Heavy metals and metallothionein in seabirds, with reference to their use as biomonitors

Fiona M. Stewart

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CANDIDATE'S DECLARATION

I declare that the work recorded in this thesis is entirely my own, unless otherwise stated, and that it is of my own composition. No part of this work has been submitted for any other degree.

Fiona M. Stewart

April 1994

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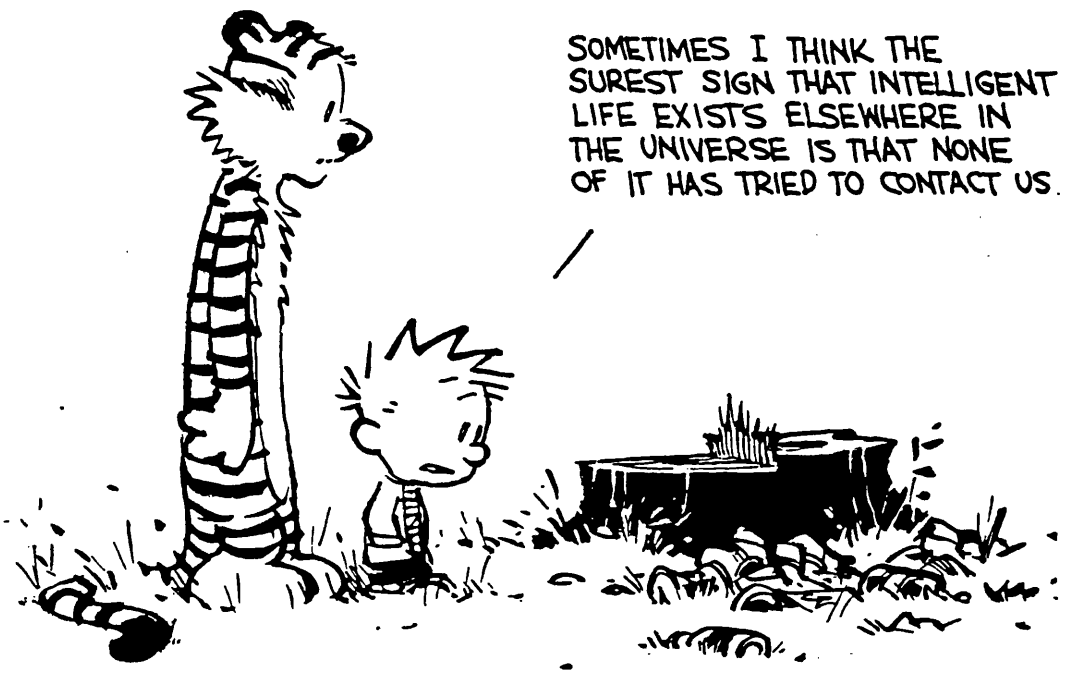

SOMETIMES I THINK THE SUREST SIGN THAT INTELLIGENT LIFE EXISTS ELSEWHERE IN THE UNIVERSE IS THAT NONE OF IT HAS TRIED TO CONTACT US.

CONTENTS

Summary

The use of seabirds as biomonitors is introduced and their applications to various ects of monitoring discussed.

A review of cadmium in birds is presented. The tissue distribution, uptake and :age of cadmium, the role of metallothionein, accumulation, excretion, toxicity and a and inter-specific variation in heavy metal concentrations are discussed. Recent lies in monitoring are summarised.

Biomonitoring of cadmium using feathers and eggs is assessed. It was concluded t feathers and eggs were of no value as monitors of cadmium in the marine ironment.

Cadmium concentrations in the internal tissues of adult lesser black-backed gulls re significantly higher than in immature gulls, but the accumulation patterns of lmium into liver and kidney of the two age classes were the same.

No increase in cadmium concentrations in internal tissues with age were detected in known age great skuas (aged 3-21 years), even when data were combined with a vious study (total n = 40).

Dietary specialisation is probably important in determining the differences between lividuals in cadmium concentrations and may mask the effects of age. To date, there : no studies demonstrating continuing cadmium accumulation with increasing age in ly grown seabirds.

The relationships between cadmium, zinc, copper and metallothionein concentrations re investigated in adult lesser black-backed gulls. A metal-binding protein was lated using gel filtration. The protein was bound to the metals cadmium, zinc, and pper, was a low molecular weight protein, and was heat resistant. This protein also und onto the Ag^+ in the silver saturation assay and it was concluded that it was nost certainly a metallothionein.

levels between April and June, and feathers were thought to be the main excretion route of mercury during moult, from June to November.

14. The fluctuations in cadmium were thought to be due to one of two hypotheses.

1. Physiological processes during breeding and moult created changes in the body burdens of metals. 2. Seasonal dietary changes were responsible for the changes. There were dietary switches during the sampling period which could create a change in cadmium concentrations in the prey items.

15. It was proposed that cadmium in seabirds could have a shorter biological half-life than previously thought, and they may have some kind of regulatory ability.

16. An experimental study of the uptake and assimilation of cadmium and its effects on other metals and metallothionein in great skua chicks was conducted.

17. The dose response of birds did not vary significantly over the range of doses administered. Mean uptake was 0.51% of the dose. Tissue distribution was dose-dependent, at low doses kidney was the preferential site of accumulation, and at higher doses the liver accumulated more than the kidney.

18. Experimentally increased cadmium concentrations in bird tissues affected the concentrations of metallothionein and the essential metals zinc and copper, all of which increased with increasing cadmium concentrations.

19. There were no consistent changes in the concentration of metallothionein in the experimental birds, when compared to the control birds. This was thought to be due to the excess metal being bound up by the metallothionein already present in the chicks' liver, kidney and duodenum.

20. Great skua chicks absorbed a low percentage of cadmium from a given dose in comparison to mammals and terrestrial birds, and this did not change with increasing dose. The possibility that seabirds are more resistant to the presence and effects of cadmium than other animals was discussed.

Chapter One General introduction

"Mankind has become the most important element in the global biogeochemical cycling of the trace metals"

(Nriagu and Pacyna 1988)

"Over one billion (10^9) human guinea pigs are now being exposed to elevated levels of toxic metals and metalloids in the environment"

(Nriagu 1988)

1.1 Introduction

The heavy metals cadmium, mercury and lead are ubiquitous in both the terrestrial and marine environment. They occur naturally but concentrations may be increased as a result of human activities. Relative to natural emissions, 'anthropogenic' emissions of cadmium, lead and mercury are high (Bryan 1984). Before the huge contemporary increase in the use of metals and production of metal waste, metals distributed throughout the environment were in a state of constant stable cycling in biogeochemical cycles. Now there is evidence that anthropogenic emissions into the environment have overwhelmed the natural cycles in many ecosystems (Nriagu 1988). Consequently there are significantly higher quantities of metals in many components of the environment.

Cadmium, mercury and lead are three of the most toxic, non-essential metals. They are all widely used in industry. Some eight times more cadmium has been consumed since the 1950's than was ever used before (Stoeppler 1991), and the consumption of mercury and its compounds has also increased greatly since the Second World War. A considerable amount of the metal produced is released into the environment (Nriagu and Pacyna 1988).

1.2 Uses of heavy metals

Metals are very useful elements and have been used for thousands of years; Pliny and Discoides Pedanius prescribed mercurials as a treatment for syphilis and skin disorders. Nowadays mercuric compounds are rarely used for medicinal purposes due to their known toxicity, but are widely used in industrial processes (Von Burg and Greenwood 1991).

