Lead exposure in an urban population of free-ranging kaka (*Nestor meridionalis septentrionalis*)

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Abstract

Anthropogenic lead use has resulted in widespread environmental lead contamination known to affect wildlife populations worldwide. Lead is a highly toxic, non-essential heavy metal recognised as a cause of morbidity and mortality in birds. Ecotoxicological investigations in wild birds have thus far prioritised waterfowl and raptor species and primarily addressed contamination in natural ecosystems. Urban areas are increasingly associated with high levels of heavy metal contamination, however the risk of lead exposure in urban wildlife is less well known.

This study aimed to identify the significance of lead exposure in a well-established urban population of kaka (*Nestor meridionalis septentrionalis*). Blood lead concentrations were assessed in adult and nestling birds to quantify exposure prevalence and magnitude. The impact of lead exposure on physiological and neurological function was assessed using behavioural and physiological parameters. Finally, lead stable isotope analysis was employed to identify the primary sources of lead in the urban environment.

Lead exposure is prevalent in this kaka population, with 43.2% of adults and 36.7% of nestlings with detectable blood lead concentrations. Blood lead concentrations in nestlings ranged from <3.3 to 42.9ug/dL, with no detectable neurological or physiological deficits. The pattern of exposure in chicks is suggestive of parental feeding of lead, however detection of lead in some eggshells suggests that maternal transfer is another route of exposure in this species. Blood lead concentrations in adult birds ranged between 3.4 to 50.7ug/dL. Although no acute clinical signs of toxicity were observed, lead exposure was associated with reduced body condition in adults. Behavioural changes were present in one individual with the highest recorded blood lead concentration. Lead isotope ratios in kaka blood samples overlap with isotope values of roof-collected rainwater, suggesting this to be an important source of exposure in this population.

The prevalence of lead exposure observed in this study suggests that lead is a threat to kaka interacting with urban areas. Wildlife intoxications largely result from anthropogenic lead sources and this study identifies a previously undescribed urban source of lead in wildlife. The well-described subclinical and persistent effects of lead highlight the need for abatement strategies to reduce lead exposure and its effects in this population.

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CHAPTER ONE

Introduction, literature review and research aims

1.1 Introduction

Kaka (*Nestor meridionalis*) are large, sexually dimorphic, forest parrots endemic to New Zealand (Moorhouse, Sibley, Lloyd, & Greene, 1999; Moorhouse et al., 2003). They inhabit low to mid-altitude forests on mainland New Zealand with larger populations on offshore islands (Berry, 1998). Kaka are episodic breeders (Powlesland et al., 2009), and breeding success is strongly influenced by food availability (Moorhouse et al., 2003). They breed between October and July and nest in tree cavities (Powlesland et al., 2009). Once abundant throughout mainland New Zealand, populations of kaka are estimated to be declining rapidly. As a result, they are classified as endangered by the IUCN and listed as nationally vulnerable in New Zealand (BirdLife International, 2014). The major threats to their population include habitat destruction, food competition and predation by introduced species, and to date research and conservation initiatives have focused on addressing these specific threats (Moorhouse et al., 2003; Greene, Powlesland, Dilks, & Moran, 2004). However, there is a lack of understanding of additional factors, such as exposure to heavy metal toxins, which can potentially contribute to the mortality and population declines observed in this species.

An established population of kaka exist in urban Wellington city as a result of a successful reintroduction programme. From 2002 to 2007, 14 captive bred adult kaka were reintroduced to Zealandia Ecosanctuary, a 225 hectare predator roof reserve on the outskirts of Wellington City (Miskelly, Empson, & Wright, 2005) (**Figure 1.1**). Control of invasive predators and provision of supplementary food has seen this population expand, with approximately 750 nestlings banded since reintroduction (Karori Sanctuary Trust, 2016). Kaka in Wellington are increasingly venturing beyond the boundaries of the reserve and are commonly sighted in urban areas (Charles, 2012; Recio, Payne, & Seddon, 2016). Their inquisitive nature, expanding population and the increased availability of food in Wellington are the primary factors implicated in their dispersal into urban areas, where there is increased risk of exposure to anthropogenic pollutants.

In recent years, there has been an anecdotal increase in the incidence of lead toxicity in kaka in Wellington. Lead is a highly toxic heavy metal with profound impacts on avian wildlife populations worldwide. There is a large body of research highlighting the population level impacts of lead exposure in free-ranging birds, of which majority

focuses on waterbirds, raptors and upland game birds (Franson & Pain, 2011). Relatively little of this published work is specific to native New Zealand avifauna. Significant lead exposure has been documented in an endemic New Zealand parrot, the Kea (*Nestor notabilis*), signifying the potential for lead exposure in New Zealand wildlife, and particularly in parrots (McLelland, Reid, McInnes, Roe, & Gartrell, 2010; Reid, McInnes, McLelland, & Gartrell, 2012).

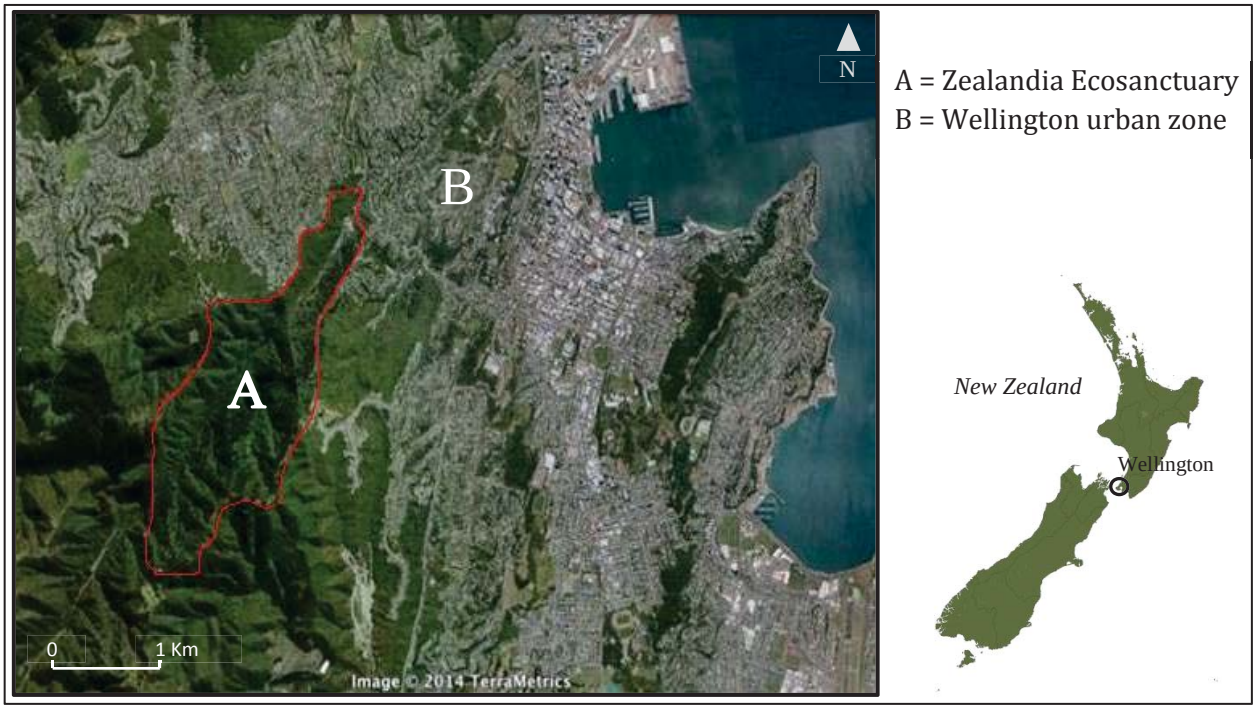

Figure 1.1 Map of Wellington showing study site (outlined in red) in proximity to the urban zone.

1.2 Overview of lead

1.2.1 Properties and use

Lead (Pb) is a naturally occurring, highly toxic heavy metal. Naturally occurring lead ores comprise 0.002% of the earth's crust (World Health Organisation, 2006). Small amounts of lead are released into the environment as a result of natural processes, such as weathering of rocks and radioactive decay, however this is incomparable to contributions from anthropogenic processes (Pattee & Pain, 2002). Being a versatile and relatively inexpensive metal, lead has been extensively used in agricultural, industrial and domestic applications (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Its use dates back to the Roman times, where it was used in water pipes and earthenware containers (Tong, Schirnding, & Prapamontol, 2000). Anthropogenic use of lead has resulted in altered distribution of lead and widespread environmental contamination.

Lead is a soft, bluish-grey metal which exists in organic and inorganic forms (Pattee & Pain, 2002). It is malleable, dense, highly resistant to corrosion, and has a low melting point and is therefore extensively used in different industries (Flora, Gupta, & Tiwari, 2012). Commercial lead metal is described as being primary, when referring to mined lead ore, or secondary, when referring to the production of recycled lead. Lead is one of the easiest metals to mine and approximately 5 million tonnes of lead ore is mined per annum (International Lead and Zinc Study Group, 2017). As most commercial lead is in a readily recyclable form, secondary lead accounts for more than half of the lead produced worldwide (International Lead and Zinc Study Group, 2017). As of 2015, the United States Geological Survey (USGS) reported China and Australia to be the largest producers of mined lead in the world (U.S. Geological Survery, 2017).

Being a versatile metal, lead has a variety of industrial applications. Since the 1960s, lead-acid batteries have accounted for the largest consumption of lead, with approximately 80% of lead used for the production of batteries (World Health Organisation, 2006). Pigments rolled and extruded products and lead shot/ammunition account for comparatively much smaller proportions of lead use (International Lead and Zinc Study Group, 2017). Lead sheet is the principal element in the category of rolled and extruded lead products and its use has increased over the last decade (World Health Organisation, 2006). The malleable and anti-corrosive properties make it ideal for use in the building industry as flashing, roof cladding and

roofing nails. Pigments account for the second largest use of lead following lead-acid batteries (World Health Organisation, 2006). Lead is traditionally used for painting steel and iron, owing to its anti-corrosive properties, but has also been used extensively in commercial decorative paints. Historically, lead was also used in gasoline as an anti-knock additive to improve efficiency and engine performance (World Health Organisation, 2006). However, in recent years, the continued use of lead in products such as paint, fuel and pipes has reduced due to health implications associated with lead exposure.

Lead is however inexpensive and continues to be used to manufacture fishing sinkers and ammunition. Although lead ammunition accounts for only 3-4% of global lead consumption (World Health Organisation, 2006), it is considered the most significant source of lead exposure in wildlife (Haig et al., 2014).

1.2.2 Lead toxicity

Lead has long been considered both a public health risk and a risk to wildlife populations worldwide. The first reports of lead poisoning in humans were recorded as early as 370 BC (Tong et al., 2000). In Europe, the addition of lead to wine as a sweetener was associated with outbreaks of colic in the 16th century (Needleman, 2004). Following the industrial revolution, lead exposure was commonly reported as a workplace hazard primarily in industrial workers (Tong et al., 2000). Increasing occupational exposures resulted in the implementation of safety protocols to prevent lead exposure in the late 19th century (Needleman, 2004). Extensive research on lead exposure in humans has demonstrated the adverse physiological effects associated with exposure and has provided a better understanding of risks of exposure in people.

Contrary to toxicity in adults, lead poisoning in children was recognised as a concern only a century ago. It was first described in children in 1892 in Brisbane, Australia, where household paint was later implicated as the primary source of toxin (Needleman, 2004; World Health Organisation, 2006). Subsequent studies have highlighted the profound implications of childhood lead exposure and the various physiological abnormalities associated with exposure to even low concentrations of lead at a young age (Tong et al., 2000; Needleman, 2004; Chandramouli, Steer, Ellis, & Emond, 2009; Hsiang & Díaz, 2011). Recent work investigating lead exposure suggests

there is no safe lower threshold for lead exposure in children (Chiodo, Jacobson, & Jacobson, 2004; Flora et al., 2012).

In wildlife, lead studies have predominantly focused on waterfowl and raptors. One of the earliest reports of lead intoxication in birds was a study in waterfowl in the 1950s (Franson & Pain, 2011). Over the years, there has been a growing body of evidence implicating lead as a toxin of threat to wildlife populations worldwide. Lead is recognised as a major contributor to population declines in multiple species, and additionally has been implicated in inhibiting species recovery efforts, such as the case of the Californian condor (*Gymnogyps californianus*) (Finkelstein et al., 2012; Haig et al., 2014).

1.3 Environmental sources of lead exposure in wildlife

Anthropogenic lead use has resulted in a wide distribution of lead in the environment and consequently a wide range of environmental exposure sources and pathways. Lead can be emitted into water, air or soil, with physico-chemical processes such as weathering and precipitation allowing for the exchange of lead between these mediums (Pattee & Pain, 2002). Compared with air and water, lead in soil is relatively immobile, whereby soils and sediments can act as lead sinks (World Health Organisation, 2006).

Lead exposure in wildlife can result from multiple sources and determination of these sources is integral to mitigating exposure. Source attribution techniques such as stable isotope analysis are used in human studies and, more recently, are being applied in wildlife studies.

1.3.1 Ammunition

Lead ammunition is the most commonly reported source of lead in avian wildlife. The first report of lead shot causing toxicity in wildlife was more than a century ago, and since, lead shot exposure has been documented in > 130 species (Tranel & Kimmel, 2008). The majority of this research has focused on waterfowl and raptors, where

ingestion is the primary pathway of exposure. Waterfowl foraging in lead shot contaminated areas directly ingest shot particles mistaking it for grit (Haig et al., 2014). Lead in soil is very stable and therefore lead shot can persist in the environment for long periods of time. Flint and Schamber (2010) identified that lead pellets can remain in the top 10cm of sediment for approximately 10 years, posing a risk to shallow foragers and can embed deeper within the sediment for >25 years potentially exposing deeper foraging birds. Therefore, although the use of lead shot has been banned in many regions around the world, the persistence of lead shot in soil continues to be a threat to wildlife (Svanberg et al., 2006).

Raptors are indirectly exposed to lead when scavenging on shot carcasses or tissue left behind in the field containing lead shot fragments (Fisher, Pain, & Thomas, 2006). Lead bullet fragments are known to disperse widely within the tissues of shot animals with deposition of lead in almost all of the viscera (Cruz-Martinez, Grund, & Redig, 2015). Un-retrieved hunter-shot carcasses and discarded offal left in the field therefore pose a risk to scavenging wildlife. Ingestion of lead-contaminated meat has also been recognised as a public health concern, especially in subsistence harvesting communities (Tsuji et al., 2008).

The significance of lead poisoning due to ammunition is conveyed through a large body of evidence highlighting the population level impacts of lead exposure in wildlife. Lead exposure has resulted in population declines in the critically endangered Californian condor (*Gymnogyps californianus*) and continues to threaten species recovery efforts (Finkelstein et al., 2012). Furthermore, waterfowl population losses resulting from lead exposure are estimated at around 2-3% overall (Haig et al., 2014). The impacts of lead shot on wildlife are primarily observed in heavily hunted areas such as waterways and subsequently, legislation restricting the use of lead shot in certain areas has been implemented in many regions around the world (Fisher et al., 2006). The types of regulation vary between countries, and range from a complete ban on lead ammunition for all forms of hunting, as implemented in Sweden and Denmark, to seasonal or regional bans (Avery & Watson, 2009). In New Zealand, 10g and 12g lead shot are banned within 200m of a waterbody or wetlands, however this does not apply to lead shot less than 12g in calibre. The negative impact of lead ammunition on avian wildlife is evidenced by studies evaluating the effectiveness of lead ammunition restrictions and demonstrating decreased exposure rates in wildlife following lead

shot bans (Flint & Schamber, 2010). Kelly et al. (2011) examined a population of raptors prior to and following a ban of lead shot, and reported a significant decrease in blood lead concentrations in raptors following the implementation of the lead ammunition ban.

1.3.2 Soil

The majority of lead in soil is derived from anthropogenic processes including industrial activity (mining, smelting), vehicular emissions, ammunition and urban waste (Komárek, Ettler, Chrastný, & Mihaljevič, 2008). Most of the lead emitted into the atmosphere by vehicular or industrial emissions is eventually deposited in soil through wet or dry deposition (Pattee & Pain, 2002). Highly populated, urbanised areas in particular, are associated with higher levels of soil contamination, owing primarily to the use of leaded-fuel and lead paint (Roux & Marra, 2007). Lead is non-degradable therefore historic emissions from leaded fuel or industrial activity, for example, can result in persistent contamination of soil (Scheifler et al., 2006). In children, ingestion of lead contaminated soil is an important route of exposure and remains a significant public health problem (Tong et al., 2000).

The bioavailability of lead in soils is dependent on the chemical composition and particle size of lead, the soil composition, and most importantly, the pH of the soil (Walraven et al., 2015). Exposure of birds to soil sources of lead is suggested to occur via accidental ingestion of contaminated soil during foraging, and therefore ground-feeding birds are considered particularly vulnerable to soil lead exposure (Beyer, Connor, & Gerould, 1994). Ingestion of lead contaminated sediment was a primary route of lead exposure in waterfowl in a polluted river system (Nelson Beyer, Blus, Henny, & Audet, 1997). Birds feeding on soil invertebrates are also considered susceptible to lead exposure as invertebrates can bio-accumulate lead in the soil (Roux & Marra, 2007). Earthworms, for example, are considered to contain approximately 20-30% of soil and are capable of accumulating lead in their tissues (Beyer et al., 1994). Scheifler et al. (2006) and Roodbergen, Klok, and van der Hout (2008) provide evidence for the indirect transfer of lead in the soil to birds through ingestion of earthworms in polluted areas. The ability of plants to uptake lead from the soil provides an additional dietary source of lead exposure in wildlife. Soil characteristics

influence the uptake of lead in plants, and uptake has also shown to vary between species of plants (Labare, Butkus, Riegner, Schommer, & Atkinson, 2004). Lead can accumulate in the roots, leaves, stems and within seeds and can therefore be transferred to animals through ingestion (Labare et al., 2004).

