samples for metal analysis, was performed on certified standard reference material (dried oyster tissue, SRM1566b) (National Institute of Standards and Technology).

### *4.3.2. Inductively Coupled Plasma Optical Emission Spectrometry* (*ICP-OES*)

The concentrations of Cu, Cr, Fe, Ni, Zn, Pb and Cd in liver (n = 26), kidney (n = 26) and muscle (n = 26) samples were determined using ICP-OES (Perkin Elmer, Optima 5300 DV), as described in Chapter 2. Calibration standards of varying concentrations were prepared according to trial measurements of each metal in the samples. The same baseline calibration curves set in Chapter 2 (Chapter 2) were used. The only metal in the tissue samples where all concentrations were below detection of the ICP-OES was Pb (DL = 0.0420 µg/g), which was therefore not presented in the results. The major limitation of ICP-OES is the high detection limit for the metal quantification. A number of measured concentrations for Cd, Ni and Cr were also below

their detection limits (DL = 0.0027 µg/g; 0.0150 µg/g; 0.0071 µg/g respectively). All metal concentrations below detection were assigned the value of the detection limit for the respective metal. Recovery rates for the metals in the standard reference material (dried oyster tissue, SRM1566b) ranged from 67% to 150%, with the exception of Cd which had a poor recovery rate of 11%. However, metal concentrations between the three replicates of oyster samples analyzed were consistent, with only 1% to 6% standard deviation between replicates. Thus, despite the wide range of recovery rates between metals, the trends across upstream, tank and downstream sites per metal should be consistent.

### *4.3.3. Statistical analysis*

Because of low sample sizes (n = 0 or 1 for some sites), statistical analyses could not be used to compare differences in tissue metal concentrations among upstream, tank and downstream sites at all three rivers. The tissue metal data for the sites could not be pooled for the three rivers because metal concentrations (all metals except Pb) measured in the water (Chapter 2) differed among rivers. Therefore, one-way ANOVAs (including Tukey HSD post-hoc tests) comparing differences among sites were performed for only the Mdloti River where $n \geq 3$ per site. Assumptions of normality and equality of variance for the metal concentration data were tested using a 1-sample Kolmogorov-Smirnov Test and a Levene's Test, respectively.

To determine the extent to which metal concentrations measured in *N. nana* tissue (dependent variable) are associated with metal concentrations in the water (independent variable), I conducted simple linear regression analyses for each tissue type. All water and tissue metal concentration data were $\log_{10}$ transformed. Assumptions of linearity, normality and equality of variance of residuals were tested. If assumptions could not be satisfied with data transformation, Spearman's rank correlation was used to assess whether there was a significant association between metal concentrations measured in *N. nana* tissue and metal concentrations in the water. Analyses were performed with SPSS 19.0, using alpha of 0.05.

## *4.4. Results*

Results for metal concentrations measured in tissue samples are presented as means (±standard deviation), range of metal concentrations (µg/g), and number of samples below detection limit (BDL) in Table 1 (kidney), Table 2 (liver) and Table 3 (muscle). Only Zn (liver: $F = 4.968$, $df = 2$, $P = 0.029$; muscle: $F = 6.420$, $df = 2$, $P = 0.014$) and Fe (muscle: $F = 6.957$, $df = 2$, $P = 0.011$) concentrations differed significantly among upstream, tank and downstream sites at the Mdloti River. Tukey HSD post-hoc tests showed that Zn in the liver was significantly higher at the upstream site than at the tank site ($P = 0.029$). Fe in the muscle tissue was significantly higher at the upstream site than at the tank and downstream site (down vs. tank: $P = 0.024$, down vs. up: $P = 0.029$), while Zn in the muscle tissue was significantly higher downstream than at the tank site ($P = 0.013$). There were no significant differences in Cu, Cd, Cr and Ni concentrations among sites.

Cu, Zn and Fe were detected in all samples of all tissue types (Table 1, Table 2, Table 3). Cd, Cr and Ni, however, were detected in tissue collected from only wastewater-polluted sites (except one occurrence of Cd at an upstream site) for all rivers. At all three rivers, the maximum Cd concentration at wastewater-polluted sites in the kidney tissue was higher than that of the upstream sites (see range, Table 1). In liver and muscle tissue, the maximum Cd concentration at wastewater-polluted sites was higher than or equal to that of the upstream sites (see range, Table 2 and Table 3). Cr and Ni were below detection in all liver samples. However, at the Mdloti River, Cr was detected in one downstream kidney sample, and in three muscle samples from the tank site (Table 1, Table 2). Ni was detected in only kidney samples from the wastewater-polluted sites of the Mdloti River, and in muscle tissue samples from the wastewater-polluted sites of the Umbilo River.