1.2.1 Uses of cadmium

The main uses of cadmium (and cadmium compounds) are in the manufacture of nickel/cadmium batteries, pigments, plastics and protective metal coatings. Cadmium is released into the environment from the combustion of coals and oils, in wastewater from mining and smelting, in fertilisers, in sewage sludge and from wastage of commercial products (Stoeppler 1991).

1.2.2 Uses of mercury

Mercury occurs in several organic and inorganic forms and human activities cause the release of a wide variety of mercury compounds. The chloralkali, wood paper-pulp, chemical and electrical industries and also the burning of fossil fuels have released mercury into the atmosphere as well as into fresh and seawater (Von Burg and Greenwood 1991). The main uses of mercury are in the manufacture of scientific instruments (thermometers, barometers), electrical equipment (switches, rectifiers, oscillators, electrodes, batteries, meters, mercury vapour lamps, x-ray tubes, lead and tin solder), dental amalgams and synthetic silk. In the chemical industry mercury is used as a fluid cathode for the production of acetic acid, chlorine and sodium hydroxide. Mercury is also still used in the extraction of gold from sediments.

1.3. Metals released into the environment

Eventually almost all of the cadmium and a considerable amount of the mercury produced will "escape" into the environment thus becoming available in the biogeochemical cycle. This unnatural mobilisation into the environment of cadmium and of mercury (both organic and inorganic forms) is estimated at around 30,000 and 11,000 tonnes/year respectively (Nriagu and Pacyna 1988). There is little information regarding the quantities of discharged metals which enter the marine environment. However waste is often discharged into freshwater rivers and into the atmosphere, both can be routes leading to the marine environment. Riverine inputs to the sea are particularly important for cadmium as it is less volatile than mercury or lead and less is deposited by atmospheric transport, whereas atmospheric deposition is the main route for mercury and lead into the marine environment. There is evidence that levels of dissolved cadmium in seas and oceans may be increasing (Nriagu and Pacyna 1988). If heavy metal levels are increasing in the marine environment then it is of the greatest importance to monitor these changes and the possible impact of chronic low level exposure to heavy metals which can bioaccumulate up through the ecosystem with effects on organisms.

A huge amount of research on heavy metals in the marine environment has already been done. This includes many aspects of the distribution, bioaccumulation and toxic effects of metals on organisms, as well as their use as monitors of pollution.

1.4 Environmental pollution incidents

There are several well known examples of heavy metal contamination of the food web resulting in disease and death or toxic effects in wildlife and people. The Minamata Bay disaster is perhaps the most notorious of these. From 1932 until 1968 it is estimated that over 80 tonnes of mercury was discharged into the bay by the Chisso corporation company. Their factory was producing acetaldehyde and vinyl chloride and using the mercury as a catalyst. This resulted in widespread poisoning of the local population, for whom seafood was a staple component of their diet. Poisoning caused deaths, as well as impaired vision, loss of motor co-ordination and other neurological abnormalities, and hormonal and enzymatic disturbances. By 1975 there were around 2800 victims of this pollution. A second outbreak in Niigata, Japan in 1965 was also caused by mercury effluent from a factory which affected fishermen living downstream (Tsubaki and Irukayama 1977, Clark 1989).

Alkylmercury was widely used as a fungicide to treat grain before planting, from the 1940s onwards. The worst incidents of mercury poisoning occurred in Iraq, where treated seed meant for planting was consumed by humans. Over 2000 people died and more than 60,000 people were exposed to the toxin. Alkylmercury treated grain also caused poisoning in Pakistan and New Mexico (Bakir *et al.* 1973, Von Burg and Greenwood 1991). The effects on wildlife were similarly fatal after granivorous birds and their predators accumulated toxic amounts of methylmercury by foraging on newly planted treated grain. This practice was later banned and the fatalities and amount of mercury in birds dropped rapidly (Jensen *et al.* 1972).

Wastewater from a zinc smelter contained high levels of cadmium and was the cause of Itai-Itai disease in Japan. Cadmium was accumulated in the rice crop from the surrounding polluted water. The disease affected bones, causing osteomalacia, severe pain in the back, legs, and joints, and severe renal damage. This resulted in around 100

deaths. Local people were ingesting as much as 300-400μg of cadmium per day (Stoeppler 1991). Since then awareness of metals as pollutants has led to the development of technologies which reduce emissions from factories. The occurence of these acute incidents has drawn attention to the hazards of heavy metal pollution. The concern now however is over the long-term chronic low-level pollution of the environment.

1.5 Heavy metals in the marine environment

Cadmium in the ocean behaves as a nutrient-like metal. This means that the cadmium will cycle in the ocean along with phosphate and nitrate. These are taken up in the surface waters by living phytoplankton and zooplankton. When these organisms die, they sink along with the faecal material and detritus. Between 500-1000 metres depth the organic particles are oxidatively decomposed, releasing the metals and nutrients which are then advected to the surface waters to re-enter the nutrient cycle. Nutrients and nutrient-like metals can undergo this cycle many times, and in each cycle only a little will sink down to the depths, to become biologically unavailable. Mean residence time of cadmium in the water column has been estimated at 450 years (Mart and Nurenberg 1986). Generally cadmium levels are very low in the central Atlantic, Pacific and Indian Oceans,(levels hardly exceeding 5ng/kg, and can be as low as 1ng/kg in some areas), with higher values occurring in more nutrient-rich areas or areas of upwelling. Elevated levels of cadmium in the North Sea, (up to 38ng/kg), Baltic Sea, (up to 34 ng/kg) Mediterranean Sea, (up to 18ng/kg), and off the North American eastern coast (up to 18ng/kg), are due to pollution from human activities by riverine and aeolian routes (Mart and Nurenberg 1986).

Mercury in the ocean is scavenged by organic complexes and so is present bound onto organic matter or within organisms themselves. Therefore mercury will accumulate in food webs. Scavenging metals are said to have a short residence time in the ocean, but this is still estimated at 350 years (Burton and Stratham 1990).

Concern about pollution and high natural levels of mercury in swordfish and tuna led to measurements of mercury concentrations in oceanic waters being made in the 1970's,

but these early results were inaccurate due to sampling difficulties and contamination. The input of mercury to the ocean is dominated by atmospheric pathways which are estimated to transport more than ten times the riverine flux (Burton and Stratham 1990).

1.6. Biomonitoring

Heavy metals are accumulated by numerous marine and terrestrial species and can be directly toxic to aquatic organisms. This has given rise to concern over the possible detrimental effects of metals on coastal resources, in terms of the potential hazards that metals pose to human health, and from a conservation point of view, the potential for damage to the marine environment and the fauna and flora within. These concerns have manifest themselves in a range of monitoring programmes to establish baseline levels, spacial and temporal trends in metal abundance and bioavailability in estuaries and other coastal environments. Although monitoring programmes may analyse metals in the waters themselves and in sediments, the use of organisms (which reflect the bioavailibility of metals) is the most widely employed method to monitor heavy metals in the environment (Phillips 1990).

Monitoring programmes have been developed on a local, national and international basis but a detailed discussion of these is beyond the scope of this thesis, and has been dealt with eloquently elsewhere. Furness and Greenwood (1993) discuss theory and practice of monitoring programmes fully, especially in relation to birds. Spellerberg (1991) summarises and discusses local, national and international monitoring approaches to ecological change, and Gerges (1994) brings the approach and strategy of UNEP (United Nations Environment Programme) for marine pollution monitoring up to date.