1.3.3 Atmospheric contamination

Anthropogenic activities account for the large majority of atmospheric lead emission worldwide (Pattee & Pain, 2002). Prior to the discontinuation of its use, leaded fuel was considered the most important source of atmospheric lead. Organic tetraalkyl lead forms are volatile and when added to gasoline, are emitted in exhaust fumes. Tetraalkyl lead is relatively stable in the atmosphere and can be deposited into soil or alternatively can be transported long distances in air (Pattee & Pain, 2002). The addition of lead in gasoline has been banned in the large majority of countries worldwide and has been accompanied by a reduction in prevalence of human cases of led exposure (Landrigan, 2002). In New Zealand, lead was removed from gasoline in 1996 (Wilson & Horrocks, 2008), and recent measures of airborne lead concentrations are considered to be well within regulatory limits (Stats NZ, 2015).

Industrial emissions also contribute to atmospheric lead pollution and are associated with proximity of the source (Pattee & Pain, 2002). There are limited reports of inhalational toxicity associated with atmospheric contamination in wildlife, however, deposition of atmospheric emissions in soil, water and plants provide a source of lead exposure. Many studies have reported higher levels of lead exposure in birds within vicinity of industrial sites (Blanco, Frías, Jiménez, & Gómez, 2003; Gómez-Ramírez, Martínez-López, María-Mojica, León-Ortega, & García-Fernández, 2011) and areas with high vehicular traffic, such as urban areas (Grue, Hoffman, Nelson Beyer, & Franson, 1986; Roux & Marra, 2007).

1.3.4 Urban environments and lead contamination

Urban environments are associated with significant levels of heavy metal pollution. Studies comparing lead exposure in wild birds in urban areas to those in natural ecosystems or rural areas consistently report higher lead exposure levels associated

with urban exposure (Roux & Marra, 2007; Orłowski et al., 2014; Hargitai et al., 2016; Naidoo, Wolter, & Botha, 2017). Prior to being discontinued, leaded fuel was considered the primary contributor to urban lead pollution, however, contributions from additional sources such as lead-based paint and lead-containing building material are becoming more relevant. The use of lead in pigments was widespread, however is now restricted in many countries (World Health Organisation, 2006). Exposure to lead paint can result from direct ingestion of paint, inhalation of paint particles/dust or ingestion of soil contaminated with paint (World Health Organisation, 2006). Finkelstein, Gwiazda, and Smith (2003), for example, report on lead intoxication in Laysan albatross (*Phoebastria immutabilis*) as a result of direct ingestion of lead paint.

The use of lead in roofing material has been identified as a public health risk in human studies where lead has been identified as a contaminant in roof-collected rainwater intended for human consumption (Simmons, Hope, Lewis, Whitmore, & Gao, 2001). Lead roof cladding, lead-headed roofing nails, lead paint and lead roof flashings are some of the elements found in roofing systems and which contribute to high levels of lead in roof-collected rain water (Stewart, Kim, Johnston, & Nayyerloo, 2016). Stewart et al. (2016) and Simmons et al. (2001) sampled rainwater tanks in New Zealand (Wellington and Auckland) and identified lead concentrations above acceptable values for human consumption (>10ug/dL; >0.01mg/L) in rainwater samples in both studies suggesting it is an important urban source of lead exposure.

Investigations of heavy metal pollution in urban wildlife are limited, outlining the need for future work, in particular exploring the varied sources of lead present in the urban environment.

1.3.5 Source attribution using stable isotope analysis

Identifying the source of lead exposure in wildlife informs mitigation, management and policy around lead use. Lead stable isotope analysis has been widely utilised in human studies and is more commonly being used in wildlife studies to trace lead exposure (Scheuhammer & Templeton, 1998). Lead is naturally present as four main isotopes, ^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb (Komárek et al., 2008). Different ores have specific isotopic ratios based on their origin, and therefore comparisons of ratios between

sources allows for the determination of the most likely source of exposure (Huang et al., 2015). Achieving accurate source attribution is dependent on a variety of factors including the number of sources investigated and variability in isotopic ratio between sources investigated (Gwiazda & Smith, 2000). Isotope analysis has been useful in identifying ammunition as the primary source of exposure in certain species (Church et al., 2006; Svanberg et al., 2006; Pain et al., 2007; Finkelstein et al., 2012), and where accurate source attribution was not possible, it has allowed for the elimination of certain sources of exposure (Scheuhammer, Bond, Burgess, & Rodrigue, 2003; Behmke et al., 2015). Stable isotope analysis has primarily been used in studies investigating ammunition as a source of exposure in wildlife. Although it has proven successful in a proportion of studies, investigations across a variety of sources and case-studies are warranted to determine the usefulness of this method in wildlife ecotoxicological investigations.

1.4 Toxico-kinetics of lead

1.4.1 Routes of exposure and absorption of lead

The proportion of lead absorption following exposure is variable between individuals and is dependent on the chemical form of lead, the dose of exposure and biological factors such as species, age and physiological state (Pattee & Pain, 2002). Inhalation, ingestion and dermal exposure are described as potential routes of lead exposure in humans and animals (Pattee & Pain, 2002). Ingestion is the primary route of exposure reported in terrestrial birds (Fisher et al., 2006). In mammals, following ingestion majority of lead is excreted in the faeces with only a small proportion of lead being absorbed systemically (Pattee & Pain, 2002). In humans, approximately 10% of ingested lead is absorbed systemically and gastrointestinal absorption rates are suggested to be similar in magnitude in animals (Pattee & Pain, 2002). In birds, ingested lead fragments undergo mechanical erosion in the gizzard, and the acidic environment allows for further chemical dissolution of lead and the release of lead salts which can be absorbed into the blood (Vyas, Spann, & Heinz, 2000). Retention times of lead fragments within the gastrointestinal tract are variable and dependent on factors such as nutritional status, diet, presence and composition of grit and size of ingested lead fragments. In a controlled study in mourning doves (*Zenaida macroura*), time of day, amount of food consumed and number of lead pellets were suggested to

influence retention time of pellets in the gastrointestinal tract (Schulz et al., 2006). Pattee, Wiemeyer, Mulhern, Sileo, and Carpenter (1981), in an experimental study on bald eagles (*Haliaeetus leucocephalus)*, reported variability in retention time, excretion and erosion of lead pellets between individuals. In raptors, the ability to regurgitate casts including lead fragments allows for an additional route of lead elimination (Pain et al., 2007). Nutritional status, in particular hypocalcaemia, has been shown to influence lead absorption in birds (Scheuhammer, 1987; World Health Organisation, 2006). Lead can bind to calcium binding proteins, which are produced in response to hypocalcaemia and allow for increased intestinal lead absorption (Dauwe, Snoeijs, Bervoets, Blust, & Eens, 2006). Dauwe et al. (2006) argued that even when exposure levels are similar, lead absorption can vary between individuals based on nutritional status.

These studies contribute to a growing body of evidence suggesting that inter-individual variability in the retention and absorption efficiency of lead contributes to the variability in response to lead exposure.

1.4.2 Distribution

Lead distribution within the body is described using a three-compartment model of blood, soft tissue and bone (Rabinowitz, Wetherill, & Kopple, 1976; Gordon, Taylor, & Bennett, 2002). Following exposure, lead is absorbed into the blood and is transported bound to proteins on the membrane of erythrocytes. Concentrations in the blood are highest immediately following exposure. Soon after exposure, lead is redistributed to soft-tissue compartments considered as an intermediate pool (Gordon et al., 2002). Blood lead concentrations therefore are a valuable indicator of acute exposure. Concentrations of lead in the blood and intermediate soft-tissue compartments are maintained at a relative equilibrium with exchange between the two compartments (Franson & Pain, 2011). The half-life of lead in the blood is highly variable and is partly dependent on dose of exposure where higher doses of lead result in more persistent blood lead concentrations (Franson & Pain, 2011). In humans, a blood lead half-life of 35-40 days is suggested (Gordon et al., 2002; Wani, Ara, & Usmani, 2015). In birds, information on blood lead half-life is not readily available, however is suggested to be similar in magnitude to human studies (Buekers, Steen Redeker, & Smolders, 2009).

An experimental study on Japanese quail chicks (*Coturnix coturnix japonica*) fed a single dose of lead pellet showed blood lead concentrations return to pre-experiment levels after 128 days (Fair & Ricklefs, 2002).

Lead concentrations in the bone are relatively stable. Lead competitively replaces calcium and is incorporated into the bone in place of calcium (Tchounwou et al., 2012). Lead bio-accumulates in bone throughout life and is therefore considered representative of chronic or life-long exposure (Franson & Pain, 2011). Bone represents 95% of the total body burden of lead and is an endogenous and labile source of lead (Gordon et al., 2002; Barbosa Jr, Tanus-Santos, Gerlach, & Parsons, 2005). In humans, the half-life of lead in the bone is considered to be 20-30 years (Rabinowitz et al., 1976). Lead retention is affected by bone type as lead in trabecular bone is more biologically active when compared to cortical bone (Barbosa Jr et al., 2005). Long-term deposits of lead in the bone can be mobilised into systemic circulation, during certain physiological states, such as during pregnancy and lactation (Garza, Vega, & Soto, 2006). Increased calcium, and therefore lead absorption, is suggested to occur in female birds as intestinal absorption increases prior to laying. An increased requirement for calcium during eggshell formation triggers increased synthesis of intestinal calcium-binding proteins and therefore increased calcium absorption in the gut (Scheuhammer, 1987). As lead competes for binding sites with calcium, during egg-laying, female birds are likely to accumulate lead as a result of increased calcium demand and subsequently increased intestinal absorption and mobilisation from bone. Svanberg et al. (2006) reported higher bone lead concentrations in female white-headed ducks (*Oxyura leucocephala*) compared to males.

Exposure dose and duration can influence distribution of lead in the body. With acute lead exposure, concentrations of lead are generally highest in the blood and soft-tissue such as liver and kidney while in the case of chronic exposure, bone lead concentrations are generally the higher.

1.4.3 Excretion

The primary route of lead excretion is via the kidneys (Gordon et al., 2002). Vostál and Heller (1968) suggest that renal excretion of heavy metals in birds occurs through

glomerular filtration and also via direct transport across the tubules. The deposition of lead in feathers and, in female birds, in the egg are alternate pathways of lead excretion in birds. During feather growth, while the feather is supplied by blood vessels, lead is bound to protein molecules and deposited in the feather (Dauwe, Bervoets, Pinxten, Blust, & Eens, 2003). Upon completion of feather growth, blood supply diminishes and the feather, and subsequently the deposited lead, are essentially biologically inert (Dauwe et al., 2003). Lead concentration in feathers is indicative of lead exposure at the time of feather formation (Franson & Pain, 2011).

Deposition of lead in the egg is an established route of toxin excretion in birds (Burger, 1994), whereby eggs are considered a bio-indicator of environmental heavy metal contamination (Mora, 2003; Swaileh & Sansur, 2006). The calcium-mimetic properties of lead result in lead being incorporated in the egg, in the place of calcium. Concentrations of lead deposition in the egg are dependent on lead burdens of the female bird at the time of laying (Dauwe, Janssens, Bervoets, Blust, & Eens, 2005). There is some debate regarding the distribution of lead in the eggshell and egg contents. Higher concentrations of lead were found in egg contents in a few species (Burger, 1994), while in vast majority of studies the eggshells are considered to better represent lead sequestration in the egg (Mora, 2003; Dauwe et al., 2005; Swaileh & Sansur, 2006; Kim & Oh, 2014). Species variability in this regard highlights the need for species and case based investigations, especially given there is a lack of knowledge in certain species groups such as in parrots.

1.5 Pathophysiology of lead toxicity

Lead is a non-specific toxin affecting multiple biological processes. The toxic effects of lead are resultant of, but not limited to, three important biochemical properties of lead. Firstly, lead has the ability to substitute for a variety of bivalent cations (Ca^{2+}, Mg^{2+} and Fe^{2+}) and competitively inhibits their actions (Gordon et al., 2002; Garza et al., 2006). Garza et al. (2006) concluded that lead induced abnormalities in calcium-regulated proteins is the major pathogenic mechanism resulting in lead toxicity. Secondly, lead has a high affinity for sulfhydryl and amide group of enzymes and by binding to them, inhibits their activities (Gordon et al., 2002). Sulfhydryl-dependent enzymes, such as glutathione, have important anti-oxidant functions and depletion of these enzymes due to lead can result in oxidative stress (Flora et al., 2012). Lastly, lead is shown to affect gene regulation and cause genetic damage through a combination of mechanisms which include oxidative cell damage and by interacting with nucleic acid binding proteins, resulting in abnormal genetic transcription (Tchounwou et al., 2012). Lead is known to affect the physiological function of multiple body systems, however, the most profound effects are suggested to be on the neurological and haematopoietic systems.

1.5.1 Neurological system

1.5.1.1 Pathophysiology

Among the body systems affected by lead, the neurological system is especially sensitive to its effects. Mechanisms responsible for neuro-toxicity are varied and complex. The interaction between lead and calcium binding sites is suggested to be the primary mechanism of action affecting the nervous system (Flora et al., 2012). In the central nervous system (CNS), lead can cross the blood brain barrier using calcium-ATPase pumps, and accumulates in and subsequently damages glial cells (Flora et al., 2012; Wani et al., 2015). Glial cells play an important role in CNS function. Oligodendroglial cells, a type of neuroglial cell, provides support to neurons in the CNS by creating the myelin sheath and inhibition of these cells by lead results in disruption of myelin and neuronal function (Hsiang & Díaz, 2011). Similar effects are seen in Schwann cells in the peripheral nervous system (PNS) resulting in altered neuronal conductivity and function (White et al., 2007). Disruption of calcium-dependent

processes results in disruption of neurotransmitter storage and release, inhibiting evoked neurotransmitter release and enhancing spontaneous neurotransmitter release (White et al., 2007). Key neurotransmitters such as protein kinase C are augmented by even low concentrations of lead, disrupting long term neural excitation and memory storage (Flora et al., 2012). Glutamate, a neurotransmitter important for learning is also negatively affected by lead.

Lead is suggested to induce apoptosis in neuronal cells in the CNS, and the hippocampus is particularly sensitive to the effects of lead (Needleman, 2004; Flora et al., 2012). Chronic exposure to lead is shown to modify neurogenesis in the adult brain (White et al., 2007). The breadth of lead-induced cellular effects results in manifestation of a variety of clinical symptoms of neurotoxicity.

1.5.1.2 Effects on the developing neurological system

The developing neurological system is more susceptible and vulnerable to the effects of lead. In children and foetuses, higher concentrations of circulating lead are absorbed by the brain compared to adults (Flora et al., 2012). In adults, lead exposure is more commonly related to peripheral neuropathies, while in children, toxic effects are more likely to manifest as central nervous system disorders (Chandramouli et al., 2009). Hsiang and Díaz (2011) state that exposure to lead during development of the nervous system can increase apoptosis and contribute to tissue damage in the CNS.

Extensive research on pre- and post-natal lead exposure in humans demonstrates two key concepts; (i) there is no lower bound toxicity threshold for post-natal lead exposure and (ii) lead exposure at crucial periods during development can lead to persistent neurological deficits. Children exposed to lead at even low concentrations have developed deficits in cognition and motor function and importantly, in many cases these deficits are considered to be irreversible (Garza et al., 2006). White, Diamond, Proctor, Morey, and Hu (1993) present one of many studies highlighting the persistence of lead associated neuropsychological deficits, where children hospitalised for lead intoxication at the age of four, showed deficits in cognitive function when tested fifty years later. Cecil et al. (2008) found that lead early-life lead exposure was correlated with reductions in grey matter volume in adulthood.

Based on research investigations of dose-response relationships, there is consensus amongst scientists that there is no apparent lower threshold for the adverse consequences of lead exposure in developing young (Canfield et al., 2003; Chiodo et al., 2004; Garza et al., 2006). Chiodo et al. (2004) identified deficits in attention in children with blood lead concentrations as low as 3 ug/dL.

1.5.1.3 Clinical effects of neurotoxicity in humans

Neurological deficits resulting from lead exposure can appear immediately following exposure in cases of acute toxicity, or present with delayed symptoms in cases of chronic toxicity (Sanders, Liu, Buchner, & Tchounwou, 2012). These symptoms include memory loss, cognitive deficits and behavioural alterations (Sanders et al., 2009). The effect of lead on cognitive function has been the focus of many human studies. Cognitive function is commonly measured using IQ tests and in children, increase in blood lead concentration of 10ug/dL was shown to decrease IQ by 4.6 points (Canfield et al., 2003). Similarly, in adults, specifically older women, lead exposure was shown to be associated with decreased cognitive function (Needleman, 2004). In addition to impairments in cognitive function, lead exposure is also associated with behavioural problems such as aggression, attention deficit disorders, hyperactivity and anti-social behaviour (Hsiang & Díaz, 2011; Wani et al., 2015). Effects of lead on the peripheral nervous system manifest as reduced motor activity, muscular weakness, specifically of the exterior muscles, and lack of muscular co-ordination (Sanders et al., 2009). In adults, foot drop or wrist drop, resulting from peripheral neuropathy, is reported as a manifestation of chronic lead exposure (Needleman, 2004).