There were no significant relationships between tissue metal and water metal concentrations at the Umbilo and Little Amanzimtoti Rivers. There was a positive significant relationship between tissue metal and water metal concentrations for only kidney samples. Metal concentrations in *N. nana* kidney increased with increasing metal concentrations in the water ($R^2 = 0.662$; $df = 1$; $P < 0.0005$; $F = 48.974$). The regression equation was $y = 1.158\ x + 2.753$, where y: tissue metal concentration, and x: water metal concentration (Fig 1).

Table 1. Means ±SD and range of metal concentrations (μg/g), and number of samples below detection limit (BDL) in the kidney of *N. nana* at upstream (U), tank (T) and downstream (D) sites of the Umbilo, Little Amanzimtoti and Mdloti Rivers.

| | | **Umbilo** | | | **L. Amanzimtoti** | | | **Mdloti** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **T** | **D** | **U** | **T** | **D** | **U** | **T** | **D** |
| No. of samples | | 3 | 1 | 1 | 6 | 1 | 2 | 6 | 6 |
| Cadmium | Mean ±SD | 2.639 ±1.582 | 1.775 | 0.003 | 0.910 ±0.948 | 0.273 | 0.070 ±0.097 | 0.614 ±0.645 | 0.228 ±0.111 |
| | Range | 0.819 - 3.686 | - | - | 0.003 - 2.594 | - | 0.003 - 0.137 | 0.137 - 0.956 | 0.137 - 0.410 |
| | BDL | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| Chromium | Means ±SD | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.120 ±0.276 |
| | Range | 0.007 - 0.007 | - | - | - | - | 0.007 - 0.007 | 0.007 - 0.007 | 0.007 - 0.683 |
| | BDL | 3 | 1 | 1 | 6 | 1 | 2 | 6 | 5 |
| Nickel | Means ±SD | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 1.375 ±3.272 | 3.289 ±8.018 |
| | Range | 0.015 - 0.015 | - | - | - | - | 0.015 - 0.015 | 0.015 - 8.054 | 0.015 - 19.656 |
| | BDL | 3 | 1 | 1 | 6 | 1 | 2 | 4 | 5 |
| Copper | Means ±SD | 4.459 ±1.060 | 4.914 | 5.187 | 4.914 ±1.209 | 3.686 | 6.552 ±0.579 | 11.102 ±4.613 | 9.805 ±1.282 |
| | Range | 3.276 – 5.324 | - | - | 3.276 - 6.416 | - | 6.143 - 6.962 | 6.552 - 18.018 | 8.054 - 11.603 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Zinc | Means ±SD | 392.256 ±17.975 | 386.022 | 465.875 | 422.559 ±101.246 | 375.375 | 513.513 ±115.052 | 471.448 ±91.292 | 496.178 ±86.807 |
| | Range | 373.055 - 408.681 | - | - | 300.846 - 577.259 | - | 432.159 - 594.867 | 352.580 - 617.799 | 434.616 - 645.372 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Iron | Means ±SD | 174.993 ±33.859 | 236.828 | 122.850 | 330.307 ±216.314 | 127.901 | 177.178 ±41.890 | 333.879 ±106.520 | 285.444 ±124.466 |
| | Range | 138.684 - 205.706 | - | - | 144.417 - 746.149 | - | 147.557 - 206.798 | 242.424 - 486.486 | 138.684 - 468.468 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 2. Means ±SD and range of metal concentrations (µg/g), and number of samples below detection limit (BDL) in the liver of *N. nana* at upstream (U), tank (T) and downstream (D) sites of the Umbilo, Little Amanzimtoti and Mdloti Rivers.