1.7 Seabirds as monitors

Seabirds accumulate cadmium and mercury, in some cases to very high concentrations, even though these metals have no known biological function and can be toxic at relatively low concentrations. Mercury has been implicated in some cases of reduced breeding success in terrestrial, freshwater and coastal breeding birds (Walsh 1990).

Since biological availability is one of the prerequisites for pollution there is a strong argument for the analysis of biological indicators. If an organism is to be used as a biomonitor then it must reflect the average bioavailable pollutant concentration (Phillips 1990). Seabirds have been used extensively as monitors of metals. Seabirds are a high profile group and there is great interest in their study and conservation, both among the general public and scientists. The long-standing interest in birds means there is a large data bank on bird breeding and ecology, and also museums have collections of bird study skins and eggshells, dating back 150 years or more, which means these can be used to monitor a few particular pollutants. Seabirds are marine predators at the higher trophic levels and can be used to monitor pollutants which bioaccumulate in the food web (e.g. mercury, cadmium, persistent organochlorines). Seabirds occur globally and thus have the potential to provide data on possible world-wide pollution, though birds which migrate long distances may be less suitable as local monitors (Furness 1993).

In order to use seabirds as effective monitoring tools, there are many factors which must be taken into account. The uptake, tissue distribution, storage, elimination and toxicity of metals must be known. Variations due to season, sex, age, and location must all be considered. Other important factors to consider are that environmental variation will be reflected by levels in the bird tissues by reflecting dietary uptake. Monitor species must accumulate the metal, be long lived to enable sampling through time and be common and accessible (Walsh 1990). Knowledge of the birds' foraging areas, diet, moult, migration and breeding can help interpretation of data. Since the 1960's many seabirds have been analysed for metals and therefore there is a large amount of data available on many species. This information could be very useful, for example in conjunction with other long term data on bird breeding ecology to follow environmental change. The analysis of seabird feathers for mercury has shown many interesting trends. The levels of mercury in seabirds around the British Isles appear to have increased over the last 150 years, due to humans' activities causing an increase in mercury in the sea (Thompson *et al.* 1992a). There is the possibility that there has been a simultaneous increase in other heavy metals such as lead and cadmium.

1.8 Seabirds and heavy metals

There is a huge published literature of heavy metal studies in seabirds. The metals most commonly analysed are cadmium, mercury, lead, zinc and copper. These analyses have been used to look at different aspects of heavy metals in seabirds;

a. To check for evidence of pollution and to quantify anthropogenic inputs into the marine environment. Brothers and Brown (1987) found that the fairy prion reflected the differences between a polluted and unpolluted area, and that the pollution of the ocean by jarosite (composed of zinc, cadmium, lead, iron, copper, mercury and arsenic) caused an increase in the metal burdens in these birds.

b. To measure the metal burdens to look at both baseline natural levels and to look for any toxic effects of the metal burdens. There have been many studies to look at baseline levels of metals in birds, and more general studies can be used for informative purposes (see Thompson 1990 for a review). Nicholson and Osborn (1983) found evidence of kidney lesions in free-living, apparently perfectly healthy seabirds, which were associated with high natural cadmium and mercury concentrations.

c. To examine geographical trends in metal distribution across the globe. Patterns of geographical variation in mercury have been shown in common guillemots. Monitoring of eggs from 16 colonies around Britain showed a 20-fold variation in mean mercury concentrations, lowest concentrations from north-west Scotland around to north-east England and much higher concentrations at five Irish Sea colonies (Walsh 1993). Anthropogenic inputs of mercury are high in the Irish Sea, reflected in the guillemots eggs. Mercury and cadmium concentrations in adult Cory's shearwater from Mediterranean colonies were higher than in birds from Atlantic colonies (Renzoni *et al.* 1986), reflecting both anthropogenic and geological influences. Cadmium also has a tendency to occur at higher concentrations in pelagic species due to naturally occurring concentrations of metals in the food web; kidney concentrations in Atlantic puffins from St Kilda were about twelve times higher than from the coastal Isle of May population (Osborn 1979b).

d. To determine changes in metal levels in the ecosystem through time, using museum specimens. The analysis of feathers for mercury in museum specimens from 150 years ago and contemporary samples has shown that the amount of mercury in seabirds has increased four-fold in that time (Thompson *et al.* 1992a). This reflects an increase in the mercury in the sea, due to human activities. There is a possibility that there has been a simultaneous increase in other heavy metals in the oceanic environment.

e. To study the accumulation and dynamics of heavy metals in seabirds. It is interesting that animals do absorb non-essential metals and may store and accumulate them. The differential accumulation of mercury, cadmium and lead into different tissues has been studied. Mercury in birds' tissues can be excreted out into the feathers and eggs and these may act as routes for detoxification. Similarly the concentrations of lead and cadmium have also been measured in eggs and feathers. A metal-binding protein metallothionein, has been found in kidney of free-living seabirds (Osborn 1978), but the role of the protein and its interactions with metals are unclear.

1.9 Aims of this thesis

The aims of this study were to investigate more fully some aspects of heavy metal accumulation, storage and dynamics in seabirds which were either understudied or notably controversial. For this reason emphasis has been placed on cadmium in seabirds. Chapter Two is a review of cadmium in birds to assess the current state of knowledge. Further chapters also look into some aspects of mercury dynamics, but as these have been comprehensively reviewed recently (Thompson 1990, Lewis 1991), they are not reviewed here.

Feathers and eggs have been used very successfully to investigate trends in mercury concentrations but the usefulness of cadmium concentrations in these tissues is more controversial. I had hoped to assess the suitability of cadmium concentrations in feathers and eggs as monitoring tissues. This is mainly dealt with in review form in Chapter Two. The accumulation of cadmium with age is also an unresolved issue and this was investigated in a sample of known age great skuas ringed as chicks and lesser black-backed gulls aged by plumage. There is very little in the literature on metal

binding proteins in seabirds and so the metal-binding proteins within bird liver and kidney and interactions with heavy metals were investigated in adult lesser black-backed gulls and fledgling Cory's shearwaters. There are no field studies on the uptake of cadmium in seabirds and this was looked at by a study of the natural accumulation of cadmium in young Cory's shearwaters and an experimental study of great skua chicks. The possibility of synergistic effects of cadmium and mercury were studied in the skua chicks with artificially elevated cadmium concentrations. Variations in metal burdens may fluctuate seasonally and the effects of this were assessed by analysis of tissues of the common guillemot, sampled at three times during one year.

1.10 The structure of the thesis

Apart from this and Chapter Three, each chapter in this thesis has been treated as a separate section, with an introduction, methods, results and discussion. References are given together in one list at the end of the thesis. All scientific names are given together in Appendix 1 at the end of the thesis (except for the chapter headings). Inevitably, the introduction and methods section in each chapter overlap somewhat with other chapters. This is because the aim is to prepare chapters in a format suitable for publication. The chapters in this thesis are as follows;

Chapter Two. A review of cadmium in seabirds.

Chapter Three. Materials and methods (outlining methods used, often in several chapters, in a more detailed form than appropriate for publication in a paper).

Chapter Four. Aspects of metal dynamics in lesser black-backed gulls, *Larus fuscus* and great skua *Catharacta skua* with reference to the use of seabird tissues as monitors of heavy metals.

Chapter Five. Heavy metal relationships with metallothionein in the lesser black-backed gull, *Larus fuscus*.

Chapter Six. The natural accumulation of cadmium in fledging Cory's shearwater, *Calonectris diomedea* chicks from the Azores.