1.5.1.4 Clinical effects of neurotoxicity in birds

Studies assessing behavioural effects of heavy metal toxins have largely been restricted to an experimental setting. Burger and Gochfeld (2000) studied herring gull (*Larus argentatus*) and common tern (*Sterna hirundo*) nestlings, and found that lead profoundly affected neurobehavioural development in chicks and that the effects were dependant on dose and time of exposure. In an experimental setting, chicks with even low concentrations of lead showed reduced learning ability, poor individual recognition, deficits in begging and feeding and deficits in motor function (Burger &

Gochfeld, 2000). Similar tests were performed on herring gull chicks in the wild with comparable outcomes (Burger & Gochfeld, 2005). Fair and Myers (2002) adopted a similar approach to the aforementioned study and using the righting reflex as a marker of neurological function in western bluebird (*Sialia mexicana*) nestlings found birds doses with high concentrations of lead showed reduced ability to right themselves. Zhong et al. (2010) investigated the effects of lead on memory in an experimental study, which demonstrated long-term memory deficits in day old chicks with in-ovo exposure to lead.

Contradictory to the results in the above studies, results from an experimental study on great tit (*Parus major*) nestlings revealed that lead exposure did not affect exploratory behaviour, learning or spatial memory in this particular species (Ruuskanen, Eeva, Kotitalo, Stauffer, & Rainio, 2015). The authors suggest that variations in the dose of exposure, duration of exposure and route of exposure are likely to explain the contradictory results in this study. The observed variability in response to lead exposure highlights the difficulty in assessing the risks of exposure and the many factors, which influence outcome following exposure.

1.5.2 Haematology

Haematological effects are one of the more commonly reported effects of lead intoxication in humans and animals. Lead inhibits several enzymes involved in haeme synthesis typically resulting in hypochromic, microcytic anaemia. δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase are both sulphydryl-dependent enzymes essential for the synthesis of haeme (Gordon et al., 2002). As discussed earlier, lead binds to sulphydryl-dependent enzymes and inhibits their function. ALAD in particular is considered highly sensitive to lead (Assi, Hezmee, Haron, Sabri, & Rajion, 2016). Additionally, lead exposure results in decreased life span of red blood cells as a result of increased cell membrane fragility (Assi et al., 2016). In birds, even low blood lead concentrations (<5 ug/dL) are associated with decreased levels of circulating ALAD (Franson & Pain, 2011). Although mild reductions of ALAD are tolerated in birds, prolonged depression of ALAD can lead to anaemia (Franson & Pain, 2011). Although ALAD is considered a sensitive bio-marker of lead exposure, the clinical significance of reduced ALAD activity is not easily determined (Franson & Pain, 2011). There is a lack

of knowledge of basal activity of ALAD levels in many species and the relationship between ALAD concentration and anaemia is not consistent across species (Gómez-Ramírez et al., 2011).

Studies in birds commonly utilise packed cell volume (PCV) and haemaglobin as criteria to evaluate the effects of lead exposure. Reviewing these studies highlights the variability in the response to lead exposure between species. A negative associated between lead exposure and PCV were identified in some studies (Hoffman, Franson, Pattee, Bunck, & Murray, 1985; Schulz et al., 2006; Katavolos et al., 2007), while in others measurable lead exposure showed no relationship to PCV (Fair & Myers, 2002; McLelland et al., 2010; Eeva et al., 2014). Species, age and physiological status can influence lead-related changes in haematological values, therefore variation between studies is not unexpected (Gómez-Ramírez et al., 2011). Additionally, comparisons across these studies are not straightforward as the markers measuring exposure levels, such as blood lead concentration or tissue lead concentration, vary between studies.

1.5.3 Effects of lead on other body systems

1.5.3.1 Renal

Studies examining the functional effect of lead on renal function in birds are limited. In humans, renal effects of lead can manifest as acute or chronic nephropathy in response to dose and duration of lead exposure (Flora et al., 2012). Acute exposure to high concentrations of lead affects proximal tubular function presenting with degenerative changes and nuclear inclusion bodies on histology (Flora et al., 2012). McLelland et al. (2010) demonstrated acid-fast intra-nuclear inclusions in kea (*Nestor notabilis*) with elevated renal lead concentrations. Chronic nephropathy manifests as glomerular and tubulo-interstitial changes and can lead to irreversible changes in renal function ultimately resulting in chronic renal failure (World Health Organisation, 2006). Although accounts of renal functional alteration in birds with lead exposure are rare, the sub-lethal effects of lead on renal and various other body systems is considered to be a contributing factor potentially affecting survival.

1.5.3.2 Reproductive system

There is some debate regarding the direct pathological effects of lead on reproductive function in birds. Human studies assessing reproductive effects of lead exposure on an individual scale describe abnormalities in spermatogenesis, changes in serum testosterone and infertility in males, and an increased risk of miscarriage and infertility in females (Flora et al., 2012). In birds, Grandjean (1976) suggests that the incorporation of lead in the egg affects egg qualities such as eggshell thickness consequently increasing risk of damage to the egg. However this is contradicted by multiple studies in birds showing no effect of lead on eggshell traits (Dauwe et al., 2005; Nam & Lee, 2006; Ruuskanen et al., 2014; Hargitai et al., 2016). Incorporation of lead in the egg is however considered a risk to the developing embryo. In the hatching chick, majority of calcium (80%) required by the embryo for development is absorbed form the shell (Simkiss, 1961). Therefore, lead deposited in the egg can outcompete calcium and can be absorbed by the developing embryo. Maternal transfer of lead has been demonstrated in mallard ducks (*Anas platyrhynchos*) whereby Vallverdú-Coll, López-Antia, Martinez-Haro, Ortiz-Santaliestra, and Mateo (2015) showed a correlation between lead concentrations in the eggs and duckling tissues. Maternal transfer of lead has also been suggested as a significant source of exposure in nestling marbled teal (*Marmaronetta angustirostris*) in Spain (Mateo, Green, Jeske, Urios, & Gerique, 2001). Providing conclusive evidence of maternal lead transfer is difficult in a natural ecological setting as both female and offspring need to be sampled at precise time intervals to correlate lead concentrations during egg laying and hatching. As a result, there are very few studies able to provide conclusive evidence of maternal transfer of lead exposure in wild birds.

Exposure to lead during critical periods of embryonic development can have negative consequences on physiological function and ultimately influence survival. Early-life lead exposure has shown to increase fledging time in great tits (*Parus major*) (Janssens et al., 2003) and in pigeons (*Columba livia*) (Nam & Lee, 2006), likely as a result of delayed development. In-ovo lead exposure, even at low concentrations where no overt physiological changes are detectable, can result in immunotoxic effects in nestlings (Lee, Chen, Golemboski, Parsons, & Dietert, 2001). In mallard ducklings, low concentrations of lead exposure early in life were related to abnormalities in immune development and reduced survival (Vallverdú-Coll et al., 2015). These studies provide evidence for the potential indirect effects of lead exposure on reproductive success on

a population scale. Maternal transfer of lead via the egg has the potential to influence survival outcomes in the chick and therefore population success.

1.6 Interpreting lead exposure and effect

In spite of the large number of existing studies investigating lead intoxication in wildlife, there is still a degree of ambiguity regarding exposure concentrations and effect. Franson and Pain (2011) suggest that tissue lead concentration alone is not a determinant of toxic effects. There are multiple factors capable of influencing outcome following lead exposure. These factors include dose and duration of exposure, species variability in response to exposure, individual fitness and the presence of concurrent environmental stressors (Franson & Pain, 2011). There are numerous studies outlining the individual and inter-specific variability in response to lead exposure. In a review of lead studies in birds, Franson and Pain (2011) describe variable toxic thresholds in Anseriformes, Falconiformes and Columbiformes, with the latter reportedly more tolerant to higher concentrations of lead exposure. Similarly, Buekers et al. (2009) reviewed experimental lead exposure studies in 12 avian species and reported considerable variability in the toxicity thresholds across species in regards to blood lead concentrations at which no effect was observed. Although the thresholds described in these studies are used as guidelines, it is important to note that many of these studies are conducted in an experimental setting and the chosen endpoints to measure effect are variable between studies, making comparisons or extrapolations to other species difficult.

The biological significance of tissue/blood lead concentration is difficult to assess particularly in cases of low lead exposure. Ecotoxicological studies in wildlife have documented a need for a more holistic approach when estimating risk of toxin exposure (Haig et al., 2014). This includes consideration of environmental and individual variables in a natural ecological setting. Even low levels of lead exposure in the presence of environmental stressors, such as reduced food availability and inclement weather, or altered physiological state due to disease or nutritional stress can increase susceptibility to the effects of lead toxicosis (Newth et al., 2016). Kelly and Kelly (2005) identified that swans with lead exposure were more susceptible to collisions with power lines resulting in morbidity or mortality. In an experimental study on mourning doves (*Zenaida macroura*) pre-treatment body mass had a

negative effect on survival whereby heavier doves had a decreased risk of death (Schulz et al., 2006). These findings argue that lead exposure, even at sub-clinical concentrations can contribute to reduced survival in free-living birds and highlight the difficulties in directly relating tissue concentrations with effect.

1.7 Biomonitoring of lead exposure

There are various biomarkers of lead exposure documented in humans and animals, and their use in wildlife studies is based on practicality, analytical accuracy and cost (Bergdahl & Skerfving, 2008). Selection of physiological biomarkers is also dependent on the purpose of the study, as certain tests are indicative of acute toxin exposure while some are more indicative of chronic exposure.

1.7.1 Blood lead concentration

Blood lead concentration is the most commonly used biomarker for lead exposure and has been extensively used as a screening, diagnostic and monitoring tool in humans (Barbosa Jr et al., 2005). Blood lead concentrations are primarily used to diagnose acute lead exposure as lead concentrations are highest in the blood immediately following exposure (Fisher et al., 2006). However bone and soft-tissue lead stores can also contribute to blood concentrations as lead is mobilised back into the blood (Bergdahl & Skerfving, 2008). Lead in the blood therefore represents a combination of exposure, both recent and long-term.

In birds, blood lead concentration provides a non-destructive and sensitive marker of exposure and has been utilised in a large number of wildlife studies (Chapa-Vargas, Mejía-Saavedra, Monzalvo-Santos, & Puebla-Olivares, 2010; Legagneux et al., 2014; Pérez-López et al., 2016). Graphite furnace atomic absorption spectrophotometry (GFAAS) or inductively coupled plasma mass spectrometry (ICP-MS) are the commonly used laboratory methods for calculating blood lead concentrations. Both methods are highly sensitive but require costly equipment and trained technicians (Bischoff, Gaskill, Erb, Ebel, & Hillebrandt, 2010). More recently, human analytical tests such as the Leadcare® II blood lead analyser system (ESA Biosciences Inc., Chelmsford, MA) are proving useful in wildlife ecotoxicological studies (Craighead &

Bedrosian, 2008). The Leadcare® II system is portable, easy to use and importantly requires a small sample size (0.05ml of blood), which is highly practical for use in birds (Bischoff et al., 2010). Studies in animals comparing GFAAS and Leadcare® II conclude that results between the two tests are comparable across a wide range of lead concentrations (Fry & Maurer, 2003; Brown, Luebbert, Mulcahy, Schamber, & Rosenberg, 2006; Bischoff et al., 2010).

1.7.2 Tissue lead concentration

Tissue lead concentrations provide an indication of sub-acute to chronic lead exposure. Tissue lead concentrations are advantageous in determining total body burdens of lead, however are limited to post-mortem investigations due to the invasive nature of sampling required.

Kidney, liver and bone are the most commonly sampled tissues in avian lead studies. Liver and kidney are considered to represent recent lead exposure, while lead concentrations in the bone represent long-term exposure (Scheuhammer, 1987). Lead is distributed from blood to soft-tissue compartments soon after exposure. The proportion of lead distributed into various soft-tissues varies between species, therefore where possible multiple soft-tissue samples should be analysed concurrently (Franson & Pain, 2011). For example, lead accumulation in mourning doves (*Zenaida macroura*) was greater in the kidney compared to the liver (Schulz et al., 2006), while in some raptors such as the red-tailed hawk (*Buteo jamaicensis*) concentrations in the liver were comparably higher (Martin, Campbell, Hughes, & McDaniel, 2008). Lead concentrations in the bone are representative of chronic, cumulative lead exposure (Barbosa Jr et al., 2005). Lead in the bone accounts for >90% of body burdens and therefore provides an estimate of total body burden of lead accumulated over the lifetime of the bird (Franson & Pain, 2011; Madry et al., 2015).

Tissue lead concentrations are useful indicators of population level exposure, however do not necessarily translate to clinical significance. Due to the degree of variability in sensitivity and response to lead exposure, between individuals and species, the concentration of lead in tissues alone is insufficient to qualify lead intoxication as a direct cause of mortality. Tissue lead levels provide an indication of exposure, however additional clinical and diagnostic information is required to identify if lead

intoxication is a primary or contributing cause of mortality. Franson and Pain (2011) recommend evaluating history, necropsy findings and pathological findings in conjunction with tissue lead concentrations to support a diagnosis of lead intoxication as the cause of death.

1.7.3 Other detection methods

Feathers are used in multiple studies to measure lead exposure levels in birds, primarily due to the ease and non-destructive nature of sampling. Lead concentration in feathers is representative of lead exposure at the time of feather growth (Franson & Pain, 2011), and correlate with lead levels in internal tissues such as the liver and kidney (Dauwe, Bervoets, Blust, & Eens, 2002). Dauwe et al. (2003) reported that lead concentrations vary significantly between feathers and the outermost primaries had the highest lead concentrations. Additionally, they reported variation in lead concentration within segments of a feather suggesting that different segments vary in their ability to incorporate lead. A further consideration when using feathers as biomarkers is the influence of external contamination on lead levels in the feathers (Scheuhammer, 1987). Thorough washing and processing methods are suggested to reduce external contaminations in feathers prior to analysis (Franson & Pain, 2011).

Eggs are considered ideal bio-indicators of environmental contamination and are more commonly being used in heavy metal studies in wildlife (Orłowski et al., 2014). Dauwe et al. (2005) promote the use of eggs as bio-indicators of lead contamination as they are easily sampled have a consistent composition and removal of a single egg in a clutch has a relatively minimal effect on the population. In birds, majority of studies primarily use eggs as indicators of environmental pollution rather than as quantitative markers of biological lead exposure in female birds or nestlings (Burger, Bowman, Woolfenden, & Gochfeld, 2004; Orłowski et al., 2014; Ruuskanen et al., 2014). Although lead concentrations in the eggs are suggestive of lead exposure in female birds, there are very few studies investigating the relationship between systemic lead exposure and lead concentration in eggs (Dauwe et al., 2005).

1.8 Lead exposure in New Zealand wildlife

Compared to Europe and North America, there are very few studies on lead exposure in wildlife in New Zealand. Marine filter-feeding organisms such as mussels, have been used as bio-indicators to assess marine heavy metal contamination in New Zealand (Chandurvelan, Marsden, Glover, & Gaw, 2015). Stockin et al. (2007) examined trace elements in liver and kidneys of stranded New Zealand common dolphins (*Delphinus sp*) and found low concentrations of lead exposure. Early studies in birds focused on determining exposure concentrations and prevalence. Thompson and Dowding (1999) surveyed pied oystercatchers (*Haematopus ostralegus*) at two locations in Auckland and found site-specific differences in blood lead concentrations. Juvenile oystercatchers were reported to have the highest blood lead concentrations compared to adults at both sites. Lock, Thompson, Furness, and Bartle (1992) conducted a survey of lead exposure in seabirds in the New Zealand region, including migratory birds, and found a low prevalence (28% of adults and 31% of juveniles) of lead exposure based on bone lead assays. The sample population in this study consisted primarily of opportunistically sampled dead birds.

More recently, in-depth investigations of lead exposure in terrestrial birds have provided information on not only the prevalence and concentrations of exposure, but also the effect of exposure in selected species. McLelland et al. (2010) and Reid et al. (2012) reported on findings in free-ranging kea (*Nestor notabilis*), an alpine parrots species endemic to New Zealand. Reid et al. (2012) sampled kea at seven locations and identified a 100% prevalence of lead exposure (n=88), repoting significantly higher blood lead concentrations in kea in populated areas. McLelland et al. (2010) reported a similar prevalence in 38 birds sampled and additionally investigated physiological parameters to estimate the risk of lead exposure in this species. The results from these studies prompted the implementation of a lead abatement strategy in populated areas accessible to kea (Kea Conservation Trust, 2016). This research group also presents evidence of lead intoxication in swamp harriers (*Circus approximans*), a raptor species considered ubiquitous throughout New Zealand, where they examined the relationship between lead intoxication and peripheral neurological deficits (McLelland, Gartrell, Morgan, Roe, & Johnson, 2011). Although these studies provide an indication of the risks of lead exposure in wildlife in New Zealand, there is limited

available information on the extent of lead contamination and subsequent risks to wildlife in New Zealand.

1.9 Research aims and thesis structure

Apart from anecdotal reports, little is known about lead exposure in kaka. Wellington sustains the only urban population of kaka in New Zealand (Charles & Linklater, 2013), therefore provides an ideal opportunity to examine direct anthropogenic influences on lead exposure in this population. It is expected that mainland kaka populations will require intensive monitoring and management to sustain their populations in the future (Greene et al., 2004). Increasing urbanisation, and overlap of urban and wildlife landscapes, increases the exposure of native avifauna to anthropogenic contaminants. Future attempts to manage lead toxicity in this urban population of kaka require an understanding of the prevalence, biological effects and potential sources of exposure.

Therefore, the primary aim of this research was to evaluate the dynamics and biological effects of lead exposure in an urban population of kaka and identify what implications this may have for the future management of this species. This was achieved by investigating:

1. the prevalence of lead exposure in free-ranging adult kaka and kaka nestlings in urban Wellington, New Zealand.
2. the physiological effects associated with lead exposure at different life-stages; in adults and in young, developing chicks.
3. the various pathways of lead exposure in nestlings and the source of environmental lead exposure in free-ranging adult kaka.