| | | Umbilo | | | L. Amanzimtoti | | | Mdloti | |
|---|---|---|---|---|---|---|---|---|---|
| | | T | D | U | T | D | U | T | D |
| No. of samples | | 3 | 1 | 1 | 6 | 1 | 2 | 6 | 6 |
| Cadmium | Means ±SD | 1.866 ±1.290 | 0.546 | 0.003 | 0.071 ±0.166 | 0.003 | 0.003 | 0.070 ±0.113 | 0.003 |
| | Range | 0.410 - 2.867 | - | - | 0.003 - 0.410 | - | 0.003 - 0.003 | 0.003 - 0.273 | 0.003 - 0.003 |
| | BDL | 0 | 0 | 1 | 5 | | 2 | 4 | 6 |
| Copper | Means ±SD | 15.925 ±8.237 | 11.057 | 9.419 | 8.918 ±0.940 | 9.419 | 17.813 ±6.274 | 14.082 ±3.152 | 13.195 ±2.862 |
| | Range | 10.374 - 25.389 | - | - | 8.054 - 10.511 | - | 13.377 - 22.250 | 8.054 - 17.063 | 9.692 - 16.380 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Zinc | Means ±SD | 268.233 ±19.508 | 269.042 | 281.873 | 266.425 ±68.643 | 254.573 | 316.953 ±43.627 | 244.381 ±10.989 | 268.700 ±35.731 |
| | Range | 252.525 - 290.063 | - | - | 191.100 - 376.194 | - | 286.104 - 347.802 | 235.053 - 262.217 | 232.460 - 325.553 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Iron | Means ±SD | 940.622 ±169.350 | 859.131 | 705.978 | 1130.220 ±195.366 | 850.205 | 1237.509 | 1103.807 ±373.536 | 1278.377 ±481.068 |
| | Range | 834.970 - 1135.953 | - | - | 875.102 - 1332.517 | - | - | 736.281 - 1584.356 | 941.441 - 2118.753 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

All Cr and Ni samples were below the detection limit

Table 3. Means ±SD and range of metal concentrations (µg/g), and number of samples below detection limit (BDL) in the muscle of *N. nana* at upstream (U), tank (T) and downstream (D) sites of the Umbilo, Little Amanzimtoti and Mdloti Rivers.

| | | **Umbilo** | | | **L. Amanzimtoti** | | | **Mdloti** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **T** | **D** | **U** | **T** | **D** | **U** | **T** | **D** |
| No. of samples | | 3 | 1 | 1 | 6 | 1 | 2 | 6 | 6 |
| Cadmium | Means ±SD | 0.047 ±0.077 | 0.003 | 0.003 | 0.025 ±0.055 | 0.003 | 0.003 | 0.003 | 0.071 ±0.166 |
| | Range | 0.003 - 0.137 | - | - | 0.003 - 0.137 | - | 0.003 - 0.003 | 0.003 - 0.003 | 0.003 - 0.410 |
| | BDL | 2 | 1 | 1 | 5 | 1 | 2 | 6 | 5 |
| Chromium | Means ±SD | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 1.209 ±2.313 | 0.007 |
| | Range | 0.007 - 0.007 | - | - | 0.007 - 0.007 | - | 0.007 - 0.007 | 0.007 - 0.956 | 0.007 - 0.007 |
| | BDL | 3 | 1 | 1 | 6 | 1 | 2 | 3 | 6 |
| Nickel | Means ±SD | 0.056 ±0.070 | 1.229 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 |
| | Range | 0.015 - 0.137 | - | - | 0.015 - 0.015 | - | 0.015 - 0.015 | 0.015 - 0.015 | 0.015 - 0.015 |
| | BDL | 2 | 0 | 1 | 6 | 1 | 2 | 6 | 6 |
| Copper | Means ±SD | 12.285 ±1.190 | 15.152 | 14.196 | 13.263 ±1.350 | 14.196 | 16.380 ±1.351 | 15.993 ±2.291 | 16.062 ±1.380 |
| | Range | 11.466 - 13.650 | - | - | 11.330 - 14.742 | - | 15.425 - 17.336 | 13.514 - 18.974 | 14.196 - 17.882 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Zinc | Means ±SD | 212.986 ±11.330 | 214.988 | 209.528 | 225.453 ±39.952 | 229.047 | 234.712 ±3.185 | 196.697 ±18.206 | 243.129 ±28.967 |
| | Range | 201.611 - 224.270 | - | - | 167.759 - 267.131 | - | 232.460 - 236.964 | 173.492 - 214.305 | 232.050 - 300.164 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Iron | Means ±SD | 118.619 ±7.517 | 154.655 | 115.206 | 134.976 ±25.347 | 84.494 | 203.590 ±3.571 | 184.048 ±35.822 | 131.131 ±24.818 |
| | Range | 113.295 - 127.218 | - | - | 91.728 - 158.477 | - | 201.065 - 206.115 | 141.960 - 220.721 | 104.423 - 159.432 |
| | BDL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