Chapter Seven. An experimental study of the uptake and assimilation of cadmium and its effects on metal-binding proteins and essential metals in great skua, *Catharacta skua* chicks.

Chapter Eight. Seasonal variation in heavy metals in common guillemots, *Uria aalge,* from North-west Scotland. This chapter is in the form of a paper, in press in The Archives of Environmental Contamination and Toxicology, and appears in exactly the same format in this thesis (including American spelling, and reference list). The guillemots in this chapter were dissected and samples prepared and dried by K Ensor, Ingrid Baber and D. R. Thompson. D.R. Thompson carried out all mercury analysis. All other analysis, and the writing of the paper was my own work.

Chapter Nine. General discussion.

Chapter Two A review of cadmium in birds

2.1 Introduction

The aim of this chapter is to review the current state of knowledge and discuss areas of controversy with regard to cadmium in birds. This then provides a background for the research undertaken in this thesis, and reported in Chapters 4-8.

2.2 Tissue metal distribution

Cadmium concentrations in bird tissues are generally highest in the kidney tissue, lower in the liver, even lower in muscle tissue and very low (often undetectable) in eggs and feather (Thompson 1990). Cadmium concentrations have also been analysed in other tissues; central nervous tissue, pancreas, spleen, blood, brain, heart, lung, testes, salt gland, uropygial gland and intestines (Nordberg 1974, Howarth *et al.* 1981, Burger and Gochfeld 1985, Honda *et al.* 1986a), but the levels are generally low and these tissues are not often analysed.

The kidney is thought to be the important organ where cadmium accumulates and is stored (Nordberg 1974, Scheuhammer 1987). Studies show that birds retain cadmium in the kidney bound to metallothionein (Osborn 1978), a low molecular weight protein induced by the presence of cadmium (Scheuhammer 1987). However it is thought that the liver concentrations can give an indication of recent exposure, and have the advantage over kidney tissue in that with high levels of exposure kidney levels of cadmium may fall due to tubular dysfunction (Scheuhammer 1987). This has been shown experimentally in rats (Goyer *et al.* 1984) but not seabirds. Kidney lesions found in seabirds (Nicholson and Osborn 1983) show this as a possibility. It would appear that measuring both kidney and liver levels can be advantageous since liver : kidney cadmium concentration ratios give information on the type of cadmium exposure. Ratios greater than one indicate acute exposure to relatively high doses of cadmium, whereas liver : kidney ratios of less than one are more indicative of a chronic low level exposure (Scheuhammer 1987).

2.2.1 Cadmium in feathers

The literature on cadmium in feathers is conflicting. Studies can generally be grouped into three categories; firstly, studies which investigated whether feathers were a valid monitoring tissues for cadmium bioaccumulated in the birds' bodies, secondly, studies which measured cadmium in feathers along with other tissues (e.g. liver, kidney) as part of a larger study, and thirdly, studies which have used the concentrations of cadmium measured in the feather to investigate problems without questioning the validity of feathers as a monitoring tool. The results of these studies are summarised in Tables 2.1, 2.2 and 2.3.

There has been no study which clearly demonstrates a relationship between concentrations of cadmium in liver and kidney tissues and the concentration of cadmium in the feather tissue. Concentrations found are generally very low, and often undetectable (see Tables 2.1, 2.2, 2.3). Osborn *et al.*(1979) measured some of the highest concentrations found in healthy vertebrates, in kidney and liver tissue, and this was not reflected by the levels of cadmium in the birds' feathers, indeed many of the feather samples had no detectable cadmium in them. One fulmar kidney contained 480μg/g cadmium (mean value 228μg/g, n = 5) with feather concentrations < 0.351μg/g (range N.D-0.503μg/g, n = 5). Many other studies have also found this (Goede and de Bruin 1984, Goede and de Voogt 1985, Honda *et al.* 1986a, Lee *et al.* 1987, Lee *et al.* 1989, Stock *et al.* 1989, Denneman and Douben 1993, own unpublished data). The very high value of 27μg/g measured in blue-grey noddy (Cheng *et al.* 1984) is unique and conflicts with data obtained by all other authors, and as such is difficult to explain.

Studies which have suggested a relationship are certainly inconclusive. Scharenberg (1989) found a correlation between feather and liver cadmium in young male grey herons, but not in any other groups in the study (young female grey herons, young and old male and female cormorants) and concluded that the concentration of heavy metals in feathers does not indicate the concentration in internal tissues. Lee *et al.* 1989 were the only other authors to report a relationship between body tissue levels and feathers. However all species data were pooled

(carnivorous birds, passerines, waders) and cadmium levels were very low (0.05-0.24μg/g) with cadmium at undetectable levels in seabird and waterfowl feathers. A recent review on metals in feathers stated cadmium has the lowest average levels (with tin) reported in the literature (Burger 1994a), and that cadmium is likely to be deposition within the feathers rather than external contamination. However the review presents no evidence for this, and reports no correlations between cadmium levels in feathers and concentrations in female soft tissues.

Experimental studies (Table 2.1) have shown cadmium will accumulate on (in?) feathers when birds are fed cadmium in their diet (Mayack *et al.* 1981, Pilastro *et al.* 1993). Pilastro *et al.*(1993), in an experimental dosing study of starlings, concluded that the source of cadmium contamination was external, since pre-moult feathers grown before diet dosing accumulated cadmium as well as newly-grown feathers. However they proposed that cadmium contamination was due to the birds excreting the metal via the uropygial gland onto the feathers, and not from the contamination of the feathers from food. Birds in that study were fed large amounts of cadmium for a long period of time and accumulated very high levels (mean liver concentration group A = 75μg/g, kidney = 116.03μg/g, mean liver concentration group B = 208.49μg/g, kidney = 308.93μg/g), and low levels were found in the uropygial gland of dosed birds (0.5-2.8μg/g). Their theory of excretion does not hold up to scrutiny. The authors also offer no explanation of the mechanism required for the cadmium, bound in the kidney and liver to be transported to the uropygial gland. Control groups in the experiment had undetectable levels in the uropygial gland. A simpler explanation of their findings is that birds were heavily contaminated (and quite possibly poisoned) throughout their bodies with cadmium, and feathers were contaminated from spilt food and by dust bathing. Other studies of cadmium concentrations in the uropygial gland found levels of < 0.02μg/g (Leonzio *et al.* 1986), and 0.21-1.29μg/g (Renzoni *et al.* 1986). Other experimental studies (see Table 2.1) clearly demonstrate that cadmium in feathers is mainly or entirely from exogenous sources. The work done in Germany rather elegantly demonstrates that feather cadmium concentrations, and many other metal concentrations

Table 2.1. Experimental studies on cadmium concentrations in feathers.