This thesis is organised into four chapters, which include two data chapters. This first chapter provides a review of the properties and use of lead, sources of lead in the environment, toxico-kinetics and physiological effects of lead exposure, biomonitoring of lead exposure and information on lead exposure in New Zealand wildlife. The second chapter investigates the prevalence, effect and source of lead exposure in free-ranging adult kaka sampled in urban Wellington between May 2015 and January 2016. Chapter three investigates the dynamics and physiological effects of lead exposure in

nestling kaka sampled over two consecutive breeding seasons (2014/2015 and 2015/2106). Prevalence of exposure and potential routes of lead exposure in nestling kaka are investigated. The fourth chapter forms the general discussion, which provides a conclusion and addresses the conservation implications of lead exposure in this population. The data chapters presented in this thesis are written in a format allowing for journal publication.

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CHAPTER TWO

Evaluating lead exposure in urban free-ranging kaka

2.1 INTRODUCTION

Lead is regarded as a toxin of threat to wildlife populations worldwide. It is considered a primary cause of mortality in certain species and implicated as a major hindrance to reintroduction and conservation programmes around the world (Pain et al., 2007; Lambertucci et al., 2011; Finkelstein et al., 2012). The physiological effects of lead exposure are diverse and dependent on dose, duration and route of exposure. Additionally, the clinical manifestation of lead toxicity can be variable across species groups, therefore calculating the risk on a population scale is not always straightforward (Haig et al., 2014).

In terrestrial birds, ingestion is regarded as the primary route of lead exposure (Fisher, Pain, & Thomas, 2006). During the immediate period following ingestion, lead concentrations are highest in the blood. Subsequently, lead is redistributed to various soft-tissue compartments such as liver, and kidney, and then subsequently can be incorporated into bone (Franson & Pain, 2011). Blood lead concentration is therefore considered an indicator of recent exposure, and provides a non-destructive and sensitive measure of exposure. Lead toxicity can manifest acutely or chronically in birds, depending on dose and duration of exposure. Exposure to high concentrations of lead can result in acute toxicity, with detectable clinical signs of toxicity, while exposure to low concentrations of lead and subsequent bioaccumulation of the toxin can result in a range of disease from chronic sub-clinical toxicity to clinical toxicity and mortality. Lack of clinical signs of toxicity does not translate to a lack of effect, as subtle physiological and behavioural alterations associated with sub-lethal lead toxicity have been shown to influence long-term survival in birds and other organisms; especially in the presence of contributing stressors present in a natural ecological setting (Fisher et al., 2006).

Lead is known to affect the neurological, renal and haematopoetic systems, resulting in physiological, biochemical and behavioural changes in birds (Fisher et al., 2006). However, physiological effects associated with exposure are not consistently recorded in birds, and while some studies provide evidence for the deleterious effects of lead exposure on physiological markers, behaviour and survival (Henny et al., 2000; Burger & Gochfeld, 2005; Schulz et al., 2006; Ferreyra et al., 2015), contradictory findings are presented in others (Janssens et al., 2003; Eeva et al., 2014; Ruuskanen, Eeva, Kotitalo, Stauffer, & Rainio, 2015). It should also be noted that majority of the studies

examining the effects of lead exposure in birds are conducted in an experimental setting, with a limited number investigating free-ranging birds, hence a case-based approach should be taken to ecotoxicological studies of the effects of lead in wildlife.

The largest proportion of work on lead exposure in avifauna has focused on waterfowl, raptors and scavenging birds. The primary source of exposure in these species is considered to be lead ammunition (Fisher et al., 2006; Tranel & Kimmel, 2008). Lead ammunition is capable of causing persistent environmental contamination and is considered a major factor in the decline of threatened and endangered species (Tranel & Kimmel, 2008). Due to the recognised consequences of the use of lead ammunition in wildlife, many countries have implemented management strategies limiting the use of lead shot in ecologically important areas (Avery & Watson, 2009). While the focus has been on reducing lead contamination in wild ecosystems, knowledge of lead exposure in urban environments is relatively limited. Urban environments are associated with a wide spectrum of contamination and studies across urban gradients show an increased lead burden amongst birds in urban areas compared to rural areas or pristine natural ecosystems (McMichael, 2000; Swaileh & Sansur, 2006; Binkowski & Meissner, 2013; Naidoo, Wolter, & Botha, 2017). Proposed sources of lead exposure in urban wildlife include lead-based paint, leaded fuel and industrial emissions (Finkelstein, Gwiazda, & Smith, 2003; Roux & Marra, 2007; Gómez-Ramírez, Martínez-López, María-Mojica, León-Ortega, & García-Fernández, 2011). In Wellington, lead contamination of roof-collected rainwater was recently recognised as a potential public health risk whereby Stewart, Kim, Johnston, and Nayyerloo (2016) identified lead-containing components in roofs as the primary source of contamination. Elevated lead concentrations were recorded in 69% of samples collected from 5 different households in Wellington, which exceeded the maximum acceptable value for lead according to the Drinking-water Standards for New Zealand (Stewart et al., 2016). Similar studies in Auckland, New Zealand and Brisbane, Australia demonstrate the leaching of lead from roofing material into roof-collected rainwater (Simmons, Hope, Lewis, Whitmore, & Gao, 2001; Huston, Chan, Chapman, Gardner, & Shaw, 2012). Lead in roofing material and subsequently in roof-collected rainwater is biologically available to wildlife and is a potential source of exposure for kaka in Wellington.

Kaka are forest parrots, endemic to New Zealand and classified as endangered by the IUCN (BirdLife International, 2014). Once well distributed throughout mainland New

Zealand, their population is estimated to be declining rapidly, subsequent to habitat destruction, predation and food competition (Berry, 1998). As part of a restoration initiative, in 2002, kaka were reintroduced to Zealandia Ecosanctuary, a 225 hectare predator proof reserve on the doorstep of urban Wellington. Their population has since expanded in part due to efficient management practices, such as habitat restoration, predator management within and surrounding the sanctuary, and due to the provision of supplementary food (Miskelly, Empson, & Wright, 2005; Charles & Linklater, 2013). Driven by an expanding population, their inquisitive nature and the increased availability of food in the urban environment, kaka are venturing into urban Wellington where they are regularly sighted on roofs and known to cause damage to roof joinery, cladding and chimneys (Charles, 2012; Charles & Linklater, 2013). Increased interaction with the urban environment is a potential risk factor for lead exposure in kaka and this study provides an opportunity to investigate lead exposure in an urban context.

Investigations of lead toxicity in wildlife are ultimately aimed at identifying the potential sources of lead in the environment as source attribution can provide valuable information to influence policy and management around lead use (Scheuhammer & Templeton, 1998). In recent years, lead stable isotope analysis is increasingly used in wildlife ecotoxicological studies to trace pathways of lead exposure (Scheuhammer & Templeton, 1998; Church et al., 2006; Finkelstein et al., 2012). There are four main naturally occurring isotopes of lead, including ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb, and the composition of these isotopes can vary between sources. This variability essentially creates a 'fingerprint', allowing for the differentiation between sources of lead in the environment; both anthropogenic and environmental in origin (Jiang & Sun, 2014). Furthermore, physico-chemical processes do not alter lead isotope ratios, allowing for accurate traceability to the original source (Komárek, Ettler, Chrastný, & Mihaljevič, 2008). Investigating the isotopic composition in environmental samples and comparing these to biological samples from wildlife can allow for exclusion of proposed sources and in some cases identification of specific sources of lead.

The aim of this study was to evaluate if lead is a toxin of importance to kaka in Wellington. This involved: (i) identifying the prevalence and concentrations of lead exposure in free-ranging adults; (ii) evaluating the acute physiological effects of lead

exposure; and (iii) identifying the source of lead in the urban environment. Kaka in Wellington will require intensive management to be sustainable in the future (Greene, Powlesland, Dilks, & Moran, 2004), and an understanding of the risks of lead exposure in this population will provide necessary information to inform management decisions.

2.2 MATERIALS AND METHODS

Blood lead survey

A total of 37 free-ranging adult kaka were opportunistically sampled at two locations in suburban Wellington, New Zealand over a 9 month period (May 2015 – January 2016). Both locations are outside of the Zealandia sanctuary, have established feeding stations and for more than 5 years have reported regular sightings of kaka. These locations were therefore chosen as capture sites, as they provided consistent and reliable access to kaka. Birds with leg-bands were preferentially targeted for capture to allow for individual identification. Birds were captured and restrained by hand, while blood samples were collected from the right cutaneous ulnar vein using a 25-gauge needle and a 1ml syringe. A volume of 0.3-0.5mls of blood was collected into lithium heparin microtainers (Becton Dickenson Vacutainer Systems, Franklin Lakes, New Jersey, USA 07417) and stored at room temperature prior to processing. Packed cell volume (PCV) and total plasma solids were measured in house by capillary tube centrifugation within 48 hours of sample collection. Blood lead analysis was performed within 12 hours of collection using a portable lead analyser (LeadCare® II, ESA Inc, Chelmsford, Massachusetts, USA 01824). The LeadCare® II testing kit uses anodic stripping voltammetry to measure blood lead concentrations and has a reportable range of 3.3 to 65 ug/dL (0.16 – 3.14 umol/L). In this study, birds with blood lead concentrations above 3.3 ug/dL (0.16 umol/L) are recorded as being lead exposed. Specifically formulated control materials, provided by the manufacturer, were used for quality control of the testing kits.

Clinical examination and neurological examination

All sampled birds were subjected to a clinical and neurological exam. Body weight divided by tarsometatarsal length was used to calculate a body condition index (BCI) for each individual. Sex was determined based on bill length (Moorhouse, Sibley, Lloyd, & Greene, 1999). Specific neurological tests were selected and modified to accommodate for the temperament of free-ranging adult birds and to minimise stress and injury to the bird. Mentation was assessed prior to capture and during handling, and was recorded as bright and alert or quiet and depressed. Gait and posture were assessed prior to capture and any abnormalities defined and recorded. Grip was

evaluated subjectively by allowing the birds to grip assessor's index finger with each foot and was subsequently graded as strong or weak. The wing withdrawal test was used to evaluate the integrity of the local reflex arc involving the brachial plexus. The test was performed by extending the wing away from the body and when released, a normal response was recorded as flexion of the wing back into normal position. A righting reflex test was used to evaluate the function of the peripheral and central nervous system and was performed at the end of sampling and handling. The test was performed by positioning the bird on its back with restraint and when released, time taken to right into an upright position was recorded.

Roof-collected rainwater samples

Roof-collected rainwater samples were contributed from a study by Stewart et al. (2016) and detailed collection protocols are outlined in the publication. Briefly, rainwater samples were collected from 5 emergency rainwater tanks installed at five different sites located in suburban Wellington. The tanks are connected to downpipes and are supplied by roof collected rainwater. Samples were collected regularly for a 12 month period between 2014 and 2015. Due to availability of samples and for the purposes of this study, samples from three time points were analysed from each of the six tanks, providing a total of 18 samples; 7th May, 27th August and 17th December 2014. Samples were collected in 250ml plastic bottles containing nitric acid preservative and analysed for lead concentration and lead stable isotope analysis.

Isotope analysis

Lead concentration and isotope ratios were determined using ICP-MS and performed at Hill Laboratories (Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton, New Zealand) according to EPA Method 200.8 (Environmental Protection Agency, 1994). For routine lead analysis, the three major isotopes of lead (206, 207, 208) are measured and averaged for calibration and measurement since natural sources of lead may vary in their compositional ratios. For the lead isotope analysis, the individual instrument response of each isotope, in counts/second, was reported. Quality control measures undertaken include the digestion of blanks (to check for contamination contributed during the weighing, digestion and filtration

steps), reference material (in-house manufactured material to check for reproducibility and bias of method) and spiked quality control material (to check for recovery of known addition of elements of interest). Duplicate analysis of randomly chosen samples was also performed to check for within bath precision and method performance.

Statistical methods

Descriptive statistics and linear regressions were calculated using Excel (Microsoft Office 2010). Univariate GLMs of the isotopic ratios of rainwater and kaka blood samples were analysed using SPSS for Windows (IBM SPSS v23). Where data was not normally distributed, non-parametric analysis was performed using a Mann-Whitney U test again using SPSS for windows (IBM SPSS v23). R (version 3.3.2) was used to plot the 95% confidence interval of isotopic ratios of lead (206/207 and 208/206). A statistical significance level of 0.05 was chosen for all tests. Results are presented as mean +/- 1 standard error (s.e.) unless otherwise stated.

Permits for this study were granted by the New Zealand Department of Conservation (permit number: 39490-FAU) and approved by the Massey University Animal Ethics Committee (MUAEC 14/76).

2.3 RESULTS

2.3.1 Blood lead survey

The prevalence of lead exposure in kaka was 43.2% (16/37) of birds sampled. Blood lead concentrations in lead exposed birds (n = 16) ranged between 3.4 to 50.7 ug/dL (0.16 to 2.45 umol/L) with a mean of 10.87 ug/dL ± 3.05. The majority of lead exposed birds (87%) had blood lead concentrations <20 ug/dL (<0.97 umol/L) with only 2 birds (12%) with concentrations > 20ug/dL (**Figure 2.1**). Males and females were equally represented amongst lead exposed birds and there was no significant difference between lead exposure in the two groups (Z = 0.472, p = 0.638).

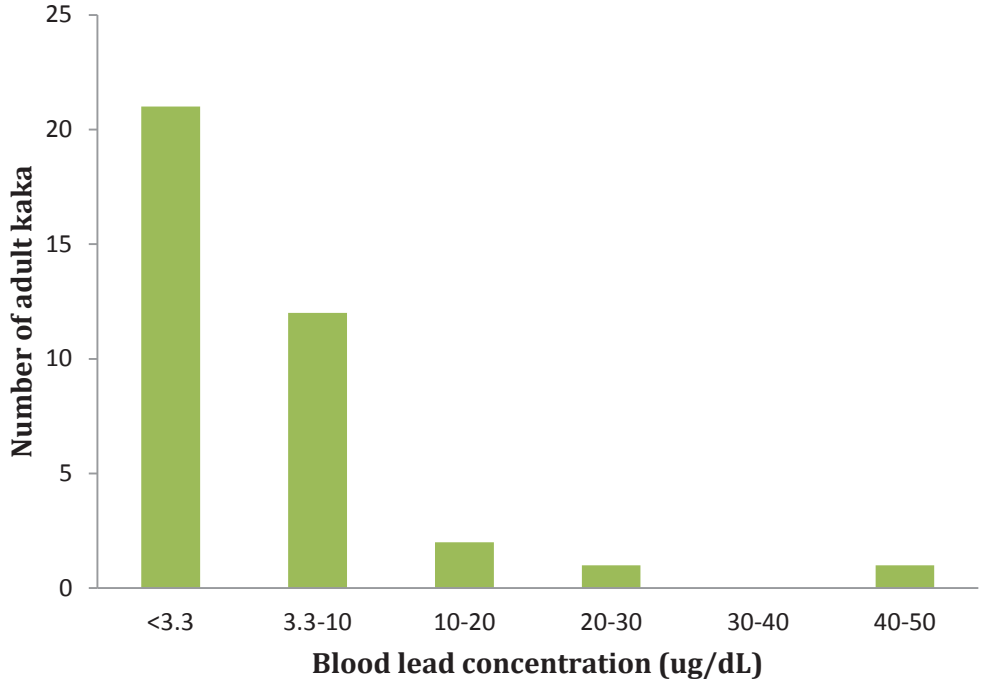

Figure 2.1 Blood lead concentrations (ug/dL) of adult kaka in urban Wellington.

2.3.2 Physiological changes

No overt clinical signs of lead toxicity were observed in sampled birds, with the exception of a single bird with the highest blood lead concentration (50.7 ug/dL; 2.45 umol/L). This particular bird (identified as GR-R) showed abnormal behaviour upon capture and release. GR-R was relatively easy to capture and approached people without apprehension, readily hand-feeding from people. Upon release, contradictory

to the behaviour seen in the rest of the sampled birds, GR-R returned to the sample site within 5 minutes of release and again approached captors without apprehension. Following capture, all other sampled birds did not return to the field site for a minimum of 5-7 days and upon return were noticeably apprehensive and actively avoided interaction with people.

The body mass of sampled birds ranged between 360g to 505g with a mean of 439.18g ± 6.86. The bird with the highest blood lead concentration (50.7 ug/dL) had the lowest body mass (360g) and BCI (8.55) and had markedly reduced pectoral muscle mass. There was a significant negative correlation between blood lead concentration and BCI (R^2 = 0.510 p = 0.0009) (**Figure 2.2**).

No significant relationship was identified between blood lead concentration and PCV (R^2 = 0.025, p = 0.58). The mean PCV of adult kaka was 50% ± 1.04%. Two samples were excluded due to insufficient blood volume.

No deficits were observed in neurological parameters assessed in this study. Mean righting time of adult birds was 0.57s ± 0.04s. There was no significant relationship between righting time and blood lead concentration (R^2 = 0.005, p = 0.463).

