Heavy metal concentrations in mallards in New Zealand

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To my Grandfather, Gerald Gibb.

(Gibb and MacMahon, 1955)

I miss your late-night phone calls about the elusive iPad off button, your well-worn stories, your passion for learning, but most of all, I miss your endless enthusiasm and support for ‘my ducks’ and their lead.

I’m not sure who should apologise the most- you for leaving right before my original due date, or me for not writing fast enough.

I’m sorry you never got to see the full story.

Abstract

This study aimed to identify the exposure of two geographically separated populations of mallard ducks (*Anas platyrhynchos)* and a cross-section of native waterbirds to three heavy metals. The concentrations of cadmium (Cd), copper (Cu), and lead (Pb) were determined in the liver, blood, and eggshell of mallard ducks and in archived liver samples from 71 native waterbirds. By using mallards as a bioindicator to establish the bioavailability and contamination of the New Zealand waterfowl ecosystems to these three heavy metals, the pollution rate and possible dangers to both environment and human heath can be established. Exposure to all three metals is widespread in mallards, with all livers and blood samples showing detectable levels of all metals. Eggshells concentrations were low for Cd (3.5%) and Pb (13.6%), and detectable levels of Cu were found in all eggshells, reflecting its status as a trace mineral.

Pb is a nonessential, highly toxic heavy metal that is of concern to waterfowl due to the use of Pb shot ammunition for hunting. Ingestion rates of Pb shot were examined through analyses of mallard gizzard contents, and while Pb ingestion was found, there was a significant decrease in both sites from rates recorded prior to Pb shot restrictions. Mallards from the Waikato site were found to have significantly higher concentrations of Pb, in both liver and blood, suggesting that the bioavailability within this region is higher than Southland. For native waterbirds, only two pāteke (*Anas chlorotis*) from Aotea (Great Barrier Island) had hepatic Pb concentrations consistent with toxic levels of exposure.

Cd is a toxic carcinogen known to adversely affect reproduction and survival. In mallards, while Cd blood concentrations were low, 32.4% of livers had elevated Cd above background levels and 16.1% of livers were above the maximum allowable threshold in offal for human consumption. Whio (*Hymenolaimus malacorhynchos*) from the West Coast of the South Island and pāteke from Aotea were found to have hepatic Cd concentrations that suggest high environmental exposure of Cd within these two regions.

Cu is an essential trace element, however in excess it can produce toxic effects. A high proportion (15.8%) of mallards were found to have hepatic Cu concentrations elevated above toxic thresholds. In contrast to the rural living mallards, native waterbirds all had only physiologically normal hepatic concentrations of Cu.

This study provides evidence for contamination of New Zealand waterfowl ecosystems with these three heavy metals, suggests that consumption of waterfowl livers may be contraindicated for human health, and that heavy metal exposure in mallards is at levels consistent with adverse effects on individual and population level health in these important game birds.

Acknowledgements

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P.S. This thesis began with a mudfish translocation, and if my grandfather hadn't stolen the show, this thesis would be dedicated to those mudfish who, tragically lost their home to the ever-straightening Northern Motorway. I'm sure this research will trickle back to you somehow.

Contents

Chapter One

General Introduction

Literature review, research objectives, and thesis aims

With global change and increasing levels of industrial, commercial and agricultural contaminants it is important to track changes within wild species, particularly species consumed by humans and those of economic importance. During the 20th century alone, the human population has grown from 1.65 billion to 6 billion, and it is estimated to reach 9 billion by 2038; this growth has increased pressure on rural land to produce more crops and support more livestock, both of which reduce natural nutrients and require pesticide and fertiliser use. The impact of land run off and bioaccumulation of specific pollutants, such as heavy metals, can be a serious threat for the stability of ecosystems. Although heavy metals are naturally occurring elements found throughout the earth's crust, the numerous uses and application by humanity has resulted in a dramatic change in the geochemical balance and bioavailability of these metals, resulting in widespread environmental contamination (Tchounwou *et al.*, 2012).

Throughout the 800 years since human arrival in New Zealand, the land has gone through a transformative change. It is estimated that forests covered all but the alpine environments and wetlands covered extensive areas in lowland and coastal areas. Currently about one-third of the forests remain and wetlands have been reduced to about 10% of their original extent (Ministry for the Environment and Statistics New Zealand, 2015). Such massive changes to the land, within a short time period, are known to induce significant impacts on the local biogeochemical systems which sustain the biosphere (Meyer and Turner, 1992). The problems of land use change, habitat destruction and human disturbance are amplified by contaminant problems, first clearly recognised in terms of pesticides (such as DDT) and heavy metals (such as mercury) (Porter and Wiemeyer, 1969, Bevenue, 1976, Wolfe *et al.*, 1998). Development of monitoring programs and indicators of exposure is thus critical to evaluating risk from increased exposure to contaminants.

1.1 Heavy metals

Heavy metal - a loose term

The definition of 'heavy metal' has been somewhat fluid in the literature throughout the last eighty years, with the most common definitions coming from a combination of one or more of five classifications: density (specific gravity), atomic weight, atomic number, chemical properties, or toxicity (Duffus, 2002). Density-based definitions class metallic elements that have a relatively high density compared to that of water (i.e. > 1 g/cm^3) as heavy; however, this can range from 3.5 g/cm^3 (Tegge, 1997) to densities above 7 g/cm^3 (Foster, 1936). Atomic weight (relative atomic mass) and atomic number based definitions are also comparative, with the general consensus that heavy metals have either an atomic weight greater than that of sodium, i.e. > 23 (Maynard, 1947, Duffy, 2011, Hawley and Lewis, 2002), or any metal with an atomic number > 20 (Phipps, 1981, Hale *et al.*, 2005). The fourth definition is based on other chemical properties such as density of crystals (Merriman, 1965), density for radiation screening (Birchon, 1968), or reaction with dithizone (Jackson *et al.*, 1997). The final definition, based on toxicity, relies on the assumption that heaviness and toxicity are related. It therefore defines heavy metals as elements that are generically toxic to aerobic and anaerobic processes, which would also include metalloids, such as arsenic (Smith and Scott, 2002, Duffus, 2002, Tchounwou *et al.*, 2012). As the present study aims to assess the toxic effects of heavy metals on the waterfowl of New Zealand, this thesis will use the toxicity-based definition put forward by Smith and Scott (2002): that heavy metals include elements commonly used in industry, which are generically toxic to aerobic and anaerobic processes, without necessarily being dense nor entirely metallic. Using this definition, the following metals are included; arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), and zinc (Zn).

Source of pollutants

Heavy metals occur naturally as they are components of the lithosphere and are released into the environment through many different routes (Fergusson, 1990a). Natural phenomena such as weathering of rocks and volcanic eruptions have also been reported to significantly contribute to heavy metal pollution (Fergusson, 1990a). However, most environmental contamination and human exposure result from anthropogenic activities (Mance, 1987) such as mining and smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds. Industrial sources include metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants. Environmental contamination can also occur through metal corrosion, atmospheric deposition, soil erosion of metal ions and leaching of heavy metals, sediment re-suspension and metal evaporation from water resources to soil and ground water (Fergusson, 1990a).

Although heavy metal pollution is associated mainly with areas of intensive industrialisation, pollution from other sources such as agricultural land and urban areas are also significant routes for the release of pollutants into the environment. Globally, a source of Pb that has caused major issues to wildlife populations through environmental exposure is spent ammunition (i.e., bullets, shotgun pellets and fragments) from hunting activities and fishing weights (Scheuhammer and Norris, 1996, Franson and Pain, 2011).

The intensive land changes within New Zealand over a short period of time after European settlement resulted in erosion becoming a major problem in the early 20th century. With this escalation of topsoil removal, soils lost the majority of their nutrients, forcing farmers and crop growers to mitigate this loss through increased fertiliser use. This resulted in a cascade of leaching nutrients from farmland into waterways, lakes and estuaries, which has grown in significance as farming has intensified in many parts of the country.

Major pathways of heavy metals in agroecosystems are soil-to-solution transfer (mobilisation) followed by plant uptake and leaching to surface water and groundwater. Erosion is also a cause of contaminant transfer to surface water (Haygarth and Jarvis, 2002). An overview of the major pathways of trace metals, including the most relevant receptors, is given in Figure 1.1

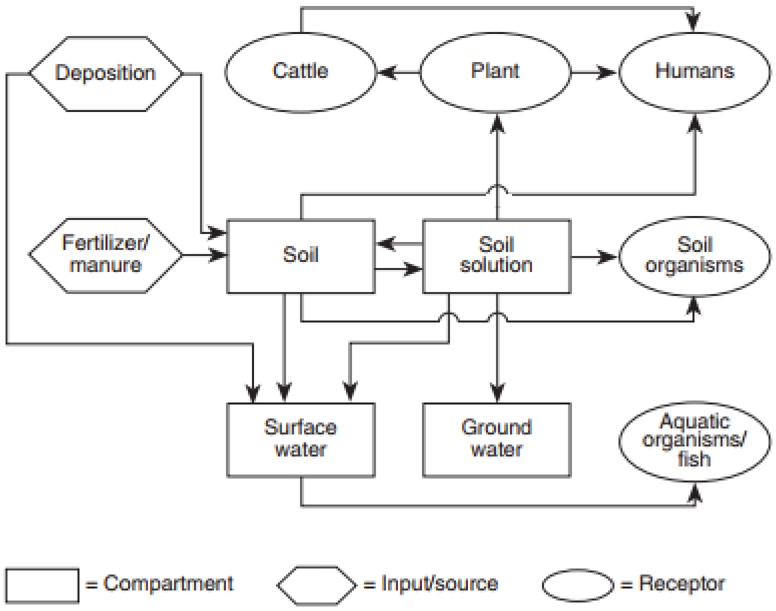


Figure 1.1 Overview of the major pathways of heavy metals in agroecosystems. Sourced from Haygarth and Jarvis (2002)

Toxicity of heavy metals

As metals are natural components of the environment, living organisms have adapted to their presence. Metals with the greatest abundance have even been incorporated into enzymes, structural features, electron transport changes, and control mechanisms (muscles and nerves), resulting in some metals—iron (Fe), Cu, manganese (Mn), and Zn—being essential micronutrients to organisms. Due to their stability and long half-life, metals can accumulate through the trophic levels, with species at the top of food chains bioaccumulating much higher concentrations of metals compared to the environmental load (Burger, 2002, Hernández *et al.*, 1999, Kraus, 1989). Organisms have developed mechanisms to protect themselves against this build-up of organic and inorganic compounds. The most important for the control of toxic compounds is the antioxidant defence, the ability to detoxify and remove toxins from the body.

Metals are present in the environment in different forms and toxicity, related to their oxidative state and reactivity with other compounds (Scheuhammer, 1987, Valko *et al.*, 2005). Unlike many organic chemicals, which can easily be metabolised into less toxic compounds, metals are more stable resulting in long residence times in soils after release into the environment. This can result in harmful effects long after the initial pollution occurred (Tiller, 1989, Elliott *et al.*, 1986). Due to the relatively high density of metals, their atoms readily lose electrons to form positive ions and are therefore very reactive, becoming toxic when interfering with metabolism and biochemical reactions (Koivula and Eeva, 2010, Scheuhammer, 1987). It has been hypothesised that the toxic effects of metals in animals are in part due to metal-induced oxidative stress (Ercal *et al.*, 2001). Oxidative stress is caused by an imbalance between antioxidant defence and the production of reactive oxygen species (ROS) causing oxidative damage to biomolecules (Sies, 2015, Koivula and Eeva, 2010). Metals are split into two groups according to their function: redox-active and redox-inactive. Redox-inactive metals such as Pb, Cd, and Hg deplete the major antioxidants of cells, particularly thiol-containing antioxidants and enzymes, while redox-active metals such as Cu, Fe and Cr undergo redox cycling by catalysing Fenton reactions. Either group may cause an enhanced generation of ROS, overwhelming the cells' antioxidant defences, resulting in cell dysfunction due to lesions on proteins, lipids and DNA (Koivula and Eeva, 2010, Valko *et al.*, 2005, Ercal *et al.*, 2001).

Wild bird populations have been shown to be greatly affected by heavy metal contamination, even when exposed to sublethal levels (Scheuhammer, 1987). The main route of heavy metal uptake is ingestion, both through diet or water consumption, and to a lesser degree inhalation. Contaminant uptake by ingestion of food becomes more important for top predators and for those elements that tend to bioaccumulate (Burger, 2002, Maedgen *et al.*, 1982, Dauwe *et al.*, 2000). Waterfowl in particular are prone to heavy metal exposure due to their ingestion of grit, soil and sediment while feeding. After absorption in the acidic proventriculus, metals enter the blood stream, where individual metals show specific affinities for tissues or organs depending on whether they bind to lipids, their solubility, and the transporters within particular cell types (Burger *et al.*, 2003, Aloupi *et al.*, 2017). Metal toxicity produces physiological and biochemical disorders, which can impair reproduction (Scheuhammer, 1987), induce behavioural changes (Peakall, 1985), and increase susceptibility to disease (Eeva *et al.*, 2005). Metal pollution can also have indirect effects on wildlife by altering habitat, food and prey abundance (Frakes *et al.*, 2008), community structure (Winner *et al.*, 1980), and between-species interactions (Lefcort *et al.*, 2002).

The toxicity of most metals follows the general dose-response relationship, meaning that even essential metals can reach toxic levels. When threshold concentrations are reached, an organism's homeostatic capacity to regulate internal concentrations breaks down, resulting in the observed adverse effects that ultimately influence survival (Nordberg *et al.*, 2014, Koivula and Eeva, 2010).

However, interpretation of tissue metal concentrations and how they translate to threshold or critical values for toxicity is difficult (Furness and Greenwood, 2013, Beyer and Meador, 2011). This has led to a wide range of metal toxicity thresholds reported within the literature, suggesting that ranges, rather than a single value, are more useful in evaluating levels or effects of contamination (Aloupi *et al.*, 2017, Pain, 1996).

1.2 The triple threat; heavy metals in New Zealand waterfowl

This thesis is an evaluation of the levels of heavy metals in the waterfowl of New Zealand, principally the mallard (*Anas platyrhynchos*). Six heavy metals were identified as possible threats: arsenic (As), mercury (Hg), zinc (Zn), cadmium (Cd), copper (Cu), and lead (Pb). A pilot study was conducted which found that three of these six were of particular concern in the study populations of mallards (Appendix 1). The main body of this study will therefore focus on Pb, Cd and Cu.

1.2.1 Cadmium

Properties, uses and environmental exposure

Cd is also considered a nonessential element, has no known biological requirement in most organisms and is classified as a human carcinogen. Although widely distributed in the earth's crust, in its natural form Cd is relatively rare with an average concentration of 0.1 $mg.kg^{-1}$ (Tchounwou *et al.*, 2012). The highest levels accumulate in phosphate, argillaceous rocks, and shale, where it is deposited as greenockite (CdS) or otayite ($CdCO_3$) (Nordberg *et al.*, 2014). Cd becomes more mobile as the pH of the surrounding environment decreases, particularly at pH values between 4.5 and 5.5 (Mulligan *et al.*, 2001). Its divalent form is soluble but can also form complexes with organics and oxides and is usually associated with Pb, Zn or Cu in sulphide form. Volcanoes are a natural source of Cd, releasing it into the atmosphere and allowing it to be spread over a wide area (Fergusson, 1990b). Emissions of this trace metal increased dramatically during the Industrial Revolution, with extensive use in steel plating, pigment stabilisation and nickel-Cd batteries. Other sources include polyvinyl chloride plastic (PVC) manufacture, solders, fungicides, textile manufacturing, electroplating, rubber, and phosphate fertilisers. Cd often pollutes the wider environment through landfill leaching, industrial effluents, mining, and household wastes (Mulligan *et al.*, 2001).

By the 1990s, anthropogenic activities were estimated to contribute 2.3 times more Cd into the atmosphere than natural sources (Pacyna and Pacyna, 2001). Although only 4-6% of Cd in soil is transferred to water, the amount of Cd released into aquatic environments from human activities is estimated to be much higher than that released into the atmosphere (Nriagu and Pacyna, 1988). Once in water Cd is known to accumulate in the sediment more quickly than in biota (Burger, 2008). In recent years, its commercial use in developed countries has declined in response to environmental concerns, further enhanced by the introduction of general restrictions on Cd consumption and effluent limits in certain countries.

Uptake

Cd is more efficiently absorbed through the respiratory route (7-40%) compared to that of intestinal absorption (1-7%) (Scheuhammer, 1987), yet inhalation appears to be of less importance in contributing to the body burden of animals, unless they inhabit areas with severe air pollution (Burger, 2008). Ingestion of Cd has a dose-dependent relationship—as consumption increases so does the rate of absorption (Koo *et al.*, 1978)—although the absorption rate can also be affected by dietary essential elements. Fox *et al.* (1984) found that in Japanese quail (*Coturnix japonica*), Zn deficiency caused an increase of Cd uptake in the liver, while Cu and Fe deficiencies resulted in higher Cd in the kidneys. Deficient Ca levels have also been found to increase intestinal uptake of Cd (Koo *et al.*, 1978).

Once absorbed, Cd is bound to albumin or proteins with higher molecular weights and transported to either the liver or kidney. Here it induces the synthesis of metallothionein (MT), a low molecular weight protein involved in Cu, Zn and Cd metabolism. MT serves as protective function, binding Cd in a stable bio-complex (Nordberg, 1984), resulting in less interference with other cellular components and preventing adverse physiological effects. Elimination of Cd from organs is slow; White and Finley (1978) found that Cd levels (dietary uptake over 90 days) in the kidney, liver, and gonads of ducks did not decrease after 30 days on a Cd free diet. However, this appears to be associated with age, as Mayack *et al.* (1981) found that Cd had a half-life of 30 days in duckling kidneys. While the main elimination route of Cd is via excretion, sequestration in epidermal structurers (feathers, skin, scales, or hair), uropygial gland, and salt gland secretion (in sea birds) are also routes of elimination (Burger, 2008). Elimination of female Cd load through egg formation is debated within the literature, with evidence both for and against this occurring. Burger (1994) found in both herring gulls (*Larus argentatus*) and roseate terns (*Sterna dougallii*) that shell Cd accounts for 83% and 32% of the entire egg burden of Cd respectively. Despite this differential deposition within the egg, the low total amounts reported suggest that eggs are not an important source of Cd elimination (White and Finley, 1978).

Effects

As there are no studies observing acute Cd poisoning in wild birds, chronic dietary exposure is considered the most harmful route by which Cd affects avian health and fitness. Kidney and liver damage is considered to be the primary pathological effect of Cd toxicity, yet a number of other physiological systems can also be affected. Cd can damage the absorption cells of the digestive tract (Richardson and Fox, 1974, White *et al.*, 1978) and as a cascading effect of this, impact the uptake of nutrients such as Ca, Cu or Fe, which in turn increases the amount of Cd absorbed. Cd is a nephrotoxin, causing damage to proximal renal tubules, Bowman's capsule, and glomeruli in the kidney (White *et al.*, 1978, Mayack *et al.*, 1981). This damage can result in renal dysfunction, impairing mineral re-uptake, and osmoregulation (Bennett *et al.*, 2000).

The effect of Cd on reproductive organs and reproduction are well documented. In males, dietary Cd can cause small or atrophied testes, decreased germinal cell maturation, and an absence of spermatozoa through the reduction or inhibition of spermatogenesis (White *et al.*, 1978). This results in a major reduction in semen volume and sperm concentration, and morphological abnormalities in sperm cells. Damage to female reproductive organs are less extensive; Nolan and Brown (2000) found haemorrhagic necrosis resulting from damage to the vasculature of hens' ovaries, while Hughes *et al.* (2000) observed a non-significant reduction in ovary mass (36%) of birds fed Cd (300 µg/g) compared to control groups. The total body load of Cd in female birds is known to affect egg development and production, with egg production being suppressed in mallard hens fed at 200 ppm of dietary Cd (White and Finley, 1978) and in chickens at 60 ppm (Sell, 1975). There has also been evidence of eggshell thinning due to Cd-induced Ca reduction (Scheuhammer, 1996).

Cd can alter behaviour and activity. For example, one week old ducklings fed 4 µg/g of Cd ran farther from adverse stimuli than both the control group and ducklings fed 40 µg/g (Heinz *et al.*, 1983). Black ducks (*Anas rubripes*) also fed a diet containing 4 µg/g of Cd were significantly more active then control ducks, although activity levels returned to normal quickly after Cd exposure ceased (Silver and Nudds, 1995). This hyper-sensitivity and responsiveness in birds has been suggested to be just as harmful for birds as failing to respond. Cd-dosed willow ptarmigan (*Lagopus lagopus*) were found to have decreased brood survival compared to those dosed with saline, suggesting that Cd can negatively impair parenting ability, although the mechanism behind this is unknown (Pedersen *et al.*, 2006).

Toxicity thresholds

Interpretation of tissue concentrations of Cd are not as well documented as Pb, however adults of most healthy waterfowl have liver Cd concentrations of <0.9 $mg.kg^{-1}$ w.w. (<3 $mg.kg^{-1}$ d.w.), which is considered a threshold for increased environmental exposure (Scheuhammer, 1987). Hepatic concentrations ranging between 2-3 $mg.kg^{-1}$ w.w. (7 -10 $mg.kg^{-1}$ d.w.) have been related to sublethal adverse effects on renal function and energy metabolism (White and Finley, 1978). Concentrations of >40 $mg.kg^{-1}$ w.w. (150 $mg.kg^{-1}$ d.w.) are consistent with clinical poisoning (Furness, 1996). There is little information available on toxic thresholds of Cd concentrations in other tissues, although White and Finley (1978) noted that the lowest Cd concentration in blood that was associated with adverse effects in adult mallards was 0.26 $\mu g.g^{-1}$. There is some evidence to suggest that juvenile birds could be more sensitive to Cd; blood values as low as 0.048 $\mu g.g^{-1}$ have been seen to induce adverse effects in the growth of pheasant chicks (*Phasianus colchicus*) (Świergosz and Kowalska, 2000), compared to the higher levels seen in adults of the same species (Swiergosz, 1991).

Cadmium in New Zealand

In New Zealand, industrial exposure to Cd is rare, and the main sources of human uptake are tobacco products and food (Järup and Åkesson, 2009). However, historical use of phosphate fertilisers have resulted in high soil concentration of Cd throughout New Zealand (Cavanagh *et al.*, 2015). Taylor (1997) found that within a 50-year time period (1947–1997) the Cd soil load in New Zealand increased from 0.39 $mg.kg^{-1}$ to 0.85 $mg.kg^{-1}$ due to fertiliser use, with over 80% of the added Cd remaining in the topsoil. Accumulation rates within soil vary between regions (Figure 1.2b) due to differences in land use (Figure 1.2a), source location of phosphate rock used in fertiliser, soil type and pH, as well as climate. Prior to the 1990s phosphate fertiliser was manufactured predominantly from Nauru Island phosphate rock, which is Cd rich. This was greatly reduced after the 1995 introduction of voluntary limits on Cd levels in phosphate fertilisers, initially set at 340 mg Cd/kg then reduced further to 280 mg Cd/kg by 1997 (Cavanagh *et al.*, 2013). A 2007 report found that the national New Zealand soil average Cd level was 0.35 $\mu g.g^{-1}$, which increased to 0.50 $\mu g.g^{-1}$ in horticulture areas, and 0.73 $\mu g.g^{-1}$ in pastoral land used for dairying. New Zealand Cd levels compared to undisturbed land can be seen in Figure 1.3.

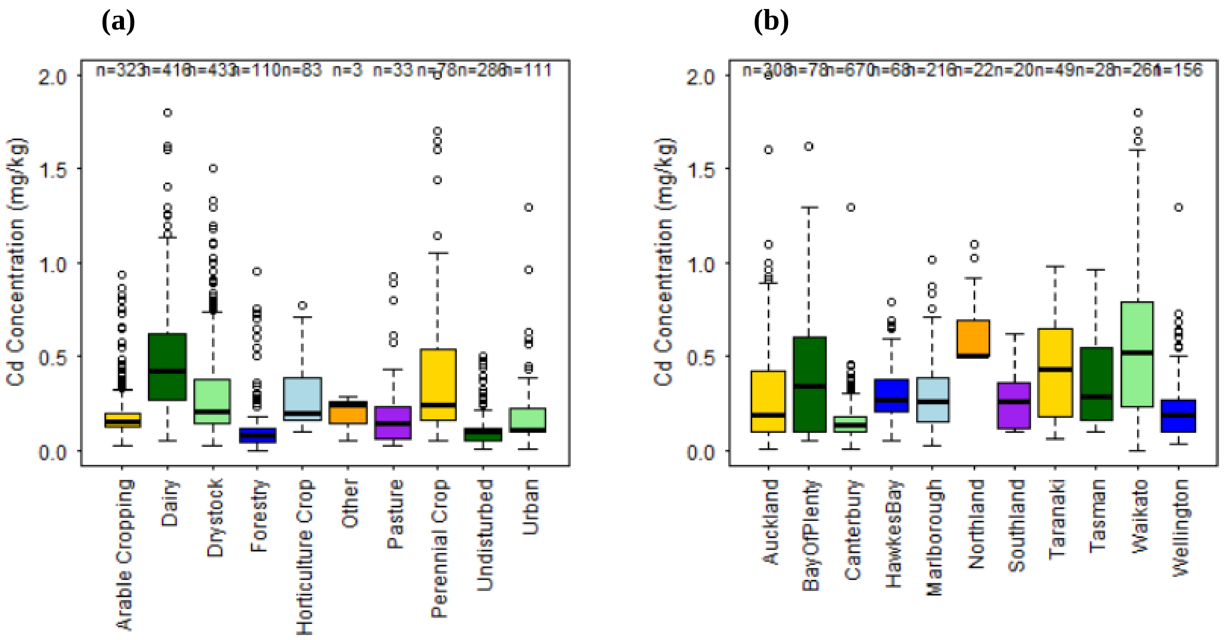


Figure 1.2 Boxplots of cadmium (Cd) soil concentrations from regional council datasets grouped by a) land use and b) region. Whiskers represent maximum and minimum observations within 1.5 x interquartile range. Source Cavanagh et al. (2015)

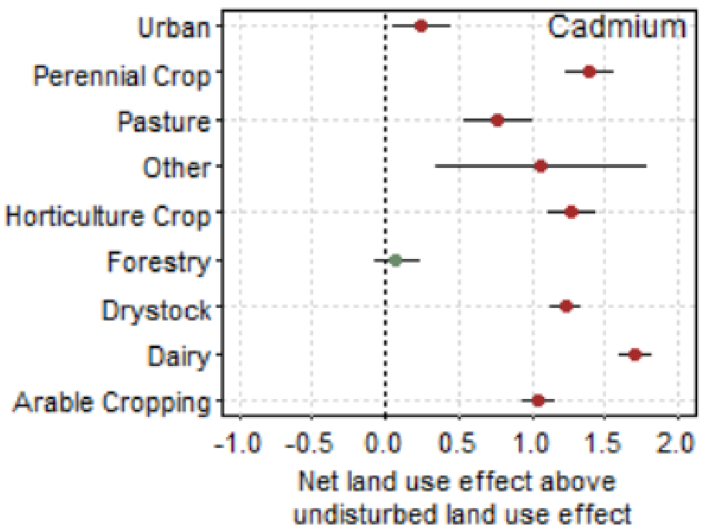


Figure 1.3 Differences in Cd soil concentrations by different land uses in relation to undisturbed land use class for the regional council dataset. 0.0 represents no difference from undisturbed land use, lines are the 95%CI of the mean. Source (Cavanagh et al., 2015)

1.2.2 Copper

Properties, uses and environmental exposure

Cu is an essential trace element, found in small amounts in all organs and cells, with the highest concentrations found in the liver (Nordberg *et al.*, 2014). Cu is involved in many biological processes, serving as an essential cofactor for several oxidative stress-related enzymes including; antioxidant defence (superoxide dismutase and catalase), cellular respiration (cytochrome c oxidases), neurotransmitter biosynthesis (dopamine β-monooxygenase), Fe homeostasis (ferroxidases), and inactivation of neurotransmitter (monoamine) (Nordberg *et al.*, 2014, Stern, 2010). It is an essential nutrient incorporated into a number of metalloenzymes involved in carbohydrate metabolism, haemoglobin formation, catecholamine biosynthesis, and cross-linking of collagen, elastin, and hair keratin (Tchounwou *et al.*, 2008). Cu can exist in both oxidised (cupric, Cu^{2+}) or reduced (cuprous, Cu^{+}) states, and is used by cuproenzymes involved in redox reactions. However, it is this property of Cu that also makes it potentially toxic. The transitions between Cu^{2+} and Cu^{+} can result in the generation of superoxide and hydroxyl radicals resulting in oxidative stress; if these ROS are not detoxified efficiently, susceptible cellular components can be damaged (Camakaris *et al.*, 1999). In addition, the high affinity with which Cu can bind to certain residues of proteins such as methionine, histidine and cysteine can result in their inactivation.

Cu is relatively abundant in the earth's crust, with concentrations of 4–150 ppm in soils. Large reserves found in Africa, China, and South America are mined for Cu ore which is passed through extensive concentration processes to produce Cu (Sverdrup *et al.*, 2014). Phosphates used in the production of phosphate fertilisers can also contain significant amounts of Cu. Due to its high conductivity Cu is used in electric wires, motors, and cables for electricity transmission. Other uses include water piping, roofing material, and nanoparticles. Cu compounds are of particular importance, used in wood preservatives and fungicides, as nutritional additives to livestock feed, and as additives to fertilisers (Nordberg *et al.*, 2014). While volcanos, decaying vegetation, and forest fires are important natural sources of Cu into the environment, anthropogenic sources such as industrial and mining effluent, domestic waste water, coal consumption, and phosphate fertiliser production make up the bulk of environmental releases (Georgopoulos *et al.*, 2001). Cu-based algaecides are also used in freshwater systems to control algal blooms and contribute to sediment loading (Salam and El-Fadel, 2008, Tsai, 2016).

Uptake

Ingestion is considered the main route of Cu absorption, with 30–50% of dietary Cu absorbed in the small intestine of mammals, and very small amounts absorbed in the stomach

(Turnlund *et al.*, 1997). A similar pattern is seen in birds, with a greater amount of absorption occurring in the duodenum compared to the proventriculus (Starcher, 1969). Once absorbed Cu is transported in the blood, bound generally to albumin or transcuprein, and either stored within hepatocytes, secreted into plasma, or excreted in bile (Gaetke *et al.*, 2014). Cu stored in the liver is predominantly bound to MT, or to a lesser extent synthesised into cuproenzymes (proteins dependent on Cu). MT may bind intracellular ionic Cu, in a similar role to MT-bound Cd, to prevent cytotoxicity and serve as an antioxidant (Nordberg *et al.*, 2014). Cu released from the liver is primarily bound to ceruloplasmin, an acute phase protein, where approximately 60–90% of the total body load of Cu is situated for transport to tissues (Turnlund, 1998). It was estimated that only 0.04% of Cu released into the environment is released into the atmosphere, thus inhalation of Cu is not considered a major route of uptake (Georgopoulos *et al.*, 2001).

Effects and toxicity thresholds

Acute Cu toxicity is well documented in sheep, where it is estimated that 20–100 mg/kg of ingested Cu can result in death within 24–48 hours (Winge and Mehra, 1990). Bubien *et al.* (1971) found that a single dose of 400 $mg.kg^{-1}$ given to male chickens was lethal within 48 hours, while 80 $mg.kg^{-1}$ per day was lethal after 10 – 34 days, and 45 $mg.kg^{-1}$ mg was tolerated without any observed damage.

Chronic Cu toxicity predominantly affects the liver, as this is the first site of deposition after bodily uptake. The pathology of Cu toxicity typically manifests as development of liver cirrhosis, which can lead to haemolysis, damage to renal tubules, and necrosis in kidneys and the brain (Gaetke *et al.*, 2014). Cu poisoning in wildlife can result in clinical signs of lethargy, weakness and anorexia in early stages, as well as pathology including erosion of the epithelial lining of the gastrointestinal tract, hepatocellular necrosis in the liver, and acute tubular necrosis in the kidney (Camakaris *et al.*, 1999, Tchounwou *et al.*, 2008). As the poisoning continues, pathology can worsen to include complete hepatic necrosis and vascular collapse resulting in coma and death (Winge and Mehra, 1990). Cu is given as a dietary supplement to broiler hens to increase growth, weight gain and for disease control, however studies show that high levels of Cu in layer hens can induce a reduction in egg production (Griminger, 1977), gizzard erosion (Fisher *et al.*, 1973), and yolk lipid concentration (Pesti and Bakalli, 1998).

Thresholds for toxic tissue concentrations of Cu have not been established for many wild waterfowl species, although field-collected studies usually have liver Cu concentrations of < 100 $mg.kg^{-1}$ w.w. (Isanhart *et al.*, 2011, Di Giulio and Scanlon, 1984a, Kalisińska *et al.*, 2004). Acute Cu toxicosis was observed in mallards exposed to synthetic acid metalliferous water, where hepatic concentrations of 81–391 mg.kg^{-1} w.w. were recorded (Isanhart *et al.*, 2011). It has been suggested that waterfowl species may be more susceptible to accumulating Cu than other species, as ducklings had a rapid uptake of Cu in the liver and much higher levels (~190 mg.kg^{-1} d.w. / ~50 mg.kg^{-1} w.w.) than chickens (~15 mg.kg^{-1} d.w. / ~4 mg.kg^{-1} w.w.) on the same diet (Beck, 1961). It is therefore noteworthy that liver concentrations of some wild canvasback ducks (*Aythya valisineria*) in Louisiana (Custer and Hohman, 1994) and wild mute swans (*Cygnus olor*) from England (Bryan and Langston, 1992) have been found to have hepatic concentrations of more than 500 mg.kg^{-1} w.w..

Copper in New Zealand

As New Zealand is a temperate country with organic-rich and sandy soils, Cu deficiency is a concern for both farmers and crop growers. An FOA (Food and Agriculture Organisation of the United Nations) global study found that a relatively high proportion of sites sampled in New Zealand were potentially Cu deficient (Sillanpää, 1990). Yields of crops such as wheat and barley, can be up to 20% lower when grown in Cu-deficient soils, while the dairy and meat industries report a reduction in animal condition and production under low Cu environments (Alloway, 2008). As low uptake by pasture plants is known to be a common source of Cu deficiency in cattle, farmers increase the planting of grasses such as chicory, over the traditional ryegrass, for its ability to uptake high Cu quantities (Liao *et al.*, 2000). Additionally livestock, especially dairy cows, are fed a combination of supplementary mineral blends (~370mg of Cu per cow per day) and palm kernel extract (~22mg per kg of dry matter) to increase uptake (Johnston *et al.*, 2014). Thus application of Cu fertilisers appears necessary for these industries, and although this may be well controlled, it can result in environment contamination. Cu-cladding contamination of storm water resulted in high levels of Cu being released into river ways surrounding Christchurch, New Zealand, during the post-2011 earthquake rebuild of the city (Adrian *et al.*, 2015).

The average Cu concentration in New Zealand non-polluted soil is 20 mg.kg^{-1}, while contaminated regions can range 100–800 mg.kg^{-1} (Roberts *et al.*, 1996). Soil levels vary (Figure 1.4b) depending on pH, cation exchange capacity, soil type, land use (Figure 1.4a), interaction with other elements, and organic matter content. New Zealand Cu levels compared to undisturbed land can be seen in Figure 1.5.

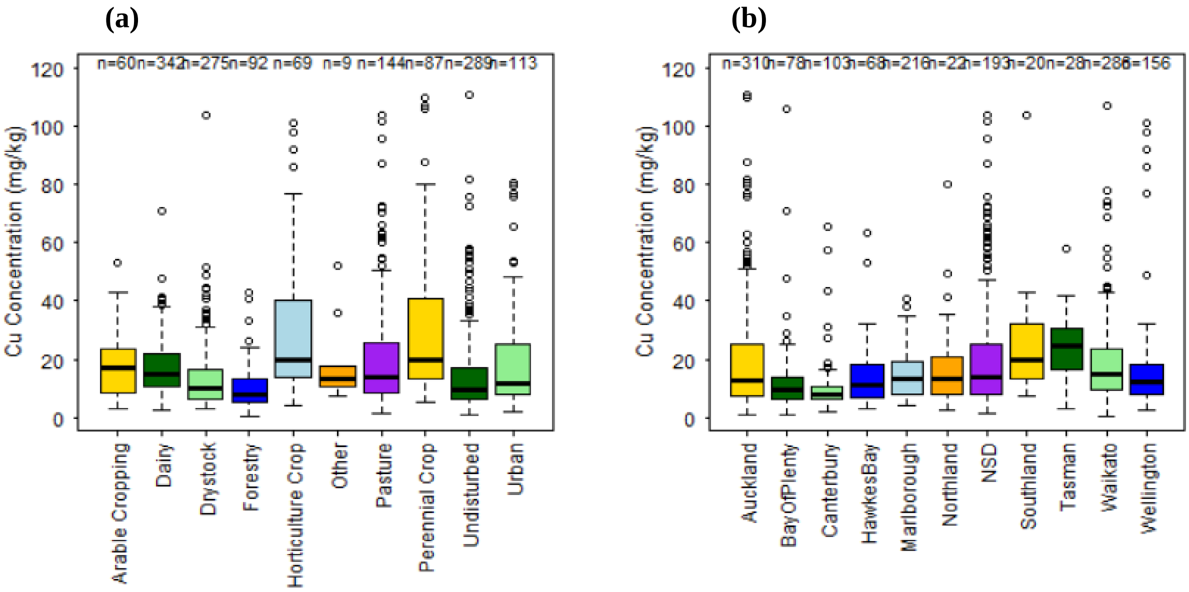


Figure 1.4 Boxplots of copper (Cu) soil concentrations from regional council datasets grouped by a) land use and b) region. Whiskers represent maximum and minimum observations within 1.5 x interquartile range. Source Cavanagh et al. (2015)

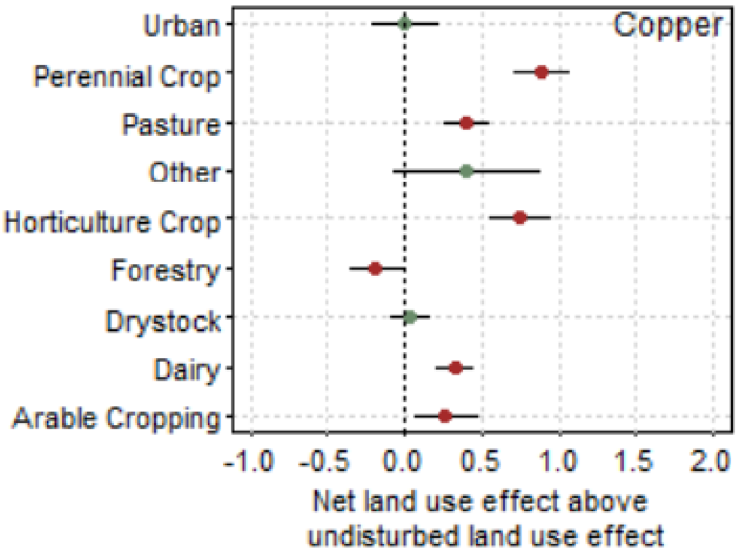


Figure 1.5 Differences in Cu soil concentrations by different land uses in relation to undisturbed land use class for the regional council dataset. 0.0 represents no difference from undisturbed land use, lines are the 95%CI of the mean. Source (Cavanagh et al., 2015)

1.2.3 Lead

Properties, uses and environmental exposure

Pb is considered to be a nonessential element as it has no known biological requirement in most organisms. It is a highly toxic pollutant that acts as a nonspecific poison affecting all body systems (Needleman, 2004). Pb has been used by humans for centuries, even being blamed for the down fall of the Roman Empire due to their excessive use of Pb in pipes, wine, and paint (Hernberg, 2000). Pb use intensified during the Industrial Revolution, to the point where anthropogenic emissions saturated the environment (Demayo et al., 1982). As a result of these emissions, most animal populations have a higher body burden of Pb compared to their pre-industrialised ancestors (Franson and Pain, 2011). Despite very low tissue levels having some measureable physiological effects, the post-industrial increase in concentration has generally not been considered to directly affect the survival of most wildlife. Since the 1970s there has been a marked decrease in Pb exposure in humans as a result of multiple efforts, including the removal or reduction of Pb in gasoline, paint, food and plumbing systems (Tchounwou *et al.*, 2012).