Ref.	Species	Tissues analysed	Washing procedure for feathers.	Feather conc. (µg/g)(dry weight)	Experimental procedure	Conclusions
Hahn *et al* 1989a	Goshawk	Primary feathers	Rinsed with acetone	0.2-0.8	Three sites with varying levels of heavy metal deposition were chosen and primaries of incubating goshawk females were collected. Values for the deposition rates of metals were obtained from the rainwater program of the Institute for Applied Physical Chemistry	Standardised moulted feathers of Goshawks were used as biomonitors to indicate heavy metal pollution of areas. There was a strong correlation between the cadmium (and lead and copper) content of the feathers and the wet deposition rate measured by samplers. Cd, Cu and Pb levels in the feathers represent heavy metal burdens in the atmosphere over time and space, shown by increasing heavy metal levels in the more exposed feather parts such as the tips and outer vane
Hahn *et al* 1989b	Magpie	Various feather parts and types	Thoroughly rinsed with acetone	Freshly grown tail feathers =0.4 One year old tail feathers =3.5	The sample areas were predetermined by sites of deposition samplers of the Institute- sites with very high deposition rates as well as less contaminated and control areas. Feathers were divided into pieces and levels of metals from base to tip were analysed	Pb, Cd, and Cu content of the feathers are mainly due to external accumulation. The rise in metal content occurs over exposure time and depends on the exposed surfaces of the feather vanes. The highest Cd and Pb levels are found in the most exposed parts of the feathers (edges, tips) and in those feathers which are most exposed i.e. primary tail feathers.
Goede and de Bruin 1984	Knot Bar tailed godwit	Adult primary flight feathers no. 8	Shaken in de ionised water for 1 min.	N.D.- 0.84	Various procedures carried out to look at concentrations with time, external exposure, secretion by uropygial gland and leaching by seawater	External contamination may occur but whether feather tissue can be used as a monitoring tissue is uncertain as only in a very few cases could detectable concentrations of cadmium be measured

Table 2.1. continued

Ref.	Species	Tissues analysed	Washing procedure for feathers	Feather conc. (µg/g dry weight)	Experimental procedure	Conclusions
Weyers *et al* 1988	Blackbird	First primaries of birds of various ages	Feathers cleaned in propanon ultra-sonic bath for 3 hours then washed under running propanon, and then again exposed to ultrasonic treatment in a solution containing equal amounts of aqueous 1 % Triton-X- 100 solution and propanon. Finally feathers were washed under a running jet of metal-free water and dried for 12 hours	Unwashed 4 days old = 0.76 Washed 26 days old = 0.50 Washed 150 days old = 0.70 Washed 400 days old = 2.03 Unwashed 400 days old = 6.07	Electron microscope photographs made of cleaned and uncleaned feathers to look at the particles adhering to the external surface	Both washed and unwashed feathers were examined with the help of SEM micrographs. Numerous small particles can be seen both between and on the surfaces of unwashed feathers- this shows that it is impossible to clean a feather perfectly. If a method could be developed which would remove all external contamination then in may be possible to detect and quantify the endogenous part of the cadmium. Feathers could be used to quantify and monitor air pollution but there are other possible sources of pollution and therefore it would be very hard to quantify.
Pilastro *et al* 1993	Starling	Primaries, secondaries, livers, kidneys and uropygial glands	Shaken in de ionised water for 40 min. then dried	A=10 µg/g B = 50µg/g OP 2.09 7.41 OS 0.65 2.24 NP 0.96 1.88 NS 0.24 0.84	Group A were fed 10 µg/g and group B were fed 50 µg/g Cd Cl_2. Birds were sacrificed at the beginning of moult (19 weeks into the experiment) and at the end of moult (22 weeks into the experiment)	The presence of cadmium in the diet of starlings resulted in cadmium accumulation in the feathers: both old and new feathers accumulated cadmium therefore external deposition is the main mechanism of feather contamination. The authors suggest that the uropygial gland plays an important role in the excretion of cadmium.
Mayack *et al* 1981	Wood duck	Liver, kidney, feather	Washed in distilled water	Control = 2.22 1.0= 2.36 10= 4.00 100= 29.56	Ducks were fed diets of 0,1,10,100µg/g cadmium as $CdCl_2$ for 12 weeks before sacrifice, then some were kept until 19 weeks.	As amounts of cadmium ingested increased larger amounts of cadmium residue were found in feather tissue. May be external as the cadmium measured from litter samples corresponded with those found in the feed.
Burger and Gochfeld 1992	Common tern	Different parts of growing flight feathers	Washed in de ionised water and acetone	Whole breast = 0.112 Wing (whole)=0.061 Wing (distal)=0.061 Wing (proximal)=0.064) Wing (blade)=0.034 Wing (sheath)=0.063	Various parts of the growing feathers were analysed	Cadmium was higher in the sheath of a feather than the whole feather. Metals may be eliminated through sheaths as well as whole feathers, although the differences between blade and sheath were not significant.

Table 2.2. Feather cadmium measured as part of a larger study

Ref	Species	Tissues analysed	Washing procedure for feathers	Feather concentration µg/g	Relationship to other tissues	Conclusions
Goede and de Bruin 1984	Knot Bar tailed godwit	F (pr.)	Shaken with de ionised water	Only detected in vane of godwit	None	Whether the feather can be used as a monitoring tissue is uncertain as only in very few cases could detectable concentrations be measured
Goede and de Voogt 1985	Waders of various ages from the Dutch Wadden sea	F (pr no. 8)	None	Only detected in some dunlin adults and one juvenile godwit		Feathers are of doubtful value when monitoring exposure to cadmium, levels seemed incidental above the limit of detection.
Denneman and Douben 1993	Barn owl	F (pr. no. 7, vane only) Mixed sample of other pr. K, L from road victims	Shaken in de ionised water for 1 min.	7 th primary = 1.8-1.7 Mixed sample = 4.2	No correlation found between cadmium in liver and kidney and feather	-
Lee *et al* 1987	20 various species from Japan and Korea .	K, L, M, Bo, F	None	N.D. - 0.45	None	-
Honda *et al* 1986b	Easter great white egret	M, B, F(remiges, coverts, abdominal, pr. both rachis and barbs)	Rinsed in tap water, distilled water and acetone	0.007-0.059	-	-
Osborn *et al* 1979	Puffin Fulmar Manx shearwater	L, K, Br, H, M, S, P, G, I, Bl, F(pr.)	None	Puffin N.D. Fulmar N.D. - 0.503 Manx shearwater N.D. - 0.963	None	Although levels of cadmium in kidney and liver were amongst the highest found for healthy vertebrates, cadmium did not go into the feathers.
Stoneburner *et al* 1980	Sooty tern	Eg, Br, Bl, Bo, Fa, F(tail),K, L, M, St, Fae	None	17.00 (n=4)	N.A.	Levels due to natural volcanic pollution.

Table 2.2. continued

Ref.	Species	Tissues analysed	Washing procedure for feathers	Feather concentration µg/g	Relationship to other tissues	Conclusions
Stoneburner and Harrison 1981a	Sooty terns	K, L, Eg, F, Br.	None	Lisianski island = 0.02 Dry Tortugas = 4.25	N.A.	Pelagic seabirds use eggs and feathers as a mechanism for detoxification. In this case metals probably come from high volcanic activity
Lee *et al* 1989	Various species of Korean birds	K, L, F.	Rinsed in tap water, acetone and distilled water	N.D.- 0.96	Cd was positively correlated among liver kidney and feather ($r=0.60$, $p<0.01$)	-
Honda *et al* 1986a	Adelie penguin	K, L, M, Pa, Sp, Bl, Bo, F, Br, H,T	Rinsed in tap water, acetone and distilled water	0.20	-	Very little cadmium was in the feather
Howarth *et al* 1981	Crested tern	L, K, Sa, M, Br, H, F, Bo	None	Non-industrialised = 0.55 Industrialised = 0.51	-	-
Stock *et al* 1989	Oystercatcher	L, K, F(tail)	Feathers were sonicated in a 0.01% Troton-X-100 solution for 2 min.	0.21	No relationship found between feathers and internal organs.	Feathers are of no value as indicators in monitoring tissue Cd, but may reflect external contamination