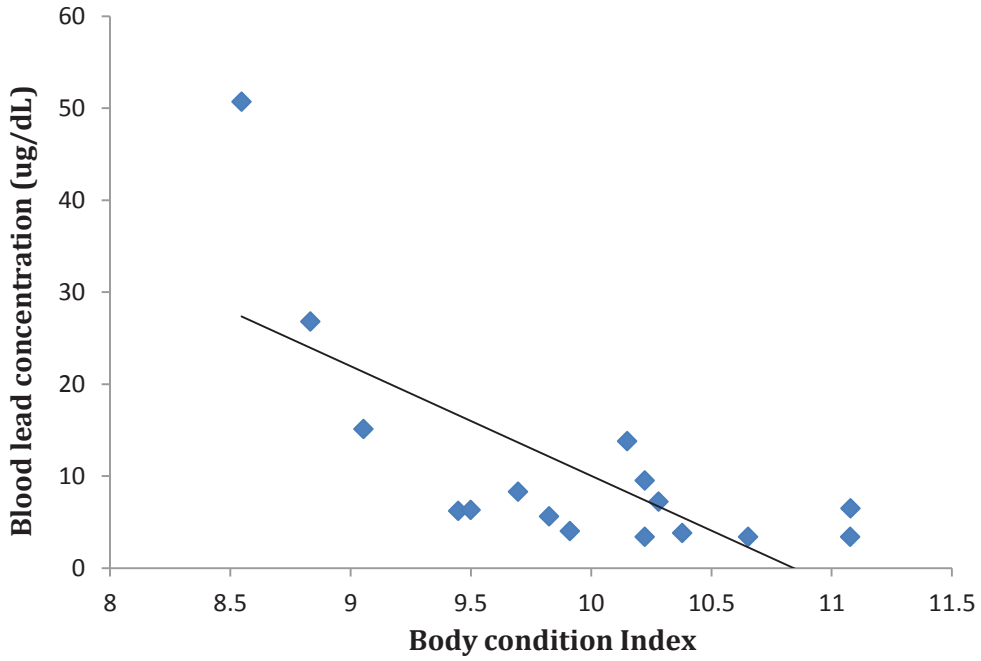

Figure 2.2 Relationship between blood lead concentration and body condition index in lead exposed adult kaka in urban Wellington

2.3.3 Rainwater and blood lead isotope ratios

Lead isotopic composition varied among rainwater samples from different sites with $^{206/207}$Pb ratios ranging between 1.042 – 1.177 (CV 3.5%) and $^{208/206}$Pb ratios ranging between 2.054 – 2.216 (CV 1.9%). The lead isotope composition of kaka blood samples showed slightly increased variability compared to rainwater samples ($^{206/207}$Pb range = 0.996 – 1.154, CV 4.5% and $^{208/206}$Pb range = 1.969 – 2.428, CV 5.3%). Isotopic ratios of kaka blood samples overlap with isotopic ratios of rainwater samples and are statistically indistinguishable ($^{206/207}$Pb t = 0.487, df = 29, p = 0.629, $^{208/206}$Pb t = -0.120, df = 29, p = 0.904) (**Figure 2.3**). There was no significant correlation between blood lead concentration and lead isotope ratios in kaka ($^{206/207}$Pb R^2 = 0.004, p = <0.001, $^{208/206}$Pb R^2 = 0.01, p = <0.001).

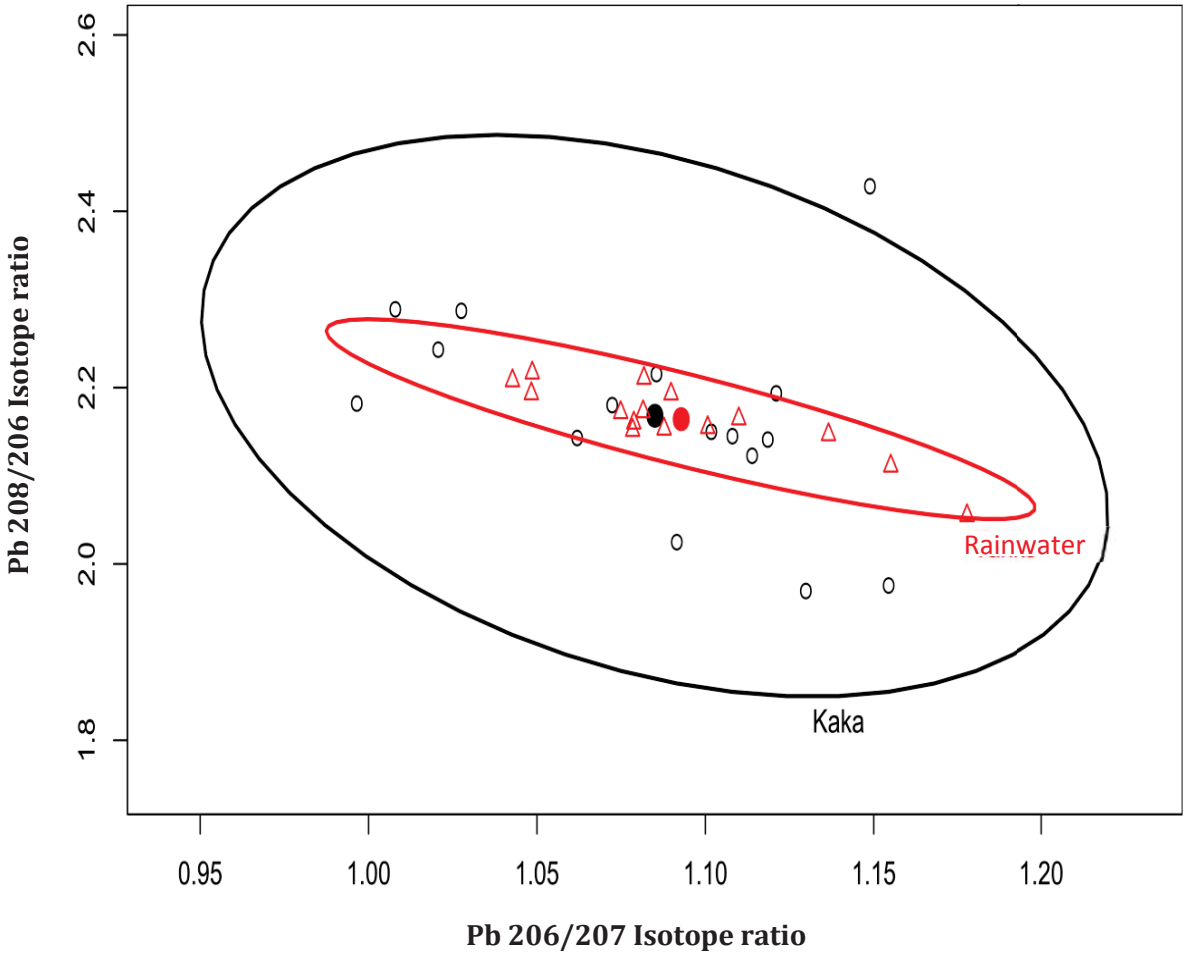

Figure 2.3 $^{208/206}$Pb and $^{206/207}$Pb isotope ratios of kaka blood samples (o) and roof-collected rainwater samples (Δ). Mean ratios of $^{208/206}$Pb and $^{206/207}$Pb found in kaka blood samples (•) and roof-collected rainwater samples (•) with ellipses indicating the respective 95% confidence intervals.

2.4 DISCUSSION

Lead affects wildlife populations worldwide, and is implicated as a major contributor to population declines in some species (Lambertucci et al., 2011). On a population scale, the impacts of lead exposure can be variable and are influenced by the availability of lead in the environment, species behaviour and life history characteristics that influence the animals' interaction with the environment and additionally, species-specific sensitivity to exposure (Haig et al., 2014). The aims of this study were to establish a baseline prevalence of lead exposure in this urban population of kaka and to evaluate if this toxin is a threat requiring management and/or mitigation. This study identified a higher than expected prevalence (43%) of lead exposure in free-ranging kaka. Prevalence not only provides a measure of population level exposure, but is also a reflection of local environmental lead contamination. Studies in areas where contamination is considered widespread show a higher prevalence of exposure, where >80% of birds sampled had detectable blood lead concentrations (Svanberg et al., 2006; McLelland, Reid, McInnes, Roe, & Gartrell, 2010; Binkowski, Meissner, Trzeciak, Izevbekhai, & Barker, 2016). The prevalence in this study although lower, is still noteworthy as free-ranging parrots are rarely reported to be affected by heavy metal contamination. To the author's knowledge, lead exposure has previously only been reported in one other species of free-ranging parrot, the kea (*Nestor notabilis*) (McLelland et al., 2010; Reid, McInnes, McLelland, & Gartrell, 2012).

Diet, foraging behaviour and interaction with the environment are considered predisposing factors to lead exposure (Roux & Marra, 2007; Chapa-Vargas, Mejía-Saavedra, Monzalvo-Santos, & Puebla-Olivares, 2010; Franson & Pain, 2011). Unlike waterfowl and scavenging birds, where ammunition is the primary source of toxin, there are few existing studies on heavy metal levels in parrots as, in the majority of cases, their foraging strategies are not considered to be a predisposing factor for lead exposure. Waterfowl are commonly exposed through accidental ingestion of lead shot mistaken for food particles or grit, while foraging in contaminated environments (Tranel & Kimmel, 2008). Ingestion of bullet fragments in hunter-shot prey is the most common route of exposure in raptors and scavenging birds (Fisher et al., 2006). In an urban setting, Roux and Marra (2007) identified land-use type and foraging method, specifically ground-feeding, to be significantly associated with lead exposure in

passerines. Kaka however have a varied diet, which consists of insects, sap, fruits and flowers (Moorhouse, 1997). They are largely arboreal, spending very little time foraging on the ground, limiting accidental exposure to the more commonly implicated sources such as soil or ammunition. Considering the diet and foraging behaviour of kaka, the prevalence observed in this study is noteworthy.

There are multiple studies highlighting the deleterious effects of acute, high-level lead exposure in birds, however it must be noted that low level exposure has the potential to influence physiological parameters and ultimately influence survival and population growth (Pokras & Kneeland, 2009; Ferreyra et al., 2015; Newth et al., 2016). While the majority of lead exposed birds in this study had blood lead concentrations <10 ug/dL (<0.48 umol/L), two birds had moderate to high concentrations (26.8 ug/dL and 50 ug/dL). These concentrations are considered consistent with toxicity in clinical avian medicine (Samour, 2016). The highest recorded concentration in this study is comparable to or above the maximum level previously recorded in studies on wild birds (Brown, Luebbert, Mulcahy, Schamber, & Rosenberg, 2006; Chapa-Vargas et al., 2010; Legagneux et al., 2014). This variability in blood lead concentration is not unexpected due to the lack of point source contaminants in the vicinity of the study site, and is likely explained by the aberrant distribution of lead in the environment rather than the uniform environmental contamination that arises from point sources such as mines or smelters (Thomas, Scheuhammer, & Bond, 2009).

There are a range of suggested thresholds of toxicity for lead exposure in birds, based on existing literature which is largely comprised of experimental studies on waterfowl and raptors (Franson & Pain, 2011). Applying these thresholds across a variety of species groups must be done with caution as tolerance to exposure can vary between species and between individuals (Haig et al., 2014). Galliformes and Columbiformes for example, are less sensitive to lead exposure compared to other species groups (Franson & Pain, 2011). Additionally, direct parallels cannot be made to studies in an experimental setting versus those conducted on free-ranging birds in their natural setting as the former does not accommodate for environmental stressors potentially influencing behaviour and physiological parameters (Haig et al., 2014). There is very little known about thresholds of toxicity in free-ranging parrots. Low level lead exposure as seen in majority of the birds in this study, when recurrent or chronic, has the potential to result in sub-lethal toxicity. Results of this study reveal a moderate

prevalence of lead exposure in this urban population of kaka, with 10% of the population reported as having a moderate to high level of exposure, suggesting there are important sources of lead in this urban environment. Longitudinal studies, over longer periods of time are necessary to understand the relationship between duration of exposure, dose of exposure and impacts on health in this population.

Interpreting blood lead concentrations is dependent on available information on the relationship between exposure dose and clinical effect. Quantifying population wide effects of lead exposure requires an understanding of the acute and chronic effects of toxicity and threshold concentrations at which these effects manifest specific to both species and their respective ecosystems. In this study, body condition index was the only measured parameter shown to be significantly affected by increasing blood lead concentration, evidenced by a negative correlation between blood lead concentration and body condition index. The bird with the highest lead concentration had the lowest body mass and was noted to have marked reduction in pectoral muscle mass as well as behavioural signs suggestive of reduced fitness to survive, in particular the increased reliance of supplementary feeding. Loss of muscle and fat reserves are one of the most consistent lesions seen in birds with lead exposure (Franson & Pain, 2011). Results from this study are corroborated by similar studies on whooper swans (*Cygnus cygnus*) and eider ducks (*Somateria mollissima*) where lead concentrations, considered to be below the toxic threshold for waterfowl, had a negative impact on body condition (Newth et al., 2016; Provencher et al., 2016). However contradictory results are presented in other species, where no effect on body condition was observed in lead exposed birds (Scheifler et al., 2006). In a study on a closely related endemic New Zealand parrot, the kea (*Nestor notabilis*), no relationship was identified between body condition and lead exposure at mean blood lead concentrations of 42.8 ug/dL (McLelland et al., 2010). This disparity, although confounding the ability to estimate the risk of exposure, further highlights the inter-species variability in response to lead exposure and the importance of a case-based approach when assessing the impacts of lead exposure.

The relationship between lead exposure and body condition is not necessarily directly causal. Sub-lethal exposure can indirectly affect body condition by altering physiological parameters ultimately affecting foraging ability, behaviour and energetics (Provencher et al., 2016). Although no overt clinical signs of toxicity or

mortality were observed in birds during this study, lack of observable clinical disease does not discount negative health impacts in kaka. Loss of body condition, in the face of additional stressors present in a particular ecological setting, can ultimately affect overall fitness. Kelly and Kelly (2005) found that mute swans with lead exposure were more likely to collide with power lines compared to non-exposed birds. Sub-lethal lead exposure can contribute to morbidity and/or mortality in the face of additional environmental stressors and ecosystem pressures (Haig et al., 2014). Results of the present study indicate that although lead exposure is unlikely to be the primary cause of morbidity or mortality in this population, it has the potential to influence fitness and survival.

No clinical abnormalities were identified in birds in this study, however, the individual with the highest recorded blood lead concentration (50.7 ug/dL) displayed distinctly abnormal behaviour during sampling. This particular bird, in contrast to the rest of the cohort, showed no signs of apprehension towards people in the immediate period following capture and handling. Although a subjective assessment, this is noteworthy as there is ample evidence for lead induced alteration in behaviour and cognition across a variety of species groups. In humans, lead exposure at even low concentrations is shown to negatively affect social behaviour, intellect and memory abilities (Canfield et al., 2003; Chiodo, Jacobson, & Jacobson, 2004; Chandramouli, Steer, Ellis, & Emond, 2009). In birds, experimental studies have revealed lead associated changes in behaviour, learning and memory (Burger & Gochfeld, 2000, 2005; Zhong et al., 2010). Altered behaviour can ultimately affect survival by influencing predator avoidance, ability to forage and affecting social behaviour and reproductive success. Although outside the scope of this study, further research to characterise the effects of lead on behaviour and cognition in this highly intelligent species is warranted.

In this study, isotope analysis was performed on roof-collected rainwater, identified as a plausible source of toxin exposure in kaka. This selection was based on an understanding of behaviour traits and feeding habits of kaka, and observation of kaka interactions in the urban environment which contributed to the deduction of likely exposure pathways. Twenty five percent of birds in this study had lead concentrations considered to be above the toxic threshold, with the highest recorded concentration (50.7 ug/dL) suggestive of a distinct source influencing isotopic ratios. The isotopic

composition of most of the kaka blood samples were consistent with the isotopic ratios of rainwater samples and the mean ratios of $^{206/207}$Pb and $^{208/206}$Pb were not significantly different between these groups, suggesting rainwater is a likely source of lead exposure in kaka. However, the variability in isotope ratios in kaka suggests there are likely to be other contributing sources. The lack of correlation between blood lead concentration and lead isotope ratios in kaka is further evidence that the source of lead is variable in this population. This variability is likely associated with the low (<10 ug/dL; <0.48 umol/L) blood lead concentrations in the majority of the birds in this study. At lower blood lead concentrations, sources of exposure are expected to be more diffuse and variable, likely resulting from background environmental contamination (Gwiazda & Smith, 2000). The variability observed in isotopic ratios in rainwater samples ($^{206/207}$Pb ranging from 1.042 – 1.177) is explained by the varied composition of lead sources in roofs, contributing to lead in the rainwater. Multiple lead containing elements were identified on sampled roofs, such as flashings, lead-headed roofing nails and lead paint (Stewart et al., 2016), and isotopic ratios of these different elements are likely to vary based on the source from which they originated.

Due to the limited number of sources investigated in this study, precise and specific source attribution was not possible. However, isotope analysis was advantageous in that it allowed for the exclusion of distinct sources such as ammunition. A large proportion of work using lead isotope analysis has involved the investigation of lead ammunition as the most important source of toxin exposure in avifauna (Church et al., 2006; Svanberg et al., 2006; Pain et al., 2007; Legagneux et al., 2014). All lead ammunition available in New Zealand is imported from other countries (S. Brown, personal communication, May 6, 2016), therefore comparisons to North American and European studies are considered to be relevant. Additionally, there are no New Zealand studies investigating stable isotopes of lead for anthropogenic or environmental sources. Composition of $^{206/207}$Pb ratios in kaka in this study are distinct from reported $^{206/207}$Pb isotope ratios of North American and European lead pellets, suggesting that ammunition is unlikely to be an important source of exposure in kaka in Wellington (**Table 2.1**). This is further supported by the nature of kaka foraging strategies, which reduce their risk of exposure to ammunition.