There are two main pathways in which Pb can enter the environment: non-particulate Pb exposure, or via spent Pb ammunition and fishing weights. Non-particulate Pb exposure occurs from the ingestion of contaminated sediment, especially from locations near mines and smelters, and the consumption of Pb-contaminated animal tissues (Gómez *et al.*, 2004, Beyer and Day, 2004, Beyer *et al.*, 1998, Henny *et al.*, 1994). Airborne sources within urban environments have also caused an increase in Pb contamination within bird populations (Johnson *et al.*, 1982, Ferreira-Baptista and De Miguel, 2005, Scheifler *et al.*, 2006, Swaileh and Sansur, 2006, Ohi *et al.*, 1981), as has ingestion of chipped Pb-based paint (Finkelstein *et al.*, 2003). Pb poisoning due to ingestion of spent Pb ammunition has resulted in widespread avian mortality worldwide (Scheuhammer and Norris, 1996). Due to the concerns for both avian and subsequent human health, restrictions on Pb shot have been implemented in more than 29 countries (Avery and Watson, 2009). Most countries, including New Zealand, restrict the use of Pb shot in waterfowl hunting and around waterways and wetlands, while some countries have banned the use of Pb shot completely (Avery and Watson, 2009).

Uptake and effects

Following solubilisation in the acidic proventriculus, Pb is absorbed into the bloodstream via the intestinal wall, before becoming deposited into the bone and soft tissues of the body, predominantly the liver and kidney. One of the major mechanisms by which Pb causes toxic effects is through biochemical processes that allows it to mimic calcium (Ca) and interact with proteins

(Bressler and Goldstein, 1991). Pb binds to important enzymes (primarily sulfhydryl groups) and biological molecules, interfering with their functions and in some cases inactivating them (Nordberg *et al.*, 2014). Many studies show that Pb induces cellular damage mediated by the formation of ROS, unbalancing the activities of antioxidant enzymes (Koivula and Eeva, 2010). Pb toxicity affects all organ systems, but is most known for its damage to the nervous, digestive, and circulatory systems. As Ca is needed for cell signalling in nerve impulse transmission, Pb interferes with functions that depend on nerve conduction, such as learning, blood pressure regulation, and muscle contraction (Pokras and Kneeland, 2009). In the blood, Pb can interfere with the function of haemoglobin, limiting oxygen to organs (Ercal *et al.*, 2001), and the formation of new red blood cells in the bone marrow, resulting in anaemia. The end point for most absorbed Pb is the skeleton, where it is incorporated in place of Ca. This store of bone Pb can be mobilised when the body requires Ca (growth, eggshell formation, healing, or dietary imbalances) and can therefore cause chronic poisoning (Needleman, 2004).

Exposure to Pb can result in acute or chronic poisoning, depending on the dose. Laboratory experiments found that if there has been a large ingestion of shot (> 10 pellets), acute poisoning rapidly ensues (Jordan and Bellrose, 1951) and the bird may die within a few days. More commonly, however, birds die of chronic poisoning following the ingestion of only a few pellets (Scheuhammer and Norris, 1996). The effects of chronic Pb toxicosis include distension of the proventriculus, weight loss, anaemia, renal failure and drooping posture. These clinical signs of toxicity appear gradually and death may take up to 3 weeks (Bellrose, 1959, Friend *et al.*, 1987). Sublethal exposure will also be responsible for many unattributed deaths, as decreased immune function, and damage to tissues, central and peripheral nervous systems, as well as the renal and circulatory systems result in biochemical, physiological and behavioural impairments (Scheuhammer, 1987). These impairments contribute to an increased risk of predation, starvation, and disease, leaving contaminated birds with an impaired ability to cope with potential threats.

Toxicity thresholds

There are well-established thresholds for the severity of Pb concentration in tissues based on consequent impacts (Table 1.1). Poisoning can be divided into three categories based on observed effects: subclinical, clinical, and severe clinical poisoning. Subclinical poisoning is defined as levels that cause physiological effects but are insufficient to impair biological functions, resulting in no outward signs of poisoning, and with the animal having a high chance of recovering if exposure ceases. Clinical poisoning is defined as the threshold where pathological manifestations of physiological effects are observed, such as anaemia, muscular incoordination, anorexia, and microscopic lesions in tissues. Without exposure removal, concentrations at this level will result in probable death. Severe clinical poisoning is defined as an approximate threshold at which levels become directly life-threatening. Any value below these levels is considered ‘background’, meaning evidence of environmental exposure not linked to a specific source of contamination.

Lead in New Zealand

Pb ammunition is one of the most common sources of Pb within humans and wildlife in New Zealand. Pb shot was banned in 2001 when hunting waterfowl using a 10 or 12-gauge cartridge for all areas within 200 m of a body of water or wetland, and this will be expanded in 2021 to include all sub-gauge shotguns. Entry of Pb ammunition into the environment is still possible through hunting of species not declared as ‘game’. For example, there are no restrictions for the use of Pb shot in wetland environments for species such as Canada geese (*Branta canadensis*), and Pb can still be used over land. The compliance rate in regard to the use of non-toxic Pb shot alternatives is another potential issue. Ranging activities conducted by Fish and Game in the Waikato indicate that on average around 90% of hunters are compliant, but this can be variable between years and location with some areas exhibiting much higher rates of Pb shot use (D. Klee, Auckland/Waikato Fish and Game, pers. comm.). Ministry of Health reports indicate that Pb-based paint as the major source for Pb toxicity in New Zealand humans, followed by exposure at rifle ranges, and ammunition (Xu, 2016). The majority of Pb in New Zealand soils is due to anthropogenic sources: mining and smelting, vehicles, and urban waste, which contaminates stormwater. Soil levels depend on the chemical composition of the Pb, soil composition and the pH, resulting in different regional (Figure 1.6b) levels based on land use (Figure 1.6a) and levels compared to undisturbed land (Figure 1.7)

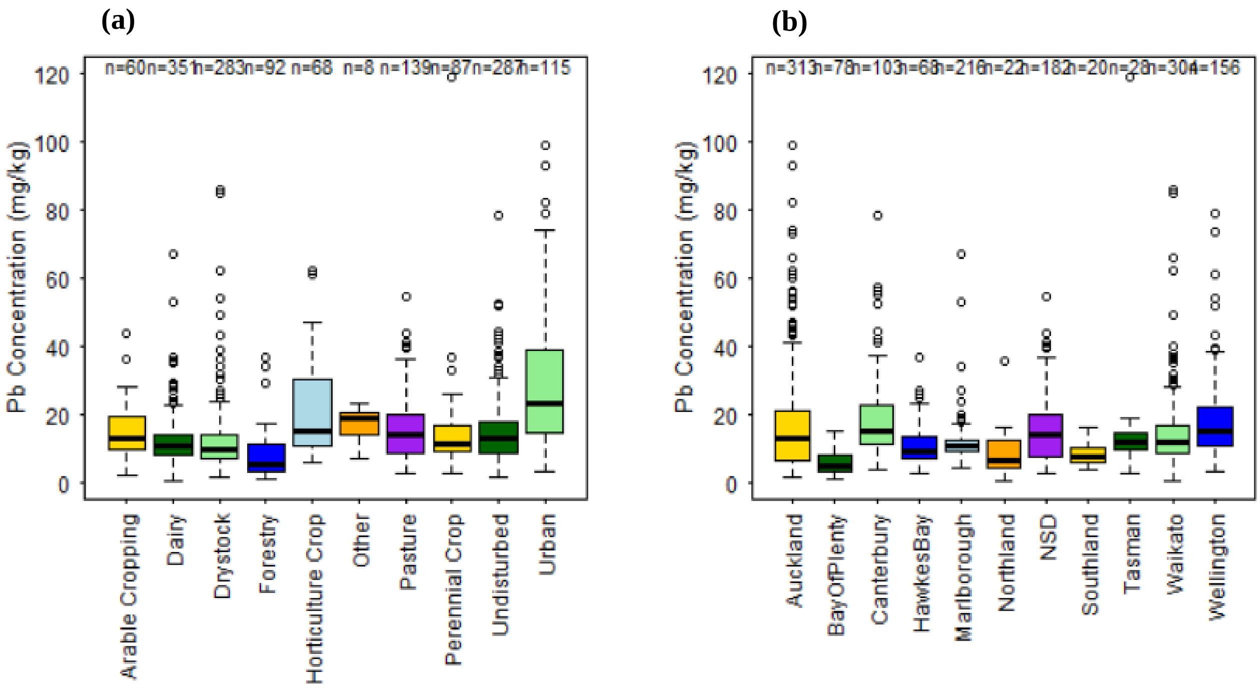


Figure 1.6 Boxplot of lead (Pb) soil concentrations from regional council datasets grouped by a) land use and b) region. Whiskers represent maximum and minimum observations within 1.5 x interquartile range. Source: Cavanagh et al. (2015).

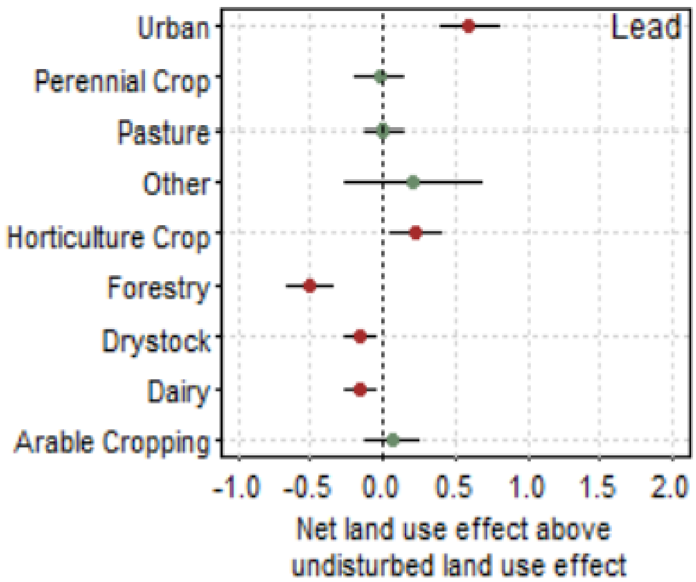


Figure 1.7 Differences in Pb soil concentrations by different land uses in relation to undisturbed land use class for the regional council dataset. 0.0 represents no difference from undisturbed land use, lines are the 95% CI of the mean. Source: (Cavanagh et al., 2015)

1.3 Waterfowl as bioindicators

The need for bioindicators

Although the presence of eco-toxicants in the environment can be determined by chemical abiotic measurements, this often gives little information about the bioavailability of the toxins. Biomonitoring of a sentinel species has become an established method to quantify heavy metal abundance and bioavailability, as pollutants accumulate in animals to levels much higher than those of the surrounding environment (Furness, 1993). Care in the selection of indicator species representative of the considered ecosystem is important to adequately assess ecosystems health, as some species have biological habits that increase their likelihood of exposure (Battaglia *et al.*, 2005). The accumulation of metals in certain species may increase with age and with each succeeding level of the food chain (Burger, 2002). Due to this, there has been a decided bias in the literature to use long-lived species from high trophic levels, such as birds (Furness, 1993).

1.3.1 Birds as bioindicators

Birds are abundant, widely distributed and, in some species, long-lived. They offer a number of advantages as indicator species – their high trophic level allows for the monitoring of the pollutants through the food chain, and their sensitivity allows for the study of many diverse factors affecting their food chains. Birds at high trophic levels, such as raptors or owls, can yield information over a large area, shedding light not only on the bioavailability of the pollutants but also on their pathway through the food chain (Jager *et al.*, 1996, Battaglia *et al.*, 2005, Pérez-López *et al.*, 2008). Migratory species can also be used to monitor both local ecosystems, and to compare exposure in different regions (Pereira *et al.*, 2009). However, the most compelling reasons for the use of birds are quite pragmatic: they are easy to identify, respond relatively rapidly to contamination events, and large amounts of data have already been collected (O'Halloran *et al.*, 2003, Taggart *et al.*, 2009).

Several traits of waterbird biology make them particularly useful as bioindicators. Firstly, they are predominately high trophic level feeders, which allows monitoring of pollutant movement through the environment and may indicate changes at lower trophic levels (Tsipoura *et al.*, 2011, Zhang and Ma, 2011, Burger and Eichhorst, 2005, Strand and Jacobsen, 2005). Secondly, waterbirds have been seen to track environmental variations at both species and community levels, over both short (months) and long (years) temporal scales (Abraham and Sydeman, 2004, Rendón *et al.*, 2008, Almaraz and Amat, 2004, Nudds, 1983, Henry and Cumming, 2016, Kingsford *et al.*, 1999, Lehikoinen *et al.*, 2013). Thirdly, waterbirds and their prey are exploited by humans (either for hunting or fisheries), implying an open pathway for contaminant transfer from the environment into humans with possible health implications (Guitart *et al.*, 2002, Scheuhammer *et al.*, 1998, Binkowski, 2012).

Numerous waterfowl species are considered convenient bioindicators of environmental contaminant pollution (Kalisińska *et al.*, 2004, Scheuhammer, 1987, Parslow *et al.*, 1982, Kim and Oh, 2012). Waterfowl, especially dabbling species, are particularly susceptible to exposure to metals due to the ingestion of soil and sediment that aids in gizzard digestive function. Species in heavily contaminated areas show a marked increase in body burdens of heavy metals. For example, waterfowl are particularly susceptible to pollution from mining waste (Beyer *et al.*, 1998, Beyer *et al.*, 1999, Hernández *et al.*, 1999), spent Pb shot (Pain, 1996, Pain *et al.*, 1992, Scheuhammer *et al.*, 1998) and fishing weights (Sears and Hunt, 2013). Species that are easily identifiable, have distinct sexual dimorphism, are relatively long-lived, and have a wide geographical range are particularly useful as indicators. The domestic duck and captive mallards have been subject to many experimental heavy metal toxicity studies (Cain *et al.*, 1983, Di Giulio and Scanlon, 1984a, White *et al.*, 1978, Johnson, 1961). Due to this extensive research not only is the physiology of both species is well understood, toxic thresholds and ranges of essential metals in regards to both age and sex are known (Scheuhammer, 1987). As a result, most metals have well established toxicity threshold ranges that allow the effects of heavy metal contamination observed in wild studies to be interpreted.

1.3.2 Effect of age

There are differences in concentrations of heavy metals between different age classes; young or immature organisms have lower tissue concentrations than that of adults or mature organisms. These age differences exist as certain contaminants accumulate in internal tissues, with uptake levels exceeding the amounts excreted, causing bioaccumulation of heavy metals to occur over time. Additional explanations for age-related variances can depend on the species, diet switching, the specific pollutant, and the ability of individuals to eliminate or excrete the metals differently (Burger and Gochfeld, 1997a). However, juveniles can also be more sensitive to toxic elements, especially Pb. Exposure to Pb, even at low doses, has been found to affect behavioural, physiological and intellectual development in juvenile humans (Gibb and MacMahon, 1955) and other animals (Burger and Gochfeld, 1993). For example, recognition of parents was delayed in herring gull (*Larus argentatus*) chicks exposed to Pb at 2–4 days, but not those exposed at 12 days of age (Burger and Gochfeld, 1993). Delayed parental recognition can be lethal, as once chicks begin to explore the surrounding area they can be killed by neighbouring adults if they approach a gull other than their parent. This result suggested the timing of exposure to Pb in relation to neurological developmental events may be as important as overall dose.

1.3.3 Effect of sex

Sex plays a role in the physiology, morphology and behaviour in organisms, which in turn can influence the uptake, tissue distribution, and effects of pollutants. Differences between sexes and the accumulation of contaminants can be due to either ecological or physiological differences in metabolism or distribution. Ecologically, there is a feed-back loop in that sex-differences in size, morphology, and behaviour can lead to niche differences in foraging, breeding, and even migratory behaviour, resulting in different rates of exposure (Burger *et al.*, 2004). While sex affects exposure and susceptibility, it can also influence the ability to eliminate the total body load of toxins. Although most methods of excretion are similar for both sexes, females can eliminate contaminants through off-loading into egg and embryo development. This extra pathway may result in lower tissue concentrations in reproductively active females but may also come with the reproductive cost of embryonic exposure to toxins (Burger, 2007). Sexes may also differ in their production of various metalloproteins that play fundamental roles in the transport, storage, and excretion of metals.

1.3.4 Tissues storage

There is variation between the deposition of different heavy metals in different soft tissue compartments of the body. An overview of hepatic and blood critical toxicity threshold concentrations cited from the literature from the three metals examined in this study are listed in Table 1.1.

Liver

Livers are frequently used as a sample for bioindication purposes, with tissue concentrations used to both identify the cause of toxicity in animals, and as a measure of the severity of the exposure. Livers are considered good indicators of environmental contamination due to the role this organ plays in detoxification of the body, with some elements are primarily accumulating here, such as Cu and Fe. While the kidneys can accumulate Cd in higher levels, the liver is considered the best measure of exposure as the Cd content is more stable compared the kidney, and it is more resistant to Cd toxic effects (Scheuhammer, 1987). A major drawback of liver use is the seasonal change in mass, which can alter the observed pollutant concentration even though the total body load is unchanged (Furness and Greenwood, 2013). Liver samples from live birds can be taken by biopsy under anaesthesia, but this is technically demanding, with a high incidence of complications (Mulcahy and Esler, 2010). Therefore, most liver sampling for bioindicator studies is done at post-mortem examination.

Blood

Blood is the preferred tissue choice for non-destructive biomonitoring when determining the recent exposure to contaminants. It is obtained quickly and easily without a high risk of permanent damage. Another benefit is that repeated samples can be taken from individuals, so exposure in response to life stage, reproductive activities, and seasonal effects can be monitored to a certain degree. Absorption of metals from the gut following ingestion normally occurs within 24 hours, where they are transported through the bloodstream and deposited in tissues (Hoffman *et al.*, 1981). Stored metals are also released from soft tissues and eliminated from the body by either excrement, deposition into eggs, or feather formation during moult (Pain *et al.*, 2005, Dolci *et al.*, 2017, Metcheva *et al.*, 2011). Therefore, blood samples reflect dietary exposure, but also the redistribution and elimination of trace metals from internal storage (Mason *et al.*, 2014). Blood collection therefore only provides an insight into the circulating amount of metals at time of sampling, rather than a complete picture of full body burden. However, studies have reported an asymptotic relationship between the concentration of both Pb and Cd in liver and blood, suggesting that blood could be a useful indicator of certain metals (Benito *et al.*, 1999, Maia *et al.*, 2017, Berglund, 2017). It should be noted that due to differences in storage and retention time in hard and soft tissue, the concentration in bloods may underestimate the total body load, especially in highly contaminated environments (Berglund, 2017). However, even with this underestimation, blood can still indicate a risk of accumulation and toxic effects in birds (Pain, 1996, Wayland and Scheuhammer, 2011).

Eggshell

Egg contents and shells are considered to be good bioindicators for some heavy metals, especially in long-term studies of geographical, geochemical, and temporal trends in environmental contamination. This is due to their reflection of female exposure to the local environmental load of trace elements (Burger, 1994), which can be eliminated by sequestration in eggs. Metals excreted to eggs derive from both stored body burden, and food choice of the female during egg formation (Burger and Gochfeld, 1993). The rate of transfer of contaminants is thought to be related to the body load of the female and ultimately to the exposure of pollutants in the female’s environment (Dauwe *et al.*, 2005). However certain metals, such as Cd and Pb, have been shown to transfer very little in waterfowl and seabirds, with only baseline exposure levels reported in eggs of birds exposed to high levels of contaminants (Furness and Greenwood, 2013). A controlled experiment in mallards found Cd concentrations in eggs were low regardless of the amount consumed by laying hens (White and Finley, 1978). While this is a major disadvantage for environmental monitoring, the use of eggs allows us to estimate the amount of pollution passed to the subsequent generations and possible routes for contamination to affect chicks.

Table 1.1 An overview of critical, threshold, or observed levels regarding metal toxicity in Anseriformes within the literature

Metal Tissue	**Species**	**mg/kg w.w.**	**Significance**	**Reference**
Pb				
Liver	Anseriformes	2 < 6	Subclinical poisoning	(Franson and Pain, 2011)
		6–10	clinical poisoning	
		> 10	Severe clinical poisoning	
Blood	Anseriformes	0.2 < 0.5	Subclinical poisoning	(Franson and Pain, 2011)
		0.5–1.0	clinical poisoning	
		> 1.0	Severe clinical poisoning	
Cd				
Liver	Mallard	< 0.9	Background levels	
		> 0.9	Elevated above environmental levels	(Scheuhammer, 1987, Di Giulio and Scanlon, 1984a)
		> 2.1	Change in energy metabolism	(White and Finley, 1978, Di Giulio and Scanlon, 1984a)
Blood	Mallard	> 0.26	Adverse effect reported	White and Finley (1978)
Cu				
Liver	Anseriformes	> 100	Accepted elevated threshold	(Taggart *et al.*, 2006)
	Mallard	81–391	Acute Cu toxicosis	(Isanhart *et al.*, 2011)
	Canada geese	56–97	Acute Cu toxicosis	(Henderson and Winterfield, 1975)
Blood	Mallard	2.73	Medium level reported in polluted Polish area.	(Binkowski and Meissner, 2013)
	Mallard	0.26	Mean found after toxic spill	(Benito *et al.*, 1999)

1.4 General Introduction

1.4.1 Research objectives

The aims of this thesis are to determine the concentrations of certain heavy metal contaminants in selected waterfowl populations in New Zealand by:

1. Quantifying the levels of Cd, Cu and Pb in two spatially discrete mallard populations, determined by:
    i. Liver concentrations from hunter-shot birds
    ii. Blood concentrations from samples collected pre-breeding season (June and July) and post-breeding season (January and February)
    iii. Eggshell concentrations collected from females caught for pre-breeding blood sampling
2. Establishing the percentage of mallards carrying embedded and ingested shot (Pb and steel) through examination of the gizzards of hunter-shot birds.
3. Investigating the exposure of native wetland and waterfowl species to heavy metal contamination by determining liver heavy metal concentrations from historical and fresh samples sent to the Massey University Wildbase for post-mortem examination.

1.4.3 Study Species

Mallards are a near-cosmopolitan species and are one of the most widely distributed duck species. Numerous researchers have considered them as good indicator species especially for heavy metal pollution, as their biology is well understood, they are considered high trophic level foragers, and they are of economic importance to humans through hunting. In New Zealand the sale of game bird licences alone generates ~$2.6 million annually (D. Klee, Auckland/Waikato Fish and Game, pers. comm.). As mallards are so widely distributed, comparative data exist on a range of heavy metal tissue and blood concentrations in various parts of the world. Although migratory within their native range (North America and Europe), mallards in New Zealand tend to be sedentary (Balham and Miers, 1959), with 85% of band returns being within 50 km of the banding site (McDougall, 2012). There is minimal genetic exchange between North and South Island populations (Guay *et al.*, 2015).

Mallards of European origin were first introduced to New Zealand in the 1867 and although not particularly successful, several distinct populations were established by the turn of the century (Nichols *et al.*, 1990). While populations continued to expand in number and range, the Auckland Acclimatisation Society imported a number of mallards from North America in the 1930s, which lead to the rapid growth and expansion of the New Zealand population. There are two major concerns involving mallards in New Zealand. Firstly, they hybridise with the native grey duck (*Anas superciliosa superciliosa*) to the degree that complete integration of the two species is now assumed and they are affectionately termed "grallards" by researchers (Dyer and Williams, 2010). Secondly, total national harvest of mallard has declined over the past decade, with populations in certain regions fluctuating. In general, the North Island population appears to be lower now than in previous decades. There are many suspected causal factors behind these population changes including climatic conditions, habitat degradation and destruction, over-hunting, waterway degradation, and land use change. The role of pollution, and in particular heavy metal contamination, as a factor in these declines has yet to be explored.

1.4.4 Structure of thesis

This thesis is organised into five chapters – this introductory chapter, three independent research chapters that are intended for publication, and a concluding general discussion. The scope of the research chapters includes:

> Chapter Two – The concentrations of Cd, Cu, and Pb in blood and eggshells of mallards.

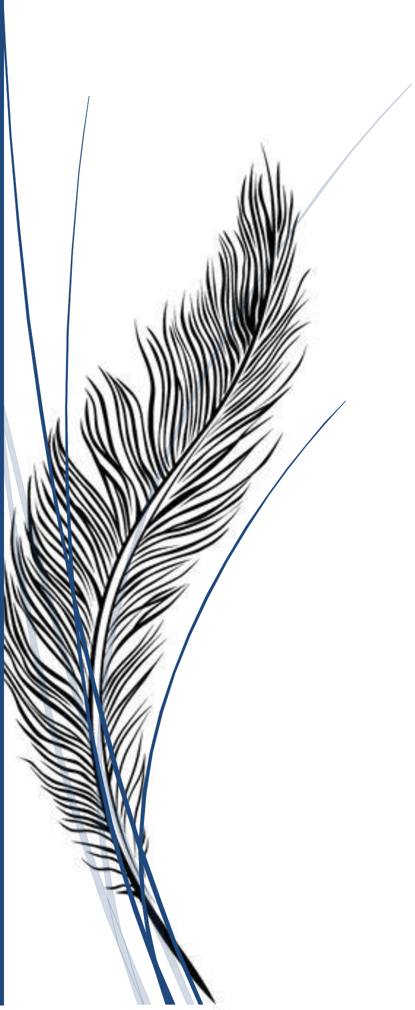
> Chapter Three – Examination of internal tissues of mallards post-mortem, for both the heavy metal concentration of Cd, Cu, and Pb stored within the liver and the rate of ingested shot in the gizzard.

> Chapter Four – The analysis of concentrations of Cd, Cu, and Pb in archived liver samples from six native waterbird species; pāteke (*Anas chlorotis*), whio (*Hymenolaimus malacorhynchos*), scaup (*Aythya novaeseelandiae*), paradise shelduck (*Tadorna variegata*), Campbell Island teal (*Anas nesiotis*), and Australasian bittern (*Botaurus poiciloptilus*).

Chapter Two

Heavy metals concentrations in the blood and eggshells of mallards (*Anas platyrhynchos*).

Using non-destructive sampling methods for biomonitoring pollutants in wildlife



2.0 Abstract

Birds have played a prominent role in the biomonitoring of different ecotoxins since the discovery of DDT accumulation and its effect on bird populations. Biomonitoring of certain heavy metals is an established method to quantify their abundance and bioavailability; pollutants accumulate in animals to higher levels than the surrounding environment. Although many biomonitoring studies have collected internal tissues to assess chronic exposure to pollutants, studies using non-destructive methods such as blood, eggs, feathers and faeces have been increasing. The concentrations of three heavy metals (cadmium [Cd], copper [Cu], and lead [Pb]) were determined in two spatially distinct populations of mallards (*Anas platyrhynchos*) in the Waikato and Southland regions of New Zealand. Levels were analysed in blood samples taken over two years of pre-breeding (June–July) and post-breeding (January–February) seasons, and in eggshells from breeding birds. Significant differences between sites were found for all three metals in pre-breeding blood samples, with Cd and Cu concentrations highest in birds at the Southland site, and Pb higher in birds from Waikato. Sample concentrations varied with year of pre-breeding sampling, with Cd concentrations being higher in 2014 than 2015, while Pb concentrations were lower over the same period. Cd concentrations decreased between the pre- and post-breeding season over both sites, while Pb concentrations increased from pre- to post-breeding season in the Waikato. Only Cu concentrations varied by sex in post-breeding bloods, with males having lower concentrations than females. Blood Cd concentrations were higher in adults in both pre- and post-breeding bloods compared to juveniles. In total, 9.4% (16/171) of pre-breeding and 7.1% (10/140) of post-breeding blood samples were elevated above the environmental background levels of Pb, with 3.6% of all sampled bloods estimated at levels consistent with clinical Pb toxicity. Thresholds for toxic blood concentrations of Cd and Cu have yet to be established, however, concentrations found here appear to be similar to concentrations found globally. The age of the hen had no effect on heavy metal concentrations in eggshells, but Pb concentration in eggshells was found to increase with nesting attempts, whereas eggshell Cu concentration increased with successive clutches from the same female (n = 5). These samples show that environmental exposure to increased levels of these three heavy metals is occurring in a proportion of the mallards at concentrations consistent with adverse effects. The concentration of these contaminants present in the mallard blood and eggshell samples suggest that there may be wider implications for other waterfowl species, the wider wetland ecosystems in New Zealand, and possibly for human health.

2.1 Introduction

Environmental pollution of heavy metals represents a threat to ecosystems as it is responsible for numerous pathologies in living organisms, leading to impaired survival and reproduction in wildlife (Lucia *et al.*, 2010). Birds have played a prominent role in the biomonitoring of different pollutants since the early 1950s with the realisation that the insecticide dichlorodiphenyltrichloroethane (DDT) was causing mass wildlife poisoning, especially of birds, through bioaccumulation (Furness, 1993). Biomonitoring of certain heavy metals is an established method to quantify pollutant abundance and bioavailability, as pollutants accumulate to higher levels in animals than those in the surrounding environment. Although many biomonitoring studies have collected internal tissues to assess chronic exposure (Aloupi *et al.*, 2017, Gochfeld and Burger, 1987, Kalisińska *et al.*, 2004, Di Giulio and Scanlon, 1984b), studies using non-destructive methods such as measuring concentrations in blood (Maia *et al.*, 2017, Binkowski and Meissner, 2013), eggs (Hashmi *et al.*, 2013, Dolci *et al.*, 2017), and feathers and faeces (Dauwe *et al.*, 2000, Karimi *et al.*, 2015, Tsipoura *et al.*, 2011) have increased in popularity, despite still being quite limited for comparison.

Researchers have found evidence of diverse heavy metal related impairments in birds. Cd is one of the most abundant nonessential metals within the environment due to its industrial uses. It became a national concern with the historical use of phosphate fertiliser in New Zealand, which up until the 1990s was contaminated with high Cd levels (Taylor, 1997). It is known to induce kidney and liver toxicity, disruption of calcium metabolic pathways, lesions of intestinal tissue, and thinning of eggshells (Cain *et al.*, 1983, Burger, 2008, Furness, 1996). Cu, although involved in many biological processes including serving as an essential cofactor for several oxidative stress-related enzymes (Stern, 2010), can result in toxicity at high doses. It predominantly affects the liver, causing hepatocellular necrosis as well as erosion of the epithelial lining of the gastrointestinal track, and acute tubular necrosis in the kidney (Camakaris *et al.*, 1999, Tchounwou *et al.*, 2008). The most extensively studied heavy metal is Pb, as its route into the environment is often linked with hunting activities (Pb ammunition). Known as a potent toxin, in poisoned individuals it causes haemolytic anaemia and behavioural impairments, results in adverse effects on reproduction, such as decreased egg production and plasma calcium, and impairs growth and survival of chicks and nestlings (Burger and Gochfeld, 1993, Scheuhammer, 1987, Taggart *et al.*, 2009, Pain, 1996).

Blood is the tissue of choice for non-destructive biomonitoring when determining recent exposure to pollutants, as it is obtained quickly and easily without high risk of permanent damage (Maia *et al.*, 2017). Blood has the potential to be a good indicator of environmental exposure due to the high correlations between many metal levels in blood and other tissues (Wayland *et al.*, 2001, Burger and Gochfeld, 1993). Unlike internal tissue collection, blood sampling allows studies of survival rate in relation to contaminant load. By allowing repetitive sampling of individuals, exposure in relation to life stage, reproductive activities, and seasonal effects can be monitored.

When interpreting metal concentrations in avian blood, the age of the bird should be considered (Garcá-Fernández *et al.*, 1996, Burger and Gochfeld, 1997a). Since juvenile (hatch year) birds feed within a more localised area compared to adults, they provide data on metal pollution for a limited period and territory. This effect is more exaggerated within altricial nestlings, although precocial juveniles are also likely to demonstrate similar patterns (Dauwe *et al.*, 2000, de la Casa-Resino *et al.*, 2014). Adults, on the other hand, are more likely to have accumulated contaminants over their lifetime; an increase with age has been reported in many studies examining internal tissues (Berglund *et al.*, 2011, Burger and Gochfeld, 1997a). Some studies suggest that sex can affect the accumulation of heavy metals in the body in birds, with females having lower concentrations due to their ability to excrete toxins into eggs (Gochfeld and Burger, 1987, Aloupi *et al.*, 2017). In contrast, a review on sex-related differences in metal loads in wildlife concluded that in most cases sex did not affect metal accumulation in birds, highlighting that significant differences are often sporadic and conflicting within the literature (Burger, 2007). Concentrations of heavy metals in blood have been reported less frequently, although Binkowski and Meissner (2013) studied the blood concentrations of seven metals (Fe, Zn, Zu, Cr, Ni, Pb, Cd) in mallards from Poland, which did not differ between the sexes. It should be noted that when differences between sexes have been reported, they are often small compared to the high level of variation between individuals (Furness, 1993).

Eggshell formation and laying is recognised as a mechanism that allows female birds to off-load environmental contaminants (Gochfeld and Burger, 1998, Burger, 1994), thus concentrations in eggs can potentially be used for pollutant monitoring. Pollutants excreted to eggs derive from both stored body burden, and food choice of the female during egg formation (Burger and Gochfeld, 1993). It is therefore assumed that eggshell concentrations can provide information on the degree of exposure within a female's breeding area. Concentrations of trace elements in eggshells have been useful in long-term studies of geographical, geochemical and temporal trends of environmental pollution (Burger, 2002, Dolci *et al.*, 2017, Kitowski *et al.*, 2017a). Although the transfer rates of contaminants are thought to be related to the body load of the female, and ultimately to the exposure of pollutants in the female's environment (Dauwe *et al.*, 2005), there is some debate about the transfer of certain metals such as Cd into eggshells. Although differential toxin offloading can be a major disadvantage for the use of eggshells as a bioindicator, eggshells remain a tool for monitoring the movement of certain pollutants passed to subsequent generations, which could have effects on chick growth and survival (Lam *et al.*, 2005, Agusa *et al.*, 2005, Burger, 1994).

Within New Zealand there is a lack of research into the movement of pollutants between the environment and wildlife. The data presented here are the results of a survey aiming to assess potential heavy-metal contaminations of two spatially distinct populations of mallards in New Zealand, using non-destructive biomonitoring methods. Concentrations of one essential heavy metal, copper (Cu), and two nonessential heavy metals, cadmium (Cd) and lead (Pb), were determined from blood samples from mallards taken at two time points and in eggshells collected from hatched or abandoned nests. The objectives of this study were to (i) obtain a baseline level of contaminants in the bloods of mallards in New Zealand, (ii) assess the rate of maternal transfer of heavy metals during egg formation, and (iii) evaluate the feasibility of using non-destructive methods for monitoring contaminants in wildlife.

2.2 Materials and Methods

2.2.1 Sample sites

The mallards studied were collected from Southland and Waikato in New Zealand (Figure 2.1). The first study area was located in the Southland Plains, centred on the Lochiel community within the Southland Region of the South Island (46°12´18.68´´S, 168°19´46.19´´E). Predominately flat, early maps (c.a. 1850) indicate a mosaic of tussock grassland, forest and bogs, while land cover now is dominated by pasture and forestry. While agricultural expansion has barely increased since 1985, transition in land use is still occurring with the expansion of dairy land into previously sheep and beef pastoral and arable land (Ledgard, 2013). The study area was made up primarily of private land with part of the Oreti River, numerous streams and man-made ponds created either for effluent settling or as waterfowl habitat. The Southland region, known as a hotspot for mallards, sells just over 15% of the game-bird hunting licences sold nation-wide (Garrick *et al.*, 2017). Long-term population trends indicate that this population is stable (E. Garrick, Southland Fish and Game, pers. comm.).

The second study site was located in the Waipa District, centred around the Ohaupo community within the Waikato Region of the central North Island (37°55´39.36´´S, 175°17´16.98´´E). Historically, this area was dominated by large peat-swamps, alluvial flats, and vast native forest. Since human arrival this area has been highly modified and is now dominated by pastoral farming (predominantly dairy) and forestry, with only 18% of fragmented pre-European vegetation left in the lowlands of the region. With the removal of farming subsidies in the 1980s, farmers mitigated their economic loss by intensifying land use and increased stocking rates (MacLeod and Moller, 2006b). Within the boundaries of this site are both the Waikato and Waipa

Rivers, 14 peat lakes, numerous streams, drainage ditches, and man-made effluent ponds. The mallard population within this region, once renowned for being abundant, appears to be unstable with fluctuating annual counts (Sheppard, 2017). Despite this, the region is still responsible for about 20% of the game-bird hunting licences sold nation-wide (D. Klee, Auckland/Waikato Fish and Game, pers. comm.).

2.2.2 Sample collection

Bloods

A total of 171 female pre-breeding season blood samples were collected in June–November 2014 (n = 77) and 2015 (n = 94), in collaboration with another study (Sheppard, 2017). Birds were caught using baited funnel traps (Bub, 1991), mist-nests (Bacon and Evrard, 1990), automatic nest traps (Weller, 1957), long-handled dip nets (Loos and Rohwer, 2002), net-gun, or walk-in traps (Dietz *et al.*, 1994). Females were implanted with a 22-g intra-abdominal VHF transmitter (Model IMP/150, Telonics, Mesa, Arizona, Rotella *et al.*, 1993, Paquette *et al.*, 1997) to be tracked for the subsequent breeding season. While birds were anesthetised for implant surgery, < 3 ml of blood was collected from the right jugular vein and transferred into 5 ml lithium heparin microtainers and kept on ice until samples could be stored at -20^{o}C until processed. Pre-breeding season females were aged by the perceived depth of the Bursa of Fabricus from cloaca examination, then verified by the characteristics of the greater secondary coverts, primaries, and general wing plumage. Two age cohorts were identified; adult (after-second year) and juvenile (after-hatch year). Procedures were approved under University of Auckland Animal Ethics Permit 001331.