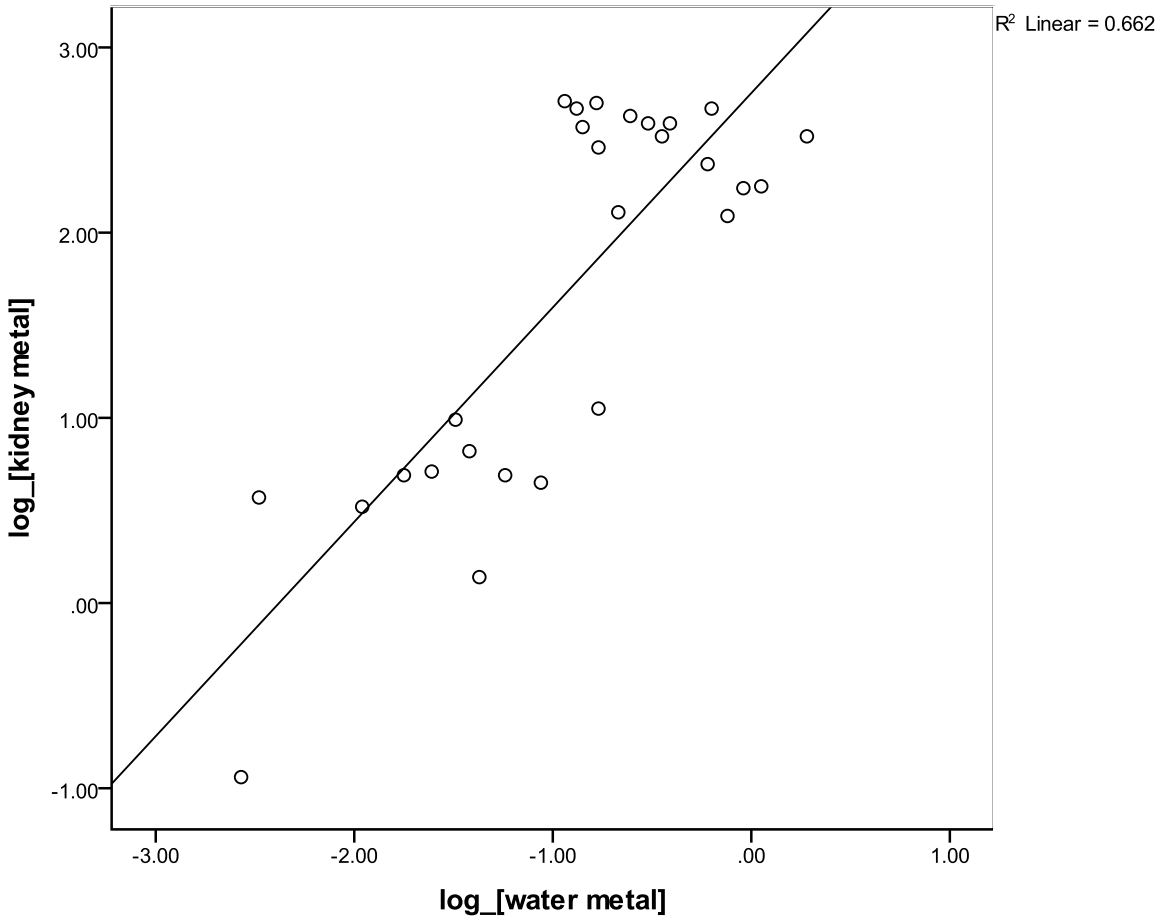


Fig. 1. Relationship between kidney metal concentration and water metal concentration (regression equation: y = 1.158 x + 2.753), in *N. nana* at upstream (U), tank (T) and downstream (D) sites of the Umbilo, Little Amanzimtoti and Mdloti Rivers

## *4.5. Discussion*

Differences in metal concentration measured in the kidney, liver and muscle tissue of *N. nana* captured at upstream, tank and downstream sites along the Umbilo, Little Amanzimtoti and Mdloti rivers varied for Cd, Cr, Ni, Cu, Zn and Fe. Consistent with my prediction, Cd, Cr and Ni were detected in tissue collected from bats foraging at wastewater-polluted sites (except one

occurrence of Cd at an upstream site). Conversely, Cu, Zn and Fe were detected in samples from bats foraging at upstream and wastewater-polluted sites.