Key L= liver, K= kidney, F= feather, H= heart, Bo = bone, Bl = blood, Br = brain, I= intestine, G= gonad, T= testes, Eg = egg, P = pancreas, Sa = salt gland, Sk = skin, M= muscle, L= lung, Sp = spleen, Fa = faeces, St = stomach contents

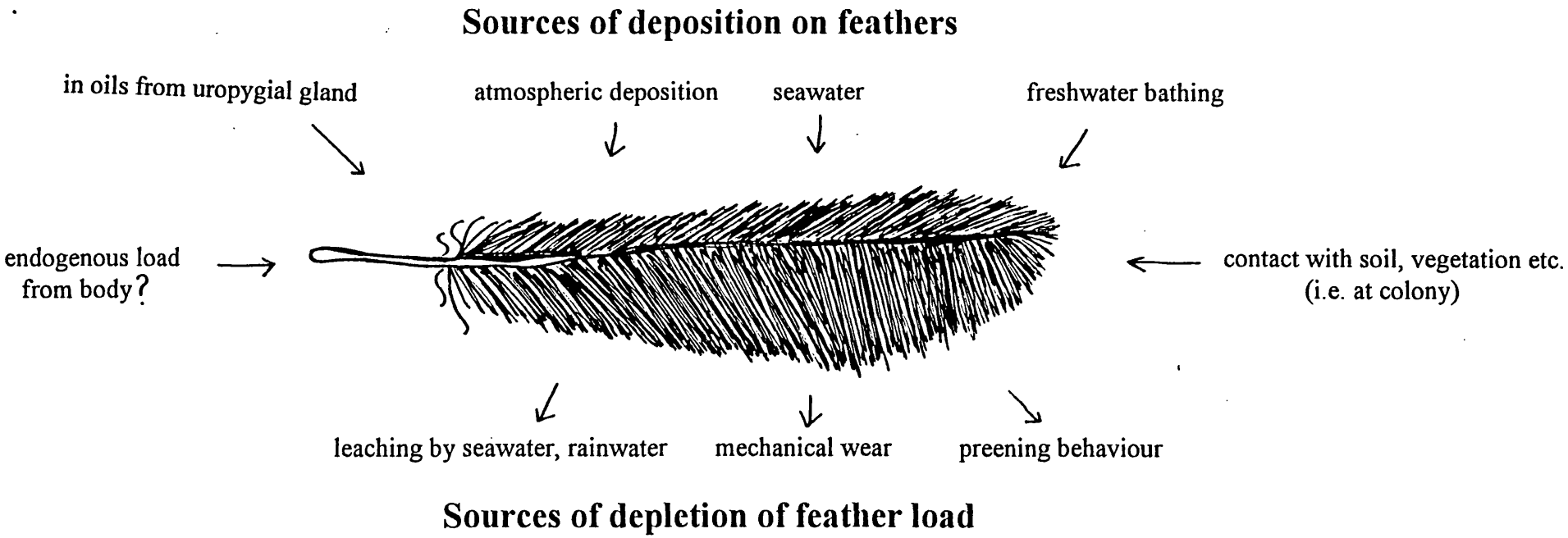
Table 2.3. Studies which use feathers as indicators of cadmium body burden.

Ref.	Species	Feather analysed	Washing procedure	Concentration µg/g	Conclusions
Janiga *et al* 1990	Feral pigeon	Throat and breast feathers	Washed in distilled water	0.95-1.65	Results of plumage accumulation in feral pigeons in Bratislava do not reflect significant regional differences in cadmium, lead or iron.
Burger *et al* 1993a	Cattle egrets from U.S.A., Puerto Rico and Egypt	Breast feathers	Washed in distilled water and acetone	Industrialised New York = 0.09 Less industrialised Delaware = 0.085 Puerto Rico = 0.425 Cairo, Egypt = 0.077 Aswan, Egypt = 0.080	-
Scanlon *et al* 1979	Wild turkey	Feathers	None	Piedmont, Virginia = 0.30 Montana, Virginia = 0.18	Showed background contamination from an unpolluted area
Burger and Gochfeld 1993a	Common tern Roseate tern Forsters tern Black skimmer Herring gull	Eggs and breast feathers	Washed in distilled water and acetone	Ct = 0.026-0.104 Rt = 0.569 Ft = 0.116 Bs = 0.056-0.182 Hg = 0.187-0.220	
Burger 1993	Brown noddys	Breast feathers	Washed in distilled water and acetone	Adults = 0.200 Young = 0.080	Young noddys had lower levels than adults. Adults have accumulated more metals with age which is endogenous in origin, as exogenous metal can be removed by washing procedure
Burger and Gochfeld 1991	Common tern	Breast feathers and eggs	Feathers rinsed with Triton -X and triple rinsed with de ionised water	Female = 0.051 Male = 0.047	Eggs had lower levels than feathers of adults. Cadmium levels in eggs too low to correlate with the feathers

Table 2.3. continued

Ref.	Species	Feathers analysed	Washing procedure	Concentrations µg/g	Conclusions
Burger *et al* 1993b	Wood stork	Breast feathers and primary feathers salvaged from the ground	Washed in de ionised water and acetone	Florida (young) = 0.203 (1992) Costa Rica (young) = 0.036 (1992) = 0.110 (1990) (adults) = 0.206 (1992)	Levels from Florida storks were significantly higher than Costa Rican storks
Burger *et al* 1992b	Common terns Roseate terns	Breast feathers	Washed in de ionized water and acetone	Initial = 0.12 Regrown = 0.15 Initial = 0.085 Regrown = 0.16	Feathers removed initially then regrowth removed 3 weeks later. Concluded both species may be more highly exposed to Cd on breeding ground than in winter quarters.
Gochfeld *et al* 1991	Common tern Sooty tern Black skimmer	Body feathers both black and white	Washed in de ionised water and acetone	White Black BS 0.11 0.202 CT 0.195 0.171 ST 0.1 0.173 PR 0.216 H 0.110 0.150	No significant difference between white breast feathers and black breast feathers therefore contour feather pigmentation does not affect metal levels
Burger and Gochfeld 1992a	Black skimmer	Breast feathers	Washed in de ionised water and acetone	Actual values not given Females = 0.098 Males = 0.031	Females had significantly higher levels of Cd , perhaps due to feedimg specialisations
Burger and Gochfeld 1992b	Sooty tern Brown noddy Wedge-tailed shearwater Brown booby Red- footed booby	Breast feathers	Washed in de ionised water and acetone	0.130-0.292	Cd levels generally low
Stoneburner and Harrison 1981b	Laysan duck	Primaries	None	0.2	Laysan duck population is practically free of metals with no biological function.

Figure 2.1 Cadmium and Feathers



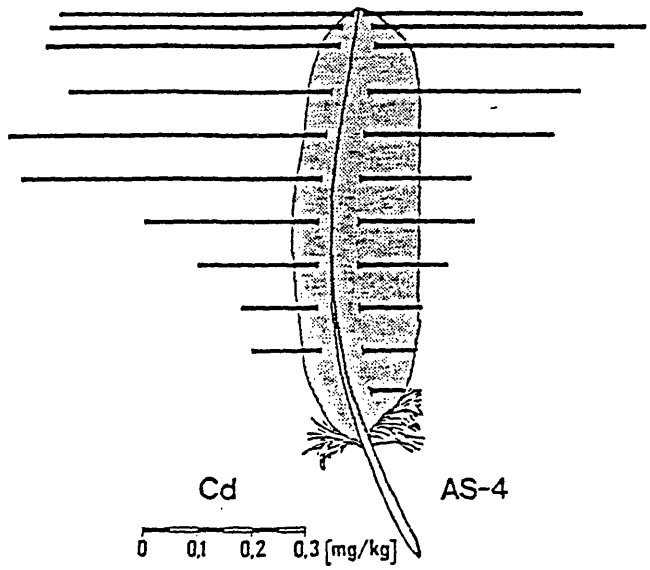


Figure 2.2 Variation in cadmium concentrations in vanes of the secondaries from a breeding female goshawk (from Hahn *et al* 1989a).