Post-breeding season bloods were collected from Waikato birds in late January 2016 (n = 70), and from Southland birds in February 2017 (n = 70). Birds were trapped using modified walk-in funnel traps (McDougall, 2012). Birds were removed from holding pens and restrained by hand while blood samples were collected from the right jugular or the metatarsal vein using a 25-gauge needle and 5 ml syringe (Figure 2.2). Pressure was applied to the sample site for at least 30 seconds or until bleeding stopped. Birds were placed in an observation pen for 10 minutes after sampling, prior to release back into wild habitat. A volume of 0.5–2 ml of blood was collected in 5 ml lithium heparin microtainers and kept on ice until samples could be stored at -20^{o}C until processed. Age was established by the perceived depth of the Bursa of Fabricus from cloaca examination (Siegel-Causey, 1990), with juveniles (hatch-year birds) able to be distinguished from adults (post-hatch year birds). Research and procedures were approved under Massey University Animal Ethics approval 16/04.

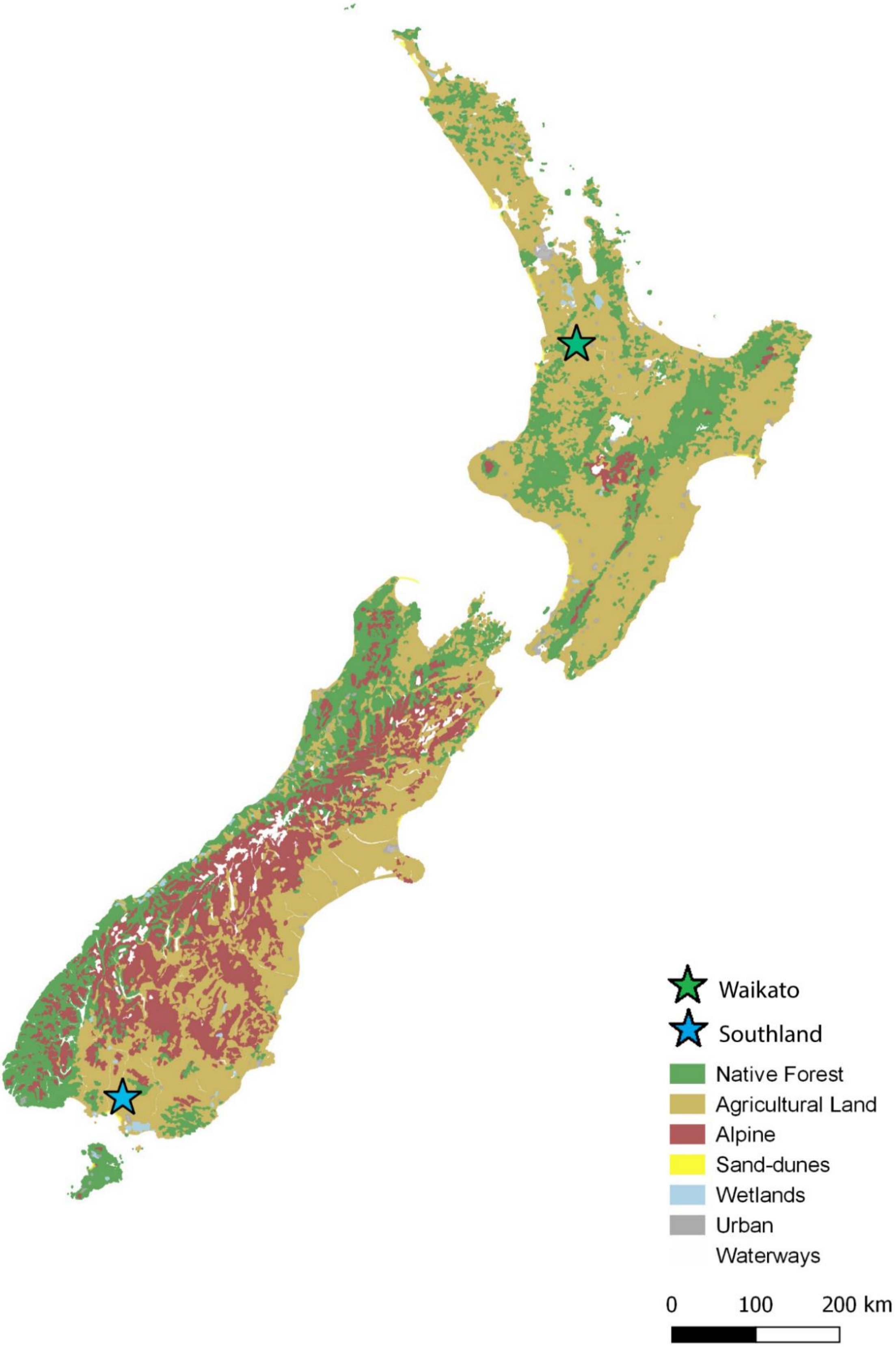


Figure 2.1 Field sites. Map created from "Vegetative cover of New Zealand" shapefile imported from LRIS (https://lris.scinfo.org.nz/layer/48420-vegetative-cover-map-of-new-zealand/) (Landcare Research Informatics Team, 2015)

All blood samples were analysed at Hills Laboratory (Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton, New Zealand). A detailed description of the methodology used can be found in American Public Health Association (APHA) "Standard Methods for the Examination of Water and Wastewater" (22nd Edition), Methods 3030Fa and 3125. In summary, an average of 1.69 g of blood (range 0.044–2.730 g) was digested with 2.5 ml of nitric and 0.5 ml of hydrochloric acid at 85ºC for one hour. Blood concentrations of Cd, Cu, and Pb were then determined by inductively coupled plasma mass spectrometry (ICP-MS). Blanks and standard reference material were processed and analysed to ensure the quality of the methodology. Limits of detection (LOD) were as follows; Cd = 0.0002 $mg.kg^{-1}$, Cu = 0.0050 $mg.kg^{-1}$, and Pb = 0.0010 $mg.kg^{-1}$. All results reported are in relation to wet weight (w.w.).

In this study, birds with blood Cd and Pb concentrations above 0.2 $mg.kg^{-1}$ were regarded as being elevated or above background levels (White and Finley, 1978, Pain, 1996), with Pb levels above 0.5 $mg.kg^{-1}$ considered to be consistent with acute clinical toxicity (Pain, 1996). Thresholds for toxicity based on Cu levels in blood are unknown.

Figure 2.2 Bleeding of the right jugular vein of a female mallard at a Waikato banding site. Photo: Southland banding team.

Eggshells

Eggshells were collected from the nests of tracked females (n = 57) throughout the 2015 breeding season, August–December (Figure 2.3). Data concerning females' nesting attempts and clutch numbers were recorded in collaboration with a research programme investigating female reproduction (Sheppard, 2017). Full clutches, both hatched shell-fragments and abandoned eggs, were collected together in plastic bags and frozen at -20^{o}c until processed. Once eggs were thawed at room temperature, egg contents and membranes were separated from the shell. External debris, nesting material, and organic matter was gently wiped or washed off. Shells from multiple eggs were pooled by clutch, and sent to Hills Laboratory for analysis, following similar methods as blood samples. Shell samples weighting at least 0.5 g (range 0.501–0.519 g) were ground and homogenised, then digested following the Nitric Acid-Hydrochloric Acid Digestion Method 3030F (Eaton *et al.*, 2005) as for bloods. Concentrations of the metals in the shells were established by ICP-MS with the following limit of detection (LOD); Cd = 0.002 mg.kg^{-1}, Cu = 0.050 mg.kg^{-1}, and Pb = 0.010 mg.kg^{-1}.

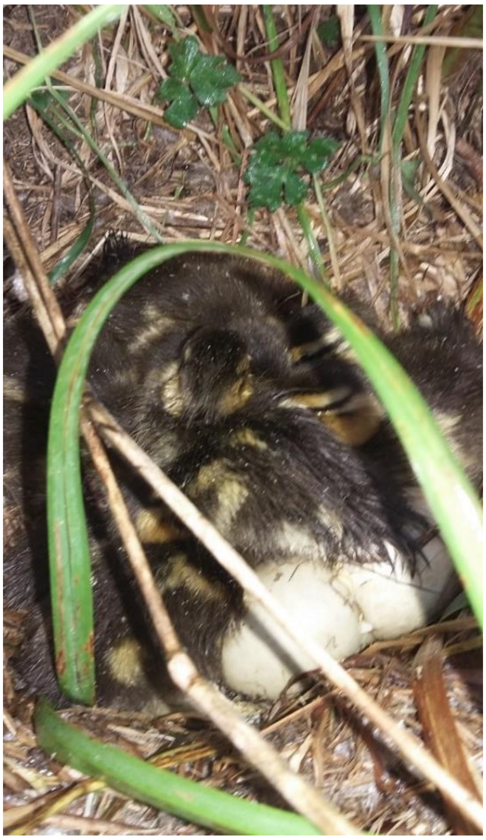


Figure 2.3 Photo of newly hatched Waikato mallard nest, from which eggshells were collected. Photo: Katie Gibb.

2.2.3 Statistical analysis

Data were analysed using the statistical software R studio (R Core Team, 2015). Values below the reported LOD were considered as one-half of the relevant LOD and included for statistical analysis, to minimise nominal type 1 error rates (Clarke, 1998). Data were tested for normality (Shapiro-Wilk), and all distributions of the data significantly differed from normal. After log transformations only Cu levels in eggshells established normality, while log-log (+10) transformation established normality in Cd and Pb in pre-breeding blood samples and Pb in post-breeding bloods. For non-normal data, nonparametric tests were used. A Mann-Whitney test was used for two independent samples to compare two groups (Waikato vs Southland; adult vs juvenile; male vs female; year 2014 vs 2015). Log-log-transformed Cd and Pb values comparing site, age cohort, year (pre-breeding), and sex (post-breeding) were analysed using an analysis of variance (ANOVA), including assessment of interactions between factors. Log-transformed shell Cu levels were analysed using a two-sample t-test for comparison between sites, but due to the small sample size of known female ages at lay a Mann-Whitney test was used for the comparison between ages (of females). Interactions between sample types were established by two different tests. Two-sample t-tests were used to establish differences between sample types: pre- vs post-breeding season bloods, and pre-breeding season bloods vs eggshells. A Spearman correlation test was used to assess the correlation between heavy metal values in pre-breeding season bloods compared to that within eggshells. Results are presented as mean ± standard deviation, median, and range for whole samples. The results are grouped by site (Waikato or Southland), age (adult or juvenile) and sex (male or female) where appropriate. Results were considered significant at $p < 0.05$.

2.3 Results

2.3.1 Pre-breeding season blood samples

Over two seasons (2014 and 2015) a total of 171 adult females were sampled as part of this study. All of the samples analysed exhibited values above the LOD for the three heavy metals tested (Table 2.1). Cd was reported with the lowest blood concentrations, ranging 0.0003–0.017 $mg.kg^{-1}$ with an overall mean of 0.0020 ± 0.0025 $mg.kg^{-1}$. There were significant differences in blood Cd concentrations between years (ANOVA: $p < 0.001$), with lower concentrations in 2014 than 2015 (Table 2.3, Figure 2.4). There was a very weak effect of age (ANOVA: $p < 0.07$) and site (ANOVA: $p < 0.09$) of sampling on Cd concentrations.

Cu presented the highest heavy metal concentration in the blood samples, ranging 0.25–0.90 $mg.kg^{-1}$ with a mean of 0.401 ± 0.071 $mg.kg^{-1}$ (Table 2.1). There were significant differences between sites (W = 4595.5, $p < 0.004$) with birds from Southland having higher blood Cu concentrations than those from Waikato (Table 2.2, Figure 2.5a). There was a very weak effect of year of sampling on blood Cu concentrations (W = 3028, $p < 0.07$).

Blood Pb concentrations in the birds ranging 0.0025–0.62 mg.kg^{-1} with a mean of 0.082 ± 0.12 mg.kg^{-1}. Elevated blood Pb concentrations (> 0.2 mg.kg^{-1}) were recorded in 16 individuals (9.4%); of these, 5 (2.9% of total) were found to have levels consistent with clinical toxicity (> 0.5 mg.kg^{-1}) (Figure 2.6). Mean blood Pb concentrations differed between sites (ANOVA: $p < 0.001$), with Waikato birds having a mean of 0.09 ± 0.11 mg.kg^{-1} compared to Southland birds 0.07 ± 0.13 mg.kg^{-1} (Table 2.2, Figure 2.5b). Blood Pb concentrations also differed between years of sampling (Table 2.3) (ANOVA: $p < 0.0001$), with birds from 2014 having higher levels than those from 2015 (Figure 2.4c).

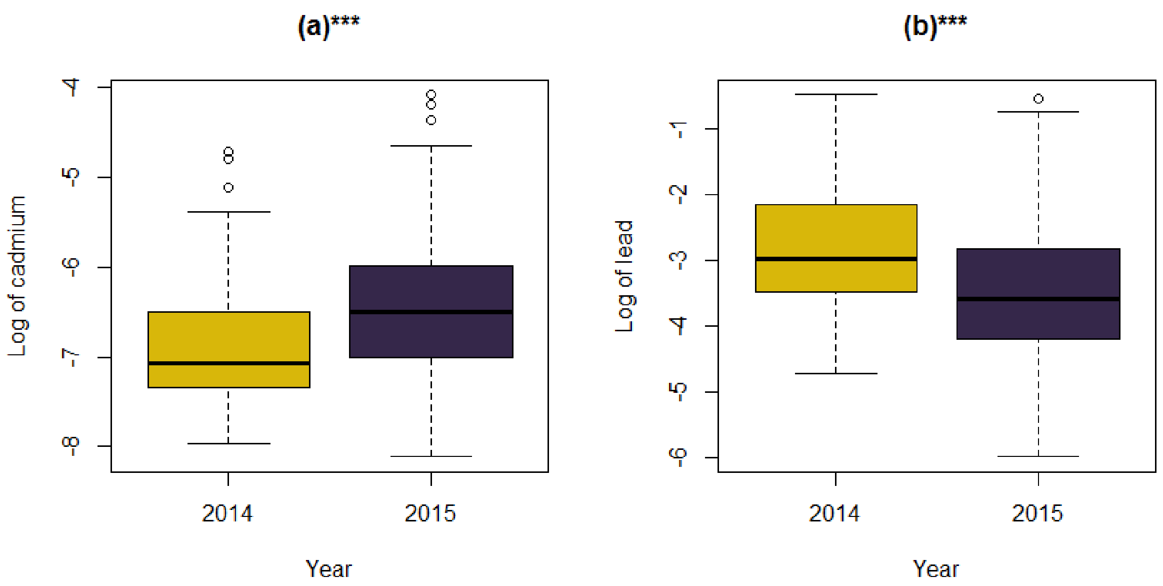


Figure 2.4 Pre-breeding season mean blood concentrations of a) Cd, and b) Pb in mallards showing statistically significant differences between year of sample, 2014 (n = 77) and 2015 (n = 94), tested by ANOVA (*p < 0.05; **p < 0.01; ***p < 0.001). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

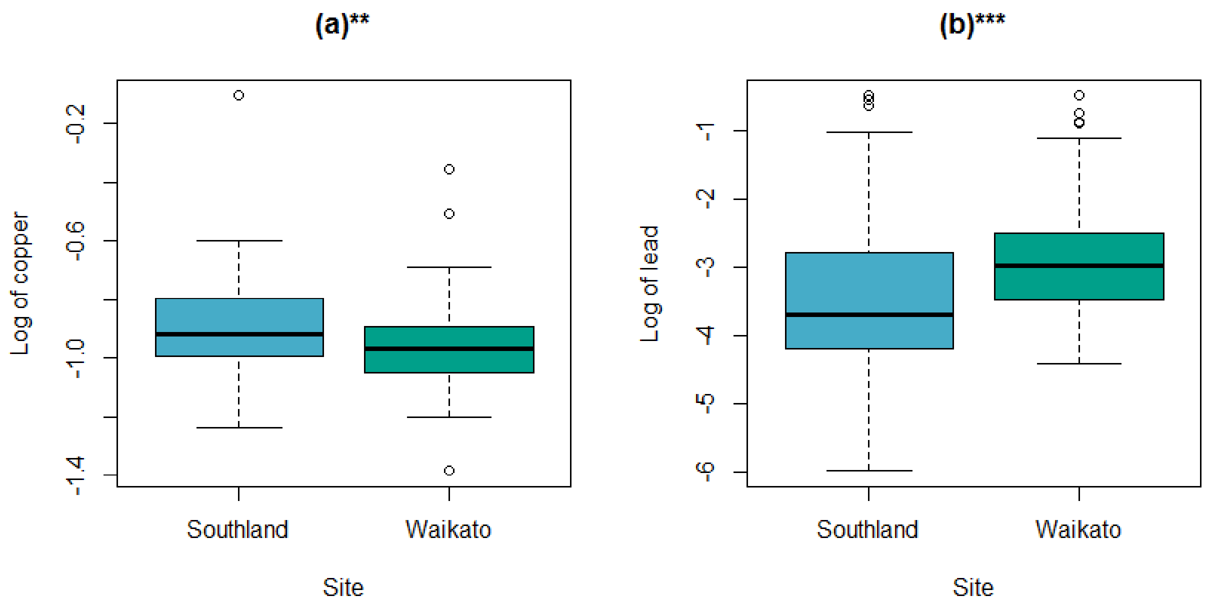


Figure 2.5 Pre-breeding season mean blood concentrations of a) Cu (Mann-Whitney nonparametric test) and b) Pb (ANOVA) in mallards showing statistically significant difference between sites, Southland (*n* = 86) and Waikato (*n* = 85), (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

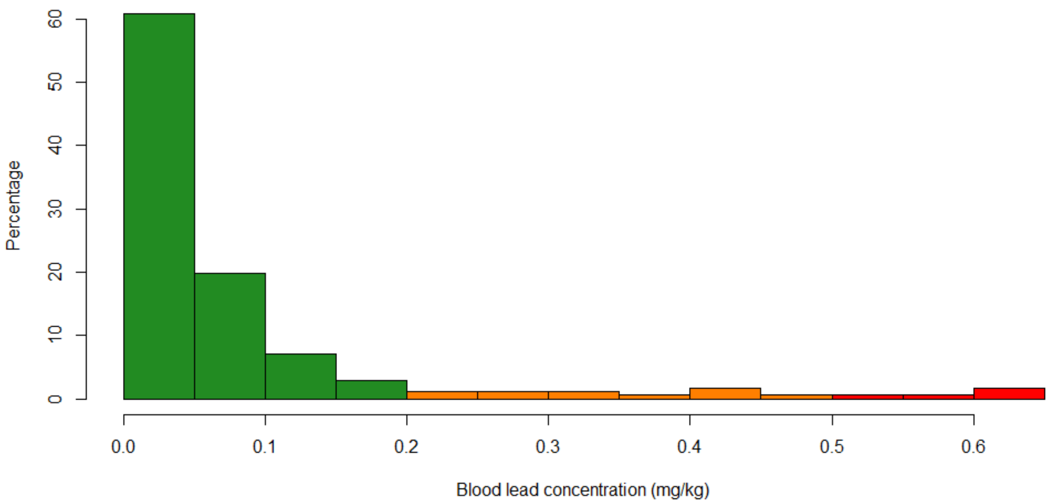


Figure 2.6 Distribution of blood Pb concentrations in pre-breeding season mallards; green: below environmental levels (< 0.2 $mg.kg^{-1}$), orange: elevated above environmental levels, subclinical poisoning (0.2 – 0.5 $mg.kg^{-1}$), red: clinical toxicity (> 0.5 $mg.kg^{-1}$).

Table 2.1 Summary of heavy metal concentrations (mg.kg^{-1} w.w.) in mallards from three different sample types (post-breeding season bloods, pre-breeding season bloods, and eggshells).

	LOD (mg.kg^{-1} w.w.)	Mean ± SD	Median	Range
Pre-breeding season				
(n = 171)				
Cd	0.0002	0.0019 ± 0.0025	0.0013	0.0003 – 0.017
Cu	0.0050	0.4011 ± 0.0710	0.3900	0.2500 – 0.900
Pb	0.0010	0.0819 ± 0.1220	0.0380	0.0025 – 0.620
Post-breeding season				
(n = 140)				
Cd	0.0002	0.0011 ± 0.0011	0.0007	0.0003 – 0.009
Cu	0.0050	0.3887 ± 0.0592	0.3800	0.0800 – 0.520
Pb	0.0010	0.1059 ± 0.3910	0.0340	0.0050 – 4.400
Eggshells				
(n = 59)				
Cd	0.0020	0.0100 ± 0.0018	0.0100	0.0100 – 0.020
Cu	0.0500	5.3100 ± 6.6700	3.4000	0.7000 – 45.00
Pb	0.0100	0.1090 ± 0.2540	0.0500	0.0500 – 1.830

Table 2.2 Summary of statistics for pre-breeding season blood samples from mallards analysed for heavy metal concentrations (mg.kg^{-1} w.w.) between two sampling sites (Waikato and Southland). Significant difference between sites tested by Mann-Whitney nonparametric tests (*p < 0.05; **p < 0.01; ***p < 0.001).

		Waikato n = 85			Southland n = 86					
	LOD (mg.kg^{-1} w.w)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	***p* value**	
Cd	0.0002	0.00197 ± 0.0028	0.00095	0.0003 – 0.015	0.00197 ± 0.0003	0.3896	0.0004 – 0.0168	ANOVA	0.085	
Cu	0.005	0.3896 ± 0.065	0.38	0.25 – 0.7	0.412 ± 0.074	0.4	0.29 – 0.90	Mann-Whitney	0.0036	**
Pb	0.001	0.091 ± 0.11	0.051	0.012 – 0.62	0.0727 ± 0.128	0.128	0.0025 – 0.6200	ANOVA	0.0001	***

Table 2.3 Summary of statistics for pre-breeding season blood samples from mallards analysed for heavy metal concentrations (mg.kg^{-1} w.w.) between the two years sampled (2014 and 2015). Significant difference between years tested by either ANOVA or Mann-Whitney nonparametric tests (*p < 0.05; **p < 0.01; ***p < 0.001).

		2014 n = 77			2015 n = 94					
	LOD (mg.kg^{-1} w.w)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	***p* value**	
Cd	0.0002	0.0014 ± 0.002	0.00085	0.00035 – 0.009	0.002 ± 0.003	0.0015	0.0003 – 0.0168	ANOVA	0.0002	***
Cu	0.005	0.393 ± 0.061	0.38	0.31 – 0.7	0.407 ± 0.077	0.4	0.25 – 0.9	Mann-Whitney	0.066	
Pb	0.001	0.109 ± 0.143	0.051	0.009 – 0.62	0.059 ± 0.099	0.0275	0.0025 – 0.58	ANOVA	0.00001	***

2.3.2 Post-breeding season blood samples

A total of 140 post-breeding season blood samples were collected, including 70 birds from each site. All of the samples analysed exhibited heavy metal concentrations above the LOD for the three heavy metals tested. Cd again presented the lowest blood concentrations with a mean of 0.0011 ± 0.00113 $mg.kg^{-1}$, ranging from 0.0003 – 0.009 $mg.kg^{-1}$ (Table 2.1). No differences in blood Cd concentration were found between sites and sexes, however a very weak effect of age on concentration level was found (W = 2913.5, $p < 0.055$) with slightly higher concentrations in adults (Table 2.4, Figure 2.8).

Blood Cu concentrations ranged from 0.08 – 0.52 $mg.kg^{-1}$ with a mean of 0.39 ± 0.059 $mg.kg^{-1}$ (Table 2.1). There was a significant difference in blood Cu concentrations between sexes (Table 2.6) (W = 2928.5, $p < 0.04$), with higher concentrations in females than in males (Figure 2.9). No differences in blood Cu concentration were found between sites and age.

Blood Pb concentrations ranged from 0.005 – 4.4 $mg.kg^{-1}$ with an overall mean of 0.106 ± 0.391 $mg.kg^{-1}$ (Table 2.1). Elevated blood Pb concentrations (> 0.2 $mg.kg^{-1}$) were recorded in 10 individuals (7.1%) and of these, three (2.1% of total) were found to have blood Pb concentrations above that of clinical poisoning (> 0.5 $mg.kg^{-1}$), including two being consistent with severe clinical poisoning (> 1.0 $mg.kg^{-1}$). There was a significant difference in blood Pb concentrations between sampling sites (ANOVA, $p < 0.0001$) (Table 2.5), with higher concentrations in birds from Waikato than in those from Southland (Figure 2.10).

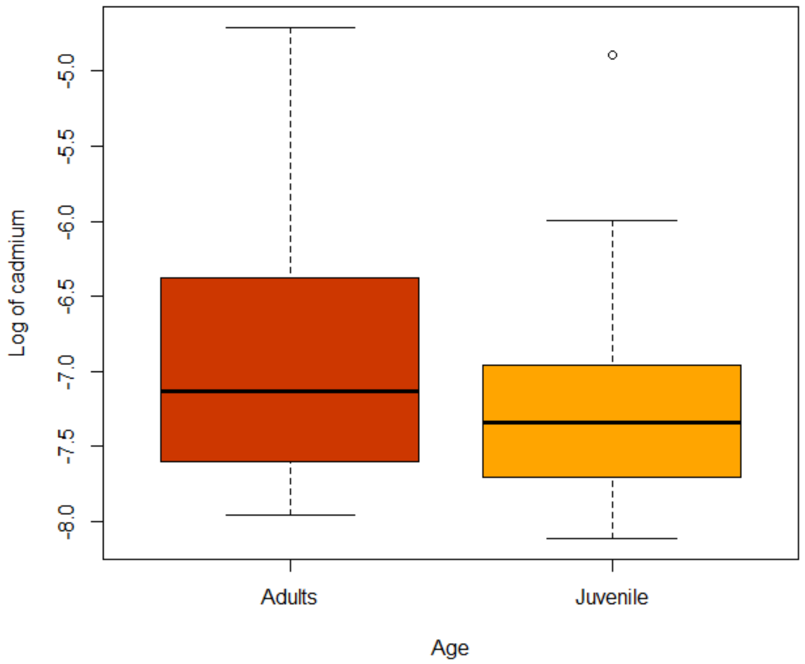


Figure 2.8 Mean post-breeding season blood Cd concentrations of mallards showing statistically significant difference between ages, adults (n = 70) and juveniles (n = 70), by Mann-Whitney nonparametric test ($p < 0.1$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

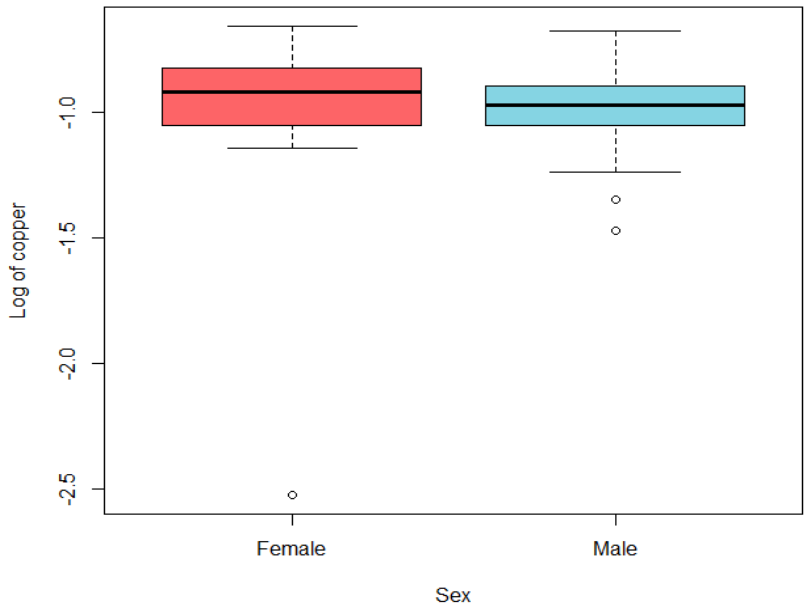


Figure 2.9 Mean post-breeding season blood Cu concentrations of mallards showing statistically significant difference between sexes, females (n = 73) and males (n = 67), by Mann-Whitney nonparametric test ($p < 0.05$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

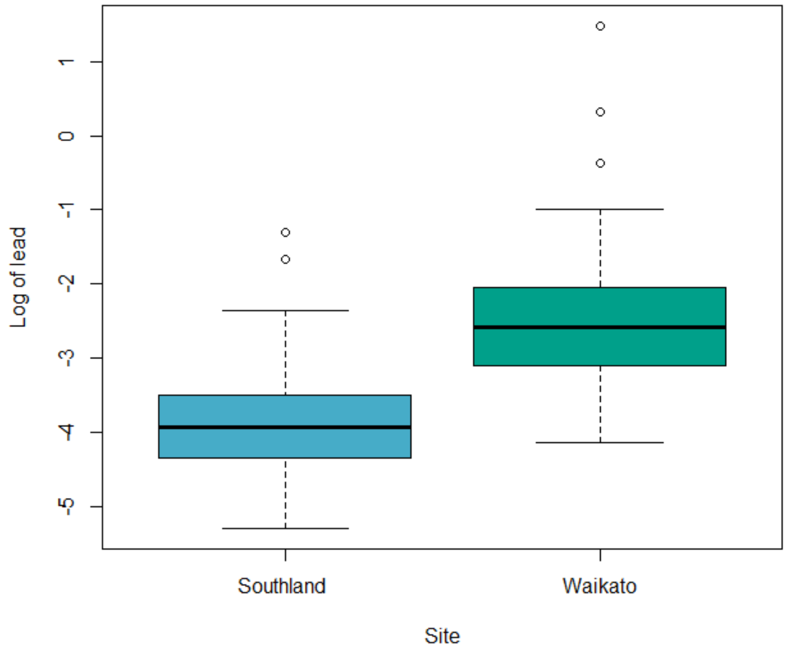


Figure 2.10 Mean post-breeding season blood Pb concentrations of mallards showing statistically significant difference between site, Southland (n = 70) and Waikato (n = 70) by analysis of variance (ANOVA) ($p < 0.0001$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

Table 2.4 Summary of statistics for post-breeding season blood samples analysed for heavy metal concentrations (mgkg^{-1} w.w.) between the two age cohorts of mallards (adult and juvenile). Significant difference between age tested by either ANOVA or Mann-Whitney nonparametric tests (*p < 0.05; **p < 0.01, ***p < 0.01).

		Adult *n* = 70			Juvenile *n* = 70					
	LOD (mg.kg^{-1} w.w)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	*p* value	
Cd	0.0002	0.001 ± 0.00127	0.0008	0.00035 – 0.00900	0.0008 ± 0.00093	0.00065	ND – 0.0075	Mann-Whitney	0.05	*
Cu	0.005	0.386 ± 0.047	0.38	0.3 – 0.5	0.391 ± 0.0696	0.395	0.08 – 0.52	Mann-Whitney	0.18	
Pb	0.001	0.06986 ± 0.0726	0.046	0.007 – 0.370	0.14 ± 0.5478	0.03	0.005 – 4.4	ANOVA	0.08	

Table 2.5 Summary of statistics for post-breeding season blood samples from mallards analysed for heavy metal concentrations (mg.kg^{-1} w.w.) between the two sites (Waikato and Southland). Significant difference between site tested by either ANOVA or Mann-Whitney nonparametric tests (*p < 0.05; **p < 0.01; ***p < 0.001).

		Waikato *n* = 70			Southland *n* = 70					
	LOD (mg.kg^{-1} w.w.)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	*p* value	
Cd	0.0002	0.001 ± 0.0014	0.0008	ND – 0.009	0.0009 ± 0.0006	0.0006	ND – 0.003	Mann-Whitney	0.18	
Cu	0.005	0.392 ± 0.063	0.38	0.08 – 0.52	0.385 ± 0.055	0.38	0.25 – 0.5	Mann-Whitney	0.41	
Pb	0.001	0.182 ± 0.542	0.075	0.016 – 4.4	0.0295 ± 0.0394	0.0195	0.005 – 0.27	ANOVA	2.0^{e-16}	***

Table 2.6 Summary of statistics for post-breeding season blood samples from mallards analysed for heavy metal concentrations ($mgkg^{-1}$ w.w.) between the sexes. Significant difference between sex tested by either ANOVA or Mann-Whitney nonparametric tests (*p < 0.05; **p < 0.01; ***p < 0.001).

		Female *n* = 73			Male *n* = 67					
	LOD ($mg.kg^{-1}$ w.w.)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	*p* value	
Cd	0.0002	0.001 ± 0.0013	0.0007	0.00004 – 0.009	0.00095 ± 0.0007	0.00065	0.00005 – 0.0004	Mann-Whitney	0.94	
Cu	0.005	0.3968 ± 0.0623	0.4	0.08 – 0.52	0.379 ± 0.0548	0.38	0.23 – 0.51	Mann-Whitney	0.04	*
Pb	0.001	0.123 ± 0.512	0.038	0.0055 – 4.4	0.0863 ± 0.187	0.032	0.005 – 1.370	ANOVA	0.56	

2.3.3 Eggshells

The results of heavy metal analysis of eggshells from the two field sites are shown in Table 2.7. Only two clutches of eggs had detectable concentrations of Cd in the eggshells (both 0.02 mg.kg^{-1}). All other clutches (*n* = 57) presented values below the LOD for Cd (< 0.02 mg.kg^{-1}), and therefore these were assumed to be 0.01 mg.kg^{-1} (half the LOD) for the purpose of analysis.

Eggshell Cu concentrations were detectable in all samples, with a range of 0.7–45 mg.kg^{-1} and mean of 5.31 ± 6.67 mg.kg^{-1}. The difference in eggshell Cu concentrations between sampling sites, with Waikato eggshells having slightly higher reported levels than eggshells from Southland, did not reach statistical significance (t = -1.97, df = 42.5, p <0.056) (Figure 2.11). The age of the hen had no effect on eggshell Cu concentrations (W = 430, p < 0.15). Overall there was no significant difference between eggshell Cu concentrations in successive nesting attempts (W = 246, p < 0.7). However, in five females with two consecutive clutches collected from Southland, eggshell Cu concentration changed between clutches (t = -4.0531, df = 4, p = 0.015), with eggshell Cu concentrations increasing on average by 1.86 mg.kg^{-1} with clutch number (Figure 2.12).

Eight of the 59 clutches (13.6%) had detectable concentrations of Pb, with a highest concentration of 1.83 mg.kg^{-1}. The rest of the clutches (51) had levels below the LOD (< 0.1 mg.kg^{-1}) for Pb and were therefore taken as 0.05 mg.kg^{-1}. Eggshell Pb concentrations differed significantly between successive nesting attempts (W = 282, p < 0.041), with higher concentrations found in second and third clutches compared to first nest attempts. No differences were found in eggshell Pb concentrations between sampling sites (W = 389.5, p < 0.3), or by age of hen (W = 379.5, p < 0.4).

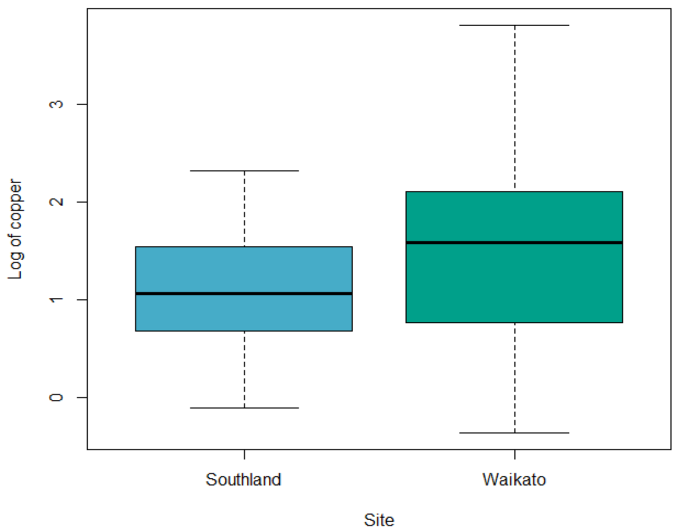


Figure 2.11 Mean eggshell Cu concentration showing statistically significant difference between sites, Southland (n = 32) and Waikato (n = 27, by Mann-Whitney nonparametric test (p<0.1). Southland (n = 32) and Waikato (n = 27). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

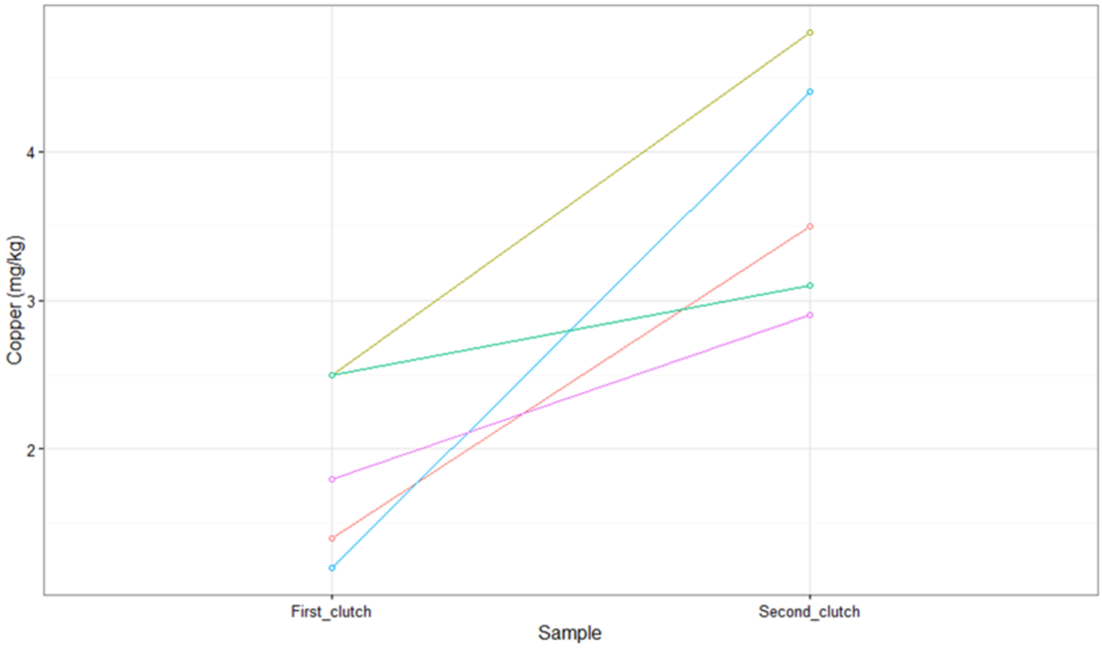


Figure 2.12 Eggshell Cu concentration increase between consecutive clutches collected from five Southland females; statistically significant difference indicated by Mann-Whitney nonparametric test ($p < 0.05$), with an average increase of 1.86 mg.kg^{-1} from first to second clutch.