While Cu, Zn and Fe are essential for normal cellular processes and bodily function, Cd, Cr and Ni are considered non-essential, and Cd is particularly harmful even at relatively low concentrations (Hoffman *et al*., 2001). The fact that Cr, Ni and Cd were most frequently detected in tissues of bats feeding at polluted sites bears important implications. When the mammalian body does not require metals, they are excreted, sequestered within protein such as metallothioneins, or deposited into intracellular granules for storage (Hoffman *et al*., 2001). The liver and kidney are adapted to the active regulation of essential metals, thus concentrations of toxic metals in the tissue reflect exposure and accumulation of those metals for a prolonged period (Mcgeer *et al*., 2000).

Cr and Ni in the kidney and muscle were detected only at wastewater-polluted sites. Cr and Ni concentrations in *N. nana* kidney ranged from 0.007 - 0.683 µg/g and 0.015 - 19.656 µg/g respectively. Cr and Ni are of less concern to mammal health than more toxic metals such as Cd, Hg and Pb, and are thus less studied. However, it is notable that in one study where the kidneys of meadow voles (*Microtus pennsylvanicus*) were collected at a site treated with municipal sewage sludge, average Cr (0.51 µg/g) and Ni (0.59 µg/g) concentrations were much lower than the upper limit of the ranges in this study (Alberici *et al*., 1989). Although detrimental physiological damage is not likely to occur from Cr and Ni concentrations in response to wastewater exposure in the current study, there is a risk of undetected sub-lethal effects. In mammals, chronic exposure to Ni may cause degenerative effects in various organs (Sheffield *et al*., 2001) while Cr has been linked to chromosomal aberrancy (at mean Cr concentrations as low as 3.053 µg/g) (Tull-Singleton *et al*., 1994) and carcinogenicity (O'Brien *et al*., 2003). In addition, it has been demonstrated in bats that both Ni and Cr are readily transferred from adult to young through lactation (Streit & Nagel, 1993). Cr and Ni were below detection in the liver of *N. nana* at all sites.

Cd, however, was present in kidney, muscle and liver. Cd is known to have deleterious effects on health (Henson & Chedrese, 2004; Burger, 2008). It is one of the metals of most concern to wildlife, and is a teratogen, carcinogen and possible mutagen (Burger, 2008). It has also been recently recognized as an endocrine-disrupting chemical (EDC), reported to mimic the effects of

oestrogen in the body (Henson & Chedrese, 2004). The concentrations obtained for tissue Cd in wastewater-polluted sites in this study ranged from 0.003 – 3.686 µg/g. This was similar to those found in the liver of *Pipistrellus pipistrellus* bats in Germany, which ranged from 0.044 to 1.53 µg/g (Streit & Nagel, 1993), but are far below the concentration of 350 µg/g (in kidney), which indicates measurable harmful effects such as kidney damage in small mammals (Cooke & Johnson, 1996). Cd however, has a high tendency to accumulate in tissue and Cd concentration is therefore strongly correlated with exposure time (Fritsch *et al*., 2010). Furthermore, the recovery rate of Cd was particularly low. This implies that Cd concentrations may have been underestimated due to low extraction from tissue samples. Metals found in lower quantities require a finer resolution and are therefore often under-detected in samples. Thus, Cd concentrations in *N. nana* tissue may in fact, be higher than those obtained.

Cd concentrations in the kidney and liver of small mammal species increase with age (Walker *et al*., 2007; Fritsch *et al*., 2010). Although Cd was below detection in the water at the sites (Chapter 2), even very low concentrations in the source can ultimately contribute to high levels in the predator because of Cd accumulation (Fritsch *et al*., 2010). Thus, despite the fact that the Ni, Cr and Cd tissue concentrations were below critical levels, there is potential for increased metal concentration over time, and the possibility of metal transfer to young.