Table 2.1 Lead 206/207 isotope ratios of ammunition from North America (Church et al., 2006; Tsuji et al., 2008) and Europe (Binkowski, Meissner, Trzeciak, Izevbekhai, & Barker, 2016)

Reference	N	$^{206}Pb/^{207}Pb$ mean ± SD	$^{206}Pb/^{207}Pb$ Range
Church et al (2006)	18	1.229 ± 0.005	1.223 – 1.241
Tsuji et al (2008)	8	1.228 ± 0.005	1.222 – 1.234
Binkowski et al (2016)	45	1.151 ± 0.009	1.133 – 1.173

Other sources of lead exposure in birds include atmospheric pollution and soil contamination resulting from urban pollution and industrial activity. As lead persists in the environment for extended periods, historical use of lead in fuel, paint and emissions from industrial activities can result in persistent environmental contamination. Leaded fuel was discontinued in New Zealand in 1996 (Wilson & Horrocks, 2008), and as a result, recent measurements of air pollution in New Zealand show low atmospheric concentrations that are reported to be well within the New Zealand Ministry for the Environment's ambient air quality guidelines (Stats NZ, 2015). Additionally industrial activity, in particular smelting and mining, is absent in urban Wellington and within the vicinity of the study area. Atmospheric pollution is therefore unlikely to be a major contributor to lead exposure in kaka. Studies investigating soil pollution highlight the higher contamination levels associated with major urban centres compared to rural areas (Scheuhammer, Bond, Burgess, & Rodrigue, 2003; Roux & Marra, 2007). Lead in the soil can be taken up by plants and be stored in leaves and seeds and subsequently transferred to birds directly via feeding on vegetation or secondarily through ingesting plant-feeding invertebrates (Roux & Marra, 2007). Ground feeding birds are at higher risk of exposure to soil contaminants as a result of incidental soil ingestion during feeding (Beyer, Connor, & Gerould, 1994; Roux & Marra, 2007). Kaka largely forage above-ground, reducing their risk of exposure to soil contaminants.

When considering the overall significance of the results of this study, one limitation to consider is the limited time frame in which this study was conducted. This cross-sectional prevalence study involved observing the population at a single point in time. Blood lead concentration provides an indication of acute exposure and is considered a poor indicator of chronic exposure resulting from bioaccumulation of lead over time

(Franson & Pain, 2011). While the results of this study provide a baseline understanding of prevalence and effects of lead exposure in this population, long-term data is required to more clearly interpret the population-level consequences of exposure. Following on from this study, future work should aim to investigate temporal trends in lead exposure providing information on frequency and chronicity of exposure. Additionally, a longer-term study would provide important information on the chronic effects of lead exposure on survival, reproductive fitness and population growth allowing for a better understanding of population-level impacts in kaka in Wellington.

An additional limitation of the study was the availability of specialised laboratories to performed precise stable isotope analysis, which limited the variety of samples tested. Isotope analysis is a useful tool to trace exposure pathways, but requires comprehensive investigations of environmental sources for accurate source attribution. In previous studies, successful source attribution was achieved when a combination of the following factors were present: (i) high concentrations of lead exposure in study species, suggestive of distinct sources in the environment (Finkelstein et al., 2012); (ii) numerous possible sources investigated (Behmke et al., 2015); (iii) few, isotopically distinct sources (Meharg et al., 2002); and (iv) case-studies where very specific sources are suspected (Finkelstein et al., 2014). Although only a limited number of environmental sources were able to be tested in this study, to the author's knowledge, this is the first study examining stable isotope ratios of lead in environmental samples in New Zealand. Testing other possible sources such as soil and air samples is warranted in future, to gain a more comprehensive understanding of sources of lead contamination in New Zealand. Given the variability observed in isotopic compositions of roof-collected rainwater samples, future work should also endeavour to increase the number of roofwater samples analysed and to individually test lead-containing elements in roofs.

The results of this study suggest that lead exposure is prevalent in kaka in Wellington. Athough no mortality was recorded during this study, the observed correlation between lead exposure and body condition highlights the potential for even low-level lead exposure to affect physiological function and ultimately influence population survival long-term. This study additionally provides evidence for a likely source of lead in kaka in Wellington, and to the author's knowledge, is the first study to

demonstrate lead contamination of roofs as a pathway of exposure in birds. To gain a comprehensive understanding of lead exposure in this population, a protracted study investigating long-term survival and trends in lead exposure is recommended.

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CHAPTER THREE

Lead exposure in nestling kaka: Investigating prevalence, source and effect

3.1 INTRODUCTION

Lead is a non-degradable, persistent environmental pollutant and is one of the most extensively studied toxic metals. It is a potent toxin with no known biological function. There is a vast body of evidence highlighting the deleterious effects of lead exposure in avifauna worldwide. In birds, lead exposure can result in a spectrum of disease from acute exposure, presenting with non-specific clinical signs of toxicity to chronic, sub-clinical disease with no overt signs of toxicity. It affects multiple body systems with wide-spread effects, targeting neurological, cardiovascular, renal and reproductive systems in birds (Fisher, Pain, & Thomas, 2006). Lead is bio-accumulated and can be stored in the body for prolonged periods, reaching toxic levels over time. In recent years, the importance of lead exposure in young, growing individuals has gained recognition due to the potential for harm during critical stages of organ development and the potential for life-long deficits. The developing central nervous system is considered particularly susceptible to lead (White et al., 2007). Post-natal lead exposure in children is associated with deficits in motor skills, memory, social behaviour and vestibular-proprioceptive control (Chiodo, Jacobson, & Jacobson, 2004). In rats and monkeys, developmental lead exposure is associated with persistent neurobehavioural deficits, affecting lifelong function (U.S. Environmental protection agency, 2006). Studies in birds showed similar results where early-life lead exposure is associated with neurological deficits, reduced growth rates, reduced haematocrit and reduced fledging success (Hoffman, Franson, Pattee, Bunck, & Murray, 1985; Burger & Gochfeld, 2000; Nam & Lee, 2006). Exposure to lead during early life stages has the potential to affect lifetime reproductive success and long-term survival and is a recognised threat to wildlife populations worldwide.

Lead is a multi-medium toxin, therefore exposure can result from ingesting water/food contaminated with lead or via inhalation of lead particles in the environment (White et al., 2007). Following exposure, lead is absorbed into the blood and blood lead concentration is high in the acute period immediately following exposure (Lumeij, 1985). Subsequently, lead levels in the blood decrease as lead is redistributed to soft-tissue compartments such as liver, kidneys, and muscle as well as to a long-term pool in bone (Gordon, Taylor, & Bennett, 2002). Routes of exposure and metabolism of lead in nestling birds are suggested to be similar to adult birds. Nestlings may be exposed to lead through inhalation in the presence of high

atmospheric pollution or via ingestion of contaminated food (Grue, Hoffman, Beyer, & Franson, 1986). Altricial nestlings spend a long period in the nest prior to fledging, where they are completely reliant on food obtained by parents. Parental feeding is therefore a significant route of toxin exposure in altricial nestlings.

In birds an additional route of exposure is via maternal transfer of lead (Vallverdú-Coll, López-Antia, Martinez-Haro, Ortiz-Santaliestra, & Mateo, 2015). Lead has a high affinity, and competes, for binding sites with calcium and is transported and stored similarly to calcium (Scheuhammer, 1987). In the female bird, lead mobilisation occurs simultaneous to calcium mobilisation during egg laying and lead is consequently incorporated into the egg (Burger, 1994). Deposited lead can be derived from recent exposure or from mobilisation of stored lead as a result of chronic exposure (Burger, Bowman, Woolfenden, & Gochfeld, 2004). Lead concentrations in the egg are therefore representative of lead exposure in the adult female. Transfer of lead to the embryo occurs as the majority of the calcium required for embryonic growth and development is absorbed from the eggshell and metals stored in the eggshells are concurrently absorbed by the developing chick (Karlsson & Lilja, 2008). Maternal transfer of lead results in exposure of chicks at an early and vulnerable stage of development, potentially resulting in higher rates of morbidity and mortality in avian wildlife. Considering the detrimental effects of early-life lead exposure in birds, research on free-living terrestrial nestlings is sparse, particularly for parrots. The majority of existing research evaluating the effects of heavy metal toxins in nestlings is experimental and provides contradictory findings.

Kaka are forest parrots endemic to New Zealand. They are classified as endangered by the IUCN, with the main threats to their population being habitat loss and predation (Greene, Powlesland, Dilks, & Moran, 2004). They have a diverse diet and are intelligent birds capable of exploiting a variety of food sources. Kaka breed between October and July and nest in tree cavities (Powlesland et al., 2009). The average clutch size is 4 chicks and kaka nestlings fledge at approximately 60 days of age (Moorhouse et al., 2003). In 2002, North Island kaka were reintroduced to Zealandia Ecosanctuary, a 225 hectare predator free nature reserve in peri-urban Wellington. There has been substantial population growth from the original founders and now the kaka at Zealandia are venturing into surrounding urban Wellington, where they are implicated

as one of three species most likely associated with avian-human conflict in the urban environment (Charles & Linklater, 2013).

The majority of research on lead exposure in avifauna concerns waterfowl, raptors and upland game birds where ammunition is implicated as the primary source of toxin. More recently urban environments have been recognised as polluted ecosystems (Scheifler et al., 2006; Swaileh & Sansur, 2006). The historical use of lead in households, fuel and industrial activities has resulted in widespread environmental pollution in urban areas. We hypothesise that interaction and exposure to the urban environment increases the risk of lead exposure in kaka. Accordingly, the primary aim of this study was to evaluate the significance of lead in urban Wellington kaka nestlings. Wellington maintains the only urban population of kaka in New Zealand therefore this study provides a unique opportunity to examine direct anthropogenic influences on lead exposure in this species. Nestlings are easily accessible and are considered to be bio-indicators of local environmental pollution levels as they are fed entirely by parent birds foraging in the local environment (Janssens et al., 2003; Swaileh & Sansur, 2006). The specific aims of this study were to (1) investigate the prevalence of lead exposure in kaka nestlings (2) identify physiological effects associated with lead exposure at different stages of development and (3) investigate routes and patterns of exposure. Understanding the risks of urbanisation in kaka will allow for adaptation of management strategies specific to this population and is vital for the future management of this urban parrot.

3.2 MATERIALS AND METHODS

Study design

The study was conducted at Zealandia Ecosanctuary. Thirty-six artificial nest boxes are maintained throughout the sanctuary and provided access to kaka nestlings. A total of 139 kaka nestlings were sampled over two consecutive breeding seasons (2014/2015 and 2015/2016) between the months of November and January. Nest boxes were inspected at least twice a week, which allowed for estimation of hatch date and therefore age of individual nestlings. Each nestling was sampled twice while in the nest, first at 3-4 weeks of age ('early') and again at 7-8 weeks ('late'). These sampling intervals were chosen based on the development of the nestlings and the expected fledgling date for this species. Chicks younger than 3 weeks of age are at risk of stress and injury from handling and manipulation due to their small body mass and premature development. Fledging age in this species is estimated to be between 9 to 10 weeks of age (Powlesland, et al., 2009), therefore chicks were sampled at least a week prior to estimated fledge to avoid stress-induced premature fledging. At 3-4 weeks of age, individual birds were marked on the rump using coloured food dye (Queen Fine Foods Pty Ltd, Alderly, Queensland, Australia 4051). This allowed identification of individuals within a particular clutch when they were once again sampled at 7-8 weeks of age. Leg bands were placed as permanent identification at 7-8 weeks of age. Nest boxes were monitored throughout the breeding season to assess fledging success.

Blood samples

Blood samples were taken from either the cutaneous ulnar vein or medial metatarsal vein using a 1ml syringe and 25 gauge needle. A minimum of 0.05mls to a maximum of 0.2mls was collected from each individual based on age and weight. Blood was transferred into lithium heparin microtainers (Becton Dickenson Vacutainer Systems, Franklin Lakes, New Jersey, USA 07417) and stored at room temperature prior to analysis. Blood lead analysis was performed within 12 hours of collection using a portable lead analyser (LeadCare II®, ESA Inc, Chelmsford, Massachusetts, USA 01824). The LeadCare II® testing kit uses anodic stripping voltammetry to measure blood lead concentrations and has a reportable range of 3.3 to 65 ug/dL (0.16 to 3.14

umol/L). Specifically formulated control materials, provided by ESA Inc (Chelmsford, Massachusetts, USA 01824), were used at regular intervals for quality control of the testing kits. For the purposes of this study, blood lead concentrations above the detection limit (> 3.3 ug/dL; >0.16 umol/L) were defined as lead exposure. Packed cell volume (PCV) was measured by capillary tube centrifugation within 24 hours of sample collection. Total plasma protein was measured using a refractometer.

Clinical and neurological examination

All sampled birds were subjected to a clinical and neurological examination conducted by the author. Body weight divided by tarsometatarsus length was used to calculate a body condition index (BCI) for each individual. Pectoral and epaxial muscle mass was used to subjectively assess body condition. Mentation was assessed subjectively and recorded as being bright and alert or quiet and depressed. Nestlings in this study were subject to a range of neurological tests which included wing withdrawal, knuckling, and righting reflex tests. Wing withdrawal and proprioceptive function testing was performed by the author and responses assessed subjectively as present or absent. The wing withdrawal test evaluates the integrity of the local reflex arc involving the brachial plexus. Both wings are extended away from the body and a positive response is recorded if the wings are replaced back to a normal position when released. Knuckling is used to assess conscious proprioception and involves displacing the foot with the dorsal surface contacting the ground. A normal response is recorded when the chick replaces the foot back to normal position. The righting reflex test evaluates the function of the central and peripheral nervous systems and provided a quantitative measure of overall neurological function. The chick is placed on its back and the time taken for it to right itself to a standing position is recorded as the righting time.

Eggshell lead analysis

Shell fragments from hatched eggs from the 2014/2015 season were collected for lead analysis. Nests were inspected at least twice a week and eggshell samples collected opportunistically. Samples were placed into clean plastic bags and frozen prior to processing. Whole eggs were thawed at room temperature and egg contents separated from the shell. External debris and egg shell membrane remnants were removed prior

to trace metal analysis. Shell samples were pooled by clutch and analysed at Hills Laboratory (Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton, New Zealand). Shells were ground and homogenised following weighing. Sample weights ranged from 3.2g to 10.0g. Samples were digested with nitric and hydrochloric acid at 85^{o}C for 1 hour. Lead concentration was determined by ICP-MS with a detection limit of 0.01 mg/kg. Spikes, blanks and standard reference material were run alongside samples as quality control measures to check for reproducibility and bias of the method. A replicate of a randomly chosen sample was also run to check for within batch precision and performance of the method.

Statistical methods

Statistical analyses were performed on SPSS for windows (IBM SPSS v22). A significance level of 0.05 was chosen for all tests. Results are presented as mean +/- 1 standard error (s.e.) unless stated otherwise. Chi-squared analysis was performed to assess frequency of lead exposure in nestlings between two seasons and t-test to compare prevalence. I performed a repeated measures GLM of righting reflex, using year of sampling as a factor and early and late blood lead concentrations as covariates. I carried out a repeated measures GLM of BCI, using year of sampling as a factor and early and late blood lead concentrations as covariates.

Permits for this study were granted by the New Zealand Department of Conservation (permit number: 39490-FAU) and approved by the Massey University Animal Ethics Committee (MUAEC 14/76).

3.3 RESULTS

3.3.1 Prevalence and blood lead concentration

Over two breeding seasons, a total of 139 nestlings were sampled as part of the study. Fifty one nestlings (36.7%) showed evidence of lead exposure based on detectable levels of lead in the blood. Descriptive statistics of samples size and blood lead concentration from the two seasons are shown in **Table 3.1**. In the first season, lead exposure was detected in 15 of 57 nestlings (26.32%). In the following year, 36 of 82 nestlings (43.9%) showed evidence of lead exposure. There was a significantly higher prevalence of lead exposure in season 2 compared to season 1 (chi square = 4.47, df = 1, p = 0.034). The majority of lead exposed nestlings in this study (90%) had blood lead concentrations below 10ug/dL (0.48 umol/L) with a range from <3.3 to 42.9 ug/dL (<0.16 to 2.07 umol/L) (**Figure 3.1**). There was a significant difference (p = 0.026) between mean blood lead concentration of nestlings between season 1 (10.89 $\pm$ s.e. 2.37 ug/dL) and season 2 (5.20 $\pm$ s.e. 0.26 ug/dL).