Table 2.7 Summary of heavy metal concentrations (mg.kg^{-1} w.w.) in clutches of eggshells from mallards at two sampling sites. ND = not detectable

	LOD (mgkg^{-1} w.w.)	Mean ± SD	Median	Range
Total (n = 59)				
Cd	0.02	0.01 ± 0.0018	0.01	ND – 0.02
Cu	0.05	5.31 ± 6.67	3.4	0.7 – 45
Pb	0.1	0.109 ± 0.254	0.05	ND – 1.83
Waikato (n = 27)				
Cd	0.02	0.011 ± 0.003	0.01	ND – 0.02
Cu	0.05	7.39 ± 9.24	4.9	0.7 – 45.0
Pb	0.1	0.163 ± 0.369	0.05	ND – 1.83
Southland (n = 32)				
Cd	0.02	0.01 ± 0	0.01	ND
Cu	0.05	3.57 ± 2.197	2.9	0.9 – 10.2
Pb	0.1	0.06 ± 0.05	0.05	ND – 0.32

2.3.4 Interactions between sample types

As pre-breeding season blood samples (n = 171) were collected from females after transmitter attachment, concentration levels were only compared to female levels from the post-breeding season (n = 73). Blood Cd concentrations changed significantly over time (W = 8578.5, $p < 0.0001$), with the post-breeding season females having lower blood Cd concentrations than those birds sampled in the pre-breeding season (Figure 2.13). Blood Pb concentrations in Waikato birds were higher in the post-breeding than pre-breeding season (W = 1184, $p < 0.03$; Figure 2.14). The five females which were recaptured and sampled in both seasons showed a significant decrease in blood Cu concentrations by 0.046 $mg.kg^{-1}$ between pre- and post-breeding seasons (t = 4.098, df = 4, $p < 0.01$; Figure 2.15), but showed no change in Pb or Cd.

Fifty eggshells were collected from females with pre-breeding season blood samples. Maternal blood levels were not strongly correlated with eggshell concentrations for any of the three metals (spearman Cu= 0.18, Pb= 0.23, Cd= -0.06). The four females in the sample that laid clutches with detectable levels of eggshell Pb had higher mean blood Pb concentrations than those whose clutch levels were below detection (means= 0.195 $mg.kg^{-1}$ vs 0.036 $mg.kg^{-1}$) although this was not significant (W = 48, p=0.12)

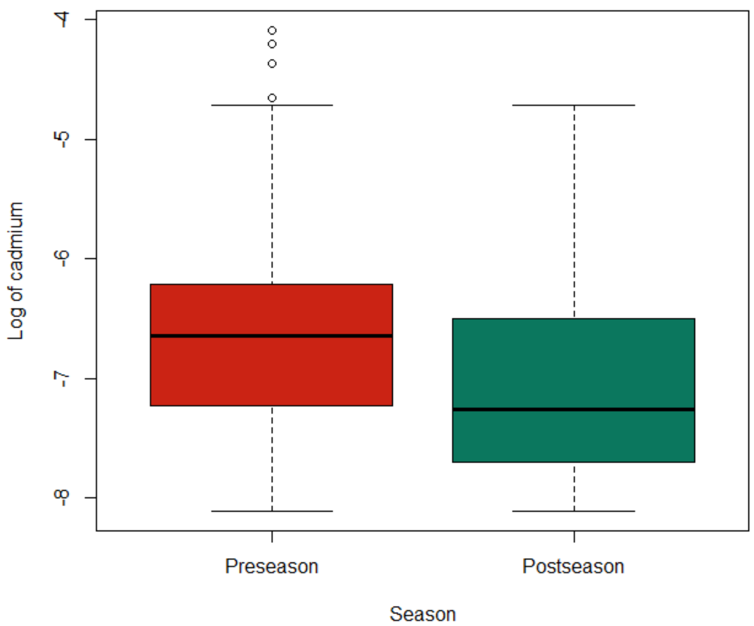


Figure 2.13 Mean Cd blood concentration of female mallards showing statistically significant difference between pre-breeding (n = 171) and post-breeding (n = 73) season levels by Mann-Whitney nonparametric test ($p < 0.0001$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

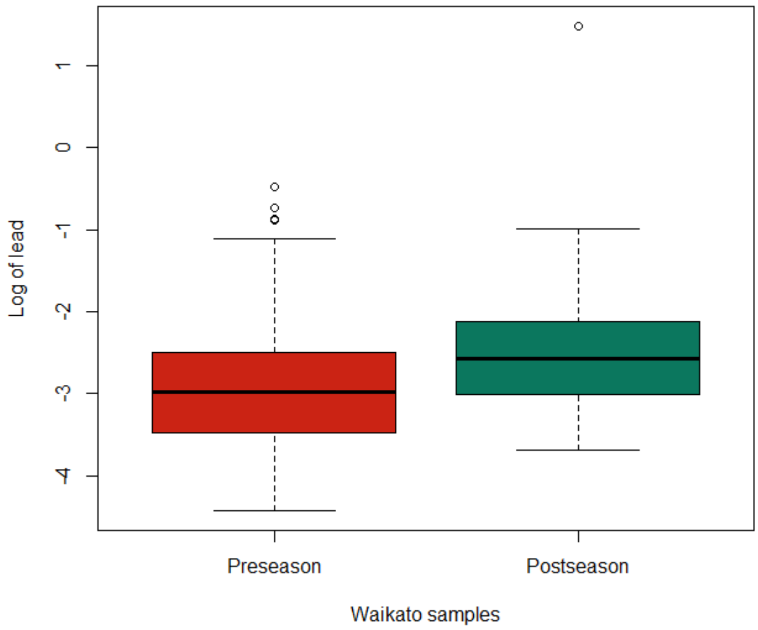


Figure 2.14 Mean Pb blood concentration of Waikato female mallards showing statistically significant difference between pre-breeding (n = 81) and post-breeding (n = 37) season levels by Mann-Whitney nonparametric test ($p < 0.05$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

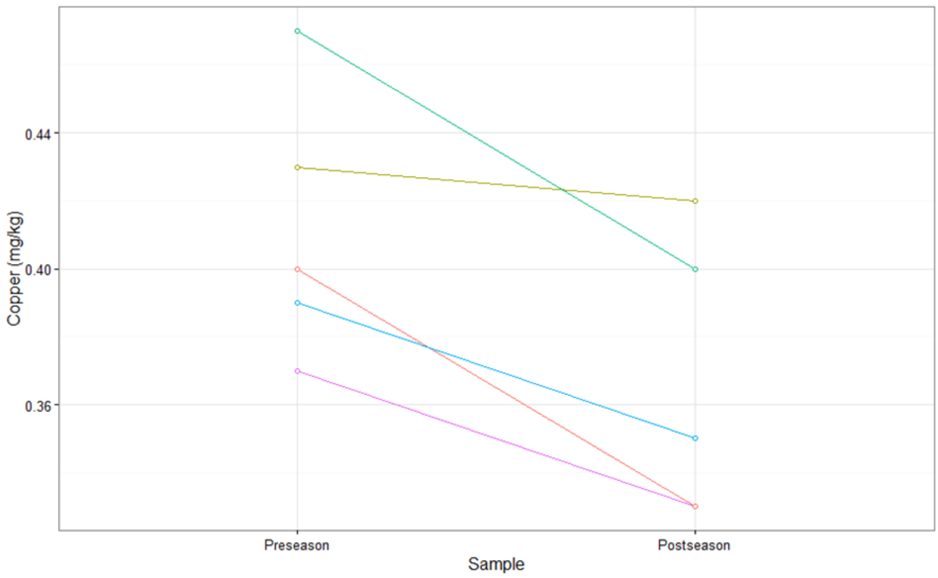


Figure 2.15 Seasonal Cu concentration decrease in bloods of five females between pre and post-breeding seasons. Significant statistical difference indicated by Mann-Whitney nonparametric test ($p < 0.01$), with an average decrease of 0.046 mg.kg^{-1} from pre- to post-breeding season.

2.4 Discussion

The bioaccumulation of heavy metal pollutants has significant detrimental effects on the survival and reproductive success of wildlife (Scheuhammer, 1987). Globally, waterfowl species are a well-studied bioindicator of pollutants due to their high trophic level, well understood biology, and economic value for hunting and importance as a food source for humans (Binkowski, 2012, Kalisińska *et al.*, 2004, Di Giulio and Scanlon, 1984b, Parslow *et al.*, 1982, Kim and Oh, 2012). However, within New Zealand there is a lack of research into the movement of pollutants between the environment and wildlife. Using blood and eggshell sampling as non-destructive methods for monitoring heavy metal bioaccumulation within waterfowl is an important tool to establish environmental contamination of ecosystems within New Zealand. In this study, the results indicate environmental contamination with three heavy metals in two widely separated areas of New Zealand.

Heavy metal concentration in bloods

Cd is toxic to several organs, mostly causing hepatotoxicity following acute poisoning and nephrotoxicity with chronic exposure. While absorbed Cd is quickly bound and stored within the liver, blood Cd concentrations indicate circulating Cd and therefore can be used as an indicator of recent exposure as well as release of Cd from tissue stores (Nordberg *et al.*, 2014). The concentration of blood Cd is a widely used biomarker of exposure in human and mammal studies (Cho *et al.*, 2010, Binkowski, 2012, Janicka *et al.*, 2015), largely due to the ease of collection. Absorbed Cd is also transported within the blood, bound mainly to red blood cells or higher molecular weight proteins (such as metallothionein) (Nordberg *et al.*, 2014). Due to the rapid uptake of blood Cd by the liver, blood samples may not reflect long-term exposure and bioaccumulation, nor take into account intermittent or infrequent doses. Moreover, due to the high individual variation of tolerance to Cd, blood concentrations alone cannot indicate its toxic effect (Cho *et al.*, 2010). Garcá-Fernández *et al.* (1996) established that only 0.5% of the total body burden of Cd in bird species was found in the blood.

Within this present study, Cd concentrations were much lower than Cu and Pb. Binkowski and Sawicka-Kapusta (2015) reported even lower levels in mallards from two areas in Poland (with the majority of samples having levels below detectable limits, and a maximum individual concentration of 0.02 mg.kg^{-1}). Other values are more similar to those reported within my study (mean = 0.001 mg.kg^{-1}). In the Canadian arctic, Wayland *et al.* (2001) found mean blood levels of Cd in common eiders (*Somateria mollissima borealis*) to be 0.66 ± 0.07 mg.kg^{-1}, whereas Maia *et* *al.* (2017) found white storks to have a mean concentration of 0.001 ± 0.002 mg.kg^{-1}. Of the 311 blood samples collected in the present study, only one pre-breeding season Waikato bird exceeded the 0.01 mg.kg^{-1} reference value established within the literature for contaminated areas (Garcá-Fernández *et al.*, 1996). In a study looking into the uptake and retention of Cd in mallards, White and Finley (1978) reported that the lowest blood Cd concentration associated with adverse effects in adult mallards was 0.26 mg.kg^{-1}. The maximum level found within this study was 0.015 mg.kg^{-1} indicating that Cd toxicity in blood is not an immediate concern for the mallard populations in the study regions. As this study determined the whole blood Cd concentrations, which includes plasma Cd, as well as erythrocyte and protein bound Cd, it is hard to extrapolate the toxic effect of this pollutant. Plasma Cd alone is likely to be the best indicator due to its increased bioavailability compared to its bound counterparts (Cho *et al.*, 2010). However, whole blood allows for a complete view of the contaminant load within the blood.

Within my study, Cu had the highest mean concentration in the blood samples, followed by Pb and then Cd. This order of blood concentration was also observed in mallards from urban areas in Poland (Binkowski and Meissner, 2013), and in the Doñana National Park, Spain (Benito *et al.*, 1999), as well as in white storks (*Ciconia ciconia*) in Spain (Maia *et al.*, 2017). Cu was expected to have the highest concentration due to its essential roles within the body, and thus would be expected to be higher than the nonessential elements tested here. Concentrations of Cu can be highly variable among species and individuals, with levels changing throughout the year that can be attributed to physiological changes (such as moulting), reproductive attempts (egg formation), and dietary change (Kalisińska *et al.*, 2004, Parslow *et al.*, 1982). Within my study, evidence was found to suggest that female Cu load is higher in the pre-breeding season compared to the post-breeding season (Figure 2.15), which could be attributed to the elimination of Cu stores through egg formation. This hypothesis is contradicted to a degree by females having higher post-breeding season blood Cu concentrations than males, although male pre-season levels would need to be sampled to effectively compare changes throughout the year (Figure 2.9). Sex difference in moult timing is well known in northern hemisphere mallards (Leafloor and Batt, 1990, Gilmer *et al.*, 1977, Lebret, 1961), with males moulting earlier and more synchronous after breeding (Boyd, 1961), while females moult time is influenced by timing, duration, and success of breeding (Gilmer *et al.*, 1977). As Cu can be sequestered into feathers during moulting, the stored concentrations in tissues are transported via the blood to the new growth feathers (Burger and Gochfeld, 1992). Therefore, if females have more recently moulted, blood Cu concentrations are likely to be higher than the males. This highlights a limitation of using blood, as it offers only a snapshot of heavy metal concentrations circulating within the body, whereas the use of organs gives a better idea of long term accumulation. Sex-differences in diet in relation and foraging behaviour could also result in different ingestion rates.

Excess concentrations of Cu in waterfowl tissues have been widely associated with environmental pollution (Parslow *et al.*, 1982, Kalisińska *et al.*, 2004, Di Giulio and Scanlon, 1984b, Hui *et al.*, 1998). While the main route of Cu exposure is still unclear, its source is hypothesised to be a secondary impact of farming activity where herbicides, fungicides, fertiliser and stock feed high in Cu are used (Longhurst *et al.*, 2004). Even though high Cu concentrations are known to be harmful to most living organisms (Nordberg *et al.*, 2014), especially waterfowl (Beck, 1961), there is a lack of experimental data on the toxicity of Cu to avian wildlife. Research on adverse effects of high Cu are focused on poultry and domestic waterfowl due to the high doses they are fed in commercial feeds used to help maximise growth (Starcher, 1969, Beck, 1961). It is assumed that environmental contamination of Cu is more likely to impact on birds by disruption of the trophic chain, where prey is adversely affected by the environmental load, resulting in limited food sources (Eisler, 1998, Frakes *et al.*, 2008). The concentrations reported within this present study (mean of 0.401 $mg.kg^{-1}$ for pre-breeding season females and 0.39 $mg.kg^{-1}$ for post-breeding birds) are comparable to those reported in the literature in contaminated ecosystems: median of 0.597 $mg.kg^{-1}$ in mallard drakes in an urban area, and 2.73 $mg.kg^{-1}$ in females from fishponds in Poland (Binkowski and Meissner, 2013); mean 0.258 $mg.kg^{-1}$ (range 0.142–0.361 $mg.kg^{-1}$) in mallards after a toxic spill into a major river system in Spain (Benito *et al.*, 1999); and mean 0.494 $mg.kg^{-1}$ in white storks in Spain (Maia *et al.*, 2017). The maximum concentration observed within the blood samples in New Zealand (0.9 $mg.kg^{-1}$) was well below levels reported in highly contaminated areas, where no apparent negative effects on survival were seen (Van Eeden *et al.*, 1996). These results suggest that environmental contamination of the habitats within the two field sites is an ongoing concern, but with no known threshold or critical range for Cu blood levels in birds it is hard to establish the extent of adverse effects.

Environmental contamination with high levels of Pb often leads to significant wildlife mortality (Bellrose, 1959). Once absorbed into the bloodstream, Pb inhibits heme-biosynthetic enzymes. Certain enzymes have been found to be inhibited at blood Pb concentrations of < 0.05 $mg.kg^{-1}$, suggesting that there may be no safe level of blood Pb (Martínez-López *et al.*, 2004). For these reasons, Pb is the most studied eco-toxin in wildlife, especially in waterfowl and other aquatic birds due to the use of Pb ammunition and fishing sinkers that accumulate in wetlands (Scheuhammer, 1987, Jordan and Bellrose, 1951, Burger and Gochfeld, 1993, Pain, 1996, Scheuhammer and Norris, 1996, Guitart *et al.*, 2002, Sears and Hunt, 2013). This body of research has resulted in clear, well accepted thresholds for Pb toxicity in most tissues of waterfowl species (Table 1.1) (Franson and Pain, 2011). Blood Pb concentrations elevated above 0.2 $mg.kg^{-1}$ are considered consistent with subclinical poisoning, while blood Pb concentrations above 0.5 $mg.kg^{-1}$ are consistent as clinical toxicity, and anything higher than 1 $mg.kg^{-1}$ is consistent with severe clinical toxicity (see toxicity thresholds of Pb in Chapter One for a more detailed explanation). Pre-breeding season blood samples showed 16 individuals (9.4%) above 0.2 $mg.kg^{-1}$, with five of these (2.9% of total) with concentrations high enough to be consistent with clinical toxicity. Post-breeding concentrations saw a slight decline, with only 10 individuals (7.14%) above 0.2 $mg.kg^{-1}$; of these only a single bird (0.7%) had concentrations consistent with clinical toxicity, whereas two (1.4%) were consistent with severe clinical toxicity. As the New Zealand game bird hunting season normally runs from May to June (prior to the pre-breeding season sample collection), this observed increase of blood Pb concentration in pre-breeding season samples could be explained by the increased exposure to newly expended ammunition, as suggested by another New Zealand study (Garrick, 2000). However, as Pb does not break down in the environment, spent ammunition can still be taken up throughout the year.

Scheuhammer (1987) established that blood Pb concentrations of 0.15 $mg.kg^{-1}$ and lower were consistent with low levels of environmental Pb exposure. Interestingly both sample periods had similar percentages of individuals that exceeded this amount: pre-breeding 12.3%, post-breeding 12.1%. Due to the global reduction in the use of Pb ammunition (Avery and Watson, 2009), especially around wetlands, these concentrations reported are comparable with the recent literature. Binkowski and Meissner (2013) reported 17% of their mallards sampled above 0.2 $mg.kg^{-1}$ in Poland, while Maia *et al.* (2017) found only 4.5% of the white storks sampled above this threshold in Spain. A Fish and Game survey of Pb blood levels was conducted in the East Coast and Bay of Plenty of the North Island in New Zealand a year before Pb shot was restricted in 1999 (Garrick, 2000). Overall, 15.4–18.5% of the 162 blood samples collected were elevated above 0.2 $mg.kg^{-1}$, with 4.9–6.8% of these elevated above 0.5 $mg.kg^{-1}$, although hunting rates, historical use, and environmental differences in these regions were not taken into consideration, making a direct comparison difficult. The results presented within this chapter show that Pb contamination of the two field sites continues and is reaching concentrations in wildlife associated with clinical toxicity.

Heavy metal concentration in eggshells

As egg formation and egg laying is recognised as a means of excreting environmental contaminants by oviparous females, egg concentrations can potentially be used as a surrogate for biomonitoring. However, Scheuhammer (1987) stated that the elimination of heavy metals to eggs could only take place when there was excessive accumulation in the organs of females. Once again, Cu was found to have the highest concentrations within the sampled eggshells, with a mean of 5.31 $mg.kg^{-1}$ (range 0.7–45 $mg.kg^{-1}$). Lam *et al.* (2005) found that the concentration of Cu in two species of birds' eggshells (little egrets (*Egretta garzetta*) and black-crowned night herons (*Nycticorax nycticorax*)) correlated with the Cu levels in the surrounding coastal marine sediment. This would suggest that eggs are useful non-invasive biomonitors of environmental contamination of Cu. The lack of published data on Cu content of mallard eggs hinders comparison of the data reported here to the literature. However, Kertész *et al.* (2006) did report that the vast majority of Cu content in mallard eggs was stored in the eggshell compared to the level in yolk and albumen. Extrapolated from a published graph, uncontaminated mallard eggshells sourced from a Hungarian waterfowl farm were estimated to have ~14 $mg.kg^{-1}$ of Cu (Kertész *et al.*, 2006). Concentrations of Cu in mallard eggs in this study were slightly higher than concentrations reported in lesser snow goose (*Chen caerulescens*) from Russia (Hui *et al.*, 1998), and similar to levels to common eider eggshells in Alaska (Franson *et al.*, 2004). Kertész *et al.* (2006) found that throughout incubation, when exposed to external sources of Cu (such as contaminated water on the incubating hen's feathers and legs), mallard eggs rapidly uptake the Cu, transferring it throughout the shell, membranes and albumen. This same study found that high Cu concentrations did not result in observable embryotic or teratogenic effects (Kertész *et al.*, 2006).

Several authors have suggested that very little Pb and Cd is actually transferred from females to eggs (Furness, 1993). This seems to be species-specific, as Burger (1994) found that herring gulls (*Larus argentatus*) used egg formation as an excretory method for both Cd and Pb. Dauwe *et al.* (2000) also found that eggshells of the great tit (*Parus major*) had higher concentrations of both Pb and Cd at a polluted site compared to an uncontaminated one, suggesting maternal transfer. The low concentration of Cd reported within the eggshells of this present study (only detectable in two clutches) suggests that Cd is not extensively offloaded. However, with eight clutches (13.6%) having Pb levels above the LOD and a similar value seen within the bloods, it is possible that females are able to offload a proportion of the Pb body load. The evidence from paired samples of female bloods and eggs suggests that any relationship between material Pb load and egg levels is weak and highly variable. Perhaps, if blood was sampled from the female closer to lay, the relationship could be more pronounced.

Maternal transfer of Pb, however, has been found in species with high prevalence of Pb shot ingestion, such as mallards (Vallverdú-Coll *et al.*, 2015, Mateo *et al.*, 2001). Medullary bones accumulate Ca before egg laying as a store for use in eggshell formation, and given the similarity of Ca and Pb, Pb is also deposited into bones (Chapter One). As females source Ca from medullary bones for eggshell formation, if bones levels of Pb are high then Pb may also be transferred into eggshells at the same time ((Franson and Pain, 2011, Simkiss, 1961). Vallverdú-Coll *et al.* (2015) found that sublethal exposure due to ingested Pb shot in mallard females can result in a significant maternal transfer through the eggs into the ducklings, which affected their developing immune system and reduced survival in early life stages. Furthermore, exposure of mallard eggs to Pb contaminated water resulted in increased embryo mortality (Kertész *et al.*, 2006). Of the eight clutches that reported Pb levels above the LOD, four (6.7%) were above the levels (0.2 $mg.kg^{-1}$)

seen to correlate with negative effects in ducklings after hatch (Vallverdú-Coll *et al.*, 2015). Laboratory experiments conducted on waterfowl showed that the Cd concentrations in eggs were low regardless of the amount consumed by laying hens (White and Finley, 1978). High Cd concentrations in eggshells are rare in the literature, and mainly reported from urbanised or industrial areas, such as with great tits (Dauwe *et al.*, 2005) and rooks (*Corvus frugilegus*) in Poland (Orłowski *et al.*, 2014). However, the addition of testing egg content as well as shell could provide more information, as Dauwe *et al.* (2000) found that while the shell Cd concentrations did not increase from control sites to polluted sites, egg content did. The lack of detectable Cd in mallard eggshells from New Zealand reported here is not unique, and many studies find very low levels of Cd in wild avian eggs (Agusa *et al.*, 2005, Morera *et al.*, 1997, Hui *et al.*, 1998, Franson *et al.*, 2004, Metcheva *et al.*, 2011). Even a single dose of Cd has been found to have a negative impact on avian reproduction for a short time, causing decreased egg production, eggshell thinning and deformed embryos (Furness, 1996, Rahman, 2007). In this present study, concentrations of Cd reported in eggshells ranged from below detection limit to 0.02 $mg.kg^{-1}$. These are much lower than the tentative threshold levels given for concentrations in internal tissues of birds that might be associated with adverse effects (liver: 40 $mg.kg^{-1}$, and kidney: 100 $mg.kg^{-1}$) (Furness, 1996). On this basis, it is possible that the low Cd concentrations in the eggshells of mallards in two areas of New Zealand are unlikely to cause adverse effect on embryo and duckling survival and development. This conclusion needs to be tempered by the caveat the eggshell may have limitations as a bioindicator, and analysing other components of the egg may be more informative for future studies.

Differences between sampling sites

Statistically significant differences related to site were detected in the pre-breeding season bloods for all three metals. Cd and Cu were found be to higher in Southland, while Pb was higher in the Waikato. However, within post-breeding samples, only Pb was found to be different between locations, with birds from Waikato once again having higher blood concentrations. No difference was observed in eggshell concentrations. The difference in uptake is interesting as published data (Land Resource Information System, 2016) suggest that the soil level of these three heavy metals are similar across both sites (Table 2.8). Therefore, these differences could be due to differences in bioavailability within the food web and/or a change in diet between the populations. In soil, metal cations are bound to negatively charged particles such as organic matter and clay. When metals detach from these particles, entering the soil solution, they become bioavailable with the potential to accumulate in plants (Gall *et al.*, 2015). Uptake rate of the heavy metals into plants may be altered due to various physical, chemical, and biological processes within the soil environment. It is known that pH plays a major role in availability of heavy metals (McGrath and Zhao, 2003), as does rhizospheric microbial activity (Peralta-Videa *et al.*, 2009), and soil microflora (Gall *et al.*, 2015). The level of accumulation of elements also varies between and within plant species (Peralta-Videa *et al.*, 2009). Within New Zealand, researchers and farmers have attempted to exploit these differences as a source of environmental maintenance and management. Farmers concerned with low Cu levels in livestock plant certain species, such as chicory and plantain, which accumulate high levels of this element (Liao *et al.*, 2000). Phytoremediation is an emerging technology, where plants are used for the clean-up of contaminated environments. Hyperaccumulator plants can not only accumulate high levels of essential elements, but also absorb significant amounts of nonessential metals, such as Cd (Peralta-Videa *et al.*, 2009). While phytoremediation will gradually remove heavy metals from soil, it can also increase uptake of heavy metals into omnivorous species such as mallards.

Invertebrates have two modes of exposure to environmental contaminants. They will either inadvertently ingest pollutants contained in the soil and plants, or come into direct contact with pollutants that can be absorbed through exoskeletons (Gall *et al.*, 2015). Accumulation is once again dependent on taxa and environment. A meta-analysis reported that Pb accumulated highest in Isopoda and Collembola, Cd accumulation was highest in Formicidae and Lumbricidae, while Cu accumulated most in Diplopoda, Isopoda, and Collembola (Heikens *et al.*, 2001). The overarching result suggests that due to diet, habitat, and physiological responses, certain invertebrate families and orders may be more likely to accumulate one heavy metal over another.

Differences in the two field sites – geographically, geochemically, and in adjacent land use –may result in more movement of pollutants throughout the ecosystems, and diet variation within the mallard populations. The geography of the Southland Plains allows for more ephemeral water to be present throughout winter months on paddocks. This leads to large aggregations of mallard flocks feeding in the wet paddocks. It is suspected that they were predominately feeding on earthworms, which could result in the increased amount of Cd and Cu seen in the blood in the winter pre-breeding birds. Different hunting rates could also be used to explain the increased level of Pb in the Waikato compared to Southland. In a Fish and Game technical report (Garrick, 2001), Waikato mallards were reported to have had a higher ingestion rate of Pb pellets than mallards in the Southland region (Waikato 10.6% vs Southland 7.5%), a result similar to what is reported in Chapter Three. No investigation into the source of Pb in mallard blood was conducted within my study, however, this this could be done with the use of stable isotopes. Isotope testing has been used with variable success to confirm the origin of Pb in wildlife and this is a possibility for future studies (Komárek *et al.*, 2008). This works by identifying the composition of the four naturally occurring isotopes ($_{204}$Pb, $_{206}$Pb, $_{207}$Pb, and $_{208}$Pb) within the environment and comparing it to the Pb found within the wildlife. The composition of these isotopes can vary between sources, creating a 'fingerprint', from which the origin source can theoretically be identified (Jiang and Sun, 2014). A limitation within this method is that sourcing of Pb for ammunition is no longer local, with many companies constantly changing the location of sourcing in response to global economic change, which results in a greater diversity of Pb isotopes being present. This complicates source attribution studies using isotopes. In my study, it would also be useful to know if the Pb accumulated by the mallards represents historical or recent Pb shot use, as this would guide policy on the effectiveness of current restrictions on Pb ammunition use in New Zealand. However, there is currently no method available to determine the age of Pb in an ecosystem.

Table 2.8 Effective median, and 95th quantile estimates of the background concentration (mg.kg^{-1}) of Cd, Cu, and Pb in the soils at both the Waikato and Southland field sites. Average taken from polygons surrounding both sites (Southland *n* = 3, Waikato *n* = 5). Predicted background soil concentrations (PBC) compiled from regional council and national soils database presented by Landcare Research (Land Resource Information System, 2016).

	Southland (*n* = 3)		**Waikato (*n* = 5)**	
	PBC (mgkg^{-1})	95th quantile	PBC (mgkg^{-1})	96th quantile
Cd	0.07	0.34	0.07	0.34
Cu	10.41	44.61	10.21	43.76
Pb	10.50	38.17	7.52	27.32

Difference between seasons sampled

Seasonal differences were present in female blood Cd concentrations with post-breeding season (January–February) females having lower concentrations than pre-breeding season birds (June–July) (Figure 2.13). Fertiliser is typically placed on agricultural pasture in late spring to help with growth of crops and grass species, after the majority of the spring rains have ceased. However, anecdotal evidence suggest that ducks are more likely to feed the paddocks in winter and early spring when the paddocks are flooded, bringing invertebrates to the surface. Within this period, it is most likely that exposure to Cd through diet and runoff is highest, resulting in this June–July increase in Cd blood concentration. Another interesting result was the increase in blood Pb concentrations in post-breeding season birds compared to the pre-breeding season birds (Figure 2.14), with Garrick (2000) noting a similar pattern in mallards from Wairoa, New Zealand. This difference could be due to the reduction in available waterways in the summer months, increasing the exposure to spent ammunition and thus increasing the likelihood of ingestion.

Influence of age and sex

Differences seen in this study between age cohorts and sexes were limited. Only Cd was influenced by age in pre-breeding ($p < 0.04$) and to a lesser extent in post-breeding ($p < 0.053$) bloods. Age difference in Cd accumulation is well documented within the literature (Maedgen *et al.*, 1982, Furness, 1996, Burger, 2008), due to its long half-life within the body allowing bioaccumulation over the lifetime of an organism (White and Finley, 1978, Nordberg *et al.*, 2014). While age of hen had no effect on the eggshell concentrations, Pb was found to increase with nesting attempt. Shell formation is reliant on the calcium (Ca) availability in the hen, either from dietary uptake or bone stores. As Pb mimics Ca, which allows it to be transferred into the bone, there is the possibility that as Ca reserves are depleted with the first clutch, more Pb is excreted in later clutches. Finley and Dieter (1978) found that laying mallard hens had a higher concentration of Pb in bones than non-laying females. Similarly, Cu concentrations in eggshell increased with consecutive clutches (Figure 2.12). Cu is known to play an important role in the shell membrane formations, which in turn influences the shell structure and shape. This observed increased could therefore be due to the liberation of soft tissue stores through the metabolic use in reproduction and egg formation. Only post-breeding season bloods included males, and only Cu ($p < 0.04$) was found to be influenced by sex, with females having higher concentrations than males. This difference could be due dietary variation, with females increasing uptake of certain foods to cope with the demands of reproduction, or due to the moult times, as males are more likely to moult after the breeding season, while females are delayed until broods are fledged (Leafloor and Batt, 1990, Gilmer *et al.*, 1977, Boyd, 1961). Alternatively, females may have enhanced gastro-intestinal uptake of Cu similar to Ca physiology. Further research is required to investigate the underlying physiology of this sex-based difference.

Conclusions

This study provides the first detailed information on the heavy metal loads within mallards in New Zealand. The results allow insights into the bioavailability of heavy metal pollutants within the environments sampled and their potential effects on wildlife. It adds to a growing body of global scientific literature establishing blood and eggshell levels of three different inorganic heavy metals in a cosmopolitan bioindicator species, and increasing the prior knowledge for future biomonitoring programs. As two spatially distinct populations were tested, an idea of the bioavailability of certain contaminants in respect to geographical, geochemical, and land use differences can be formed. Differences in the concentrations between pre-breeding season blood concentrations between the sites suggests that the availability of the metals sampled within these two environments are different depending on season. Further studies would be needed to establish a more complete view of the metals' movement through food webs. Cd showed differences in accumulation within blood samples in respect to age groups, while Cu concentrations were found to differ in regard to sex. Published threshold figures or critical levels of metals in non-destructive tissues of waterfowl relating to health and survival impairment are quite variable depending on sample type, species, metal, and literature source. However, on the basis of this existing information, these samples show that environmental exposure to increased quantities of Pb is occurring at levels consistent with adverse effects to the resident populations of mallards in New Zealand. The concentration of these contaminants present in the mallard blood and eggshell samples suggest that there may be wider implications for other waterfowl species in New Zealand, and possibly for human health where mallards are consumed as game meat. These issues are explored in the following chapter.

Chapter Three

Hepatic concentrations of heavy metals in mallards (*Anas platyrhynchos*).

Using hepatic concentrations of heavy metals in mallards to monitor bioavailability in the New Zealand environment, including an assessment of lead shot ingestion.

3.0 Abstract

Mallards (*Anas platyrhynchos*) are one of the most commonly studied aquatic bird species used as a bioindicator to monitor the pollution of their habitat. This is due to their wide global distribution, large populations, marked sexual dimorphism, and longevity, which allows for observations and assumptions of long-term exposures to pollutants and their bioavailability within the environment. The concentrations of three heavy metals (cadmium [Cd], copper [Cu], and lead [Pb]) were determined in the livers of two spatially distinct populations of mallards in New Zealand (Waikato and Southland). Both populations inhabited predominantly rural areas, where the main use of the land is either dairy or other livestock farming. Hunter-shot birds (n = 336) were collected over the 2016 waterfowl hunting season (May–August). Significant differences among birds from different sites were found for both Cd and Pb, with Southland birds having higher hepatic Cd, while Pb concentrations were higher in Waikato birds. Hepatic metal concentrations were different between sexes, with both Cu and Pb higher in males, while Cd was higher in females. Only Cd increased with age, with adults having higher hepatic concentrations than juveniles. Hepatic concentrations of heavy metals changed throughout the hunting season in Southland, with Cd increasing from May to August, while both Pb and Cu decreased. Thirteen individuals (3.9%) had hepatic concentrations of Pb elevated above background levels (> 2 mg.kg^{-1}), with four elevated to concentrations consistent with clinical poisoning (6–10 mg.kg^{-1}) and four consistent with severe clinical poisoning (> 10 mg.kg^{-1}). One hundred and nine birds (32.4%) had hepatic Cd concentrations elevated above background environmental levels (> 0.9 mg.kg^{-1}), with 17 (5.06%) showing concentrations > 2.1 mg.kg^{-1} suggestive of sublethal adverse effects. Fifty-three birds (15.8%) had hepatic concentrations of Cu > 100 mg.kg^{-1}, suggesting elevated background environmental exposure. Pb shot ingestion was detected in eight of the 343 gizzards collected (2.3%). Only half of these with ingested shot showed elevated hepatic concentrations of Pb, suggesting that the others might have ingested the shot too recently for transfer into tissues. Spent Pb shot is still a current route of Pb exposure to waterfowl in New Zealand, even with present restrictions on its use. This study of hepatic concentrations of heavy metals in two mallard populations in New Zealand clearly indicates that there is significant environmental exposure to all three heavy metals, which has implications for both wildlife and human health.

3.1 Introduction

In eco-toxicological studies concerning heavy metal accumulation in waterfowl, the most frequently tested organ is the liver, due to its crucial role in the detoxification process (Wayland *et al.*, 2001, Mateo and Guitart, 2003, Van Eeden *et al.*, 1996, Kim and Oh, 2012, Taggart *et al.*, 2009, Hui *et al.*, 1998). Even with the abundance of literature, interpreting metal tissue concentrations and establishing either a threshold or a critical value for adverse effects is difficult, due to the wide variation that is seen between and within species (Garcá-Fernández *et al.*, 1996, Gochfeld and Burger, 1987, Furness and Greenwood, 2013). This explains, in part, the wide diversity of critical values given for avian species found in the literature, supporting the argument for the use of critical ranges instead of a single threshold value (Pain, 1996). An overview of the observed hepatic critical concentrations found in the literature for the three metals examined in this study is shown in Table 1.1. It should be noted that the effort to establish diagnostic levels for Pb has been more thorough compared to other pollutants (Cd, Hg or Cu) due to the severity of Pb poisoning in birds globally (Mateo, 2009).

Pb poisoning, through ingestion of spent Pb shot, was first documented in the late 1800s (Hough, 1894, Grinnell, 1894) and since then it has been documented globally, resulting in at least 29 countries restricting the use of Pb shot, especially around waterways (Avery and Watson, 2009). Waterfowl, particularly dabbling and diving species, often mistake expended shot pellets as either food particles or grit (which is ingested and retained in the gizzard to aid in the mechanical breakdown of food). Once ingested, the grinding action of the muscular gizzard, coupled with the acids of the avian stomach (pH 2.5) erode and dissolve the pellet. The toxic Pb salts produced from this are rapidly absorbed into the bloodstream (Pain, 1996) and result in acute or chronic Pb poisoning. If a high number of pellets are ingested (> 10), acute poisoning will rapidly result with mortality occurring within a few days. More commonly, fewer pellets are ingested and chronic poisoning can result, causing an extended period of decreasing condition with affected birds dying 2–3 weeks after ingestion (Scheuhammer and Norris, 1996). Retention of ingested Pb particles is, however, variable. They can be excreted quickly with little to no Pb absorption, retained with partial erosion with significant Pb absorption, or retained until fully dissolved and absorbed (Franson and Pain, 2011). If not fatal, the majority of the shot will be removed from the gizzard within 20 days, either eroded and absorbed, or passed through the gastrointestinal tract (Sanderson and Bellrose, 1986). Surviving birds often exhibit sublethal poisoning, which affects many tissues, primarily in the peripheral and central nervous systems, as well as in the kidneys, and the circulatory and immune systems (Scheuhammer, 1987). This damage causes biochemical, physiological, and behavioural impairments, resulting in increased risk of predation, starvation, and disease in affected birds. Before shot restrictions were placed, Pb shot ingestion was judged to be the primary source of Pb exposure and subsequent poisoning in waterfowl (Sanderson and Bellrose, 1986). A national New Zealand study reported an average ingestion rate of 8.1% within mallards across six different regions the year before Pb restrictions were put in place; this included both Southland (7.5% ingestion) and Waikato (10.6% ingestion) (Garrick, 2001). While Pb shot used in hunting waterfowl has been banned in New Zealand for 10- and 12-gauge shotgun since 2001, it is still allowed for 22-gauge, a loophole that will be closed by 2021 (D. Klee, Auckland/Waikato Fish and Game, pers. comm.).

The movement of contaminants through the trophic chain is a potential source of exposure to humans. While the most consistent transfer of heavy metals is from agricultural food to humans, wildlife loads can also have an impact. Wild-shot game can be an important component of the diet of certain social and economic groups within societies, including subsistence and sports hunters, as well as those with an ethical preference for wild meat (Pain *et al.*, 2010). Within New Zealand, the sale of game-bird hunting licenses generates approximately $2.6 million NZD per annum (D. Klee, Auckland/Waikato Fish and Game, pers. comm.) and while Pb shot is restricted for certain species of waterfowl hunting, some species are exempt from this (e.g. Canada goose [*Branta Canadensis*]) and the national compliance rate is unknown. Adverse health effects to humans caused by exposure to heavy metals is well documented. Cd is known to cause kidney and liver damage, and bone demineralisation (Janicka *et al.*, 2015); Cu toxicity causes liver cirrhosis and damage to renal tubules, the brain and other organs (Johnston *et al.*, 2014); Pb poisoning may result in abdominal pain, nervous system disorders, and reduction in mental development (Needleman, 2004, Gibb and MacMahon, 1955). Pb shot residues embedded in hunted meat have been shown to increase the tissue concentration of game birds, even if the bulk of the shot is removed (Pain *et al.*, 2010). Moreover, studies have found that many hunter-shot carcasses will have small Pb fragments remaining, even when the shot exits the body (Mateo, 2009, Binkowski, 2012).