Cu concentrations in kidney, liver and muscle tissue did not significantly differ among sites at the Mdloti River, where statistical analyses were performed. The highest mean tissue Cu concentration across the rivers in the current study (17.813 µg/g) fell into normal Cu ranges found in other bat species (Hoenerhoff & Williams, 2004; Allinson *et al*., 2006). Cu concentrations are only toxic when in elevated concentrations. Acute liver toxicity caused by copper may be fatal. For example copper-associated hepatopathy occurred in a Mexican fruit bat (*Artibeus jamaicensis*) at a Cu concentration of > 4000 µg/g) (Hoenerhoff & Williams, 2004). Cu homeostasis is generally very efficiently regulated in small mammals regardless of environmental concentrations **(**Hunter & Johnson, 1982**).** Similarly, Zn and Fe are utilized in biological processes in the body and are thus well controlled by homeostasis (Johnson *et al*., 1978).

Concentrations of Zn in the liver and muscle, and Fe in the muscle differed significantly among sites at the Mdloti River. Zn in the liver and Fe in the muscle was highest at the upstream site.

Zn in the muscle was highest at the downstream site. Although Zn and Fe do not pose serious health risks, they may affect the regulation of other metals. For instance, high Zn concentrations inhibit Cu absorption in rats, which may lead to Cu deficiency (Oestreicher & Cousins, 1985). Significant differences were not found for Cd, Cr, Ni or Cu. This may be because of the high variability in the data obtained for these metals. Contaminant concentration data obtained from tissue samples often contain some degree of natural variability between individuals due to genetic variation and physiological fluctuations (Rothery, 2000). The major caveat of this chapter was the small sample sizes which prevented statistical analysis. A larger sample size and detailed age determination would have accounted better for the noise in the data from natural variability. The recovery rate of Cd in standard reference tissue was low, implying that future methods of metal extraction from tissue samples should be optimized. In addition, using methods with a lower detection limit for metal determination than ICP-OES, such as ICP-MS or differential pulse anodic stripping voltammetry (Pikula *et al*., 2010) would be better to obtain high resolution for the metals detected in lower concentrations.

There was a positive significant relationship between all concentrations of metals in the kidney tissue and metals in the water. This is an important result because the kidney is the main storage site of toxic metals including Cd (Hunter & Johnson, 1982). This suggests that there is potential for transfer of metals through the food chain. Although I did not test metal concentrations in the insects captured at the sites, chironomid midges at metal-contaminated sites usually contain high concentrations of metals (Krantzberg & Stokes, 1990). Park *et al*. (2009) sampled aerial invertebrates (including chironomid midges) which take up various wastewater-associated endocrine disrupting chemicals, and calculated exposure levels for a bat species that forages on them. It was suggested that the contaminant intake rates of bats may be sufficient to cause physiological effects (Park *et al*., 2009). Future studies should aim to quantify metal levels in the tissue of the main insect prey items of *N. nana* at wastewater-polluted sites to demonstrate metal transfer from prey to predator. Few studies have managed however, to conduct a full-scale food chain analysis. In addition to prey data, factors including biological processes, detoxification rates, biotransformation and physiological functioning of the predatory species of interest, must be determined (Linder & Joermann, 2001).

To conclude, this chapter provides an indication of the potential risk of metal contamination for the high abundance of *N. nana* bats foraging at wastewater-polluted rivers (Chapter 3). Based

on concentrations in target organs, it can be concluded that metals from wastewater may be passing through the food chain to *N. nana*. More specifically, important toxic metals, Cd, Cr and Ni, may be accumulating in organs and thus pose negative long-term health effects for *N. nana*. Further research should investigate specific physiological effects, such as quantifying lesions from metal exposure in the kidney and liver, and consequent health effects. In addition, the interactive effect of metals (amongst each other and with other pollutant types) has not received sufficient attention, and it is unclear as to whether the effect of a mixture of metals may, in fact, be greater than the sum of the toxicity of the components (Peraza *et al*., 1998). Nevertheless, the presence of toxic wastewater metals in *N. nana* suggests a risk of sub-lethal effects in both adult bats and their offspring, which could negatively affect growth and reproduction.