Table 3.1 Total numbers of nests and chicks, average clutch size, fledging success and blood lead concentrations of lead exposed chicks in season 1 and season 2

	Nests (n)	**Chicks (n)**	**Blood lead concentration (ug/dL) Mean $\pm$ s.e. (Range)**	**Clutch size Mean $\pm$ s.e**	**Survival to fledge**
Season 1	18	57	10.89 $\pm$ 2.37 (0 - 42.9)	3.16 $\pm$ 0.27	100%
Lead +ve	8	15			
Season 2	24	82	5.20 $\pm$ 0.26(0 - 10.3)	3.41 $\pm$ 0.24	100%
Lead +ve	16	36			

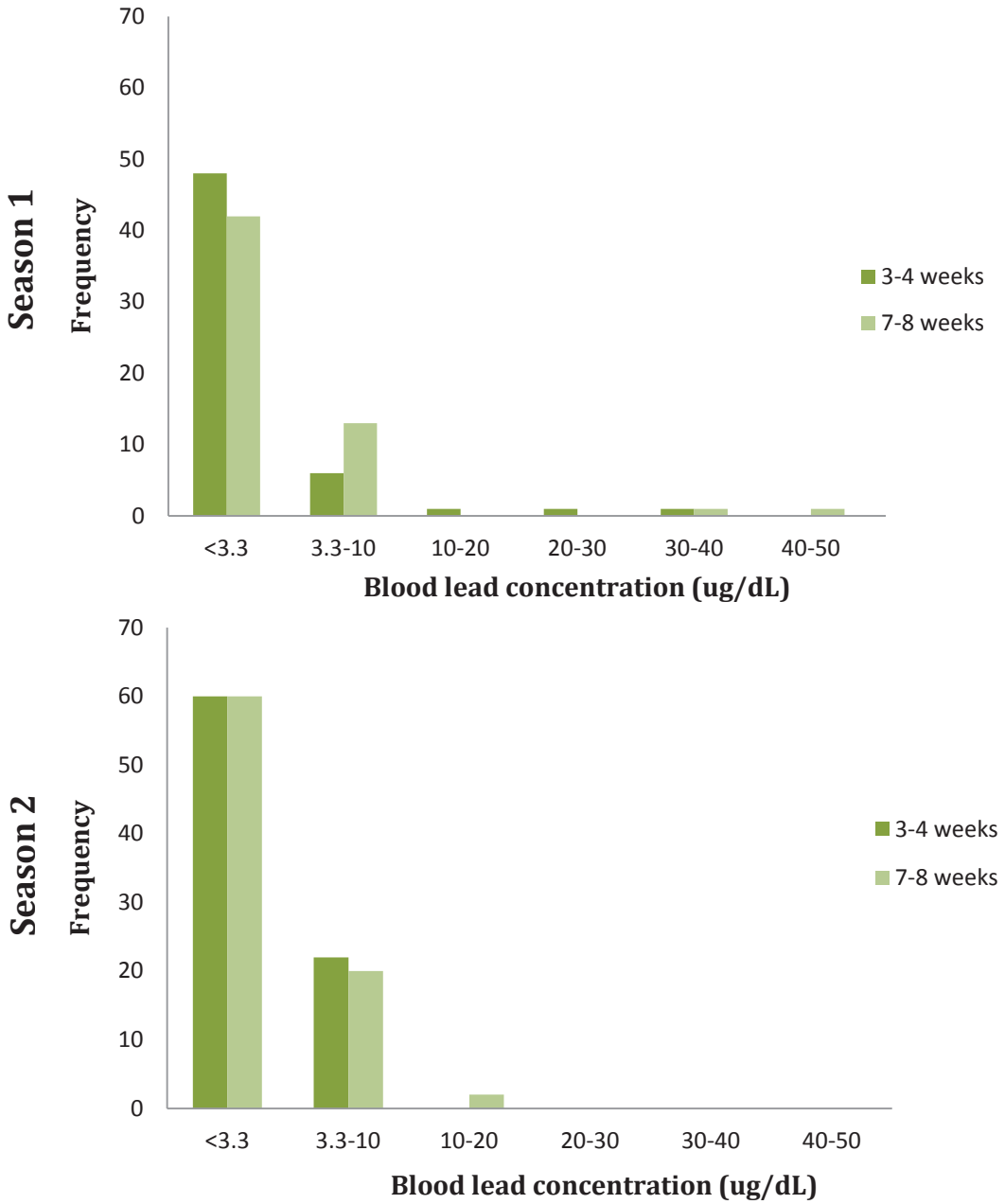

Figure 3.1 Blood lead concentration (ug/dL) of kaka nestlings sampled at 3-4 weeks and 7-8 weeks in season 2014/2015

3.3.2 Patterns of exposure

Lead exposure was detected at early sampling in 9/57 chicks (15.79%) in season one, and 22/82 chicks (26.83%) in season two. The majority of nestlings (60%) in season 1 showed lead exposure at both sampling intervals (early and late), whereas in season 2 the majority (77.77%) of nestlings were exposed only once, either early (38.88%) or late (38.88%).

The patterns of exposure within a clutch varied, with some clutches having only a single chick with evidence of lead exposure (9 of 42 nests (21%)). There were a proportion of nests (10 of 42 nests (24%)), across both seasons, where the entire clutch was lead positive at either point in sampling. The variable blood lead concentrations within and across clutches are depicted in **Figure 3.2**.

Of the 15 lead exposed chicks in the first season, the majority (73%) showed an increase in blood lead concentration between early and late sampling. In the second season, 18 out of 36 nestlings (50%) showed an increase in blood lead concentration between sampling points.

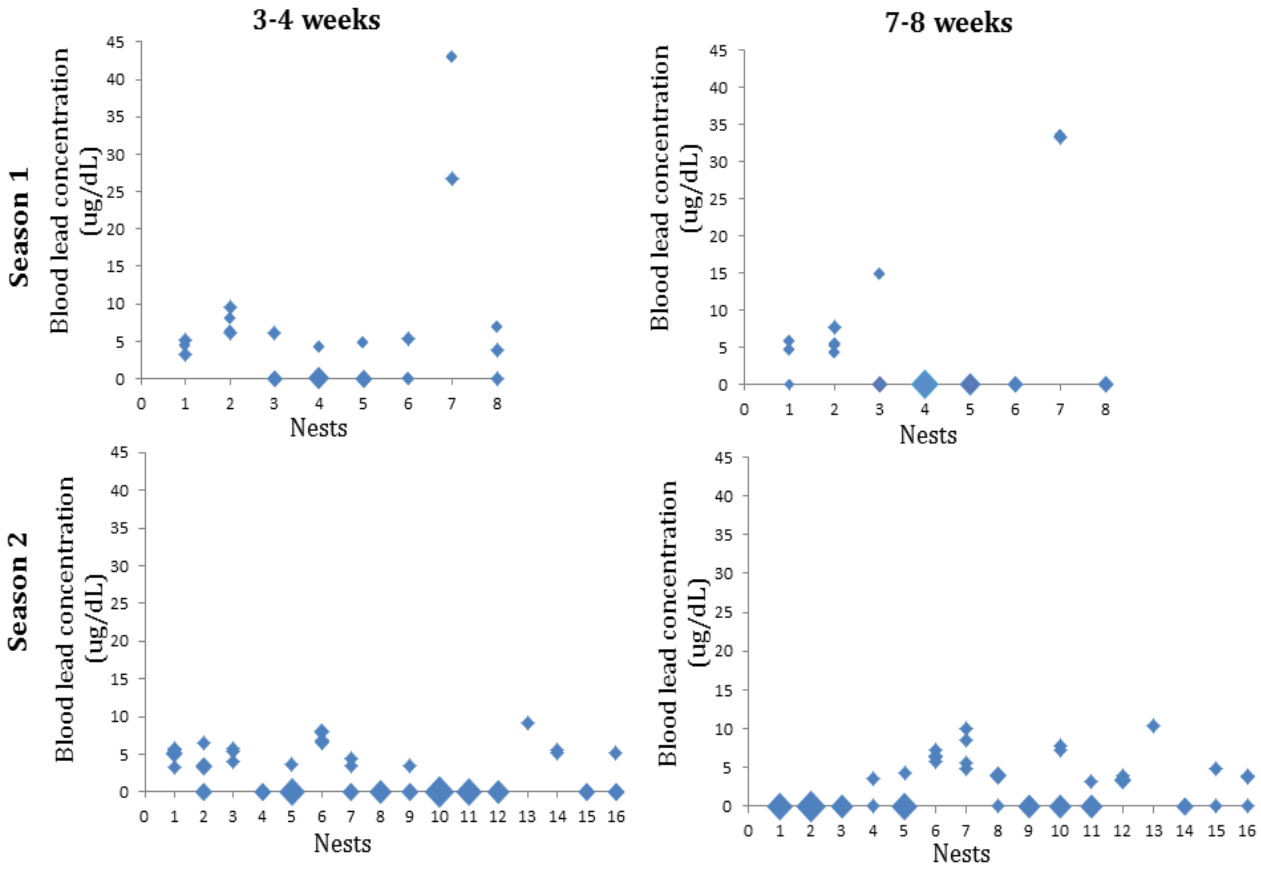

Figure 3.2 Intra- and inter-clutch variability in lead exposure in kaka nestlings in Zealandia Ecosanctuary that is located within urban Wellington. Individual nests are represented on the x-axis with the size of the symbol indicative of the number of chicks.

3.3.3 Nestling survival and condition

None of the nestlings in this study showed clinical signs associated with lead toxicity at the time of sampling. All nestlings survived to fledge. The two individuals with the highest recorded blood lead concentration in this study (42.9 ug/dL and 33.3 ug/dL), were sighted and known to survive for at least 12 months following fledging.

Packed cell volume was calculated from blood samples collected from nestlings at 7-8 weeks of age. Five nestlings were excluded due to insufficient blood sample volume. The average PCV of nestlings in season 1 was 39.11% $\pm$ 0.79, and in season 2 was 40% $\pm$ 0.49. There was no significant correlation between blood lead concentration and haematocrit ($R^2 = 0.005$, $p = 0.7$).

As kaka show sexual dimorphism in regards to body mass (Moorhouse, Sibley, Lloyd, & Greene, 1999), body condition index was used as a measure of comparison. There was no significant relationship between blood lead concentration and BCI in either season, at 3-4 weeks of age ($R^2 = 0.002$, $p = 0.89$) or at 7-8 weeks of age ($R^2 = 4.2 \times 10^{-7}$, $p = 0.71$). Mean BCI of birds, across both seasons, at 3-4 weeks of age was 9.60 $\pm$ 0.12 and at 7-8 weeks of age was 12.02 $\pm$ 0.08.

No overt neurological deficits were observed during this study. The righting times in nestlings were not normally distributed. There was a significant decrease ($F = 29.609$, $df = 1,123$, $p = <0.001$) in the righting reflex time of chicks between the early (mean = 1.85 sec, SD ± 1.12 sec) and late (mean = 1.20 sec, SD ± 0.61 sec) sampling periods, that was not affected by year of sampling ($p = 0.169$), early blood lead concentration ($p = 0.901$) or late blood lead concentration ($p = 0.993$). Lead exposure did not significantly influence righting times in the early or later stages of development in nestlings in this study.

3.3.4 Eggshell lead content

Two out of 8 lead positive nests had detectable levels of lead in the eggshells (2.9 mg/kg and 0.16 mg/kg). Eggshell lead concentration was positively correlated with blood lead concentration in kaka chicks at 3-4 weeks of age, although this relationship was not statistically significant ($R^2 = 0.974$, $p = 0.12$).

3.4 DISCUSSION

Evaluating individual and population level impacts of lead exposure requires an understanding of the prevalence of exposure within a population and the potential for toxicity as a result of exposure. The results of this study indicate that lead exposure is common in this urban population of kaka: more than a third of the population, sampled over two years, had detectable levels of lead in the blood. It is rare to find such a high prevalence of exposure in wild terrestrial birds, with exceptions being in cases where there is a point source of toxin such as smelting sites or mining sites (Eeva et al., 2014). Lack of point source contaminants within proximity of the current study area suggests an alternate source of toxin in this population. There was a significantly higher prevalence of exposure in nestlings in the second season, but a lower mean blood lead concentration compared to the first season. This significant variability between seasons indicates a sporadic pattern of exposure. The increasing prevalence seen over two years, although not adequate to make inferences about temporal patterns, highlights the importance of continued monitoring to determine significant temporal trends in this population.

This study is one of only two known studies reporting prevalence of lead exposure in free-ranging parrots, therefore comparisons across other species groups are necessary. **Table 3.2** provides a comparison of blood lead concentrations in nestlings from a variety of studies. Mean and maximum blood lead concentrations of lead exposed nestlings in season 1 in this study (mean = 10.78 $\pm$ 2.28 ug/dL, range = 3.3 – 42.9 ug/dL) are comparable to studies on populations of wild birds inhabiting polluted areas, suggestive of the presence of lead contamination in the environment. Black kite (*Milvus migrans*) nestlings and white stork (*Ciconia ciconia*) nestlings inhabiting highly polluted areas such as landfill sites and industrial sites, for example, showed mean blood lead concentrations of 8.41 and 14.6 ug/dL respectively (Blanco, Frías, Jiménez, & Gómez, 2003; de la Casa-Resino, Hernández-Moreno, Castellano, Pérez-López, & Soler, 2014).

Table 3.2 Blood lead concentrations in nestling birds inhabiting polluted and non-polluted sites.

Species	**Reference**		**Blood lead levels (ug/dL)**	
			Polluted area	**Non-polluted area**
White stork (*Ciconia ciconia*)	Pérez-López et al. (2016)	Mean	-	3.69
		Min-max		0.83 – 18.6
		(n)		44
White stork (*Ciconia ciconia*)	de la Casa-Resino et al. (2014)	Mean	14.6	2.37
		Min-max	5.3 – 50.2	0.55 – 11.9
		(n)	10	27
Eurasian eagle owl (*Bubo bubo*)	Gómez-Ramírez et al (2011)	Mean	8.61	3.18
		Min-max	0.49 – 25.61	ND – 18.37
		(n)	22	196
Booted eagle (*Hieraaetus pennatus*)	Martínez-López et al. (2004)	Mean	-	3.21
		Min-max		1.10 – 10.57
		(n)		27
Black kite (*Milvus migrans*)	Blanco et al. (2003)	Mean	8.41	-
		Min-max	0.72 – 22.1	
		(n)	69	

ND non-detected values (below 0.3ug/dL)

Investigating the prevalence of exposure is important to assess the potential effect of toxins at a population scale, however increased prevalence of lead exposure within a population does not directly relate to morbidity or mortality in wildlife. Evaluating the effects of lead exposure on an individual host scale is complex, as significant individual variability exists in response to lead exposure, therefore associations between exposure dose and clinical disease are not easily definable (Milković-Kraus, Restek-Samaržija, Samaržija, & Kraus, 1997). Additionally, there are no established toxicity thresholds for lead in nestlings. Blood lead concentrations of nestlings in this study ranged from 3.3 to 42.9 ug/dL. Reference values for lead reported in previous studies suggest that in birds blood lead concentrations <20 ug/dL (0.97 umol/L) are considered as 'background' levels and of no clinical significance and levels >20 ug/dL are associated with acute clinical toxicity (Brown, Luebbert, Mulcahy, Schamber, & Rosenberg, 2006; Stauber, Finch, Talcott, & Gay, 2010). These reference ranges are

primarily applied to adult birds and are applied across multiple species groups. Increasing research over the years has seen the toxicity threshold for lead exposure constantly lowered, with an understanding that there is no threshold at which there is 'no effect' (U.S. Environmental protection agency, 2006). Chiodo et al. (2004) claim there is no apparent lower bound threshold for postnatal lead exposure in people. Burger and Gochfeld (1997) have shown that levels of exposure as low as 10 to 30ug/dL (0.48-1.45 umol/L) in chicks result in reduced survival due to abnormal behaviour and cognitive function. When taking into consideration that early-life lead exposure creates a body-burden capable of exerting long-term toxic effects, even low levels of lead exposure in nestlings must be considered relevant.

Selecting diagnostic tests to measure subtle physiological changes resulting from low level lead exposure is difficult. In working with free-ranging wildlife, accessibility and duration of handling are additional factors which need to be considered when selecting tests; particularly with nestlings as they are less resilient to prolonged handling and manipulation. In this study, haematocrit, body condition index and neurological function tests were used to investigate the physiological effects of lead exposure in nestlings and results showed there was no association between blood lead concentration and measured physiological parameters.

Body condition index and haematocrit are commonly used as indicators of lead toxicity since lead is known to inhibit haeme synthesis and to negatively affect growth in mammals and birds (Hoffman, Franson, Pattee, Bunck, & Murray, 1985; Fisher et al., 2006). Studies assessing these physiological parameters in response to lead exposure in nestling birds present contradictory findings. Experimental lead exposure in nestling American kestrels (*Flaco sparverius*) for example resulted in reduced growth, decreased haematocrit values and, in cases with high concentrations of lead exposure, mortality (Hoffman, Franson, Pattee, Bunck, & Anderson, 1985). Alternatively, experimental studies on great tit (*Parus major*) nestlings showed no effect of lead exposure on growth rates, haematocrit or survival (Eeva et al., 2014). Fair and Myers (2002) study on Western bluebirds (*Sialia mexicana*) found no effect of lead exposure on weight, growth rates or haematocrit. Unfortunately inferences cannot be made for correlations between blood lead levels and physiological or clinical effects, as none of the studies cited here reported blood lead concentrations. Comparisons between

studies are hindered by a lack of standardised approaches when evaluating exposure and effect across different species.

Identifying tests appropriate to evaluate neurological function in altricial nestlings was challenging, especially given that tests were applied to a wild population. Nestlings of altricial species show poor locomotor function prior to fledging when compared to precocial species, which are relatively mature at hatch (Dial & Carrier, 2012). Therefore assessing motor function is comparatively difficult in altricial nestlings. In this study, wing withdrawal and knuckling tests were used to evaluate proprioceptive and motor function. No abnormalities were identified in these parameters among the studied kaka nestlings. The righting reflex test, adapted from the study by Burger and Gochfeld (2005), provided a quantitative measure of neurological function in nestlings. No significant relationship was found between lead exposure and righting times in kaka nestlings. Age however did influence righting times, whereby older nestlings (7-8 weeks of age) were able to right faster than younger nestlings (3-4 weeks of age). This is likely a result of progressive development of motor function with age, resulting in a quicker response to righting in the older nestlings. Lead is a neurotoxin and its detrimental effects on the developing neurological system of juveniles is well established. In humans and some animal models, these deleterious effects are known to occur consistently even at low levels (<10 ug/dL; 0.48 umol/L) of lead exposure (Chiodo et al., 2004; Hsiang & Díaz, 2011). In children, alterations in social behaviour, visual-motor reasoning skills, attention and vestibular-proprioceptive control were identified at blood lead levels <10 ug/dL (Chiodo et al., 2004). Studies evaluating the direct effect of lead on neurological function in birds are limited. Burger and Gochfeld (2000) conducted an experimental study in herring gulls (*Larus argentatus*) and showed that lead profoundly affected food begging behaviour, behavioural thermoregulation, individual recognition and treadmill learning and these effects persisted in lead exposed chicks compared to control individuals. In a follow up study, Burger and Gochfeld (2005) found a significant effect of lead exposure on righting reflex time, balance and depth perception in nestling herring gulls and common terns (*Sterna hirundo*). These effects are suggested to be dependent on dose and time of lead exposure during chick development.