This chapter aims to evaluate the potential heavy-metal contamination (Cd, Cu and Pb) and ingestion rate of spent ammunition in two spatially distinct populations of mallards in New Zealand. While the implications of these results on waterfowl and human health are the primary drivers for the research, the primary objectives of this study were to: (i) determine contaminant load in livers of hunter-shot mallards in two locations in New Zealand; (ii) examine differences in hepatic contaminant load between sexes and age classes (juvenile vs adult), and the month of collection and site; (iii) establish the ingestion rate of Pb shot in mallards by examination of the gizzard contents.

3.2 Materials and Methods

3.2.1 Sample sites

The mallards studied were donated by hunters from two spatially distinct areas in New Zealand, Southland and Waikato (Figure 3.1). These sites were selected due to their importance to game bird hunting, with differing observed population trends, as well as their use in other mallard studies (Sheppard, 2017, Garrick *et al.*, 2017). The Southland site is located on the Southland Plains in the southernmost region of the South Island of New Zealand (46°12´18.68´´S, 168°19´46.19´´E). Historically it was a mosaic of tussock grassland, native forest and bogs, modified to pasture after the arrival of Europeans; land use now is dominated by livestock (beef, sheep and deer) as well as dairy (Miskell, 1993). Within the Southland region, there are an estimated 500,000 resident mallards (E. Garrick, Southland Region Fish and Game, pers. Comm.). Five hunters from seven locations throughout the Southland field site contributed to this study.

The Waikato site is located in the Waipa District in the central North Island of New Zealand (37°55´39.36´´S, 175°17´16.98´´E). Historically it was dominated by large peat-swamps, alluvial flats, and vast native forest. Since human arrival this area has been highly modified and is now dominated by pastoral farming and forestry (Macleod and Moller, 2006a). The mallard population has fluctuated in the past 10 years and has only recently started to increase after reaching a low point in 2009. Hunters collected ducks from 12 locations within the Waikato site.

3.2.2 Sample collection

From the two field sites, 343 individual mallards were collected between May 4th and July 9th 2016. All 343 samples were obtained from the hunters who had shot them, so location and hunter are known. Sex was determined by examination of the gonads, while age cohort was determined by the presence of the Fabricius Bursae (Siegel-Causey, 1990), size of testes (Johnson, 1961), and appearance of ovaries and oviduct. Wing plumage of mallards was not used for identification due to the change in characteristics through hybridisation with the New Zealand grey duck (*Anas superciliosa*). Two age cohorts could be distinguished: juvenile (hatched the previous spring) and adult (birds older than 6 months).

All carcasses were stored at -20^{o}c until processed. The preparation of the ducks and samples was carried out with care taken to avoid contamination; scalpels, surgical equipment, and gloves were cleaned between each bird as was the surface used for dissection. Both gizzards and livers were removed and placed individually in plastic bags with identification and stored again at -20^{o}c until processed.

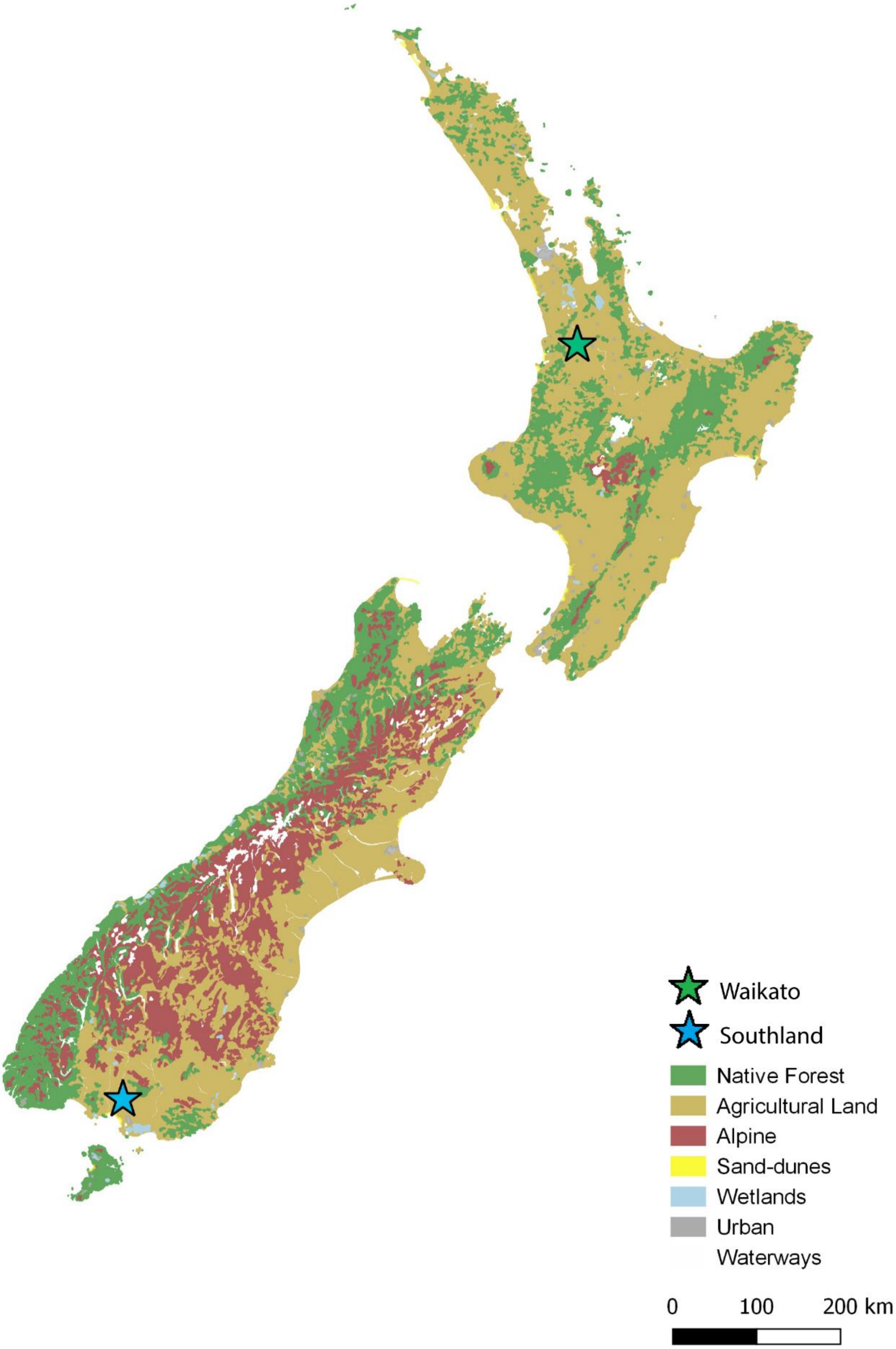


Figure 3.1: Field sites. Map created from "Vegetative cover of New Zealand" shapefile imported from LRIS (https://lris.scinfo.org.nz/layer/48420-vegetative-cover-map-of-new-zealand/) (Landcare Research Informatics Team, 2015)

3.2.3 Ingested shot analysis

Gizzards were radiographed to identify radio-dense silhouettes of metallic density that resembled gunshot pellets or metal fragments (Figure 3.2). Those showing signs of ingested metal were dissected for confirmation. Gizzards were cut in half along the sagittal plane with a scalpel, and the contents washed out with water into a shallow basin. The lining of gizzards was carefully inspected for the presence of shot entry holes to help eliminate those pellets which had entered ballistically. Vegetation was removed by decantation. A magnet was then passed over the fluid to attract steel pellets. The remaining material was placed in a petri dish and examined under a dissecting microscope (4–16x magnification). Identification of Pb shot was verified by scratching the surface with a scalpel. A positive result was recorded when a shot or piece of shot was recovered from the gizzard, without evidence of it entering ballistically.

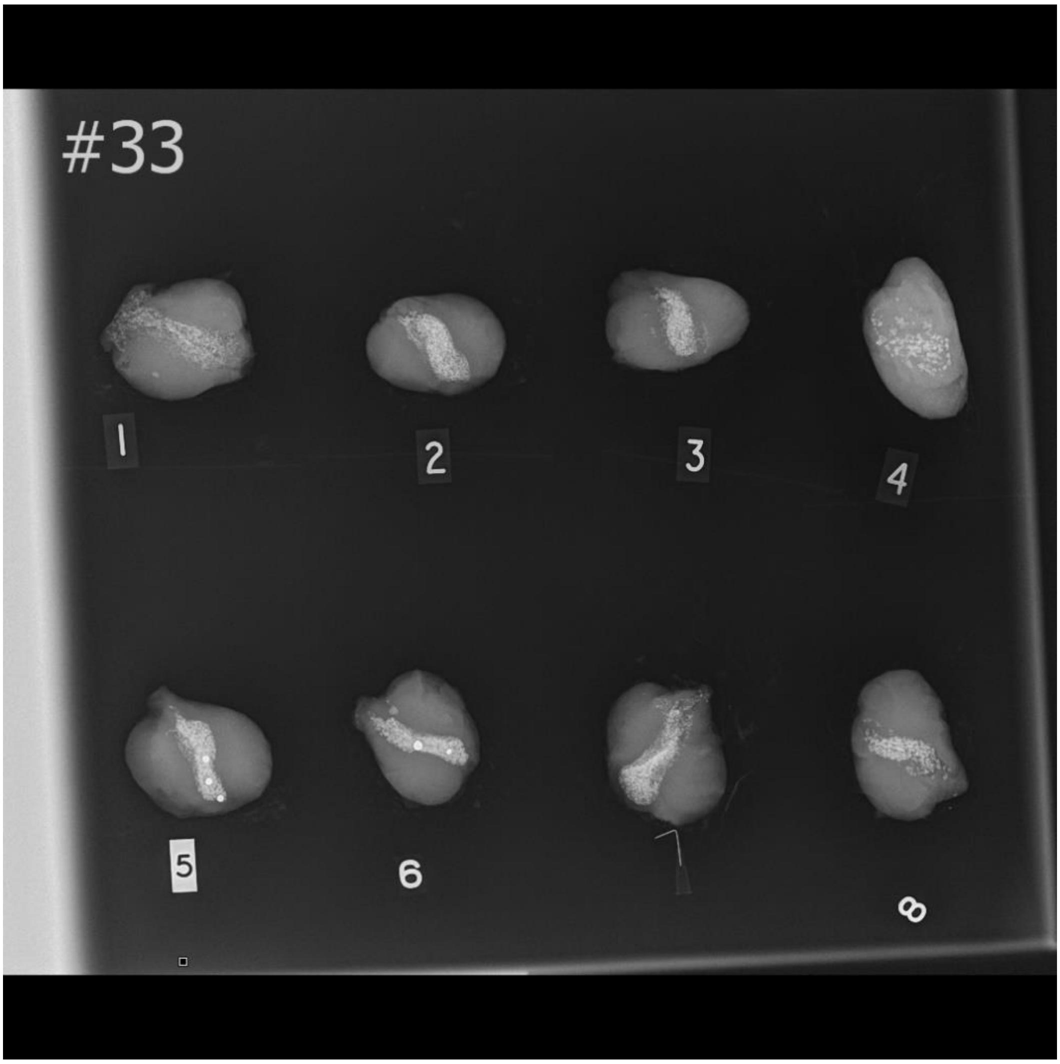


Figure 3.2 Radiograph of eight mallard gizzards – shot in the lumen of the gizzard can be seen in samples #5 and #6.

3.2.4 Toxicology

In total, 336 livers were collected from the carcasses, 163 from Southland (90 adult – 55 male and 35 female – and 73 juveniles – 40 male and 33 female) and 173 from Waikato (75 adults – 48 male and 27 female – and 98 juveniles – 42 male and 56 female). Livers were analysed for heavy metal concentrations at Hills Laboratory (Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton, New Zealand). A portion of approximately 2.5 g (range: 2.501–2.735 g) of ground and homogenised sample was digested with 2.2 ml of nitric acid and 0.5 ml of hydrochloric acid at 85ºC for one hour. Liver concentrations of Cd, Cu, and Pb were determined by inductively coupled plasma mass spectrometry (ICP-MS) using method 3125 described in *Standard Methods* (Eaton *et al.*, 2005). Blanks and standard reference material were processed and analysed to ensure the quality of the methodology. Limits of detection were 0.0004 $mg.kg^{-1}$ for Cd, 0.010 $mg.kg^{-1}$ for Cu and 0.002 $mg.kg^{-1}$ for Pb. Replicates of randomly chosen samples were also analysed to compare within-batch precision and performance of methods used. Hepatic concentrations are expressed as $mg.kg^{-1}$ wet weight. The analyses were performed on fresh tissue sample as figures related to animal tissue and foods standards are reported as wet weight, allowing reliable comparisons between datasets.

3.2.5 Statistical analysis

All statistical tests were performed using R studio (R Core Team, 2015). Values below the reported LOD were considered as one-half of the relevant LOD and included for statistical analysis, to minimise nominal type 1 error rates (Clarke, 1998). All three metal distributions in the livers were found to deviate from normal according to the Shapiro-Wilk test at the 0.05 confidence level. Cd was the only metal to conform to log normal, due to the extreme or outlying values of both Pb and Cu. Therefore, log-transformed Cd values for each location, sex, age cohort, and month of collection were studied using an analysis of variance (ANOVA) followed by a Tukey's honestly significant difference (HSD) ($p < 0.05$), and test interactions between factors were also studied. Non-parametric Mann-Whitney tests were used on Cu and Pb data, due to the skewness of the distributions of concentrations, to evaluate the differences between site, sex, and age cohort. Differences in concentration of Cu and Pb in regards to the month of shot were explored using non-parametric Kruskal-Wallis test, followed by *post hoc* Dunn's test to identify changes in concentrations. Due to the statistical differences documented between months in adults (see Results) and the lack of juveniles collected outside of May in Southland, difference in age is compared using May data only. Correlations between elements were examined by Spearmen rank correlation tests. However, no significant associations were found, with all $r_s > 0.2$ and thus will not be reported on. Data were considered statistically significant at $p < 0.05$. Pellets of both Pb and steel ingested shot were counted, a Chi-squared test performed to compare sites, and a Fisher's Exact test used to establish any association between location and the frequency of shot type found (Pb vs steel).

3.3 Results

3.3.1 Variation between site, sex, and age cohorts

All 336 livers had detectable levels of all heavy metal elements assayed. Hepatic concentrations of Cu showed the highest concentration load with an overall range of 5.2–870.0 $mg.kg^{-1}$, with no difference between sites (W = 15505, $p > 0.1$; Table 3.1). Hepatic Pb concentrations were significantly higher in the Waikato (W = 8688, $p < 0.0001$; Figure 3.3a, Table 3.1). Hepatic Cd concentrations in comparison, were significantly higher in Southland (ANOVA, $p < 0.0001$; Figure 3.3b, Table 3.1). Interaction plots for site vs age, and site vs sex clearly show that both age classes and sexes show elevated hepatic Cd concentrations in Southland compared to the Waikato (Figure 3.4).

Concentrations of all three elements differed significantly between the sexes (Table 3.2). Cu and Pb were higher in males than females (Figure 3.5b & c) while Cd was higher in females (Figure 3.5a). Hepatic concentrations of Cd were significantly higher in adults than juveniles (Table 3.3, Figure 3.6). There was no difference between age cohorts for Pb and Cu overall (Table 3.3), but suggested that adults had higher Pb levels than juveniles (W = 7198, $p < 0.10$).

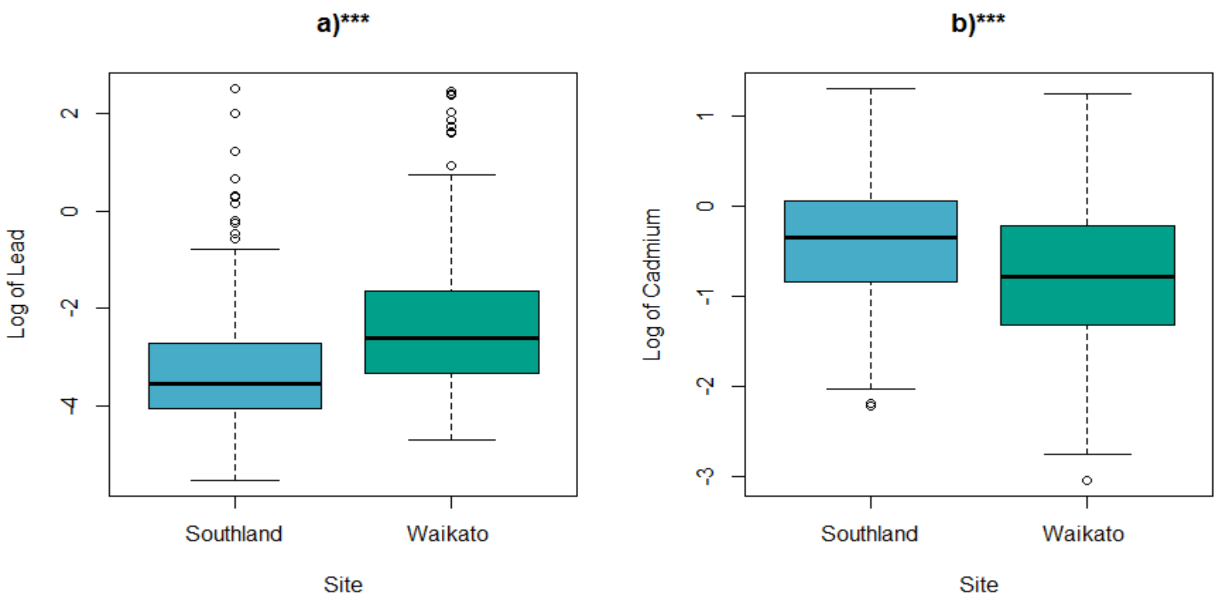


Figure 3.3 Mean mallard hepatic concentrations of a) Pb (Mann-Whitney nonparametric test), and b) Cd (ANOVA) showing statistically significant differences between sampling sites, Southland (n = 163) and Waikato (n = 173), (*p < 0.1; **p < 0.05; ***p < 0.01). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

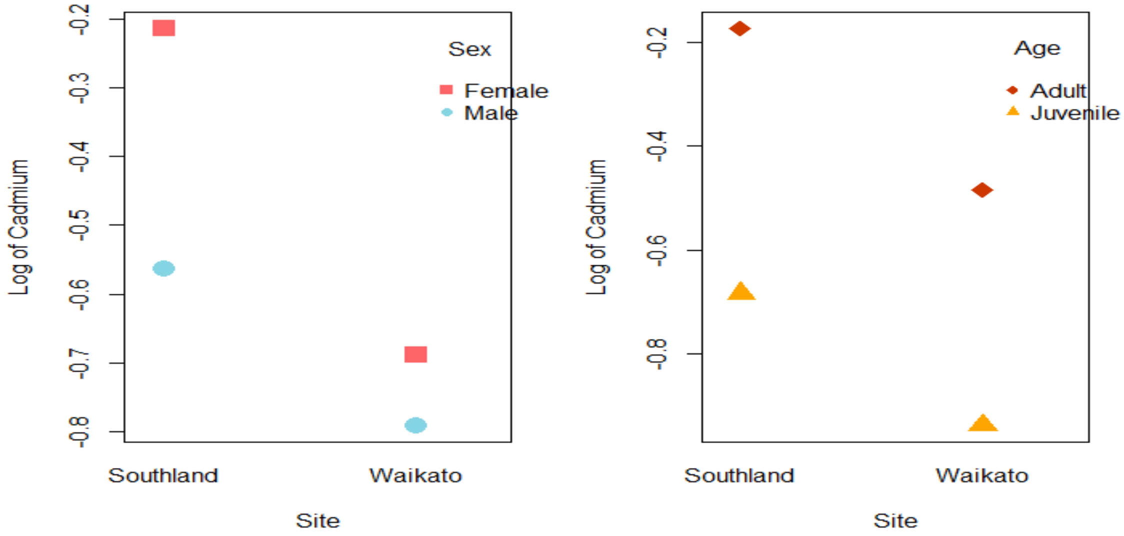


Figure 3.4 Interaction plots showing hepatic concentrations of Cd in relation to sex and age between sites.

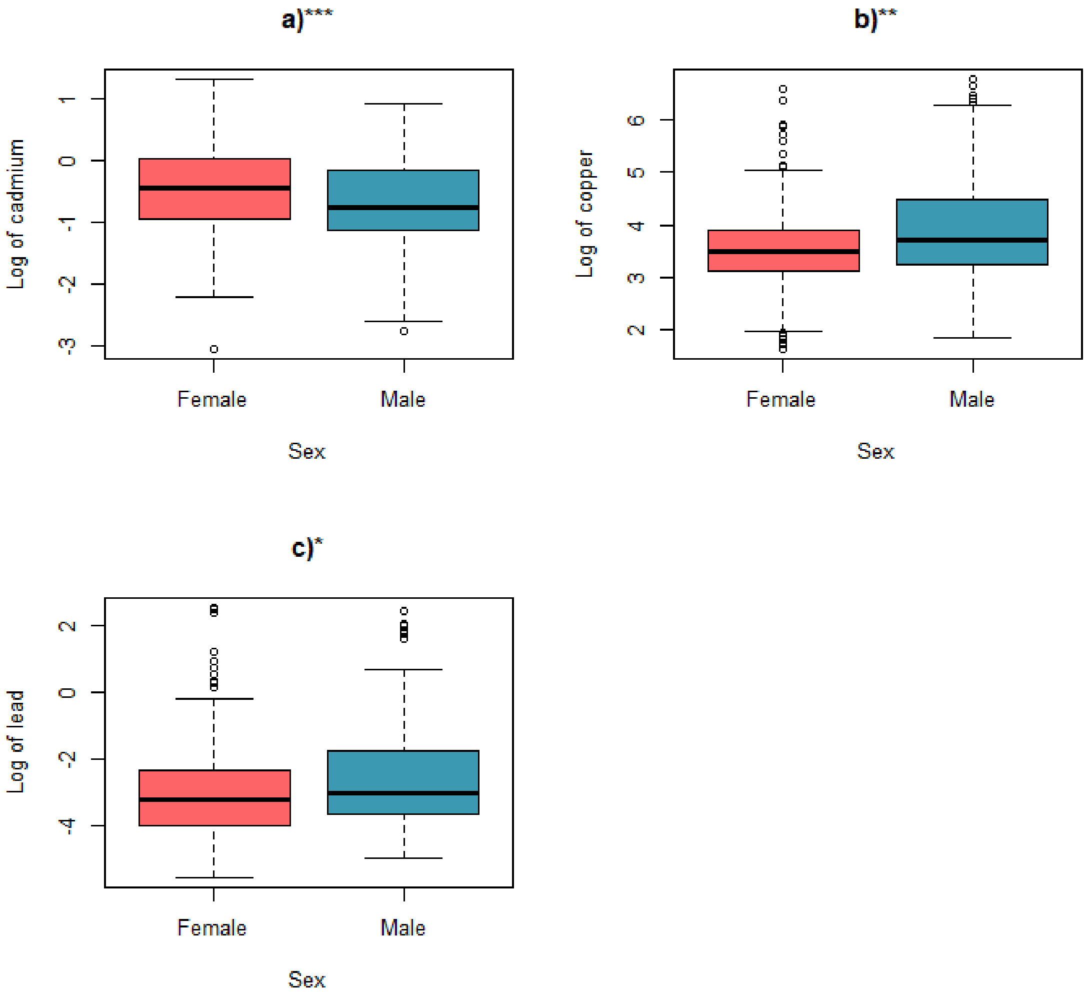


Figure 3.5 Hepatic concentrations of a) Cd (ANOVA), b) Cu (Mann-Whitney nonparametric test) and c) Pb (Mann-Whitney nonparametric test) of mallards showing statistically significant difference between sexes; females (*n* = 158) and males (*n* = 178), (*p < 0.5; **p < 0.01; ***p < 0.001). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

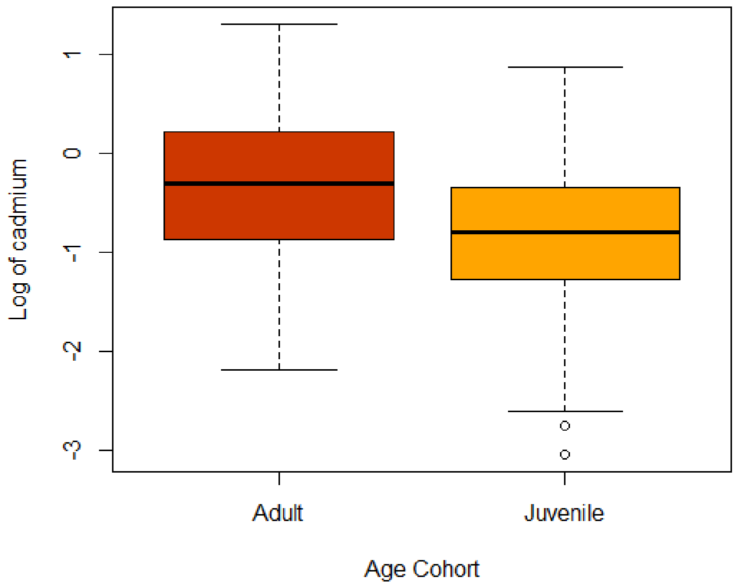


Figure 3.6 Hepatic concentrations of Cd in mallards showing statistically significant difference between age; adults (*n* = 167) and juveniles (*n* = 169), tested by ANOVA ($p < 0.001$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

3.3.2 Variation between month of collection

Adult Southland birds were collected over four months: May (29), June (36), July (15), and August (4). All donated juveniles were shot in May and were thus removed from this analysis. There were significant variation in hepatic concentrations of Cu (Kruskal-Wallis, $p < 0.02$) and Pb (Kruskal-Wallis, $p < 0.01$) by month of collection. *Post hoc* investigation (Dunn's test) identified that the detected difference seen in both metals resulted mainly from changes from May to June. Hepatic concentrations of Pb (Figure 3.7) were significantly lower in July than those collected in May (Z = -2.93, *p* [*adjusted*] < 0.05). *Post hoc* investigation on hepatic Cu concentrations (Figure 3.8) found the decreased levels in July compared to May did not reach significance (Z = -2.41, *p* [*adjusted*] < 0.09). Waikato birds were collected over a restricted period, with only two months being represented: May (139), and September (6). No significant differences in concentration were seen between these two months (Mann-Whitney, $p > 0.05$).

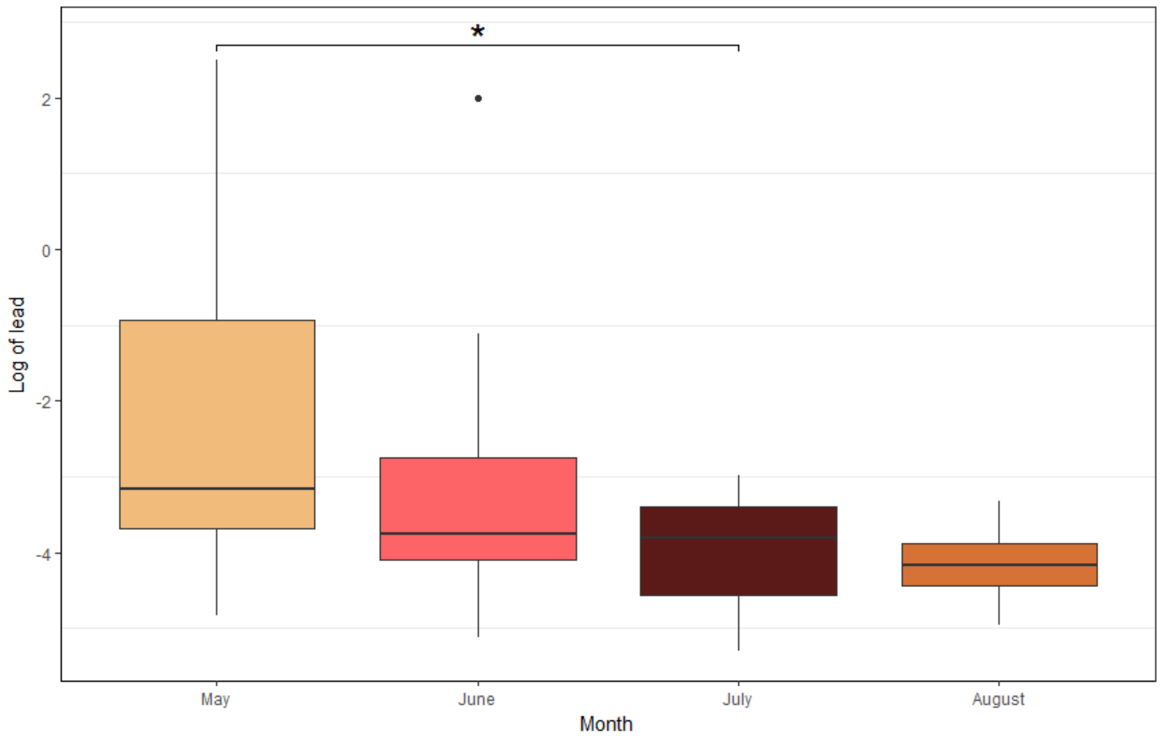


Figure 3.7 Change in hepatic Pb concentrations in mallards from Southland seen over the four months of collection. Statistically significant difference was found using Kruskal-Wallis analysis and Dunn's test. (*$p < 0.05$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

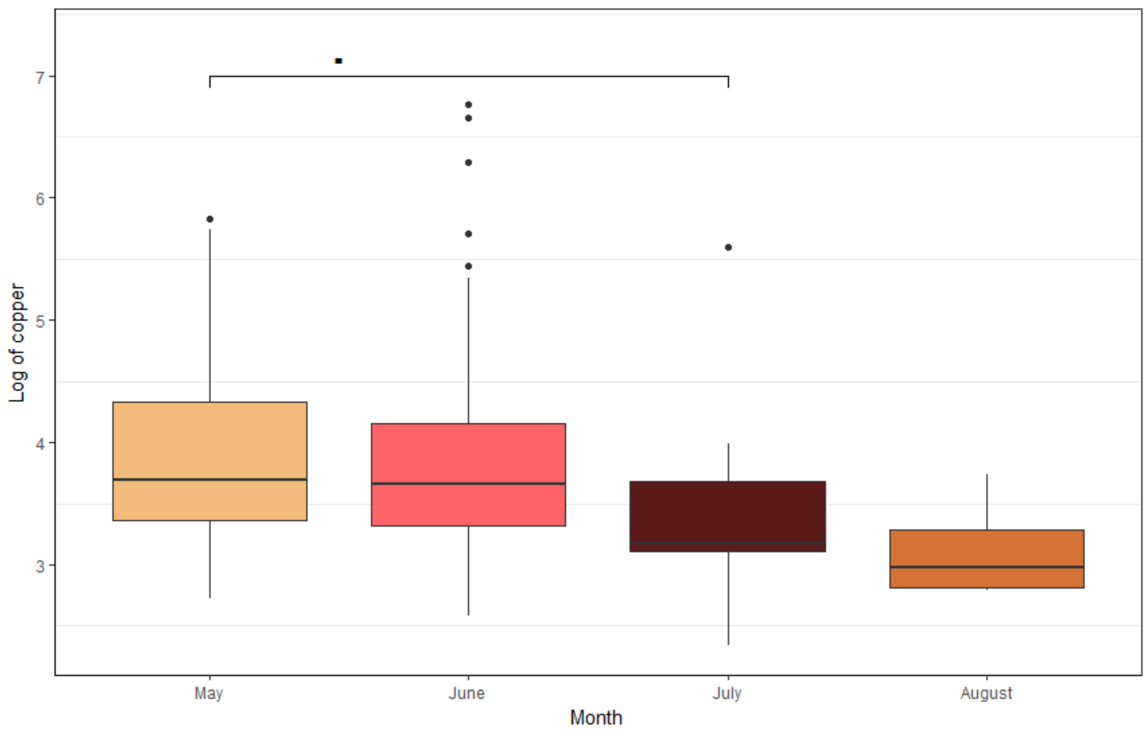


Figure 3.8 Change in hepatic Cu concentrations in mallards from Southland seen over the four months of collection. Statistically significant differences were found using Kruskal-Wallis analysis and Dunn's test ($^{\cdot}p < 0.1$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

Table 3.1 Hepatic concentrations of three heavy metals (Cu, Cd and Pb) (mg.kg^{-1} w.w.) obtained from hunter shot birds, by site (Waikato = 173, Southland = 170). LOD refers to limit of detection of the assay used. Significant difference between site tested by ANOVA for Cd, and Mann-Whitney nonparametric tests for Cu and Pb (*p < 0.1; **p < 0.05; ***p < 0.01)

		Waikato *n* = 173			Southland *n* = 170					
	LOD (mg/kg w.w)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	*p* value	
Cd	0.0002	0.65 ± 0.59	0.46	0.048 – 3.5	0.84 ± 0.602	0.71	0.109 – 3.7	ANOVA	0.000001	***
Cu	0.005	70.5 ± 114.32	35	5.2 – 790.0	89.43 ± 140.96	39	5.6 – 870.0	Mann-Whitney	0.11	
Pb	0.001	0.55 ± 1.75	0.073	0.009 – 11.7	0.25 ± 1.16	0.029	0.004 – 12.2	Mann-Whitney	0.000000001	***

Table 3.2 Hepatic concentrations of Cd, Cu, and Pb (mg.kg^{-1} w.w.) obtained from hunter-shot mallards, according to sex (male = 178, female = 158). Significant difference between sex tested by ANOVA for Cd, and Mann-Whitney nonparametric tests for Cu and Pb (*p < 0.1; **p < 0.05; ***p < 0.01).

		Female *n* = 158			Male *n* = 178					
	LOD (mg/kg w.w)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	*p* value	
Cd	0.0002	0.84 ± 0.70	0.64	0.048 – 3.7	0.66 ± 0.48	0.475	0.064 – 2.5	ANOVA	0.001	***
Cu	0.005	55.86 ± 88.9	33	5.2 – 730.0	100.83 ± 151.95	41	6.4 – 870.0	Mann-Whitney	0.002	**
Pb	0.001	0.38 ± 1.62	0.04	0.004 – 12.2	0.42 ± 1.39	0.049	0.007 – 11.1	Mann-Whitney	0.02	*

Table 3.3 Hepatic concentrations of Cd, Cu, and Pb ($mg.kg^{-1}$ w.w) obtained from hunter shot birds, according to the age cohort (adult = 167, juvenile = 169). Significant difference between age tested by ANOVA for Cd and Mann-Whitney nonparametric tests for Cu and Pb (*p < 0.1; **p < 0.05; ***p < 0.01).

		Adult n = 167			Juvenile n = 169					
	LOD (mg/kg w.w)	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Stat**	p value	
Cd	0.0002	0.94 ± 0.71	0.74	0.112 – 3.7	0.55 ± 0.396	0.45	0.048 – 2.4	ANOVA	0.000001	***
Cu	0.005	81.16 ± 141.14	34	5.2 – 870.0	78.23 ± 114.16	39	6.4 – 730.0	Mann-Whitney	0.11	
Pb	0.001	0.43 ± 1.57	0.043	0.004 – 12.2	0.38 ± 1.44	0.048	0.004 – 11.7	Mann-Whitney	0.51	

Table 3.4 Prevalence of shot ingestion in the gizzards of mallards in two spatially distinct populations in New Zealand including total ingested shot (both Pb and steel) and Pb shot only.

	Total	**Waikato**	**Southland**
#	343	173	170
Total ingested shot	55	32	23
Pb shot	8	6	2
% Ingested	16.04	18.5	13.5
% Pb Ingest	2.3	3.5	1.2

3.3.3 Thresholds for toxicity

Accepted thresholds for toxic metal concentrations in waterfowl livers do not exist for all elements studied. Toxic concentrations of Pb are well studied and displayed in Table 1.1 (Pain, 1996)**.** Thirteen individuals (3.9%) had hepatic Pb concentrations elevated above background exposure levels (> 2 $mg.kg^{-1}$ w.w.). These were 10 Waikato birds (four adult males, two juvenile males and four juvenile females) and three Southland birds (one adult female, one adult male and one juvenile female). Of these only eight had hepatic concentrations of Pb elevated enough to be considered consistent with clinical poisoning (6-10 $mg.kg^{-1}$ w.w.) and of these eight, four were elevated >10$mg.kg^{-1}$ w.w. consistent with severe clinical poisoning.

Cd threshold levels are not as well defined, but 67.6% of the birds had hepatic concentrations below the threshold for increased environmental exposure (< 0.9 $mg.kg^{-1}$ w.w.) (Scheuhammer, 1987). One hundred and nine birds (32.4%) had Cd levels > 0.9 $mg.kg^{-1}$ w.w., among which 17 individuals (5.06%) had > 2.1 $mg.kg^{-1}$ w.w., consistent with sublethal adverse effects (White and Finley, 1978). The maximum hepatic concentration of Cd found was 3.7 $mg.kg^{-1}$ w.w., which is well below the 40 $mg.kg^{-1}$ w.w. threshold considered indicative of Cd poisoning (Furness, 1996).

Toxicity thresholds for Cu are difficult to establish due to the high within-species variation of the hepatic concentration (Mateo and Guitart, 2003). However, 53 birds (15.8%) had concentrations of 100 $mg.kg^{-1}$ w.w. or higher, which is considered elevated in wild mallard studies (Di Giulio and Scanlon, 1984b, Kalisińska *et al.*, 2004). The proportions of birds in my study categorised into toxicity thresholds for the three heavy metals are displayed in Figure 3.9.

3.3.4 Prevalence of ingested shot

A total of 55/343 (16.03%) gizzards contained ingested shot (Table 3.4), with Waikato birds (n = 32/173) having a slightly higher count than Southland birds (n = 23/170). Of the 55 positive samples, eight (2.3%) were confirmed as Pb shot (six in Waikato and two in Southland). However, no statistical difference was found between location and ingestion rates (Chi-squared, $X^2 = 1.57$, $p = 0.21$). No association between location and the frequency of shot type could be established (Fisher's exact, $p = 0.45$). Of the eight individuals with ingested Pb, four had hepatic concentrations of Pb that are considered below background exposure (< 2 $mg.kg^{-1}$), two had concentrations consistent with sub-lethal poisoning (2–6 $mg.kg^{-1}$), and two had concentrations consistent with severe clinical poisoning (> 10 $mg.kg^{-1}$). Livers from birds with ingested Pb shot (n = 8) were found to have significantly higher concentrations of Pb than those without (W = 96, $p < 0.001$; Figure 3.10).