(lead, copper, zinc, cobalt, and nickel) are due to atmospheric deposition (Hahn *et al.* 1989 a,b). Weyers *et al.*(1988) demonstrate that this cannot be removed even under the most vigorous cleaning routine. All other cleaning procedures documented are much less vigorous, so must be ineffective in removing contamination.

Cadmium concentrations on feathers could be used to monitor atmospheric pollution (Hahn *et al.* 1989a). However using the concentrations of cadmium found on the feather as a monitor of air pollution must be approached cautiously. There are many sources of contamination and/or leaching of metals for feathers of seabirds are frequently exposed to sea water and spray, (see Figure 2.1). It would be difficult to standardise seabird feathers in order for any measurement of cadmium to be meaningful. Hahn *et al.* (1989a) standardised the use of goshawk feathers by analysing all the primaries, then analysing small sub-sections of each feather, and found the metal content was greater in the more exposed parts of the feather (see Figure 2.2 from Hahn *et al.* 1989a). Concentrations of cadmium found in pelagic seabird feathers are very low, (Tables 2.2 and 2.3) and probably reflect the fact that there is very little cadmium contamination of the air above the oceans, and in sea water.

Therefore I conclude that feather cadmium concentrations are of no use as an indicator of body burden or dietary intake of this metal. Feathers could only be used as a monitor of air pollution if feathers from each species used were carefully standardised by the technique of Hahn *et al.* (1989a). For pelagic seabird feathers there is no value in this type of monitoring since cadmium levels on pelagic seabird feathers are extremely low, and would be almost impossible to standardise.

2.2.2 Cadmium in eggs

Experimental work shows very little, or a complete absence of transfer of cadmium into the egg contents (Sell 1975, Leonzio and Massi 1989). Seabird data also show this, with cadmium concentrations in eggs ranging from undetectable to $< 0.7\mu g/g$ wet weight (Anderlini *et al.* 1972, Blus *et al.* 1977, Parslow *et al.* 1975, Furness and Hutton 1979, Hulse *et al.* 1980, Maegden *et al.* 1982, Reid and Hacker 1982, Becker *et al.* 1985, Honda *et al.* 1986a, Ohlendorf and Harrison 1986, Renzoni *et al.* 1986,

Scheuhammer 1987, Becker and Sperveslage 1989, Burger and Gochfeld 1991, Burger 1994b). No study has demonstrated any relation between these low levels measured and the body burden of the female. Very high levels (75.04μg/g) found in sooty tern eggs (Stoneburner and Harrison 1981b) of are difficult to explain, given that Ohlendorf and Harrison (1986) could not detect any cadmium in eggs of sooty terns also from the Hawaiian area. Presumably earlier data are erroneous.

Cadmium concentration in eggshell may be slightly higher. Concentrations of 1.39 and 1.75μg/g wet weight were measured for two laughing gull populations on the Texas coast (Reid and Hacker 1982), but very low concentrations of 0.003μg/g in Louisiana heron and 0.002 μg/g in cattle egret were reported in eggshells (Cheney *et al.* 1981), and no relationship to female body burden has been reported. Burger (1994b) measured cadmium concentrations in egg content and shell for roseate terns and herring gulls. Concentrations were very low (mean herring gull concentration = 0.01μg/g in egg, 0.05μg/g in shell, tern = 0.2μg/g in egg, 0.1μg/g in shell). The author concluded that cadmium may be excreted in this manner, but no relationship to body burden was reported. Herring gull eggshells had undetectable cadmium as measured by atomic absorption spectrophotometry (detection limits for cadmium = 0.014μg/g, own unpublished data). I conclude therefore that neither egg content nor eggshell can be used as monitors of cadmium to indicate the body burden in female birds.

2.3 Uptake of cadmium

The intestinal absorption of cadmium in rats from a given dose is around 0.4-2.0 %. Most of this is localised in the intestinal epithelium and only 0.1-0.5 % of the dose is taken up by the other tissues (Lehman and Klassen 1986, Scheuhammer 1987). The absorption of cadmium seems to be dose dependent: as the dose increases, less cadmium remains in the intestinal mucosa and more is transferred on to internal tissues. These results suggest that cadmium accumulation is diet dependent although there has been no studies on dose or diet dependency in wild birds.

There is only one study to date on cadmium uptake by captive birds. Scheuhammer (1988) orally dosed groups of Japanese quail, with a range of cadmium levels (0.01,

0.10, 1.0, 50, 500, 5000 and 50,000, μg/kg/day), for four days. The birds were killed four days after the final dose. For doses less than 20 mg/kg in total, 0.7 % of the dose was taken up by liver plus kidney plus duodenum, and in doses of 200 mg/kg, 2.0 % of the dose was absorbed into the liver plus kidney plus duodenum. The tissue distribution of cadmium was also dose dependent. At the highest dose the uptake of cadmium was greater in the liver than the kidney. At lower doses the kidney was the more important accumulation site, which is what would happen under natural conditions of cadmium intake from lower concentrations in the food. Experimental studies on the oral dosing of mammals found that the liver accumulated higher levels than the kidney, but over a period of time there was a gradual transfer of cadmium from liver to kidney (Gunn and Gould 1957, Cherian *et al.* 1978). This is now thought to reflect toxic effects on the intestinal epithelium; at higher cadmium doses the cadmium will pass directly into the blood and become associated with plasma albumen and high molecular weight proteins and be transported initially to the liver rather than the kidney (Scheuhammer 1987).

The mechanism of cadmium absorption is unclear (Lehman and Klassen 1986). However it is thought that endogenous metallothionein sequesters cadmium to saturation point. At low doses of cadmium, all the cadmium may be bound onto metallothionein, but at high doses endogenous cadmium may be completely saturated and cadmium may be absorbed as a free metal, resulting in increased absorption (Lehman and Klassen 1986, Scheuhammer 1988).

Laboratory studies on mammals and quail also show that uptake can be affected by other factors. These include injury to the intestinal epithelium by disease, by dietary content of calcium, zinc, copper, vitamin C, selenium, or iron as well as the direct toxic effect of cadmium (Scheuhammer 1987, Rambeck and Kollmer 1990). Other laboratory studies show biomagnification of cadmium in tissues of birds dosed over a period of time (Di Giulio and Scanlon 1984a, 1985, Scheuhammer 1989). There have been no studies on the uptake of cadmium by wild seabirds and it is unclear how the responses of wild birds would compare.

2.4 Storage in the body

Around 90 % of the body burden of cadmium in birds is contained in the liver and kidneys, half of this in each organ (Scheuhammer 1987). About 80 % of this cadmium is bound to a low molecular weight protein, called metallothionein, and a small amount of cadmium is bound onto higher molecular weight proteins (Osborn 1978). Cadmium tends to accumulate along with zinc as these metals are both bound by metallothionein. As a result cadmium and zinc concentrations often correlate in the livers and kidneys of birds (Osborn *et al.* 1979, Hutton 1981, Nicholson 1981, Norheim 1987, Muirhead and Furness 1988).