# CHAPTER 5:
# SYNTHESIS, CONCLUSIONS AND FUTURE WORK

## *5.1. Synthesis/ Conclusions*

River pollution is known to have negative effects on biodiversity (Nedeau *et al*., 2003; Azrina *et al*., 2006; Vörösmarty *et al*., 2010); specifically notable decreases in population abundances and acute health effects in study organisms (Lydeard *et al*., 2004; Oberholster *et al*., 2008). Although the results of this study revealed a high abundance of *N. nana* at wastewater-polluted sites, and no obvious acute health problems in individuals, there was evidence of heavy metal content in different tissues of bats foraging at these polluted sites. These results suggest that *N. nana* may benefit from WWTWs in the short-term but in the long-term there may be negative implications for this species and for other river biota exposed to wastewater pollution.

For some bat species, pertinently urban exploiters, increased activity has been noted at wastewater-polluted sites along rivers (Vaughan *et al*., 1996; Kalcounis-Rueppell *et al*., 2007). Similarly, *N. nana* abundance was significantly higher at wastewater-polluted sites (tank and downstream) than at sites located upstream of effluent discharge into rivers (Chapter 3). This was related to the increased abundance of chironomid midges captured at wastewater-polluted sites (Chapter 3), and in the diet of *N. nana* (Chapter 3). Although chironomid midges are able to tolerate polluted environments, metal pollutants accumulate in the body of this organism (Krantzberg & Stokes, 1990). Indeed, the concentrations of metals associated with wastewater pollution (Cr, Cu, Fe, Ni, Zn, Cd and Pb) were generally lower in the water at upstream sites than at wastewater-polluted sites, with the highest concentrations occurring at the tanks (Chapter 2). Because metal pollutants accumulate in midges, *N. nana* foraging on them at sites polluted with effluent from WWTWs have a high chance of being exposed to these metals.

I found evidence that metal pollutants at WWTWs were transferred to *N. nana* (Chapter 4). There was a significant positive relationship between the concentrations of metals in the

kidney tissue samples and in water samples. The kidney is the primary target organ for the accumulation of toxic metals including Cd (Hunter & Johnson, 1982). In addition, the non-essential metals Cr, Ni, and Cd (except for one occurrence at an upstream site) were detected in *N. nana* at only wastewater-polluted sites. Notwithstanding the low sample size and high detection level of the instrument, the presence of the more toxic metals in *N. nana* tissue samples collected from bats foraging at polluted sites is particularly notable. Cd has a tendency to accumulate in target organs over time, and Ni and Cr may be transferred from adult to young through lactation (Streit & Nagel, 1993). Thus, my results show the accumulation of specific metals in the tissues of *N. nana* at sites polluted by WWTWs. These results, in combination with the increased foraging behaviour of *N. nana* along wastewater-polluted rivers, imply long-term risks for the health of individuals.

## *5.2. Potential consequences for N. nana populations and the local ecosystem*

Pollutant exposure and the accumulation of toxic metals in organs and tissue may result in negative effects on reproduction over time, as discussed in Chapter 4. Therefore, pollutant effects on the reproductive health of *N. nana* individuals may potentially extend to the population level. Furthermore, the stable equilibrium of a population is also negatively impacted by an increase in death rate (Krebs, 2008). Increased mortality is particularly significant for slow reproducing, long-lived species such as bats (Fairbrother, 2001).

Mortality rates are affected by a number of factors including predation and parasitic/ infectious diseases. Although increased mortality from chronic health problems related to sub-lethal pollutant exposure may be insignificant in the short-term, it may have dire consequences over a long time period. Moreover, pollutant exposure has been linked to stressors that regulate mortality (Fairbrother, 2001). For example, impaired immune system functioning from exposure to metals and other pollutants has been associated with outbreaks of parasitic/ infectious diseases (Fairbrother, 2001; Boyd, 2010). This „contaminant-pathogen synergy' has been, for example, linked to the phocine distemper epidemic that struck the seal population in the Wadden Sea in the early 1990s, which coincided with depressed immune response from polychlorinated biphenyl contamination (Ross *et al*., 1995). Bat populations

exposed to pollutants remain to be tested for parasitic/ infectious diseases. This may be particularly important for urban exploiters, such as *N. nana*, that are active at polluted sites.