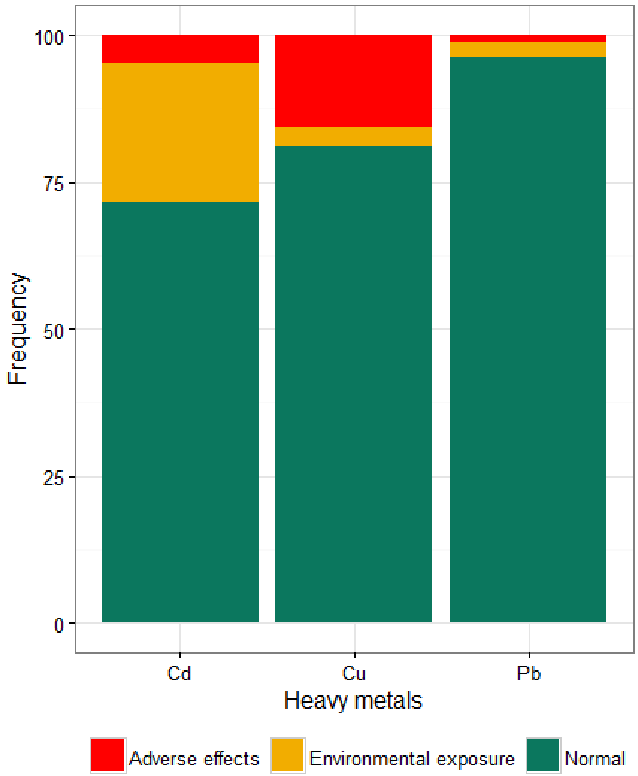


Figure 3.9 The proportion of birds categorised by threshold concentrations for toxic impact of heavy metals. Normal: indicates hepatic concentrations reported under values considered elevated. Environmental exposure: indicates hepatic concentrations considered elevated above assumed unpolluted background environmental levels. Adverse effects: indicates hepatic concentrations at which negative health effects have been reported.

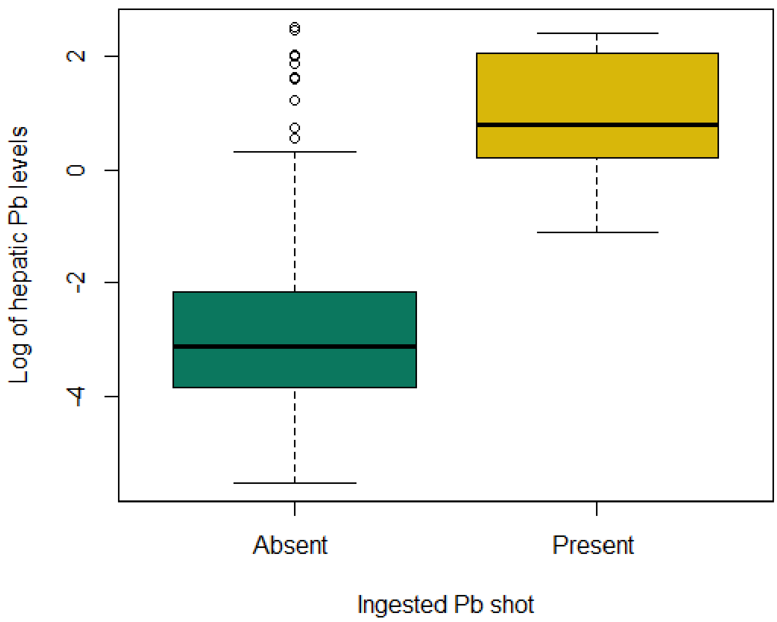


Figure 3.10 Hepatic concentrations of Pb in mallards with or without Pb shot in gizzards. Statistically significant difference found by Mann-Whitney test ($p < 0.001$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

3.4 Discussion

In many ecosystems, human-induced changes have overwhelmed the natural biogeochemical fluxes of trace elements, leading to the bioaccumulation and exposure of organisms to higher concentrations of some heavy metals. Little is known about the movement or the bioavailability of heavy metal contaminants from the ecosystem to wildlife within New Zealand. Indicator species, such as mallards, provide a useful tool for following heavy metal exposure over time as well as serving as a sentinel for early warnings of high environmental contamination. This chapter documents the hepatic concentrations of three heavy metals (Cu, Cd, and Pb) in mallard ducks from two spatially distinct populations within New Zealand. Ingestion prevalence of spent Pb ammunition by examination of the gizzards was used to help establish a possible source of exposure of Pb within the ecosystem. The results indicate that hepatic concentrations of Cu and Cd, and to a lesser degree Pb, were elevated within mallards during the months in which the two populations were studied.

Cadmium

Cd is, in addition to Pb and mercury, considered one of the most toxic metals to both humans and wildlife. Known as a nephrotoxin, Cd mainly concentrates in the kidney and to a lesser extent the liver, with up to 90% of the total body burden being contained in these two organs (Wayland and Scheuhammer, 2011). However, the kidney Cd concentration is considered unstable, as it decreases significantly after the production of Cd-induced tubular dysfunction (White and Finley, 1978). Hepatic concentrations of Cd are more stable and hepatocytes are generally resistant to Cd toxicity. The liver is therefore considered the better organ for indication of total body exposure to Cd (Scheuhammer, 1987). Hepatic stability comes from Cd binding to metallothionein within the liver, which provides a protective function by binding Cd into a stable complex with a long biological half-life (~20 years in humans). Thus Cd has a tendency to accumulate with age, even in organisms exposed to low background levels (Scheuhammer, 1987). Cd accumulation with age in waterfowl is well documented in the literature (Aloupi *et al.*, 2017, Mateo and Guitart, 2003, Kalisińska *et al.*, 2004). This pattern was also confirmed by the mallards collected in this present study, with adult levels 0.18 $mg.kg^{-1}$ higher than juveniles. However, young growing birds are more sensitive to the effects of Cd than adults, meaning that lower doses of Cd during development can have lasting adverse effects on juveniles (Cain *et al.*, 1983, Scheuhammer, 1987). Although no study has yet suggested liver threshold concentrations for adverse or toxic effects which would be applicable to immature, growing birds, this increased juvenile susceptibility must be taken into consideration when evaluating tissue concentrations (Wayland and Scheuhammer, 2011).

There was a difference between sexes in hepatic Cd concentrations, with females having significantly higher concentrations than males, although on average only by 0.11 $mg.kg^{-1}$. Sex-related foraging strategies or sexual-dimorphism are two hypotheses proposed to explain this observed sex-related difference (Stewart *et al.*, 1999). Although there is sexual dimorphism in mallards with males being on average 135 g heavier in the summer months (Sheppard, 2017), there appears to be no evidence for a substantial difference in diet or foraging behaviour. Higher liver levels in females may be due to the increased intestinal absorption of Ca during eggshell formation. The increase in Ca absorption involves an enhanced production of Ca bind protein (CaBP) within the mucosa cells (Wasserman *et al.*, 1971). The binding affinity of CaBP for Cd is similar to that of Ca, and consequently uptake of Cd may increase during eggshell formation (Bredderman and Wasserman, 1974). As results from Chapter Two suggest, with restricted efficiency of the additional excretion mechanism of Cd into eggshells, the increased intake of Cd would therefore be stored, which in turn could be a possible explanation for the higher female load. There are no consistent literature reports of sex-related Cd concentration differences (Gochfeld and Burger, 1987, Kitowski *et al.*, 2017b, Castro *et al.*, 2011, Hoshyari *et al.*, 2012), although on occasion females are found to be higher (Burger and Gochfeld, 1992, Hutton, 1981). This sporadic and conflicting difference in the literature supports Burger (2007) conclusion, that gender-related difference are rare.

Although better defined than for Cu, threshold levels for Cd toxicity are still dependent on the species studied, tissue collected, and age (Wayland and Scheuhammer, 2011). Most adult waterfowl have liver Cd concentrations of < 0.9 $mg.kg^{-1}$, thus concentrations ≥ 0.9 $mg.kg^{-1}$ are considered a threshold that demonstrates increased environmental exposure (Scheuhammer, 1987). Hepatic concentrations of > 2.1 $mg.kg^{-1}$ have been linked to sublethal adverse effects, such as testicular damage, suppressed egg production, altered energy metabolism, and disruption of hormone synthesis (White and Finley, 1978, Di Giulio and Scanlon, 1984a, Wayland and Scheuhammer, 2011, Scheuhammer, 1996). Concentrations of > 40 $mg.kg^{-1}$ are considered consistent with clinical Cd toxicity (Furness, 1996). In this chapter, 32.4% (109 of 336) of the birds had concentrations consistent with increased environmental exposure (> 0.9 $mg.kg^{-1}$), among which 5.06% (17 individuals) had levels consistent with adverse effects > 2.1 $mg.kg^{-1}$, with a maximum of 3.7 $mg.kg^{-1}$. These are well below the suggested ≥ 40 $mg.kg^{-1}$ threshold, for clinical toxicity. Toman *et al.* (2005) found that in pheasants (*Phasianus colchicus*), prolonged exposure to Cd-contaminated water (1.5 mg Cd^{2+}/L over 3 months) resulted in an adult hepatic Cd concentration of 3.53 $mg.kg^{-1}$ and an increased in the percentage of unfertilised eggs, but also an increase in hatch weight of chicks. Chronic exposure to Cd is known to suppress immune responses in birds, increasing their susceptibility to disease and other kinds of stress (Di Giulio and Scanlon, 1984a). Although exposure to Cd can adversely affect reproduction and survival, all of our knowledge of

Cd toxicity in birds comes from experimental studies, which dose birds at high levels over short periods of time (weeks and months) (Richardson and Fox, 1974, Sell, 1975, White and Finley, 1978, Świergosz and Kowalska, 2000, Rahman, 2007). Therefore, toxic effects associated with the low-level chronic exposure that is more likely to occur under natural conditions are undetermined in the literature.

Copper

Cu is essential for normal metabolic functions such as cellular respiration, antioxidant defence, and neurotransmitter function, however excess amounts can overwhelm the homeostatic control of the organism, resulting in toxicosis (Nordberg *et al.*, 2014). It has been suggested that waterfowl species may be more likely to accumulate Cu than other species, due to the rapid increase in liver concentrations seen in ducklings, which is maintained at a much higher levels (190 $mg.kg^{-1}$ d.w. / ~50 $mg.kg^{-1}$ w.w.) compared to that of chickens (15 $mg.kg^{-1}$ d.w. / ~4 $mg.kg^{-1}$ w.w.) fed on the same diet (Beck, 1961). Cu concentrations in waterfowl livers also vary among species, site of collection, and season (Hui *et al.*, 1998, Kalisińska *et al.*, 2004, Isanhart *et al.*, 2011, Parslow *et al.*, 1982), which makes establishment of background and threshold levels of Cu difficult.

Much like with the blood and egg samples (Chapter Two), Cu was found to have the highest hepatic concentrations of the three metals tested. This was expected due to the essential roles performed by Cu as a trace element in living organisms. Liver Cu concentrations were found to differ between sexes, with males having a higher average hepatic Cu concentration than females. This sex difference has also been found in other studies of mallards, and also in black ducks (*Anas rubripes*) (Gochfeld and Burger, 1987). However the opposite has also been found in other waterfowl species, with females having higher body loads in wigeon (*Anas penelope*) (Aloupi *et al.*, 2017), while other studies have found no relationship between Cu level and sex (Kalisińska *et al.*, 2004, Taggart *et al.*, 2009). These conflicting findings are supported by a critical review which concluded that there are few consistent sex-related differences in the accumulation of heavy metals in birds (Burger, 2007).

The season of collection has also been found to influence hepatic Cu concentrations. Parslow *et al.* (1982) found that mallards shot in October (UK Autumn) contained 40–50% more Cu than those in the following three months. Livers of San Francisco Bay (USA) surf scoters (*Melanitta perspicillata*) had higher concentrations in March (Boreal Spring) than February (Boreal late winter) (Henny *et al.*, 1991), while hepatic concentrations of canvasbacks (*Aythya valisineria*) from Louisiana (USA) were lower in November (northern hemisphere late autumn) than later months (Custer and Hohman (1994). Within the present study, hepatic Cu concentrations decreased from May (Austral late autumn) to August (Austral winter). This could reflect changes in environmental levels, as Hemingway (1962) reported higher concentrations of Cu in clover and grasses, both food sources for mallards, in Autumn than Spring, which could explain the higher level in May than August.

Although there are no widely accepted threshold guidelines for Cu toxicity in waterfowl, most wild mallard studies have reported liver concentrations of > 100 mg.kg^{-1} w.w. as elevated. Acute Cu toxicosis was reported in mallards treated with synthetic acid metalliferous water at liver concentrations of 81–391 mg.kg^{-1} (Isanhart *et al.*, 2011), although it should be noted that this study was evaluating the interactive effects of a number of toxic metals and acids. An accidental fatal poisoning of Canada geese (*Branta canadensis*) by an algaecide containing $CuSO_4$ resulted in hepatic concentrations of 56–97 mg.kg^{-1} w.w. (Henderson and Winterfield, 1975). Both of these studies reaffirm the wide range of hepatic Cu concentrations that may be associated with Cu-related mortality. Within this study a wide range of liver concentrations were reported (5.2–870 mg.kg^{-1}) with 15.8% (56 individuals) elevated above 100 mg.kg^{-1}. The population as a whole showed no evidence of differing levels between the two field sites, suggesting that the bioavailability of Cu is similar at both sites. As this study was based on hunter-shot birds, no live observations of these birds are available to correlate hepatic concentrations of Cu with clinical signs of toxicity. This, however, could be done in the future by examination of the gizzards and proventriculus for erosion, and staining of liver cells for Cu residues (Poupoulis and Jensen, 1976). Taggart *et al.* (2009) found 38.9% of white-headed ducks (*Oxyura leucocephala*) from El Hondo (Spain) had hepatic Cu concentrations above 100 mg.kg^{-1}, with a maximum value of 750 mg.kg^{-1}. While the Spanish study reported higher concentrations than the results of this present study, both report high values compared to other reports in the literature. An earlier study (Taggart *et al.*, 2006) in another Spanish wetland reported only 9.6% birds had a hepatic Cu concentration >100 mg.kg^{-1}, while Aloupi *et al.* (2017) reported such levels in only 1.8% of waterfowl from Evros (Greece).

The lack of correlation, or at least the varied relationship between hepatic concentration and toxicity, has been found in many species such as cattle (*Bos taurus*) (Todd, 1969), mute swans (*Cygnus olor*) (Kobayashi *et al.*, 1992), and rainbow trout (*Salmo gairdneri*) (Lanno *et al.*, 1985). It has therefore been suggested that the elevated liver concentrations merely provide a predisposition, and toxicity is triggered by other factors. Stress has been suggested as a cause, and has been observed in domestic duck studies when death by Cu toxicosis occurred only on days after handling (Wood and Worden, 1973). Regardless, the number of mallards with elevated levels reported here suggests that birds are exposed to Cu in high levels within their environment. The potential sources responsible for these elevated concentrations are numerous, due to the high proportion of New Zealand soils that are considered Cu deficient (Sillanpää, 1990) and consequently enriched for agriculture. Farmers and horticulturists use Cu-rich fertilisers, while also supplementing with mineral blends and palm kernel extract to increase uptake in livestock (Johnston *et al.*, 2014). Additional methods, such as the use of certain plants which are known to have elevated uptake of Cu are also planted, to increase bioavailability and mobility within the food chain (Liao *et al.*, 2000). Due to these practices, rural environments have the potential to be saturated with bioavailable Cu, which could explain the high levels seen within this study. Another potential source from dairy farming is Cu sulfate footbaths, which are used to control digital dermatitis in dairy cattle (Reichenbach *et al.*, 2017). This condition and treatment is common in New Zealand pasture systems (Yang *et al.*, 2017). Waste Cu sulfate solution from these footbaths is pumped out onto paddocks and will therefore enter the wider ecosystem.

Lead

Pb is a highly toxic pollutant with no known biological requirement that acts as a nonspecific poison affecting all body systems. Absorption, even at low concentrations, can result in a wide range of sublethal effects in living organisms, while high concentrations result in mortality (Demayo *et al.*, 1982). Pb is predominantly absorbed through the digestive tract into the bloodstream, where it is deposited rapidly into the soft tissues (primarily kidney and liver), as well as growing feathers and bones. Relative concentrations in individual tissues depend on time since exposure and absorption rates (Franson and Pain, 2011). As the end point for absorbed Pb is the skeleton, where it is incorporated in place of calcium, bone concentration is normally considered the best indicator of chronic and historic Pb exposure, with concentrations generally increasing with age (Pain *et al.*, 2005, Scheuhammer *et al.*, 1999). As Pb is removed far more quickly from soft tissues than bone, wild birds rarely have lower Pb concentrations in bone than tissue (Franson and Pain, 2011). Therefore, liver is seen as an indicator of more recent exposure, which could be the reason why no effect of age was found in this study when it is established in the literature that total body concentrations of Pb generally increase with age (Pain *et al.*, 2005, Scheuhammer *et al.*, 1999, Franson and Pain, 2011)

Sex and reproductive stage are other factors that are known to affect both absorption of Pb and its deposition in tissues. While males were found to have higher hepatic levels of Pb compared to females in my thesis, Finley *et al.* (1976) found that laying mallard hens dosed with Pb ammunition accumulated significantly higher bone, kidney, and liver concentrations than males. It should be noted that the major difference in accumulation happened in the bones, with females having levels more than 11 times that of the males. The reproductive stage is also important, with laying females found to have four times as much Pb in the femurs than non-laying females (Finley and Dieter, 1978). Experimental studies suggest the higher bone Pb levels in females could be related to the calcium requirements and active bone metabolism during egg laying (Finley and Dieter, 1978, Scheuhammer, 1996, Christian Franson *et al.*, 1998). Taggart *et al.* (2006) reported that female mallards had higher hepatic concentrations of Pb, however Mateo and Guitart (2003) found the opposite, reporting male mallard liver concentrations of Pb as being higher, with both studies conducted in Spain. Many studies have also reported no sex-related differences in hepatic Pb concentrations (Aloupi *et al.*, 2017, Gochfeld and Burger, 1987). This disparity once again supports Burger (2007) conclusion that due to the sporadic and conflicting literature, the relationship between sex and heavy metal uptake is hard to establish.

Birds from the Waikato were found to have significantly higher liver Pb concentrations than birds from Southland, a result which was mirrored in the blood concentrations (Chapter Two). With both studies agreeing over different seasons and reproductive stages, it can be assumed that the bioavailability of Pb in the Waikato region is higher. As the estimated soil load of Pb is actually lower in Waikato (7.52 $mg.kg^{-1}$ compared to 10.50 $mg.kg^{-1}$, Table 2.8), other sources may be contributing to this observed difference. Worldwide, the most common source of Pb for waterfowl remains lead-based ammunition (Avery and Watson, 2009), which I will discuss as a possible source in the next section but here I will focus on non-ammunition based alternatives. Atmospheric inputs to the environment can be a significant contributor to the levels of heavy metals in the environment. In comparison to other input forms (fertilisers, irrigation water, livestock manure and agrochemicals), atmospheric inputs have a more rapid effect on terrestrial environments, due to the direct contamination of plants by atmospheric fall-out on which animals graze. This pathway eliminates the involvement of both soil and plant uptake as the contaminant is ingested directly with the plant. Gray *et al.* (2003a) monitored seven rural sites to determine the rate of atmospheric deposition of heavy metals in New Zealand. Their Southland site had an average Pb deposition of 9.9 g ha^{-1} y^{-1} over a two-year period (the lowest over the seven sites), while their Waikato site had the second largest, 31 g ha^{-1} y^{-1}. It was also reported that soluble Pb comprised a significant proportion of the total Pb deposition, ranging between 8% and 47%, with a mean of 33% (Gray *et al.*, 2003a).

Land use is also known to influence the levels of trace elements within the environment (Gaw *et al.*, 2006). As heavy metals do not break down, historical use of agrichemicals (pesticides, fertilisers, fungicides and soil amendments) has resulted in the build-up of trace elements in both agricultural and horticultural soils. In New Zealand, Pb -arsenate was widely used as a pesticide for codling moth (*Cydia pomonella*) in orchards from the early 1900s till the late 1960s (Atkinson *et al.*, 1956). Gaw *et al.* (2006) linked the use of this to the high soil loads found in three locations in New Zealand (Waikato, Auckland and Tasman). They reported that Waikato horticultural soils were significantly higher in Pb than grazing soils (means of 48.3 and 24 $mg.kg^{-1}$ respectively). It should be noted that this paper also reported a mean Waikato background level of 29 $mg.kg^{-1}$

sampled from under indigenous vegetation, a value that is much higher than the figure given by Landcare Research (Table 2.8). Inputs of Pb into the New Zealand environment would be expected to be lower now than reported previously, as no Pb-based agrichemicals are currently in use and unleaded petrol has been used since 1996. Current residual inputs would include Pb as a contaminant of certain fertilisers and sewage (Gaw *et al.*, 2006).

The Pb load of freshwater is a possible path of contamination for waterfowl. The speciation and subsequent bioavailability of Pb in freshwater systems depend on many parameters: pH, hardness, alkalinity, salinity, and the concentration and quality of natural organic matter and dissolved organic carbon. However, it is thought that pH plays the biggest role in determining Pb solubility (Mager, 2011). Waters of high pH and alkalinity Pb will bind strongly with CO_3^{-2} and OH^-, while low pH and alkalinity have a far greater percentage of the free ionic form, Pb^{2+}, and are therefore more toxic. The shift between these two states is quick, resulting in a dramatic change in the speciation and solubility of Pb as the pH approaches neutrality (6.5–7.5). An Environment Waikato report (Vant and Smith, 2004) found that the pH in seven freshwater sites within the Waikato region significantly decreased over a ten year period, becoming more acidic at all sites although never dropping below pH 6.5. While unpublished data from Environment Southland indicates a trend toward alkaline conditions in many Southland streams (Environment Southland unpublished data). The more likely route of uptake in the freshwater systems is through Pb bound in the sediment ingested either by active grit intake or as a consequence of foraging (Tsipoura *et al.*, 2011). It has been shown that ingestion of Pb-contaminated sediments from mining activities is an important route of exposure that can result in the accumulation of Pb in waterfowl tissues (Hoffman *et al.*, 2000). Heavy metals in the sediment can also be taken up by aquatic plants and are redistributed in the leaves and root systems (Weis and Weis, 2004). Species such as mallards that feed on these plants will therefore play an important role in the transfer of contaminants through the aquatic food web.

Defining the source of Pb in the mallards in this study was not possible with the data available. Source attribution studies are important in reducing ecosystem contamination and minimising the effects of Pb on human and wildlife health. In particular, for the New Zealand ecosystems, defining the role of historic and more recent Pb shot use is important for the duck hunting community. As previously discussed in Chapter Two, isotope analysis has been used with varying success in identifying possible sources of Pb in wildlife (Komárek *et al.*, 2008). Successful identification of the source through isotope analysis is highly dependent on its application. Studies report higher success occurring when a large number of sources are examined, and when those sources are isotopically different (Gwiazda and Smith, 2000). Case studies where specific sources were suspected have also been successful (Finkelstein *et al.*, 2014), meaning that isotopes associated with ammunition could be investigated if this study was taken further. However, as stated in Chapter Two, Pb sourced for ammunition is no longer consistent due to the global market which results in greater variations of isotope ratios, making source attribution studies more difficult.

Pb is perhaps the only metal studied here for which widely accepted values of tissue concentrations in waterfowl exist (Table 1.1). Hepatic concentrations elevated above 2 $mg.kg^{-1}$ are classed as subclinical toxicity, meaning levels are known to cause adverse physiological effects but if the source is removed individuals have a high chance of survival (Pain, 1996). Of the 336 samples collected 13 (3.9%) were reported with levels above this value. These thirteen samples were predominantly from Waikato birds, with only three from Southland, and a relatively even split between sexes (seven males, sex females) and ages (sex adults and seven juveniles). Of these, eight (2.4%) had liver Pb concentrations indicative of clinical poisoning, passing the threshold where pathological manifestations of physiological effects are observed (6–10 $mg.kg^{-1}$). Four (1.2%) were elevated above 10 $mg.kg^{-1}$, indicating severe clinical poisoning, an approximate threshold of expected mortality. Once again, due to the global movement to reduce Pb ammunition around freshwater systems, the elevated concentrations reported here are comparable to the recent literature. Aloupi *et al.* (2017) reported 17.5% of waterfowl species from Evros (Greece) elevated above 2 $mg.kg^{-1}$; 9.5% were subclinical, 1.6% clinical, and 6.3% severe clinical. Taggart *et al.* (2006) found that only 2.4% of waterfowl in the Doñana National park (Spain) had hepatic concentrations elevated >2 $mg.kg^{-1}$, and a more recent study found 8.5% of waterfowl from El Hondo (Spain) elevated above background levels (Taggart *et al.*, 2009). The lack of knowledge regarding hepatic concentrations of Pb in waterfowl prior to the partial restriction of Pb based ammunition in New Zealand makes it difficult to be conclusive when discussing the effect of the current Pb ammunition management strategy on the survival of New Zealand waterfowl. The results presented in this chapter indicate that Pb toxicity is a continuing issue for a proportion of mallards in both study populations.

Ingested shot

Waterfowl are known to be particularly susceptible to Pb poisoning due to their philopatry for wintering grounds, migration routes and breeding sites (Kalisińska *et al.*, 2004, Robertson and Cooke, 1999, Arnold *et al.*, 2002). In New Zealand this is intensified due to the behavioural loss of migration in mallards; it is estimated that mallards in New Zealand, on average do not move further than 50 km from their hatching ground (McDougall, 2012). Therefore, the foraging areas are well known to hunters who have hunted waterfowl in these areas for generations, resulting in an accumulation of spent shot pellets over the years. Of the 343 collected gizzards, 55 had ingested shot, but only eight of these (2.3% of total gizzards sampled) contained Pb shot (3 females, 5 males; 3 juveniles, 5 adults). Waikato birds had the highest ingestion rate of 3.5%, while Southland birds had only 1.2%. This is a marked difference from a Fish and Game survey the year before Pb shot was restricted, which reported an ingestion rate of 7.5% (17/226) in the Southland region and 10.6% (41/385) in the Waikato (Garrick, 2001). Both sites showed a lower rate of Pb shot ingestion in my study than in the Fish and Game survey (*chi-square* test of independence; Waikato- X^2 = 7.07, df = 1, p<0.01, Southland- X^2 = 7.22, df = 1, p<0.01), suggesting a reduction in Pb shot used in both regions. The ingestion rate reported here is similar to other mallard and black duck studies in areas where Pb was either banned or restricted where reported ingestion prevalence is <10% (Mudge, 1983, Sanderson and Bellrose, 1986, Daury *et al.*, 1994). Of the eight birds reported in this study with ingested shot only half had corresponding liver Pb concentrations indicative of subclinical poisoning (2 $mg.kg^{-1}$). While most studies do see a clear relationship between ingested shot and high hepatic Pb concentrations (e.g. 97% > 2 $mg.kg^{-1}$ (Taggart *et al.*, 2009), 80%> 2 $mg.kg^{-1}$ (Mateo *et al.*, 2001)), all eight ducks within this study were shot early in the hunting season (in May) and thus the shot may not have had enough time to be digested. It should be noted that ingestion rates have been found to have high temporal variability, with birds collected in summer months finding higher ingestion of spent pellets (Garrick, 2000). Therefore, the limited period of collection within my study could be seen as a limitation, which could be rectified by seasonal collections in future studies.

Although there was no significant difference in ingestion rates between the two sites, Waikato was found to have three times as many incidences of Pb shot. This could be an indication of a possible route of Pb exposure, resulting in the differences seen within the blood and liver samples between the two sites. The potential for Pb contamination of surface water due to the presence of spent Pb shot must also be considered, as most waterfowl hunting in New Zealand occurs in enclosed private ponds. Ducks inhabiting areas where intense hunting occurs, where Pb pellets are introduced to the environment over many years are known to be at high risk of Pb exposure (Mateo *et al.*, 2007, Pain, 1990, De Francisco *et al.*, 2003). Pb pellets can remain in soil between 15 to 300 years, depending on physiochemical conditions such as temperature, moisture and rainfall (Jørgensen and Willems, 1987). In soil and substrate Pb is oxidised from metallic Pb to Pb^{2+}, which is present in the form of different chemical species of increasing stability until reaching highly stable forms (most commonly: litharge/massicot [PbO], cerussite [$PbCO_3$] or hydrocerussite [$pb_3(CO_3)_2(OH)_2$])(Cao *et al.*, 2003, Romano *et al.*, 2016, Hashimoto, 2013). These transformations result in an increase of Pb in the soil, water, and vegetation increasing its bioavailability for its incorporation into the food chain (Cao *et al.*, 2003). Craig *et al.* (1999) reported that streams draining a shooting range had Pb concentrations of 473 μgL^{-1} compared to levels of 0.5 μgL^{-1} further upstream and concentrations quickly fell downstream. While concentrations of Pb in other biota were not tested within my study, Pb bioaccumulation and biochemical effects were documented in fish collected in a Pb-shot contaminated stream in Denmark with a total Pb concentration ranging from 15–45 μgL^{-1} (Heier *et al.*, 2009). This could be an issue for hunting ponds, which have often been used for generations and are vulnerable to shot accumulation.

While Pb shot used in hunting waterfowl is banned in New Zealand for 10- gauge and 12-gauge shotguns, it is still allowed for 20-gauge shotgun ammunition and shooting of non-game species (Canada geese). It should also be noted that both sites have a high presence and involvement of Fish and Game rangers within the hunting community, and it is possible that the hunters will have a higher rate of compliance in these regions compared to other locations in New Zealand. Another bias could result from the collection – hunters were made aware of the aim of this study, and many birds were donated by the same individuals, making it unlikely that noncompliant hunters would offer up their carcasses.

Risks to the food chain and human food consumption

Increased contaminant levels in birds in New Zealand can impact on the predators that consume them, including humans. Although the concentrations of most metals were not elevated above background levels (Figure 3.13), the high hepatic concentrations reported for all three metals gives some cause for concern. The bioaccumulation of Pb is well established, with increased levels of Pb in the tissues of prey resulting in increased levels by orders of magnitude in the predators which consume them. A wide range of raptor species have been exposed to Pb due to predation or scavenging of Pb-shot game and waterfowl (Hunt *et al.*, 2006, Battaglia *et al.*, 2005, Pain *et al.*, 2005, Clark and Scheuhammer, 2003, Mateo *et al.*, 2003). In New Zealand, wild Australasian harriers (*Circus approximans*) have been documented with clench claw paralysis associated with Pb toxicity (McLelland *et al.*, 2011). There are also anecdotal reports of Pb toxicity in the endangered New Zealand falcon (*Falco novaeseelandiae*) but the source of the Pb in these raptors is yet to be definitively established.

Hunters and their families are also susceptible to ingestion of Pb from wildlife, with studies showing that people who consumed wild game had higher blood Pb levels that those who didn't (Iqbal *et al.*, 2009). Scheuhammer and Templeton (1998) found that the stable Pb isotope ratios in eagles and Canadian waterfowl closely matched that of Pb ammunition, supporting the view that the main source of exposure to Pb in these species is through ingestion of Pb shot. Levels of Pb in the liver of mallards may be of concern for human consumption, as 10.4% had with levels ≥0.5 mg.kg^{-1}, the maximum level of metal contamination allowed in edible offal of cattle, sheep, pig and poultry in New Zealand (Australia New Zealand Food Authority, 1987). However, most countries reduce this to ≥0.1 mg.kg^{-1} for food intended for infants and children (Food and Drug

Administration, 2006, Food Safety Authority of Ireland, 2009), which would suggest that 29.5% of the mallard livers in my study were unsuitable for consumption by children.

Cd concentrations have also been found to progressively increase along trophic levels (Croteau *et al.*, 2005) and therefore high Cd levels should also be considered as potentially harmful for human consumption. Within New Zealand, the maximum level of Cd allowed in the livers of cattle, sheep and pig used for human consumption is 1.25 $mg.kg^{-1}$, however, this does not include poultry. Other countries that include poultry in their standards implement a maximum level of 0.5 $mg.kg^{-1}$ (Food Safety Authority of Ireland, 2009, Commission Regulation (EC), 2006). Within the New Zealand standards, assuming waterfowl would be held to the same standard as livestock, 16.1% of the livers exceed the maximum allowable threshold. However, if the more conservative value of 0.5 $mg.kg^{-1}$ is used, then 55.4% of the mallard livers in my study exceeded the safe threshold for human consumption. While not a large numbers of hunters will eat the internal organs, the high number of livers which exceed the maximum levels of metal contaminants in food is a major concern for the transfer of these pollutants into humans. The liver concentrations of Cd are likely to be much higher than that in the pectoral muscles which are preferentially consumed by hunters, but further studies to investigate this potential health concern are justified based on the results presented here.

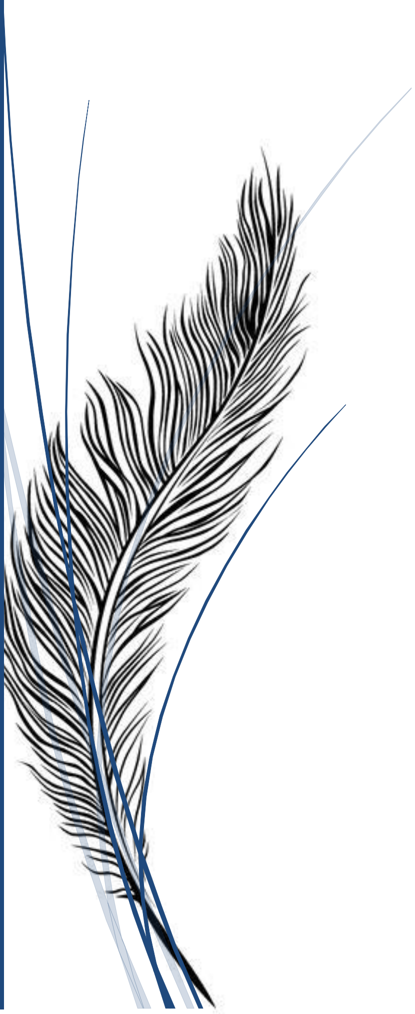
Conclusions

The present study of hepatic concentrations of Cu, Cd and Pb in two mallard populations in New Zealand clearly indicates that there is regular environmental exposure of the birds to all three heavy metals. Determining the bioavailability to pollutants in organisms is a critical component of ecological risk assessment. While published values of thresholds or critical levels of metals in tissues of waterfowl relating to adverse health effects are variable regarding sample type, species, metal, and literature source, on the basis of the existing figures the results presented in this chapter suggest a degree of exposure of toxicological concern to the birds, their predators and to humans. This study offers a more complete assessment of the internal body load of the three heavy metals than Chapter Two, with hepatic concentrations indicating accumulation over the lifetime of the waterfowl compared to the blood which reflects recent exposure. The difference seen between the two sampling sites was similar in the liver concentrations of heavy metals to the differences seen in the blood, both of which suggest that the bioavailability of these metals is different between these sites. While all three metals showed different hepatic loads in compared to sex, only Cd was found to differ between age groups, highlighting the importance of using a bioindicator species in which both sex and age can easily be distinguished. Ingestion of spent Pb shot was found to have decreased from a survey conducted in both regions of New Zealand in 2000, the year before the restrictions on Pb shot were implemented. However, spent Pb shot is still a continuing route of Pb exposure to waterfowl species in New Zealand

Chapter 4

Heavy metal in native freshwater birds of New Zealand

Using post-mortem stored samples of six species of native water birds to assess the environmental bioavailability of three heavy metals in New Zealand freshwater habitats



4.0 Abstract

Global populations of freshwater birds are declining, with heavy metal contamination one of the many potential stressors implicated in these demographic changes. Within New Zealand, over half of the native freshwater bird species are classified as Threatened or At Risk. Five major threat categories to freshwater biodiversity have been established: overexploitation, flow modification, destruction and degradation of habitat, invasion by exotic species, and water pollution. This study aimed to assess the exposure of native wetlands birds to the toxic metals (cadmium [Cd] and lead [Pb]) and the essential trace metal (copper [Cu]). The hepatic concentration of these metals was determined in 71 stored tissue samples representing six native bird species – whio (*Hymenolaimus malachorhynchos*), pāteke (*Anas chlorotis*), scaup (*Aythya novaeseelandiae*), paradise shelduck (*Tadorna variegata*), Campbell Island teal (*Anas nesiotis*), and Australasian bittern (*Botaurus poiciloptilus*). Both formalin-fixed and frozen fresh liver samples were collected and assessed separately. Comparison of individuals with both fixed and fresh tissue stored suggest that leaching of metals into the formalin storage matrix may have occurred. Significant differences were found between species for both sample types, with pāteke having significantly higher hepatic concentrations of both Cd and Pb than whio, in formalin fixed samples. Whio were found to have significantly higher hepatic concentrations of both Cd and Pb than bittern, in fresh samples. Cd liver concentrations increased with the age of the bird only in fixed pāteke samples, and there was no reported sex difference in hepatic heavy metal concentrations within any species. Twenty-five birds (35.2%) had hepatic Cd concentrations elevated above background exposure levels (< 0.9 $mg.kg^{-1}$), with 14 birds (19.7%) showing hepatic Cd concentrations > 2.1 $mg.kg^{-1}$ suggestive of sublethal adverse effects. Only whio from the West Coast of the South Island and pāteke from Aotea (Great Barrier Island) were found to have Cd concentrations > 2.1 $mg.kg^{-1}$, suggesting high environmental exposure of Cd within these two regions of New Zealand. Only two pāteke from Aotea had hepatic Pb concentrations consistent with subclinical toxicity (> 2 $mg.kg^{-1}$), and one of these was consistent with clinical toxicity (6–10 $mg.kg^{-1}$). Cu levels were all found to be well below toxic ranges, with no variation between species, age, or sex. These results show that freshwater native birds in New Zealand are ingesting concentrations of Cd and Pb that may be affecting at risk or declining populations. Further research is needed into the sources of these contaminants in freshwater systems and the links between metal contamination and the water bird population declines within New Zealand.

4.1 Introduction

Freshwater birds are declining globally due to a range of anthropogenic threats, with a number of species classed as threatened or endangered (Dehorter and Guillemain, 2008). Five major threat categories to freshwater biodiversity have been established: overexploitation, flow modification, destruction and degradation of habitat, invasion by exotic species, and water pollution (Dudgeon *et al.*, 2006). Global-scale environmental changes such as warming, nitrogen deposition, and a shift in precipitation and runoff patterns are considered to be incorporated into all five categories (Poff *et al.*, 2002). While overexploitation affects predominantly vertebrates, including fish species, reptiles, birds, and some amphibians, the other four categories result in consequences for all freshwater species from microbes to megafauna. Freshwater ecosystems are particularly vulnerable, as fresh water is a major resource for humans that may be diverted, extracted, contained, or contaminated, all of which compromise its value as a habitat for organisms. It is now assumed to be unlikely that there are many remaining substantial water bodies that have not been irreversibly altered by human activities (Lévêque and Balian, 2005).

Wetland ecosystems in New Zealand are highly threatened, with less than 10% of their original extent remaining, coupled with the fact that modification and degradation of other freshwater systems is still occurring (Ausseil *et al.*, 2011). This rate and extent of wetland loss is recognised as among the highest in the world (Mitsch and Gosselink, 2000). Waterways are considered as a product of their catchment, with land use and streamside vegetation having a major influence on the health of the aquatic ecosystem (Quinn *et al.*, 1997). Land modification in New Zealand has been most significant in the last 150 years, the main period of European settlement (Myers *et al.*, 2013), with over two-thirds of indigenous land cover converted to farmland, exotic forestry. and settlements, adding increased pressure on the ecosystem services of the remaining wetlands (Barbier *et al.*, 1997, Taylor and Smith, 1997). The high rate of agricultural land use together with intensification of dairy farming has resulted in a cascade of leaching nutrients and heavy metals into waterways, lakes, and estuaries (Duncan, 2014). Native species reliant on these habitats have noticeably declined, with 67% of native freshwater fish and 60% of freshwater-dependent bird species considered threatened or at risk (Allibone *et al.*, 2010, O'Donnell *et al.*, 2015).