In this study, no effect of lead was observed on any of the parameters tested for kaka nestlings. Moreover, all the nestlings survived to fledge. Given the vast body of evidence regarding lead as a potent toxin, the lack of morbidity or mortality observed in this study warrants discussion. One plausible explanation for the lack of observed effect is simply the low blood lead concentrations observed in this study. Almost all individuals apart from two had blood lead concentrations below the 20 ug/dL (0.97 umol/L) threshold, which is recognised by many authors as the threshold for acute clinical lead toxicity. This suggests that exposure levels in this study were low and did not result in acute clinical toxicity. However, this does not account for the lack of effect observed in two nestlings with blood lead concentrations > 30ug/dL (1.45 umol/L), which both successfully fledged and were sighted and therefore known to survive 1 year post-fledge. Individual variation in susceptibility to lead toxicity can influence outcomes of exposure (Gochfeld, 1997). In many instances, birds present to veterinary care facilities showing clinical signs consistent with lead exposure while others with higher blood lead concentrations are asymptomatic. This disparity in response exists on an individual level and also on a species level. American black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*) for example were shown to be more resistant to the toxic effects of lead compared to more sensitive species where low doses of lead exposure can result in mortality (Behmke et al., 2015). The results from the current study therefore could indicate that kaka are more resilient to lead exposure at the concentrations reported in this study.

An additional factor contributing to the lack of observed clinical disease, and a limitation of this study, is the sensitivity of tests and bio-markers used to measure the effects of lead exposure. Body condition, haematocrit and neurological functional testing are commonly utilised to assess effects of heavy metal exposure, however given the low blood lead concentrations in this study, these markers were potentially poorly sensitive and failed to highlight the subtle effects associated with low level lead exposure. An alternative biomarker of physiological function, such as delta-aminolevulinic acid dehydratase (ALAD), should be considered in future studies where low blood lead concentrations are predicted. The enzyme ALAD is involved in the haeme biosynthesis pathway and inhibition of ALAD is considered a sensitive and reliable measure of sub-clinical toxicity associated with low level lead exposure (Lumeij, 1985; Gil & Pla, 2001). Furthermore, although beyond the scope of this study, future work to evaluate sub-clinical and neuro-behavioural effects of lead exposure in

this population should include assessment of cognitive function and behavioural parameters. The known effects of low level lead exposure on these parameters warrant inclusion in follow-up studies.

Time, dose, frequency and route of exposure are all factors which influence outcome following intoxication. Time of exposure is relevant as exposure during critical windows of development can influence morbidity and mortality. Repeated sampling of nestlings in this study allowed for estimation of age, frequency and route of exposure in a natural ecological setting. Frequency of exposure varied between the two seasons. As the half-life of blood lead in kaka is unknown, only nestlings with an increase in blood lead concentration between the two sampling points were considered as repeat exposures for the purpose of this study. In season 1, lead exposure was detected at both early and late sampling periods in the majority of nestlings, suggesting repeat exposure. In the second season, in the majority of nestlings, blood lead levels were detected at only one point in sampling. Repeat lead exposure in nestlings results in accumulation of lead over time, resulting in chronic lead toxicity. In this study, there were no changes in physiological parameters tested in relation to frequency and time of exposure in kaka nestlings. Although no acute signs of clinical toxicity were observed, accumulation of lead from a young age results in an increasing body burden with the potential to influence long-term survival. Swaileh and Sansur (2006) studied house sparrows (*Passer domesticus*) in an urban environment comparable to this study, and provide evidence for the bioaccumulation of lead over time where concentration of lead in the liver increased with age when comparing nestlings to adult birds.

Investigating changes in blood lead concentration between two points in time also provided evidence for likely source of exposure in the nestlings. Detectable or increased blood lead concentrations in nestlings at 7-8 weeks of age, where previously blood lead concentrations were lower or undetectable, confirms an exposure event occurring between sampling intervals. The most plausible explanation for exposure while in the nest is via parental feeding. The dynamics of blood lead concentrations between sampling periods and the variability in exposure between individuals and between seasons suggests that active parental feeding is the primary route of exposure in kaka nestlings in this population. However, maternal transfer of lead through the egg is also recognised as a potential route of exposure. Two nests with

detectable levels of lead in the egg shells were identified in the first season and these nests were the only two where the entire clutch showed evidence of lead exposure. This correlation between lead exposure in nestlings and eggshell lead concentration provides evidence for the possibility of maternal transfer as a route of exposure in nestling kaka. In chickens experimentally exposed to lead while in the egg, distinct windows of lead exposure and toxicity are recognised, where lead-induced effects on immune function vary based on age at which exposure occurs (Lee, Chen, Golemboski, Parsons, & Dietert, 2001). Results from this study show that lead is incorporated into kaka eggs and poses a potential risk for the developing embryo at very early stages in development.

Eggs are suggested to be ideal indicators of lead pollution in birds as they can be non-destructively sampled, provide an indication of maternal exposure, and in non-migratory birds can be representative of local environmental pollution (Kim & Oh, 2014; Orłowski et al., 2014). A large number of studies have examined lead levels in bird eggs, in both polluted and non-polluted environments, and highlight the variability between species in the deposition of lead in the egg (Mora, 2003; Dauwe, Janssens, Bervoets, Blust, & Eens, 2005; Ayaş, 2007). This study is the first to report on eggshell lead concentration in a wild parrot population. Although some studies suggest eggs to be good indicators of environmental pollution, the low detection rate observed in kaka eggs questions their validity as bio-indicators of lead contamination at a population level, since the prevalence of lead exposure as determined by blood sampling of chicks is indicative if higher. However, considering the non-invasive nature and ease of sampling of eggs, further research to correlate egg levels with maternal lead exposure is advocated to better validate these samples as bio-indicators and to identify temporal trends in lead exposure in this population.

When interpreting results from this study, the time frame of sampling must be taken into account. Although this study provides a baseline of prevalence in this population, the two year data-set is inadequate to evaluate temporal patterns in exposure and effect in this population. Studies investigating long-term effects of lead exposure in wildlife are limited. In humans, exposure to low concentrations of lead over time can result in chronic toxicity as a result of bioaccumulation of the toxin. Chronic subclinical toxicity and associated sub-lethal effects can influence long-term survival and fitness on an individual and population scale. In this study, lack of observed effect in the

short-term therefore does not exclude long-term deleterious effects of lead, ultimately affecting survival.

3.5 CONCLUSION

A high prevalence of lead exposure was observed in kaka nestlings in an urban population in this study, with the primary route of exposure being parental feeding, although maternal transfer in eggshells was also documented. Lead exposure did not influence physiological parameters measured in this study, however the data from this study alone is insufficient to discount lead as an eco-toxicological concern in this population. Long-term monitoring of exposure, population growth and survival of these birds is required to understand the full extent of effect of lead exposure in this population.

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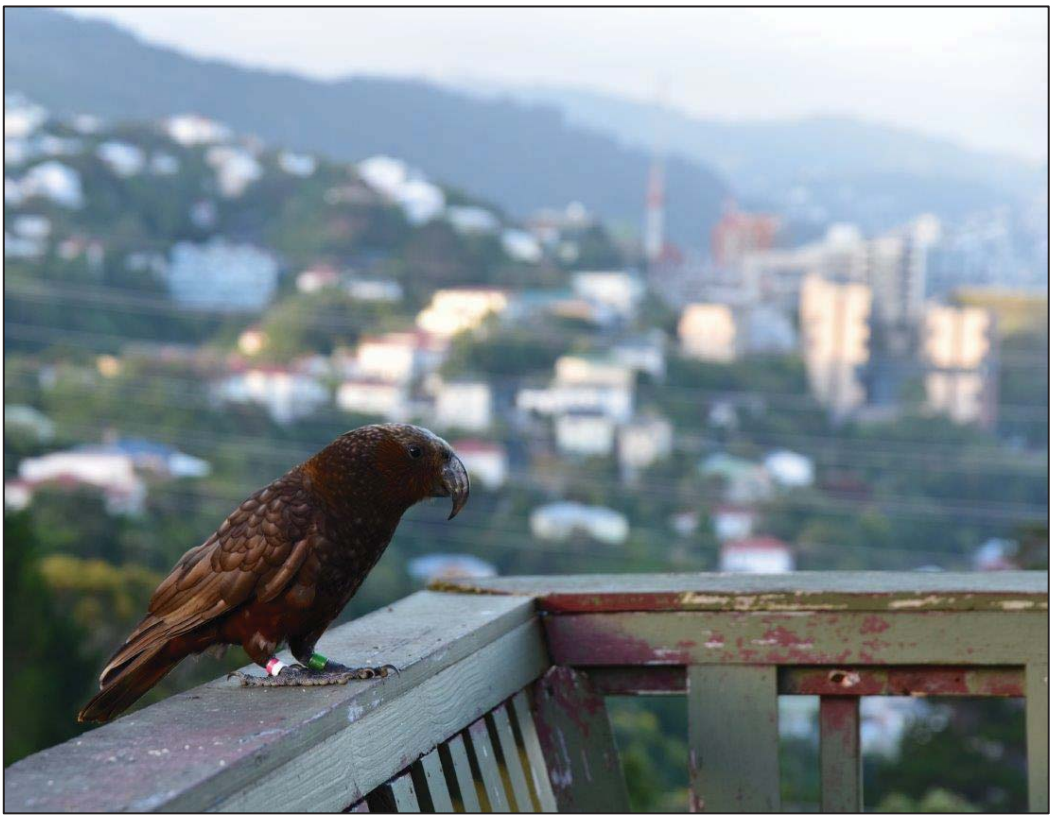
CHAPTER 4

General discussion



4.1 General discussion

Environmental lead contamination is one of many threats confronting wildlife inhabiting urban environments. This study examines an urban population of kaka and investigates the prevalence, effect and source of exposure in adult and nestling birds. Lead exposure was detected in almost half of the adult birds in this study, and blood lead concentrations found in the majority of birds is consistent with low level lead exposure (Franson & Pain, 2011). Adult birds exposed to lead showed no overt clinical signs of toxicity and no mortality was observed during this study. However, lead exposure was associated with reduced body condition, and in one individual with high level exposure, behavioural changes. These findings contribute to a growing body of evidence highlighting the significance of sub-lethal lead exposure. In birds, sub-lethal lead exposure is associated with behavioural abnormalities (Burger & Gochfeld, 2005), reduced reproductive success (Provencher et al., 2016) and poor survival rates (Kelly & Kelly, 2005). In a natural ecological setting where wild birds are subject to multiple environmental stressors, lead exposure can be a contributing factor to morbidity and mortality (Franson & Pain, 2011). In kaka, reduced body condition and behavioural abnormalities can influence their ability to forage and make them more susceptible to disease or predation, ultimately influencing survival. The high prevalence of lead exposure in free-ranging kaka and physiological effects associated with sub-lethal lead toxicity suggests that lead exposure has the potential to cause population level impacts in kaka.

Lead exposure in free-ranging adult kaka is of importance to nestlings, as the primary pathway of exposure for chicks is via parental feeding of lead. Transfer of lead from parent birds to nestlings can also occur through the egg, and is an additional route of exposure identified in this species. Lead exposure in free-ranging adults is therefore highly relevant to exposure in nestling birds. The adverse consequences of early-life lead exposure are emphasised by researchers across human and animal health disciplines (Needleman, 2004). Exposure to lead during critical periods of development can affect the physiological function of various body systems and result in persistent neurological deficits, ultimately affecting survival (Chiodo, Jacobson, & Jacobson, 2004). Given the significance of early-life lead exposure, the prevalence of exposure detected in kaka nestlings in this study is considered significant. Lead was detected in nestlings as young as three weeks of age, with evidence of recurrent

exposure in a small proportion of nestlings. Lead exposure in nestlings was not associated with detectable physiological abnormalities or mortality. This is not unexpected as studies in nestling birds provide inconsistent results, where clear effects of lead exposure are described in some studies and limited responses are observed in others (Haig et al., 2014). Response to lead can be variable depending on dose of exposure, duration of exposure, individual factors such as the health of the bird and species variability in response to exposure (Franson & Pain, 2011). Lack of detectable clinical toxicity, however, should not be interpreted as a lack of effect. Sub-clinical exposure can have significant effects on health parameters in birds, particularly in nestlings. There is consensus amongst scientists that there is no safe threshold of lead exposure during critical developmental life-stages (Chiodo et al., 2004). Neuro-behavioural deficits have been detected in children at blood lead concentrations of <10 ug/dL suggesting that even low concentrations of lead exposure should be considered a risk to health (Sanders, Liu, Buchner, & Tchounwou, 2009). In birds, early-life lead exposure is shown to be associated with deficits in feeding behaviour, individual recognition, learning and memory (Burger & Gochfeld, 2005; Zhong et al., 2010). In kaka nestlings, early-life lead exposure creates a body burden of lead, with the potential for continued exposure following fledging. The bioavailability of environmental lead sources and the increasing prevalence of lead exposure reported in kaka nestlings in this two-year study, suggests that lead exposure is a growing threat to this population.

The prevalence of lead exposure in kaka provides an indication of the magnitude of environmental lead contamination in the urban environment. Birds are commonly used as bio-indicators of environmental heavy metal pollution (Binkowski & Meissner, 2013; Burger & Gochfeld, 1997; Dmowski, 1999). The wide range of blood lead concentrations detected in adult and nestling kaka indicates that there are important and bioavailable sources of lead in the urban environment and that these sources are likely sporadic in distribution. Lead stable isotope analysis was employed to determine lead sources in the environment and indicated that lead contaminated roof-collected rainwater, originating from lead material in roofs, is a likely source of lead exposure in kaka. Isotope analysis is frequently utilised in wildlife studies and it is evident that the success of this method is highly dependent on its application. This method is most valuable when a large number of sources are examined and when these sources are isotopically different (Gwiazda & Smith, 2000). Although precise

source attribution was not achieved in this study, isotope analysis allowed for the exclusion of important sources such as ammunition. The suitability of this method for wildlife ecotoxicological studies should be examined on a case by case basis. Wildlife intoxications largely result from anthropogenic lead sources and this study identifies a previously undescribed urban source of lead in wildlife. In kaka, interaction with the urban environment is likely to increase risk of exposure to lead. There is a significant population of native wildlife in Wellington city and surrounds (Miskelly, Empson, & Wright, 2005) and the risks of exposure to urban sources of lead should also be considered in these species. The majority of research on lead in wildlife has examined lead exposure in natural ecosystems and subsequently advocated for lead abatement in these ecosystems. This study emphasises the importance of urban pollution, and the need for eco-toxicological assessments of urban areas.

The results of this study provide a platform for future work necessary to better understand the population level effects of lead exposure in kaka in Wellington. Lead sources in the urban environment are proven to be accessible to kaka. Further research should aim to investigate exposure over a longer period of time, particularly evaluating the incidence and chronicity of exposure in free-ranging birds. Additionally, a protracted study will allow for the investigation of long-term survival in birds, evaluating the impacts of early-life lead exposure on survival following fledge, and in adults, investigating the impacts of chronic lead exposure on survival. Future work should additionally explore the use of finer scale measures to detect the effects of lead exposure in kaka in a natural ecological setting. Given the significance of early-life lead exposure on neuro-behavioural function, assessing the effects of lead on cognition and behaviour in nestlings is warranted. Lastly, this is the first study in New Zealand to report lead stable isotope values of environmental lead sources, documenting a need for further work in this field. A better understanding of environmental lead sources and in particular a detailed understanding of lead sources in roof components will be beneficial in informing lead mitigation strategies in urban environments.

4.2 Conservation and management implications

The growing presence of kaka in Wellington city is a topic of controversy, involving various conflicting public opinions. While some residents welcome the presence of native wildlife in their backyard and provide food and interact with kaka, others perceive kaka as a nuisance; causing damage to trees and properties. Partially for these reasons, kaka are implicated as one of three species most likely to cause human-wildlife conflict in New Zealand (Charles & Linklater, 2013). Managing wildlife in urban areas requires a considered and case-based approach, which includes a thorough understanding of the risks and threats of urban exposure in wildlife. Heavy metal pollution is one of many threats confronting wildlife species inhabiting urban areas, and population success is dependent on overcoming or adapting to these challenges.

This study identifies lead as a toxin of importance to kaka populations in Wellington. Mitigation of lead risk in this population requires a two-fold approach. The first involves regulating exposure to lead sources in the urban environment, and the second involves reducing environmental lead contamination. Increased food availability is an important factor encouraging kaka into urban environments where the risk of anthropogenic lead exposure is increased. Further research should endeavour to better understand the relationship between urban interaction and lead exposure in kaka. Initiatives aimed at mitigating lead exposure in wildlife have largely been focused on reducing environmental lead contamination, illustrated by the implementation of lead shot restrictions in ecologically important areas. However, in an urban environment such as Wellington lead abatement may not be easily achieved. Due to cost and practicality, the widespread removal of lead from roofing and building material is improbable. However, given the public health and wildlife health risks associated with lead exposure, regulations to discourage the future use of lead in household building materials should be considered.

This study has identified that lead exposure is widespread in kaka in Wellington and provides a baseline understanding of exposure levels at multiple life-stages in this population. The prevalence of lead exposure in this study, in both adults and nestlings, is indicative of wide-spread toxin exposure which has the potential to influence individual survival with larger scale population-level impacts.

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