Exposure to pollutants may also elicit behavioural changes (Boyd, 2010). Metals in particular, have been shown to affect predator-prey interactions by modifying both prey response behaviour and predator capture ability (Boyd, 2010). In predatory fish, respiration rate and swimming performance to capture prey is impaired by exposure to metals (Atchison *et al*., 1987). Loss of co-ordination, for instance, in bats, would greatly impair their hunting ability. In insects, metals result in behaviours that increase susceptibility to predation (Mogren & Trumble, 2010). For instance, metals induce phototaxis where organisms move to areas with a high risk of predation (Mogren & Trumble, 2010). Metals may also reduce locomotive ability, resulting in decreased escape ability from predators (Mogren & Trumble, 2010). In addition, infochemical disruption by metals may prevent the organism from detecting approaching predators (Klaschka, 2008). Thus, the effects of metal pollutants affect multiple trophic levels within the local ecosystem.

Furthermore, there are both direct (physiological functioning) (Long *et al*., 1995) and indirect (modifications to the food web) effects that may arise as a result of exposure to pollutants (Fleeger *et al*., 2003). With the majority of urban rivers becoming polluted, the resident biodiversity is under serious threat (Vörösmarty *et al*., 2010). To preserve river biota in landscapes altered by anthropogenic pollution, it is important that the mechanisms of chemical-induced damage are understood. A detailed analysis of the food chain is rarely attainable due to the range of variables that have to be taken into consideration (Linder & Joermann, 2001). By unravelling pollutant effects in higher predators such as *N. nana*, much insight into these processes can be acquired.

## *5.3. Future work*

The measurement of metal concentrations in the main insect prey items of *N. nana* (chironomid midges) would clearly show the transfer of metals from the pollutant source to the predator. One important caveat of this study was that metal concentrations could not be

determined in chironomid midges, mainly due to the low abundance of the insects at upstream sites. In future studies, control midges that are not exposed to pollution should be laboratory-bred to compare with those captured at polluted sites. In addition, stable isotope analyses of *N. nana* fur and its food items could complement dietary analysis by tracking nitrogen and carbon isotope signals through the trophic levels (Hobson, 1999). Stable isotope analyses can determine the precise quantity of specific prey items assimilated into the body of *N. nana* (Hobson, 1999). This will further contribute to the calculation of metal bioconcentration (*BCF*) and bioaccumulation factors (*BAF*) (Linder & Joermann, 2001).

To provide a better understanding of how metal pollutants affect *N. nana* through the food chain, further research should aim to quantify sub-lethal physiological effects such as lesions in target organs and DNA damage in blood cells, which may serve as biomarkers of exposure (Zocche *et al*., 2010). Metal exposure at the cellular level should also be explored by the quantification of metallothioneins (metal-binding proteins). There is currently only one study that evaluated metallothionein levels in bats (Pikula *et al*., 2010). High metallothionein levels, related to high metal content in organs, were found in aquatic-insect-foraging vespertilionid bat species (Pikula *et al*., 2010). Hormone responses to pollutants are also poorly understood in bats; therefore there is an especially urgent need for research into endocrine disruption (Ringer, 2001). In the USA, insectivorous tree swallow nestlings showed altered plasma corticosterone and thyroid hormone levels at metal-contaminated sites along a river (Wada *et al*., 2009). Wastewater pollution is of particular importance because it contains metals that are endocrine disruptors, and other endocrine disrupting compounds from pharmaceuticals, personal care products and illicit drugs (Kasprzyk-Hordern *et al*., 2009). Abnormally small testes and bacula from chemically-induced endocrine disruption have been observed in male river otters (Clark & Shore, 2001). Male bats are ideal candidates for investigating bacula abnormalities associated with endocrine disruptors (Clark & Shore, 2001).

To conclude, the results of this study establish a link between the foraging behaviour patterns and the presence of toxic metals in *N. nana*. The results show that *N. nana* is an ideal model to pursue further research into pollutant transmission from water to prey to predator, and the subsequent health effects. Furthermore, many exciting and productive research avenues can be extrapolated from this study. Further research into the effects of river pollution on urban

biodiversity is vital particularly because of the increasing rate of urbanization. In addition, pollutants in rivers pose an even greater threat to the resident fauna in the light of global warming (Clements *et al*., 2008). With increased water evaporation from rivers, it is estimated that the toxicity of metals and other pollutants will increase significantly due to lower dilution (Clements *et al*., 2008). Thus, the future functioning of river ecosystems faces serious threat unless the mechanisms of pollutant transfer and effects are elucidated.

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