New Zealand has an estimated 33 indigenous bird species that characteristically inhabit, forage, breed, or shelter in lakes, wetlands, and river systems, or other freshwater systems throughout New Zealand and its surrounding islands (Heather and Robertson, 2000, O'Donnell *et al.*, 2015). This includes 10 extant waterfowl species, half of which are considered At Risk, Nationally Critical or Nationally Vulnerable, with a further 16 from other families considered

Threatened or At Risk species. As an example, the Australasian bittern (*Botaurus poiciloptilus*) conservation classification was changed from Nationally Endangered to Nationally Critical this year due to continued declining populations (Robertson *et al.*, 2017). New Zealand has three of the rarest duck species worldwide: the whio (blue duck, *Hymenolaimus malachorhynchos*), is the sole member of the genus and is classified as Nationally Vulnerable and Endangered; the pāteke (brown teal, *Anas chlorotis*) is management-dependent and classified as At Risk-Recovering; and the flightless Campbell Island teal (*Anas nesiotis*) is classed as Nationally Vulnerable (IUCN, 2017). Freshwater bird populations have been steadily declining since human settlement, resulting in 14 extinctions. This is thought to be a result of both the introduction of exotic predatory species, and the extensive modification of freshwater systems (Allibone *et al.*, 2010, O'Donnell *et al.*, 2015). This continued decline is of great concern and the possibility that heavy metal pollution may be contributing to the demise of these species has yet to be investigated.

It is known that even closely related species may show differences in metal accumulation, excretion, and sensitivity (Beyer *et al.*, 1999, Burger and Gochfeld, 2008, Berglund *et al.*, 2011, Van Eeden *et al.*, 1996, Gochfeld and Burger, 1987). The concentrations of trace metals and their effect on host organisms are influenced by numerous factors relating to diet and foraging methods, geographical range, habitat, life-history strategies, as well as physiological and ecological species-specific trace-element requirements (Peakall and Burger, 2003, Berglund *et al.*, 2011). The bioaccumulation within successive trophic levels has been well documented for a range of organisms and metals (Gall *et al.*, 2015, Park and Curtis, 1997, Burger, 2002, Besser *et al.*, 1993, Scheuhammer and Graham, 1999). The general assumption is that the lower the trophic level of an organism, the lower the exposure to metals, although this can vary due to certain species of plants which can bioaccumulate high levels of specific metals (Peakall and Burger, 2003). The geographical range of organisms will obviously play a large role in the exposure, with behaviours such as migration resulting in the movement from one ecoregion to another where bioavailability of metals and other anthropogenic exposures can vary dramatically (Blomqvist *et al.*, 1987). Unusually, most New Zealand wetland species are not migratory, which may increase their sensitivity to local pollution events. Species or families may be more vulnerable not only due to their trophic level, but also as a result of foraging methods, habitat, or the breeding grounds used. Differences may also relate to internal toxicodynamics, resulting in species differences even within closely related organisms (Lock and Janssen, 2001, Peakall and Burger, 2003). There are two well examined and explored examples of this in avian species. Firstly, Cu is known to accumulate in waterfowl to a much higher degree than in other birds. Mute swans (*Cygnus olor*) in particular can tolerate remarkably high hepatic concentrations of Cu (> 1000 $mg.kg^{-1}$ w.w.) which would be highly toxic in other species (Beyer *et al.*, 1998, Aloupi *et al.*, 2017, Frank and Borg, 1979). Secondly, seabirds appear to be able to survive exposure to methylmercury better than other bird species, due to their ability to demethylate mercury, allowing survival with tissue levels exceeding values expected to produce lethal effects in experimental settings in other species (Thompson and Furness, 1989, Burger and Gochfeld, 1997b, Eisler, 1985).

Sentinel species provide a useful tool for monitoring environmental contamination by heavy metal exposure over time as well as serving as an early warning sign (Peakall and Burger, 2003). Mallards have been used globally as a bioindicator species, as they are cosmopolitan, valued as game birds, have clear sexual dimorphism and are considered high trophic level foragers (Kalisińska *et al.*, 2004). However, as New Zealand is known for its diverse and often unique bird life, the use of an introduced generalist species such as the mallard as a bioindicator, while useful for indicating levels of primary wetland pollution with heavy metals, may be less useful for determining the environmental levels of heavy metal pollution in the habitats of specialised native waterfowl species. A major challenge for conservation managers is determining precise causes of population declines, to allow resources aimed at recovering species to be targeted appropriately.

The objective of this study was to investigate the degree of exposure of native New Zealand freshwater birds from different habitats to three heavy metals: Cd, Cu, and Pb. The main objectives of this study are (i) determine the hepatic contamination load in stored samples of native New Zealand freshwater bird species; (ii) examine differences in concentration load between sample type and species, while also investigating both age and sex differences.

4.2 Material and Methods

4.2.1 Database use and sample collection

Archived records from the Wildbase Post-mortem database (the Institute of Veterinary, Animal and Biomedical Sciences, Massey University), were searched for all wild native waterfowl species and bitterns which had been submitted for post-mortem between 2003 and 2017. Captive birds were not considered, while captive-reared or rehabilitated birds were only considered if the death had occurred at least 8 months after release allowing for ample exposure time to the pollutants within the environment. This was to ensure that only birds in native and wild habitats were sampled. Tissues belonging to individuals that matched these criteria were extracted from storage; all collected tissues had been fixed in 10% neutral buffered formalin and stored in plastic sample bags at time of the post mortem. Liver tissue was identified and removed into individual sterile 70 ml polypropylene specimen containers with enough formalin to cover the sample.

Wild waterfowl and bitterns that were submitted for post mortem examination under contract to the Department of Conservation (DOC) between 2015 and 2017 also had archived fresh liver tissue frozen and stored at -20ºC (five bittern, three whio, two scaup (*Aythya novaeseelandiae*) and one pāteke). These samples were retrieved for processing.

4.2.2 Laboratory analysis

Seventy-one liver samples comprising both formalin-fixed (n = 58) and frozen (n = 13) tissues were sent to a commercial laboratory (AsureQuality Limited, 7a Pacific Rise, Mt Wellington, Auckland, New Zealand). Approximately 1 g of the homogenised sample was digested with 5 ml of concentrated nitric acid and 0.1 ml of hydrofluoric acid at 100ºC for one hour and made up to 50 ml. The digest was then analysed by ICP MS (Inductively Coupled Plasma Mass Spectrometry) along with appropriate Blank and Standard Reference Material. Limits of detection (LOD) were: Cd = 0.002 $mg.kg^{-1}$, Cu = 0.11 $mg.kg^{-1}$, Pb = 0.01 $mg.kg^{-1}$. All results reported are in relation to wet weight (w.w.).

4.2.3 Statistical analysis

Statistical analyses were conducted using R studio (R Core Team, 2015). Values below the reported LOD were considered as one-half of the relevant LOD and included for statistical analysis, to minimise nominal type 1 error rates (Clarke, 1998). Sample types were analysed separately, due to the known potential for leaching of heavy metals into formalin over time (Gellein *et al.*, 2008). Fixed samples were able to be normalised by log-transformation (Shapiro-Wilk test, 0.05 confidence level). Therefore, one-way ANOVAs were used to evaluate differences between species, followed by a Tukey's honestly significant difference (HSD) (p < 0.05) test when appropriate. Due to the small sample sizes, intra-species differences relating to sex, age, season, and year of collection were assessed by Kruskal-Wallis or Mann-Whitney U-tests where appropriate, followed by *post hoc* Dunn's test to identify significant differences. The small number of fresh frozen samples collected were also analysed by Kruskal-Wallis or Mann-Whitney U-tests, followed by a Dunn's test. Data were considered statistically significant at $p < 0.05$. Comparisons of hepatic concentrations of the three heavy metals to toxicological threshold values or critical ranges in the scientific literature use the same thresholds as Chapters One and Three (Table 1.1).

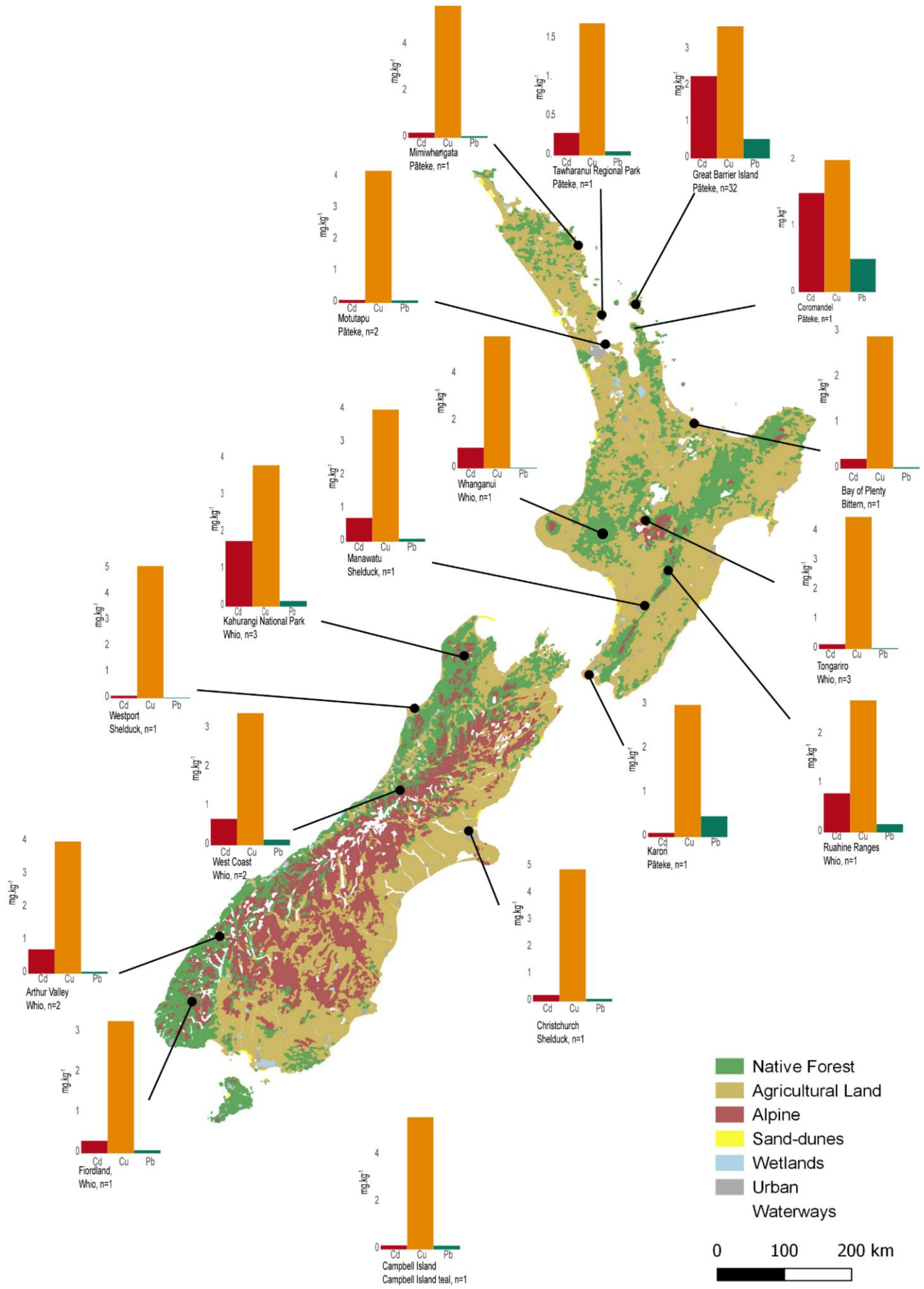


Figure 4.1 Locations (n = 17) of formalin fixed samples (n = 58) collected from archived samples from the Wildbase Post-mortem laboratory. Samples from same species collected from the same area are presented as the mean value. Location, species and number of samples are presented under the graphs. Map created from "Vegetative cover of New Zealand" sha pefile imported from LRIS (https://lris.scinfo.org.nz/layer/48420-vegetative-cover-map-of-new-zealand/) (Landcare Research Informatics Team, 2015)

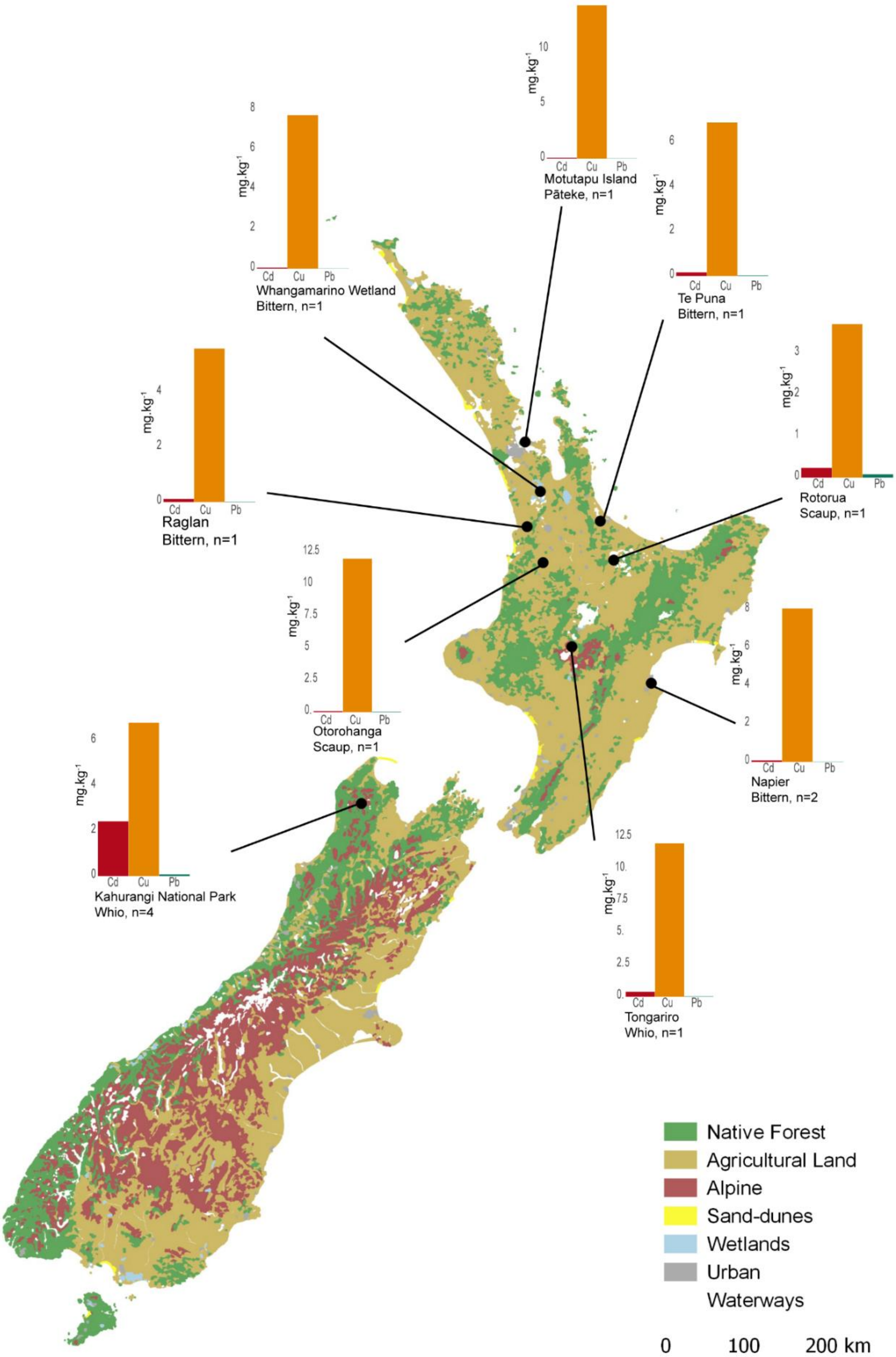


Figure 4.2 Locations (n = 9) of frozen samples (n = 13) collected from archived samples from the Wildbase Post-mortem laboratory. Samples from same species collected from the same area are presented as the mean value. Location, species and number of samples are presented under the graphs. Map created from "Vegetative cover of New Zealand" shapefile imported from LRIS (https://lris.scinfo.org.nz/layer/48420-vegetative-cover-map-of-new-zealand/) (Landcare Research Informatics Team, 2015)

4.3 Results

Samples from five native waterfowl species and one native bittern species were available for this study (Table 4.1). Seventy-one livers were analysed (58 formalin-fixed [Figure 4.1] and 13 frozen [Figure 4.2]) from 20 locations around New Zealand. Most of the samples analysed exhibited values above the LOD for the elements evaluated, except for Pb with 7.04% (5/71) of samples below the LOD (Table 4.2 & 4.3). Pāteke and whio dominated numerically due to more intensive management by DOC (Table 4.1). Four birds (3 whio, 1 pāteke) had both frozen and fixed tissue samples stored, and in these samples there was no significant difference in heavy metal concentration between the sample types (Mann–Whitney U test: Pb: W = 12, p = 0.31; Cu: W = 1, p = 0.06; Cd: W = 8, p = 1). Due to a consistent trend for lower concentration of Cu in fixed liver samples compared to frozen, and higher concentrations of Pb in fixed liver samples compared to frozen, each sample type is presented separately (Figure 4.3). Descriptive statistics showing fixed sample concentration in mg.kg^{-1} in relation to species are shown in Table 4.2.

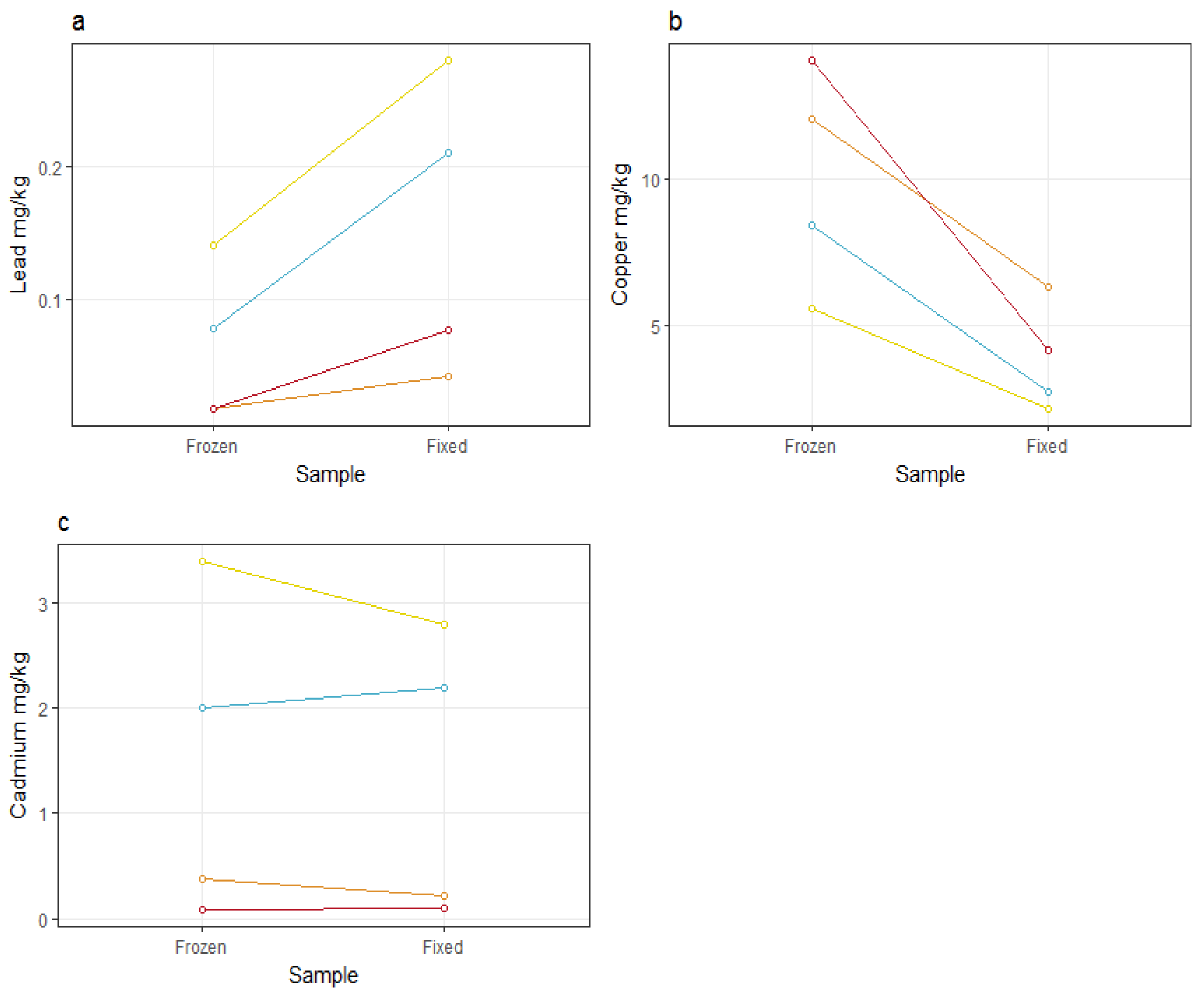


Figure 4.3 Difference in a) Pb, b) Cu, and c) Cd levels between four individuals with two sample types: fixed in formalin vs frozen. No differences were statistically significant.

Table 4. 1 Characteristics of waterfowl species sampled. *Campbell Island teal are isolated to Campbell Island, an uninhabited sub-Antarctic island south of New Zealand, and a small population on Codfish Island/Whenua Hou. **Paradise shelduck are New Zealand's most widely distributed waterfowl species, and are ubiquitous in habitats throughout New Zealand, although predominantly found in the pastoral landscape.

Family	**Species**		***N total***	***Fixed vs Frozen***	**Type of food**	**Habitat**
	Common name	Scientific name			(trophic level)	
Anatidae	Pāteke	*Anas chlorotis*	38	37:1	Omnivorous	Agricultural land/wetlands
	Whio	*Hymenolaimus malacorhynchos*	21	16:5	Insectivorous	Alpine rivers
	Campbell Island teal	*Anas nesiotis*	1	1:0	Omnivorous	Shore line/wetlands*
	Scaup	*Aythya novaeseelandiae*	2	0:2	Insectivorous	Lakes
	Paradise shelduck	*Tadorna variegata*	3	3:0	herbivorous	Agricultural land**
Ardeidae	Australasian bittern	*Botaurus poiciloptilus*	6	1:5	Fish predator	Mineralised wetlands

4.3.1 Metal toxicity thresholds

None of the examined individuals had hepatic Cu concentrations above levels consistent with adverse effects (> 100 mg.kg^{-1} w.w.), with the maximum liver concentration a sample from a Motutapu Island pāteke male at 14 mg.kg^{-1}. Only two pāteke individuals (2.8%, both from Aotea) had hepatic Pb concentrations elevated above background exposure levels (> 2 mg.kg^{-1}), with one adult female pāteke having a concentration of 8.5 mg.kg^{-1}, consistent with clinical poisoning (6–10 mg.kg^{-1}) (Pain, 1996).

Cd was elevated in 35.2% (25/71) of the individuals studied to levels above the threshold consistent with increased environmental exposure (< 0.9 mg.kg^{-1}) (Scheuhammer, 1987), though these were all from two species: whio (n = 8) and pāteke (n = 17). In the fixed pāteke samples (Figure 4.4a), 47.4% (18/38) were elevated to concentrations consistent with increased environmental exposure, with 28.9% (11/38) having levels > 2.1 mg.kg^{-1} consistent with sublethal adverse effects (White and Finley, 1978). In the fixed whio samples (Figure 4.4b), 23.5% (4/17) were elevated above 0.9 mg.kg^{-1}, with two of these samples (11.7%) having hepatic Cd concentrations > 2.1 mg.kg^{-1}. Of the five frozen whio (Figure 4.4c) samples, four (80%) had hepatic Cd concentrations elevated > 0.9 mg.kg^{-1}, of which two (40%) had concentrations > 2.1 mg.kg^{-1}.

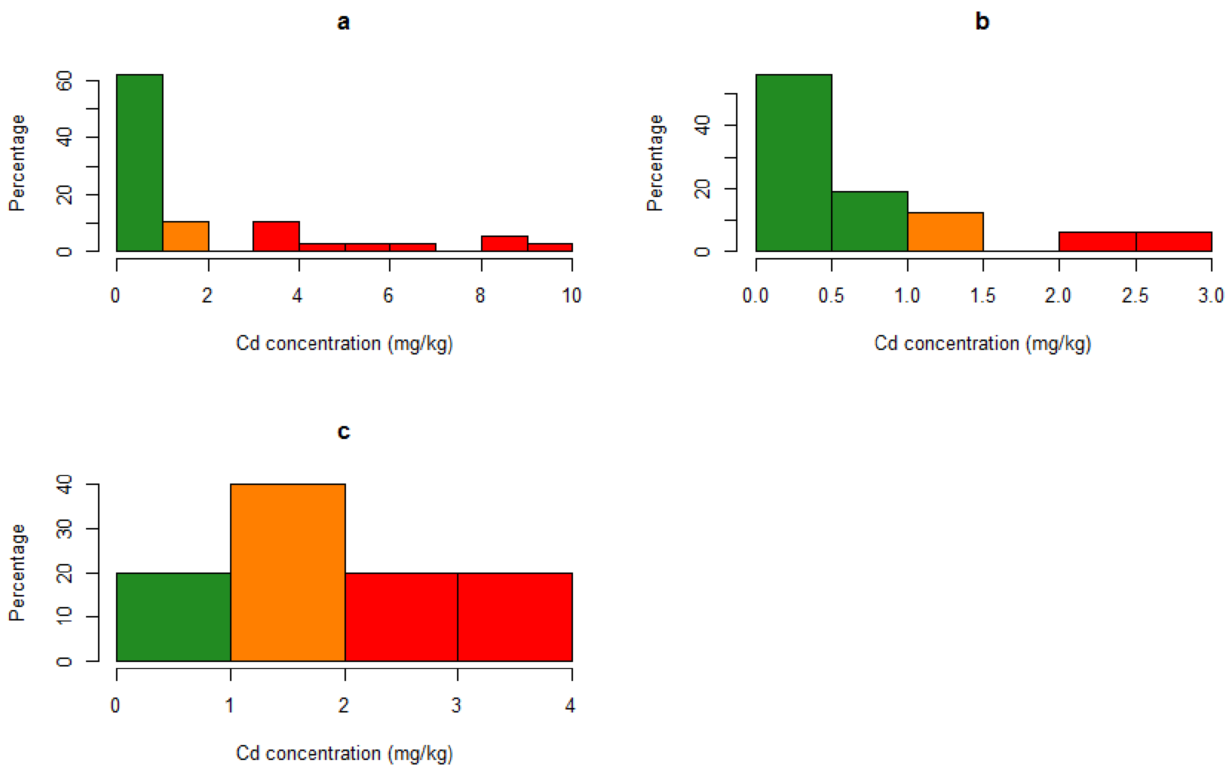


Figure 4.4 Distribution of hepatic Cd concentrations in a) fixed pāteke (n = 38), b) fixed whio (n = 17), and c) frozen whio (n = 5); green bars represent concentrations consistent with background environmental levels (< 0.9 mg.kg^{-1}), orange bars represent concentrations consistent with increased environmental exposure (0.9–2 mgkg^{-1}), and red bars represent concentrations consistent with sublethal adverse effects (> 2.1 mg.kg^{-1}).

4.3.2 Variation between species

Descriptive statistics showing fixed sample concentration in mg.kg^{-1} in relation to species are shown in Table 4.2. Bittern and Campbell Island teal were excluded from examination of interspecific variation due to the small sample size, so comparisons were only made between whio, pāteke and paradise shelduck. Significant differences in heavy metal concentrations between species were observed for Cd ($F_{2,53} = 4.013$, $p < 0.05$; Figure 4.5) and Pb ($F_{2,53} = 5.464$, $p < 0.01$; Figure 4.6), with both Cd and Pb concentrations higher in pāteke than whio (Tukey's HSD, respectively, $p = 0.046$ and $p = 0.007$).

A total of 13 frozen samples were collected from four different species (see Table 4.3 for descriptive statistics). Pāteke were excluded from the analyses due to the small sample size ($n = 1$). Similar to the fixed tissues, significant differences in hepatic concentrations were found for both Cd (Kruskal-Wallis, $p < 0.05$; Figure 4.7) and Pb (Kruskal-Wallis, $p < 0.05$; Figure 4.8). Post hoc investigation identified that the detected difference seen in both Pb and Cd resulted mainly from the difference between bittern and whio. Due to the small sample sizes, no difference was found in relation to age and sex within species.

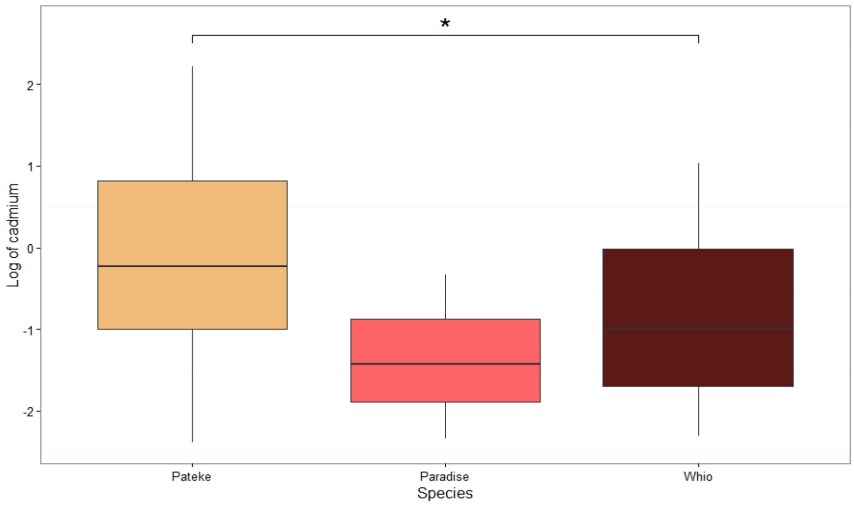


Figure 4.5 Fixed hepatic Cd concentration difference between species: pāteke (n = 37), paradise shelduck (n = 3), whio (n = 16). Statistically significant differences were found after the ANOVA and Turkey HSD test (*$p < 0.05$). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

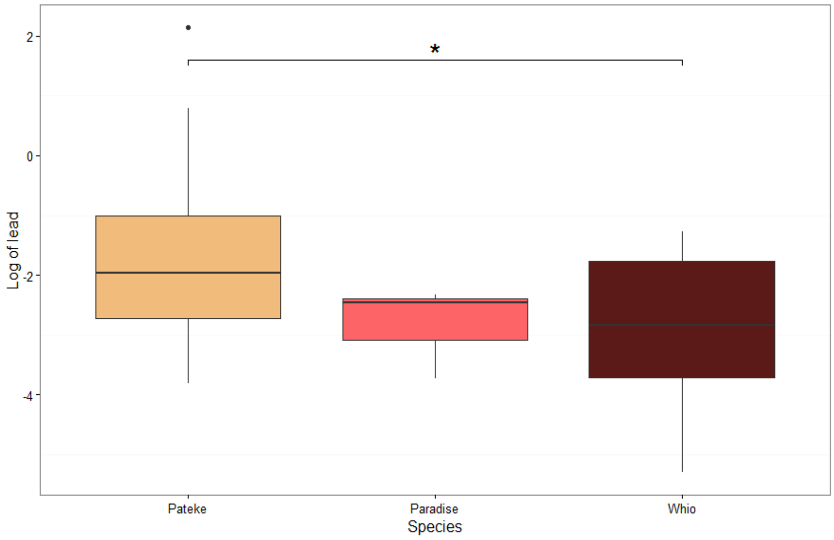


Figure 4.6 Fixed hepatic Pb concentration difference between species: pāteke (n = 37), paradise shelduck (n = 3), whio (n = 16). Statistically significant differences were found after the ANOVA and Turkey HSD test (**p* < 0.05). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

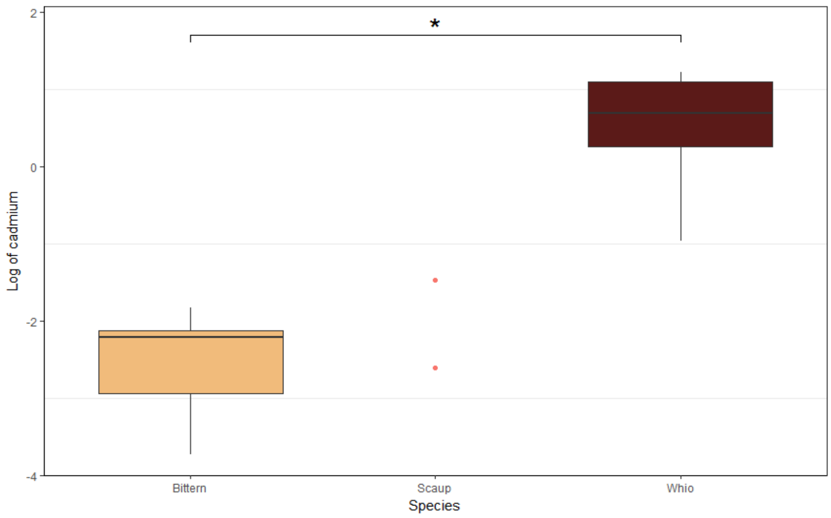


Figure 4.7 Frozen hepatic Cd concentration difference between species: bittern (n = 5), scaup (n = 2), whio (n = 5). Statistically significant differences were found using a Kruskal-Wallis analysis and a Dunn's test (**p* < 0.05). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

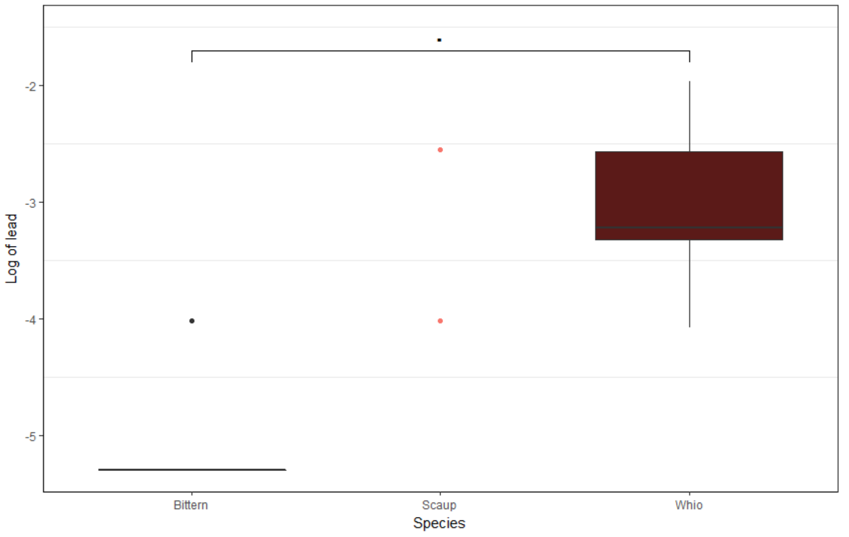


Figure 4.8 Frozen hepatic Pb concentration difference between species: bittern (n = 5), scaup (n = 2), whio (n = 5). Where statistically significant differences were found using a Kruskal-Wallis analysis and a Dunn's test (˙*p* < 0.1). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

Table 4.2 Summary of heavy metal hepatic concentrations (mg.kg^{-1} w.w.) obtained from archived samples, stored in formalin. Main statistics (mean ± standard deviation, median, and range (min–max)). *species removed from analysis due to small sample size.

		Fixed Cd			**Fixed Cu**			**Fixed Pb**		
Species	***N***	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**
Pāteke	37	2.022 ± 2.61	0.79	0.092 – 9.2	3.6 ± 1.56	3.4	1.6 – 7.1	0.498 ± 1.41	0.15	0.22 – 8.5
Whio	16	0.704 ± 0.79	0.345	0.062 – 2.8	3.419 ± 1.21	3.1	1.9 – 6.3	0.092 ± 0.09	0.05	ND – 0.28
Campbell Island teal*	1	0.17			5.6			0.16		
Paradise shelduck	3	0.352 ± 0.33	0.24	0.096 – 0.72	4.667 ± 0.59	4.9	4 – 5.1	0.069 ± 0.04	0.086	0.024 – 0.097
Australasian bittern*	1	0.21			2.9			0.026		

Table 4.3 Summary of Heavy metal hepatic concentrations (mg.kg^{-1} w.w.) obtained from archived frozen samples. Main statistics (mean ± standard deviation, median, and range (min–max)). *species removed from analysis due to small sample size.

		Frozen Cd			**Frozen Cu**			**Frozen Pb**		
Species	***N***	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**	**Range**
Pāteke*	1	0.082			14			0.017		
Whio	5	2.016 ± 1.23	2	0.38 – 3.4	7.84 ± 2.57	7.2	5.6 – 12	0.062 ± 0.05	0.04	0.017 – 0.14
Scaup	2	0.152		0.074 – 0.23	7.85		3.7 – 12	0.048		0.018 – 0.078
Australasian bittern	5	0.093 ± 0.054	0.11	0.024 – 0.16	7.26 ± 1.09	7.6	5.6 – 8.5	0.008 ± 0.006	0.005	ND – 0.018

4.3.3 Variation between sex, age, season, and year of collection.

Only pāteke and whio were included in these further analyses due to their larger sample sizes compared to paradise ducks, bittern, and Campbell Island teal. In pāteke, hepatic Cd concentrations were higher in adults than juveniles (W = 220.5, $p < 0.05$; Figure 4.9), while age was found to only have a weak effect on hepatic Cd concentrations in whio (W = 40.5, $p = 0.052$). There was no difference between sexes and season of sample for any of the three metals examined in either pāteke or whio. Levels of Cd in pāteke varied with year of collection ($X^2 = 11.71$, $p < 0.05$). Concentrations were higher in samples from 2010 than in 2003 (Dunn's test, $p = 0.02$; Figure 4.10).

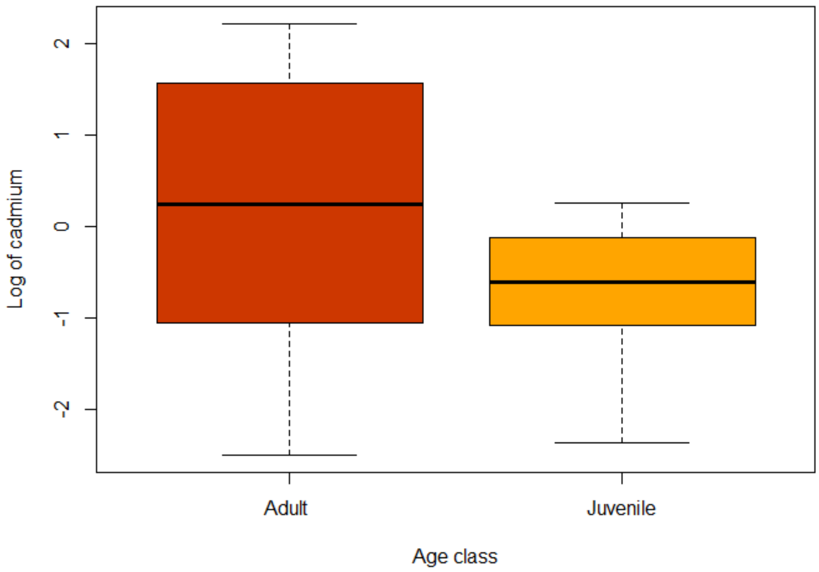


Figure 4.9 Fixed hepatic Cd concentration difference between age cohorts in pāteke: adults (n = 21) and juveniles (n = 15). Statistically significant difference found by Mann-Whitney test (p < 0.05). The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

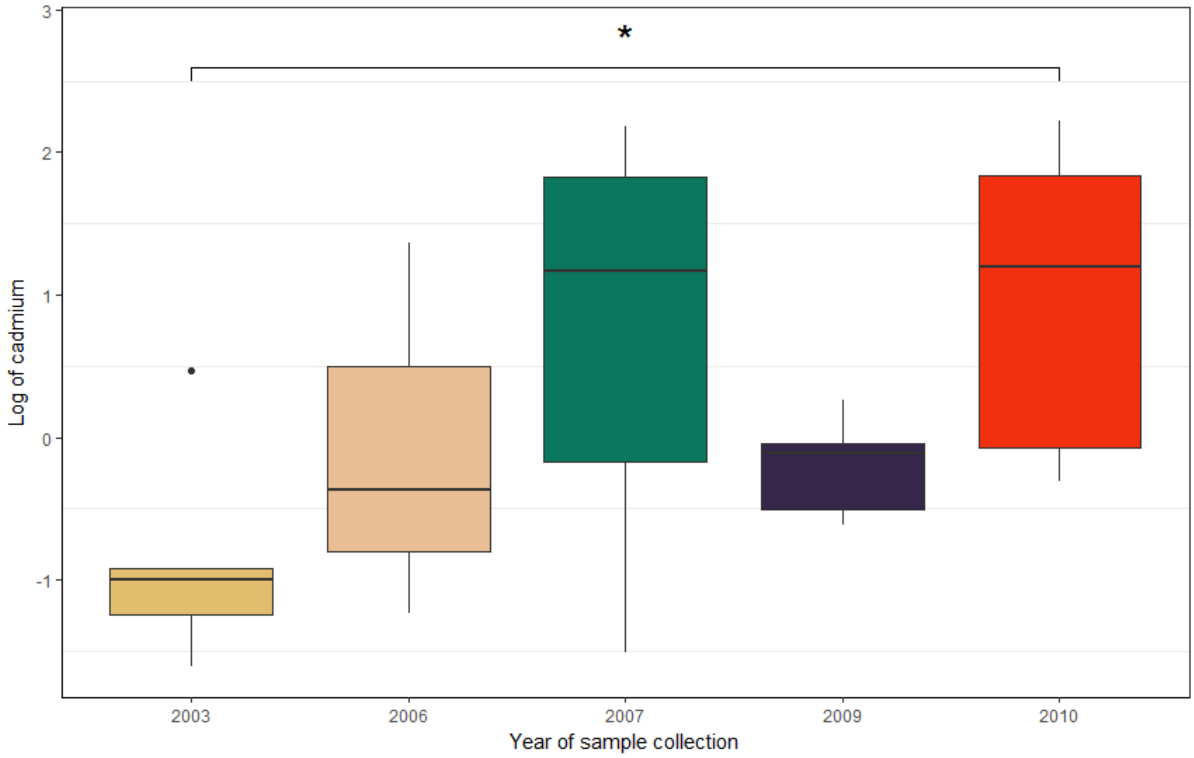


Figure 4.10 Fixed hepatic Cd concentration of pāteke sampled in different years: 2003 (n = 7), 2006 (n = 3), 2007 (n = 7), 2009 (n = 6), 2010 (n = 8). Statistically significant differences were found after an Kruskal-Wallis and a Dunn's test. *p < 0.05. The boxes represent 25th, 50th (median) and 75th percentiles. Whiskers are 1.5 times the box length (interquartile range [IQR]). Circles represent values outside that range.

4.4 Discussion

Freshwater quality in New Zealand has recently become a major national issue, with the past decades of agricultural intensification and population growth resulting in an acceleration of environmental problems centred around water quality (Larned *et al.*, 2016). Soil deterioration as a result of compaction, erosion, reduction in organic matter, and contamination with pesticides and heavy metals are closely related to the impacts on water (Stoate *et al.*, 2001). Populations of fresh waterbird species have been declining steadily within New Zealand since the settlement of Europeans, and it is assumed that drainage and degradation of freshwater systems coupled with the introduction of exotic predators are to blame (O'Donnell *et al.*, 2015). Using mallards as a bioindicator of the heavy metal contamination in rural habitats has many benefits (Chapters Two and Three), however, their use is limited to the modified landscapes they inhabit. New Zealand native freshwater birds are diverse in both their ecological needs and foraging behaviours, which could expose these species to different contamination levels and threats than the introduced mallard. There are no studies that have looked into the heavy metal load carried by the native freshwater birds of New Zealand.

Metal Concentration

Difference between species

A significant difference in metal concentrations between species was found for Cd and Pb in both fixed and frozen samples. Formalin-fixed samples from pāteke had higher concentrations of Pb and Cd than from whio and paradise shelduck, while frozen samples from whio had higher concentrations than bittern and scaup. No difference in Cu concentration was seen between species for either fixed or fresh samples. Many factors affecting interspecific variation of metal concentrations in birds have been recognised: longevity, habitat, physiological mechanisms related to metabolism, storage and elimination, foraging behaviour, energy expenditure and seasonal diet switching, changes in nutritional requirements for reproductive states, phylogeny, migration paths and duration, as well as the various environmental conditions faced by different species (Di Giulio and Scanlon, 1984b, Parslow *et al.*, 1982, Scheuhammer and Templeton, 1998, Lovvorn and Gillingham, 1996, Aloupi *et al.*, 2017, Arnold *et al.*, 2002, Lucia *et al.*, 2010, Gómez *et al.*, 2004). However, the effect of these factors may be diminished in part due to the fact that these samples originated from birds which were found dead, and not from random sampling of apparently healthy birds, or those shot by hunters. Which could result in a sampling bias, if the high levels of Pb and Cd contributed to the cause of death. Nonetheless, it is unlikely that this is the case for both metals, suggesting that some of these interspecific differences are affecting uptake.

High levels of Cd reported in the fixed samples of pāteke are interesting, as the birds are generalist omnivores (Moore *et al.*, 2006), much like mallards. However, the mean and range documented (mean 2.02 $mg.kg^{-1}$, range 0.092–9.2 $mg.kg^{-1}$) are higher than for the mallards reported in Chapter Three (mean 0.74 $mg.kg^{-1}$, range 0.048–3.7 $mg.kg^{-1}$), though this comparison must be tentative due to the difference in sample type (fixed vs fresh), the method of collection (hunted vs found dead), and the different laboratories used for analysis. Most (86.5%, 32/37) of the pāteke samples were collected from Aotea (Great Barrier Island), the largest island off the coast of the North Island. Aotea is still predominantly (90%) covered in native forest, with the developed land mainly used for livestock farming (Great Barrier Island Charitable Trust, 2010). Natural accumulations of Cd are known to occur in sulphide mineral deposits such as galena, sphalerite, chalcopyrite, and bornite, all of which Aotea has (Brathwaite and Rabone, 1985), and it is therefore likely that this island would have high natural levels of Cd (Kim, 1990). Soil samples collected from four different rock group classifications on Aotea indicate an average soil concentration of 0.18 $mg.kg^{-1}$, which is much higher than the levels of Waikato and Southland (both 0.7 $mg.kg^{-1}$; Table 2.8; (Land Resource Information System, 2016). Therefore, exposure to Cd on Aotea is more likely from natural sources rather than anthropogenic sources. While Pb levels were also higher in the pāteke compared to whio and paradise shelduck, these were lower than for mallards, suggesting that birds inhabiting rural areas of the New Zealand mainland have higher exposure than native species in less modified and unpopulated areas.

Aotea is considered the stronghold of the national pāteke population and intensive predator control and monitoring by the Department of Conservation (DOC) has been in place since 2000. An analysis of the causes of death from 2001 to 2010 found that 16% of the deaths were attributable to starvation, with 26% assumed harrier predation/scavenging and a further 29% of unknown cause (Watts *et al.*, 2016); many of those birds were included in this present study. Di Giulio and Scanlon (1985) reported that mallards that experience prolonged food shortages may be more sensitive to Cd exposure compared to birds which are not food-stressed. They found that in food-restricted birds with high Cd ingestion (dosed), resulted in the reduction in plasma concentrations of thyroid hormones (triiodothyronine and thyroxine), while increases in plasma non-esterified fatty acids and plasma and adrenal concentrations of corticosterone were reported. This suggests that energy expenditure is exacerbated in Cd-exposed animals, which could have resulted in a lower survival after periods of food-restriction for pāteke on Aotea.

In the analysis of frozen tissues, whio had the highest Cd levels, which are once again higher than those reported for mallards but are comparable to the fixed pāteke samples (mean 2.02 $mg.kg^{-1}$, range 0.38–3.4 $mg.kg^{-1}$). As whio are fast flowing riverine, predominantly insectivorous ducks that are mostly confined to high altitude mountain rivers in the North and South Islands, it would be assumed that pollution of their habitat from anthropogenic sources would be unlikely. However, four of the fresh whio samples were collected in the Kahurangi National Park, situated at the Northern end of the West coast, and had hepatic Cd concentrations elevated above 0.9 $mg.kg^{-1}$, which is considered elevated above background environmental levels. A recent study on the scavenging of atmospheric trace metal pollution found evidence of anthropogenic Cd enriched dust being present along the West Coast of the South Island, with evidence of it being wind-transported from the eastern Australian seaboard, where the bulk of Australia's industry and population are located (Marx *et al.*, 2008). While Australian- sourced metal pollution is deposited to a degree throughout New Zealand, the majority is likely to be concentrated in the West Coast region due to orographic precipitation washing the majority of the dust (as well as trace metals transported independently of dust) from the atmosphere on the western side of the Southern Alps (Marx and McGowan, 2005). Cd added to agricultural land in New Zealand as fertiliser has been found to be fairly immobile, with only 5–15% of the annual application of Cd leaching and most Cd staying within the topsoil (Gray *et al.*, 2003b), so the low levels seen in the bittern and the scaup, both which solely feed in freshwater, may reflect a lack of Cd within the aquatic environment.

Age difference and year variations in metal levels

Age-related differences in accumulation of Cd were documented in mallards and other species (Chapters Two and Three). Similarly, pāteke and to a lesser extent whio also exhibited an age-related difference of Cd. Only fixed pāteke samples showed a change in Cd hepatic concentrations increasing over the 7 years. This could be the result of two factors – leaching of Cd from the formalin (discussed in the next section) or an increase of Cd exposure between 2003 to 2010. An increase in Cd exposure could be the result of land use change or diet switching. As mentioned before, Watts *et al.* (2016) found 16% of the pāteke on Aotea died of starvation, suggesting that the island was either at carrying capacity or that it had a low quality of habitat. As it is expected that Aotea has an increased soil Cd load due to sulphate deposits (Kim, 1990), this natural load coupled with the addition of Cd from farming practices (fertiliser load) could be resulting in legacy issue of Cd build up in the top soil (Taylor *et al.*, 2017). Given that a high proportion of the Aotea pāteke population now inhabits pastoral/scrub/swamp areas, which is considered poor quality habitat (Moore *et al.*, 2006), exposure of the population to Cd is likely to increase.

Metal toxicity

The percentage of birds in which concentrations of Cd exceeded the threshold of increased environmental exposure (36.6% $\geq$ 0.9 $mg.kg^{-1}$) suggests that a significant proportion of native freshwater birds in New Zealand, especially whio and pāteke, may be affected by Cd exposure. While 21.1% had hepatic concentrations elevated above > 2.1 $mg.kg^{-1}$, which have been linked to sublethal adverse effects (Chapter Three, discussion), suggesting that the level of exposure is high in certain environments (White *et al.*, 1978, Di Giulio and Scanlon, 1984a, Wayland and Scheuhammer, 2011). Interestingly, these birds came from either Aotea or the West Coast of the South Island, both of which are known to have high environmental levels of Cd, resulting in the trophic level accumulation seen here. However, further studies on the food web movement of this metal would allow for better understanding of its movement through the trophic chain.

The percentage of pāteke with hepatic Pb concentrations indicative of sub-clinical poisoning (5.3%) would suggest that the exposure of Pb in New Zealand is highest in omnivorous dabbling ducks (similar to mallards) as no other species showed elevated levels. This could be due to the pāteke's foraging behaviour, which includes ingestion of sediment and grit to aid in digestion, and which inadvertently increases the likelihood of spent Pb ammunition uptake. It is important to note that the toxicological thresholds used were extrapolated from various waterfowl, which might not totally reflect toxicity to native New Zealand water birds. There is also a chance that due to leaching during tissue storage, measured metal levels are not representative of the actual load in the birds.

Formalin as a storage solution

Formalin fixation of tissues is a widely used method for storage of samples used by museums and research institutions that presents a potential source of retrospective analyses of contaminants and the variations of exposure across time (Campbell and Drevnick, 2015). It is evident that the leaching of heavy metals from fixed samples is not a simple function of the elemental concentration within the tissues, but it is assumed that the strength and mode of binding of each different element to the tissue is responsible for the rate of leaching observed (Gellein *et al.*, 2008). For example, elements such as Pb, which strongly bind to sulfhydryl groups that are present in proteins, have been found to leach from formalin-fixed tissue at a lesser rate than other metals (Gellein *et al.*, 2008). The pH is known to influence the dissociation and the binding of metals, and it is possible for formalin to oxidise to formic acid which would result in a lowered pH; this could result in an extraction of metals from the stored sample into the preservation medium (Simmons, 2014). Other factors such as dilution variation, evaporation, contamination from other sources, and length of storage have also been reported to effect metal leaching (Hamir *et al.*, 1995, Schrag *et al.*, 2010, Gellein *et al.*, 2008, Bush *et al.*, 1995). In my samples, where there were duplicate fixed and frozen liver samples, there was no significant difference between any of the heavy metal concentrations. However, given the known variable effect of formalin on heavy metal concentrations and the high possibility of a type II error in my limited comparisons, separation of the results by sample type was considered to be the most valid way of presenting the results. The concentrations of heavy metals reported in the fixed samples may therefore underestimate actual concentrations due to the potential leaching in the preservative solution. However, the number of both pāteke and whio sampled provides conservative information comparable with hepatic concentrations of heavy metals in mallards reported in Chapter Three.

Importance of metals in relation to declining freshwater bird populations

A high proportion of pāteke and whio showed elevated hepatic concentrations of Cd, which have been found in other species to cause such sublethal adverse effects as testicular damage, suppressed egg production, altered energy metabolism, and disruption of hormone synthesis. This is a concern for the management of any species, let alone two species, which are classified as At Risk and Endangered. Waterbirds are exposed to a large range of environmental stressors such as climate change, habitat destruction, degradation of water quality, and the invasion by exotic species. Thus, a better understanding of these stressors and their interactions on the wild populations would be beneficial to waterbird conservation in New Zealand. Further research is needed on the potential connections between body loads of trace metals and population declines, as well as the importance of heavy metal pollution and their movement throughout the food webs of the native environments. Further investigation into the thresholds at which such heavy metals as Cd and Pb affect the unique endemic waterbirds of New Zealand is warranted, as sensitivity differences between even closely related species to these pollutants are documented within the literature. Knowledge of the thresholds will be an important management tool for selection of suitable habitat for translocations and release sites for the recovering populations of both the whio and pāteke.

Conclusion

This present study of hepatic concentrations of Cd, Cu and Pb in six species of native New Zealand freshwater bird clearly shows that some species are exposed to environmental contamination of Cd and to a lesser extent Pb. Fresh or frozen samples are more reliable for ecotoxicology studies, but the results presented here would suggest that while formalin-fixed avian liver tissues might report slightly underestimated concentrations due to leaching, they will still provide conservative information, comparable with other studies. The results reported within this chapter allow insights into the bioavailability of heavy metals within the freshwater environments with varying degrees of modification by humans. In a context of diverse environmental stressors affecting waterbird populations, this analytical study shows the importance of metal contamination as an additional stressor that could be influencing the recovery of declining populations. This study highlights the need for further investigation into Cd contamination reported in habitats such as the West Coast and Aotea, and their uses as suitable habitats for endangered waterfowl species.

Chapter Five

General discussion: The heavy load to bear

A major challenge facing environmental conservation is how to balance anthropogenic development and impacts, with environmental stability. The continued growth of the human population has resulted in a massive increase in pressure on the agricultural sectors to produce more food at a quicker rate. This intensification has required the use of agrichemicals, modification of farming practices, and drastic changes to the landscape. One by-product of this is the increased amount of trace metals accumulating within the local ecosystems. Exposure to these metals is known to have significant detrimental effects on organisms' survival and reproductive success. Our understanding of this has been accumulating since the 1800s with the recognition that ingested Pb shot resulted in Pb poisoning in waterfowl (Hough, 1894). While there are still holes in the knowledge of effects and critical thresholds of specific metals and species, the literature indicates that globally, exposure to heavy metals is increasing in all ecosystems (Tchounwou *et al.*, 2012). This, coupled with the numerous other pressures facing wildlife, such as habitat degradation and invasive predators, is resulting in a continued decline of many species throughout the world. This study focused on identifying possible pressures from heavy metals and determining the concentrations of three metals in selected waterbird species within New Zealand.

Wild avian populations have been shown to be highly sensitive to heavy metal environmental contamination, with chronic exposure often resulting in the accumulation of sublethal tissue concentrations of heavy metals that have been found to cause adverse effects to both reproduction and survival (Scheuhammer, 1987). Exposure is predominantly through dietary uptake, and to a lesser extent water consumption and inhalation. Dietary exposure is particularly important for predatory species, and for pollutants that are known to bioaccumulate (Burger, 2002). Waterbirds are highly vulnerable due to their habitat and foraging behaviour. The bioavailability of pollutants is higher in aquatic sediments than terrestrial soils (Reid *et al.*, 2000). The amount of sediment that is ingested by prey, or directly, results in a constant open pathway for stored heavy metals to enter the food chain. Birds inhabiting areas of high pollution are particularly susceptible, including areas associated with mine sites and farming runoff (Hernández *et al.*, 1999, Kingsford *et al.*, 1999, Gómez *et al.*, 2004), as well as areas of intensive hunting that results in spent Pb shot (Scheuhammer *et al.*, 1998), and fishing weight contamination (Scheuhammer and Norris, 1996).

Globally, waterfowl species are a well-studied bioindicator of pollutants due to their high trophic level, their well understood biology, coupled with their economic value for hunting and importance as a food source to humans (Binkowski, 2012, Kalisińska *et al.*, 2004, Di Giulio and Scanlon, 1984b, Parslow *et al.*, 1982, Kim and Oh, 2012). The body load of metal contaminants within birds is largely affected by metal bioavailability, food quality, and environmental pollution (Di Giulio and Scanlon, 1985, Beyer *et al.*, 1998, Kalisińska *et al.*, 2004). Large data sets focused on a single species, with well-defined groups (different populations, or age groups) may in time offer a useful tool for establishing specific standards useful for not only the assessment of avian body load of metals but also of the habitat and environment where the bird is situated. The use of a bioindicator species specifically allows the monitoring of levels of bioavailable metals, which can be different to the levels seen in solely environmental assessments. Within New Zealand, there is a distinct lack of research into the movement of pollutants in the environment and wildlife, especially in freshwater species exposed to the leaching and runoff from farming, agricultural, and urban areas. This thesis offers an insight into the bioavailability of three heavy metals which may be having a detrimental effect on the waterbird populations of New Zealand.

5.1 Cadmium

Cd is considered one of the most toxic heavy metals to organisms. While exposure to Cd is known to adversely affect reproduction and survival in experimentally dosed birds, little is known about the low-level chronic effects of Cd-exposed wildlife. In this thesis, plasma concentrations of Cd were found to be slightly higher than a similar study using mallards to biomonitor the heavy metals within the Polish environment (Binkowski and Sawicka-Kapusta, 2015). The liver concentrations of Cd were also higher in New Zealand birds compared to mallards from Poland (Kalisińska *et al.*, 2004), Illinois (USA) (Levengood, 2003), and Korea (Kim and Oh, 2012). A common pattern established within the literature is the accumulation of Cd with age, with adults reporting higher Cd levels compared to juveniles. This pattern was also confirmed within the two populations of mallards, regardless of sample type, and also in the native waterfowl species pāteke, and to a lesser extent whio. It is noteworthy that the liver Cd concentrations in the fresh whio and fixed pāteke samples were higher than mallards. This comparison needs to be tempered by biases in the collection method, i.e. the native bird samples were taken from birds found dead, while the mallards were hunter shot birds.

Although Cd is found naturally, it is also released into the environment via anthropogenic sources, with global emissions coming from industrial sources such as mining and smelting. However, in New Zealand industrial pollution of Cd is rare, with the most likely route of environmental exposure assumed to be due to the use of phosphate fertilisers. Historically these fertilisers where sourced from Nauru Island which is Cd-rich, causing the Cd soil load in New Zealand to vastly increase over a 50-year period (Taylor, 1997). Cd does not readily leach from the soil, and the transfer of Cd from terrestrial to aquatic systems is not efficient, with an estimated 94–96% Cd remaining in the soil (Peakall and Burger, 2003). This creates a legacy issue for many agricultural regions, with a retention time of 200 – 700 years depending on soil type, land use, and fertiliser concentration. As it is not commercially viable to remove Cd from phosphate fertilisers, a move to less Cd-rich phosphate deposits has been made, although some ongoing pollution is still expected. Two other possible environmental sources of Cd were discussed in this thesis. Firstly, the Cd concentrations reported in pāteke from Aotea and the Coromandel are hypothesised to be due to the naturally high level of Cd found within the sulphide mineral deposits, which are abundant within that region of New Zealand due to volcanic activity. However, the most interesting Cd result was the elevated levels of whio from the West Coast of the South Island. This exposure is hypothesised to be from anthropogenic Cd, which has been transported in wind-blown dust from the eastern Australian seaboard (Marx *et al.*, 2008). While exposure of species which inhabit agricultural lands was expected, the levels seen in two regions of New Zealand which were thought to be pristine is of concern. The amount of unlogged native forests along the West Coast of the South Island has resulted in a stronghold of many threatened native species populations within this region; this includes two species of kiwi (*Apteryx australis australis* and *Apteryx rowi*), kea (*Nestor notabilis*), kotuku (white heron, *Ardea modesta*) and the mohua (yellowhead, *Mohoua ochrocephala*). The result reported within this thesis should be of interest to the recovery programs centred on these species.

Further research is needed to understand the full consequence of the elevated soil Cd levels within the agricultural regions of New Zealand and the movements of Cd throughout the ecosystems. For this, investigation of the wider ecosystem is suggested, as the levels reported in these waterfowl species are likely to be bioaccumulating up the food chain. This is a concern to human health as mallards in New Zealand are a major food source for hunters and their families. Further investigation into the population of pāteke would also be advised, as birds that experience prolonged food restrictions are thought to be more sensitive to Cd exposure. Aotea is considered poor habitat for waterfowl, with a proportion of the population dying due to starvation. Adverse effects of Cd could thus be exacerbated, decreasing metabolic activity (increased energy drain) which could result in increased predation.

5.2 Copper

It is thought that birds are relatively resistant to Cu when compared to other vertebrate classes, such as amphibians and fish (Eisler, 1998). It is therefore assumed that environmental contamination of Cu is more likely to impact avian species by disruption of the trophic chain rather than by direct toxic effects. This is particularly thought to apply where prey is affected by the environmental load, and can lead to a limitation of food sources (Eisler, 1998, Frakes *et al.*, 2008). However, a common problem noted when reporting Cu concentrations, is that there is a distinct lack of data available on the toxicity of Cu in avian wildlife, which is especially true for blood concentrations. Blood Cu concentrations presented within this present study are comparable with levels reported in the literature from contaminated environments, and a high proportion of the hepatic concentrations were found to be elevated above thresholds for Cu toxicity in mallards. Although Cu is an essential metal needed for normal metabolic functions, in excess it can produce a series of hepatic, pulmonary, metabolic, and renal toxic effects.

Within this study, it is hypothesised that the main anthropogenic source of Cu pollution is due to secondary impacts of farming activities, where herbicides, fungicides, fertiliser, recycled effluent, and stock feed high in Cu are used. The potential sources which may be responsible for the high load of Cu reported in this thesis are numerous, due to the high proportion of New Zealand soil which is reported as Cu deficient. Horticulturists and farmers use Cu-rich fertilisers and supplement with mineral blends to increase uptake in plants and livestock. These practices are coupled with additional methods, such as the use of certain plant species within fields and pastures that have elevated Cu uptake, increasing the bioavailability and mobility of Cu within New Zealand wetland ecosystems. This saturation of the rural environment with bioavailable Cu could explain why mallards from these areas had high Cu concentrations, while the native water birds in Chapter Four reported levels well below the tentative toxicity threshold established within the literature. In order to gain a wider appreciation of Cu movement through the New Zealand ecosystems, it would be useful to examine other avian species which predominantly use the rural environment such as pukeko (*Porphyrio melanotus*), spur-winged plover (*Vanellus miles novaehollandiae)*, weka (*Gallirallus australis*), turkey (*Meleagris gallopavo*), and a range of introduced passerine species.

As the Cu-deficient soils continue to be used for pastoral agriculture and horticulture in New Zealand, it can be expected that these farming practices will continue, increasing the amount of Cu within the rural environment. To understand the direct effects of this pollution to the survival of wildlife using this modified environment, further investigation is suggested. Initially, the physiological effects of these elevated Cu concentrations on the resident mallard populations should be further quantified. This could be done in a number of ways. Firstly, pathological changes which occur in functional liver lobules can be quantified through histological methods. Secondly, as Cu exposure and toxicosis has been found to erode the gizzard and proventriculus of broiler chicks (Poupoulis and Jensen, 1976), a similar examination of the collected gizzards to investigate the frequency of this pathological change in the mallards could be conducted. Lastly, similar to Cd, a wider investigation of the movement of Cu through the ecological food web would help build understanding of the movement of Cu throughout the New Zealand ecosystem.

5.3 Lead

Pb is a nonessential heavy metal which is highly toxic, affecting all body systems, and it has been implied that there is no safe or no-effect level for Pb exposure (Martínez-López *et al.*, 2004). Pb exposure was found in all the liver and blood samples tested within this study, with a proportion of these samples above thresholds consistent with sub-clinical exposure (Franson and Pain, 2011). The levels of plasma and tissue Pb reported in this thesis are comparable or lower than global levels reported from other counties with restricted use of Pb shot. Some evidence of Pb transfer from hens to eggshell within mallards in New Zealand indicates that ducklings may be exposed to Pb before hatch, which is known to affect embryonic development, chick survival, and egg production (Somashekaraiah *et al.*, 1992, Nyholm, 1998, Edens and Garlich, 1983).

The prevalence of Pb exposure reported within this thesis indicates the widespread nature of environmental Pb contamination within New Zealand. Due to human historical and contemporary Pb use and pollution, Pb is ubiquitous in soil, water, and air, in both urban and rural environments, with exposure to wildlife predominantly from environmental contamination. In relation to waterfowl, the most likely direct source is the ingestion of spent Pb pellets from hunting. Dabbling and diving ducks, in particular, are known to have an increased susceptibility due to their foraging behaviour and the ingestion of grit and stones to aid digestion. The input of Pb into the environment can occur from a variety of other sources, including ammunition, the application of biosolids, agrichemicals, irrigation water, and atmospheric deposition. Atmospheric inputs have a rapid effect on terrestrial environments, as the atmospheric fall-out directly contaminates the plants on which animals graze, avoiding the involvement of plant uptake from soil (Gray *et al.*, 2003a). Due to the stability of Pb, the historical use of agrichemicals containing Pb has resulted in its accumulation in agricultural and horticultural soils (Gaw *et al.*, 2006). Studies suggest that the physicochemical soil properties (soil structure, pH, organic matter content) are the major factors controlling the bioavailability of Pb (Magrisso *et al.*, 2009). The Pb load and solubility within water is another possible route of uptake for waterfowl, however again the physicochemical properties of the water play a prominent role in the solubility and consequently the bioavailability of Pb (Mager, 2011). Mallards from the Waikato site were found to have significantly higher concentrations of Pb, regardless of sample type, suggesting that the bioavailability within this region is higher than Southland. Studies conducted within both the Southland and Waikato regions have found this to be true for both atmospheric deposition (Gray *et al.*, 2003a) and soil levels (Gaw *et al.*, 2006).

This is the first study to investigate Pb exposure in New Zealand waterfowl since the 2001 restriction of Pb shot was implemented. While levels have decreased slightly from studies conducted before the restriction, this thesis indicates the Pb toxicity and exposure is a continuing issue for waterfowl in New Zealand. To understand the full effect of Pb on the waterfowl of New Zealand, this survey should be extended to encompass all regions of New Zealand, especially in areas with low regulatory monitoring from Fish and Game. This would create a better picture of the exposure rate of waterfowl nationally. Source of Pb could also be gauged with a stable isotope analysis, although due to the many global sources of Pb that ammunition companies use, the accuracy of is tool for source attribution is becoming more limited.

5.4 Mallards as bioindicators of the New Zealand environment

Numerous species of aquatic birds have been used as bioindicators to monitor habitat pollution. Of these, the mallard is one of the most commonly studied due to their large populations, observable sexual dimorphism, and longevity, all of which allow observation of long-term pollutant exposure (Kalisińska *et al.*, 2004). These studies have predominantly taken place within the native range of mallards, that is across Europe and North America, covering nearly the entire Palaearctic ecozone (Binkowski *et al.*, 2016). Although within New Zealand mallards are fairly ubiquitous, their habitats are not, primarily inhabiting modified landscapes such as pastoral environments and urban areas, although populations are increasing in more remote and unmodified areas (Dyer and Williams, 2010). While sampling mallards in New Zealand may result in a bias towards habitats of high environmental modification, this allows the monitoring of the areas of highest risk for anthropogenic pollution. While there are several other native aquatic bird species which could potentially be used as bioindicators (Chapter Four), native species are protected to varying degrees by the Department of Conservation, resulting in major restrictions in sample types and numbers which could be accessed. Mallards on the other hand are well established with a large national population and as they are not native, sampling is largely unrestricted. In addition, as they are a well-studied species, thresholds for toxicity of a variety of heavy metals are known specifically for mallards. The use of a globally distributed species allows for international comparisons of environmental pollution. Finally, as mallards are one of the most popular game species in New Zealand, knowing the levels of toxic metals in mallards is important in terms of food safety and human health.

5.5 Management and public health recommendations

Heavy metals are increasingly saturated within the environment, and their entrance into the food chain through soil, water, and air circulation is an important environmental issue that includes risk to humans. To date, no regulations have been established to monitor or regulate game meat for fitness for personal consumption in New Zealand. The data presented within this thesis identifies the threat to human health through the exposure of heavy metals to mallards, a nationally hunted bird. The levels in liver reported within this thesis are elevated (Pb = 10.4%, Cd = 16.1%) above the maximum allowable threshold in offal for human consumption. However, according to the World Health Organisation (WHO, 2015) there are no defined safe levels of Pb intake in humans as the toxic effects are numerous and largely irreversible. It is a well-established pattern that people who frequently consume game shot with Pb ammunition are at risk from high dietary Pb exposure due to the bullet fragments that contaminate the carcass (Hunt *et al.*, 2006, Iqbal *et al.*, 2009). Legislative actions to reduce the use of Pb ammunition came into effect in 2001 in New Zealand, however the restrictions still allow for the use of Pb shot in certain situations and non-compliance with regulations may also be an issue. While the levels of Pb reported within my thesis cannot be conclusively linked to spent Pb shot, the ingestion rates seen (Chapter Three) would imply that spent Pb shot is still a viable route of exposure to waterfowl. In other countries, restrictions have been ineffective due to their partial nature, lack of compliance, and lake of enforcement (Cromie *et al.*, 2014, Green and Pain, 2016). New Zealand regulation surrounding Pb shot use are to be further restricted in 2021, when sub-gauge shot guns will be required to use non-toxic shot in open water waterfowl hunting; this however still allows for Pb use for hunting of certain species, and with certain types of guns. With the evidence highlighted in the literature and the results from my thesis, a total restriction on Pb shot for hunting waterfowl would be recommended.

The levels of Cd reported in the liver study (Chapter Three) raise major concerns over the human consumption of offal of wild mallard in New Zealand. Coupled with the Pb levels, a recommendation would be to improve the education and awareness of hunters about the health impacts of consuming wild waterfowl offal with high Cd and Pb. Hunters should be advised to avoid consumption of internal organs of waterfowl, and further research should be conducted into the levels of these heavy metals in the pectoral muscles, a more frequently consumed part of the duck. Further research is required into the movement of these metals in the environment, especially identifying source and mapping food webs, to identify vulnerable parts of the environment. Understanding where and how these elements enter the ecosystems will be paramount to reducing their levels and the damage to the environment done by their presence.

5.6 Concluding remarks

This thesis has identified that environmental exposure to three heavy metals may be of threat to the waterfowl species of New Zealand. The results suggest the widespread bioavailability of heavy metal pollutants within the New Zealand environments sampled, and their potential effects on wildlife. While the current restrictions of Pb shot still allows this ammunition to be used under some circumstances, a significant reduction in Pb shot ingestion was seen in both field sites since the restrictions were placed in 2001, which may reflect an overall reduction of environmental exposure by spent Pb shot in the last 17 years. The unexpected high levels of both Cd and Cu which were above background exposure levels in the waterfowl studied, suggest that contamination of the New Zealand environment is occurring at levels high enough to cause adverse health and reproductive effects in a proportion of New Zealand wildlife. My results suggest that the concentrations of heavy metals may be affecting the population dynamics of mallard and other native waterbirds, although further studies are required to investigate this hypothesis. The concentrations reported within this study also suggest wider concerns for the ecosystem health and human health for those who hunt and consume wild waterfowl in New Zealand.

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Appendix 1: Pilot study

Initially the intention of this study was to conduct a broad survey of multiple heavy metals within two population of mallards in New Zealand. Elements were selected due to the prior knowledge of heavy metal contamination within New Zealand, known toxicity of metals in both humans and waterfowl and those that were comparable to the published literature. The six selected were; mercury (Mg), cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and arsenic (As). Analysis cost per sample increased with each metal, therefore the number of heavy metals tested for came at the cost of the number of samples. The objective of the pilot study was to assess the exposure of heavy metal levels in each of the four sample types in order to prioritise the heavy metals that were elevated and most likely resulting in adverse effects on the mallard populations.

Method

Samples collected for the pilot study were collected from both the Southland and Waikato study sites (see Figure 3.1). Pre-breeding season bloods (n=21) were collected from hens in June and July of 2014 and 2015. Eggshells (n=19) were collected from hatched or abandoned nests of tracked females in 2015 (for more detailed method of blood and eggshell collection see Chapter 2). Livers (n=20) were collected from donated hunter shot birds between May and August of 2015 (for more detailed method see Chapter 3). All sample types were sent to Hills Laboratory (Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton, New Zealand) for analysis. Heavy metal concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS).

Results

Most of the blood samples analysed reported values above the LOD for all six elements evaluated, except for As (Table 6.1). Only two elements reported levels above LOD in every clutch, Cu and Zn. Pb was elevated above the LOD in 4/19 (21%) of the clutches, while As, Cd, and Hg had no clutches above the LOD (Table 6.2). All elements tested for in the livers reported levels above the LOD, except As (Table 6.3).

Table 6.2 Summary of heavy metal concentrations (mg.kg^{-1} w.w.) in pre-breeding season bloods from mallards. ND- non detection

Element	LOD	Mean ± SD	Range
As	0.01	0.028 ± 0.016	ND-0.07
Cd	0.0002	0.003 ± 0.005	0.0006-0.0168
Cu	0.005	0.399 ± 0.059	0.31-0.5
Pb	0.001	0.105± 0.172	0.015-0.62
Hg	0.001	0.042 ± 0.046	0.0025-0.141
Zn	0.1	5.43 ± 0.702	4.4-6.9

Table 6.3 Summary of heavy metal concentrations (mg.kg^{-1} w.w.) in eggshells from mallards. ND- non detection

Element	LOD	Mean ± SD	Range
As	0.01	ND	ND
Cd	0.002	ND	ND
Cu	0.05	9.616 ± 10.266	1.7-45
Pb	0.01	0.81 ± 1.54	ND-1.82
Hg	0.01	ND	ND
Zn	1.0	11 ± 3.512	10-25

Table 6.4 Summary of heavy metal concentrations (mg.kg^{-1} w.w.) in livers from mallards. ND- non detection

Element	LOD	Mean ± SD	Range
As	0.02	ND	ND
Cd	0.0004	0.563 ± 0.433	0.179-2.0
Cu	0.01	87.395 ± 144.33	13.1-650.0
Pb	0.002	0.695 ± 2.59	0.009-11.7
Hg	0.002	0.101 ± 0.121	0.003-0.43
Zn	0.2	45.15 ± 13.26	31.0-87.0

Conclusion

In this study Zn was found to have the highest blood concentrations, followed by Cu, Pb, Hg, As, and Cd had the lowest concentration. The Zn levels reported here are well below the 15 mg.kg-1 reported in plasma which correlate with zinc poisoning (Eisler, 1993). Blood Cu levels were also lower than those reported in literature from contaminated sites (Chapter 2) (Binkowski and Meissner, 2013). Three (14.3%) samples reported Pb levels elevated above background levels (0.2 mg.kg-1) (Franson and Pain, 2011). No Hg values in this present study had levels close to 1.0 mg.kg-1, which is has been reported as the threshold for adverse effects in birds (Alvárez *et al.*, 2013). Authors have reported As values of 0.02 mg.kg-1 as a reference value for uncontaminated areas (Burger and Gochfeld, 1997a), within this study 52% (11/21) of this present study exceed this. Other than Cu and Pb (see Chapter 2), the only additional element to be reported in the eggshells was Zn, an essential element. Levels of Zn reported here are much lower than levels reported in common eiders (Franson *et al.*, 2004).

Livers showed a similar level of concentration to the bloods, however Cu had the highest concentrations followed by Zn, Pb, Cd, and Hg. No liver had a detectable level of As. Levels of Cu above 100 mg.kg-1 are considered elevated and can result in Cu toxicosis, 20% (4/20) were found to be elevated above this. Mallard hepatic Zn concentrations of 50 mg.kg-1 are accepted as average in controlled settings, while adverse effects were observed when liver concentration were 400 mg.kg-1 (Gasaway and Buss, 1972). No livers had values close to this, with the maximum concentration showing 87.0 mg.kg-1. Pb concentrations elevated above 2 mg.kg-1 are considered elevated above background levels, and within this study only one individual (5%) was found to exceed this threshold. However, the value reported was 11.2 mg.kg-1 which is considered consistent with severe clinical poisoning. Two (10%) of the livers had concentrations elevated above background levels (>0.9 mg.kg-1). Concentrations elevated above 1 mg.kg-1 for Hg have been found with adverse effects on behaviour (Zillioux *et al.*, 1993). No samples exceeded these levels; with the highest level found was 0.43 mg.kg-1 with an overall average of 0.1 mg.kg-1.

From the results presented here, it was decided to prioritise Cu, Cd, and Pb as the frequency and magnitude of the other metals suggested they were less important in the New Zealand context.