Metallothionein was first isolated by Margoshes and Vallee (1957) and later characterised by Kägi and Vallee (1960). Since then it has been found in many species of mammals and birds, and in a range of tissues (Roejestadi 1992). Osborn (1978) isolated a cadmium and zinc-binding protein in a free-living fulmar which had high natural concentrations of cadmium. This protein had a molecular weight of around 10,000 and was thought to be a metallothionein. Cosson (1989) measured a metallothionein-like protein in flamingos, the amount of which correlated with cadmium, copper, zinc and mercury levels in the kidney, but only with zinc and copper levels in the liver. In little egrets, only zinc concentrations correlated with levels of this metallothionein-like protein in the liver. Cosson (1989) concluded that zinc was the most important factor influencing the protein's production. Elliot *et al.*(1992) in a study of Canadian seabirds found relationships between cadmium and metallothionein concentrations in kidney, but only in Leach's petrel was there a correlation between cadmium and zinc concentrations in the kidney, showing perhaps that there are species-specific responses to heavy metal dynamics. Scheuhammer and Templeton (1990) dosed ringed turtle doves with chronic low levels of cadmium and found the responses of the liver and kidney in regulating metallothionein production to be similar. They considered the effects of cadmium, zinc and copper to be additive, and that all should be taken into account when looking at metallothionein production.

gulls showed a steady renal and hepatic cadmium increase up to three years of age, thereafter birds aged four to eleven did not show any age-related increase, and Hutton (1981) also found no evidence for lifetime accumulation in known-age herring gulls. Furness and Hutton (1979) have provided the only study I am aware of that demonstrates accumulation with age in a sample of known-age great skuas. Stock *et al.* (1989) in a study of oystercatchers and Nicholson (1981), studying herring gulls concluded from their findings that these birds had some form of regulatory mechanism for the pollutant which would mean it would not be accumulated with age, although the nature of this mechanism was not discussed. Blomqvist *et al.*(1987) found a difference between adult and sub-adult birds and calculated a half-life for cadmium in bird kidney of 1.5-2.0 years. Other authors (Schneider *et al.* 1985, Muirhead and Furness 1988, Stock *et al.* 1989) concluded that cadmium concentration in the kidney and liver is probably regulated on account of the statistical normality of distribution of levels of cadmium in the samples of birds analysed. Further studies on birds of known age are needed to determine cadmium status with age, and investigate the possibility of species specific responses.

Some studies have analysed the salt gland and the uropygial gland for heavy metal concentrations, (Howarth *et al.* 1982, Burger and Gochfeld 1985, Pilastro *et al.* 1993), and have suggested that these may act as an excretory route, but there is no evidence for this, and no relationship has been demonstrated between these glands and internal tissue concentrations.

2.8 Toxicity

The kidney is the organ that is most susceptible to the toxic effects of cadmium. With continuous exposure at low dietary levels the cadmium content of the renal cortex increases with a simultaneous increase in metallothionein concentration. When the cadmium levels reach a certain value (thought to be 100-200μg/g wet weight in humans and experimental mammals studied in the laboratory) then cadmium induced kidney damage occurs with proximal tubule cell necrosis, proteinuria, glycosuria, increased urinary cadmium, decreased cadmium in the kidney and the presence of metallothionein

in the plasma (Goyer *et al.* 1984). This type of response could also occur in birds; Nicholson *et al.*(1983) found high cadmium levels associated with patchy kidney necrosis in both free-living seabirds and metal-dosed starlings, although no damage was detected in Canadian seabirds with comparable metal burdens (Elliot *et al.* 1992). It is thought that toxic effects may become more important in free-living birds as they have the stress of bad weather, food shortages etc. and these may emphasise and enhance effects (Nicholson and Osborn 1983, Di Giulio and Scanlon 1984a). Also critical levels may be attained after gradual accumulation with time in long-lived birds. This visible damage is difficult to assess. The kidney has a large spare capacity and a large regenerative capability and the cost of damage and replacement for the apparently healthy bird is not known (Nicholson and Osborn 1983). The study by Di Giulio and Scanlon (1984a) investigated the single and combined effects of cadmium ingestion and food restriction on mallard ducks. Obviously food restriction alone resulted in weight loss and other deleterious effects but combined with cadmium ingestion, the results suggested the ability of cadmium to exacerbate food restriction and induce alterations in energy metabolism that are by themselves without apparent effect. Other experimental studies have shown cadmium-induced kidney damage (Nordberg 1972, Mayack *et al.* 1981, Nicholson and Osborn 1984) and also testicular damage (White *et al.* 1978, Cain *et al.* 1983). A dietary level of 200µg cadmium per gram of food for 60 days caused renal tubular necrosis with kidney cadmium levels reaching 130-140µg/g wet weight and testicular damage was found in 20 % of adult males (White *et al.* 1978). Studies by Richardson *et al.* (1974) showed that dietary levels of 75µg cadmium per gram of food affected testes maturation and induced several kidney lesions in four weeks post-hatching Japanese quail and 20µg cadmium per gram of food fed to mallard ducklings led to kidney lesions and anaemia by 8-12 weeks though otherwise birds behaved normally and appeared healthy (Cain *et al.* 1983). Richardson *et al.* (1974) also found significant toxic effects on a range of organ systems. Some of the effects e.g. anaemia, bone marrow hyperplasia and cardiac hypertrophy were similar to effects produced by iron deficiency, and the testicular hyperplasia was similar to that caused by zinc

value and gives a measure of the amount of variation within a sample. The cv can be used to look at the regulation of different metals within birds (Muirhead and Furness 1988). Zinc and copper, two essential metals have a low cv, usually below 25, as concentrations of these are regulated in the tissues. Mercury, cadmium and lead have much higher cv values, reflecting the wide range of concentrations found in any one sample and the fact that concentrations of these metals are not thought to be regulated in birds.

2.9.1 Variation within a species

This may be caused by several factors: age, sex, dietary specialisation, and seasonal influences.

2.9.1.1 Age

This has been discussed previously and must be taken into account when analysing a sample of birds.

2.9.1.2 Sex

In many metal studies the influence of sex on the metal levels accumulated is not tested. Some seabirds show sexual dimorphism in size and diet, which may result in differences in metal burdens. Birds can excrete metals into feathers and females may excrete pollutants into eggs, as is seen for mercury, but to a lesser extent for cadmium and other metals (Scheuhammer 1987). This may mean sex-related differences are less likely for cadmium, as it is not excreted into eggs or feathers, and will only occur in species with marked size and diet differences. Sex-related differences in metal levels were found in oystercatchers, and related to feeding ecology (Hutton 1981, Stock *et al.* 1989). Brothers and Brown (1987) studied fairy prions with a view to use them as monitors and found the influence of sex and season unimportant, thus concluding that they would be useful monitors. Cadmium will probably not accumulate differently in the sexes, unless species show marked size and dietary differences.

2.9.1.3 Dietary specialisation

Within a species there may be dietary differences which can result in different metal loads. For example, in Shetland adult great skuas feed mainly on fish, but there are

for nestlings at several different colonies varying in exposure. With this kind of information attempts at using seabirds to quantify environmental variation of metals may be more useful.

2.9.2 Variations between species

Inter-specific studies can be more useful when looking at bioavailability of metals within different food chains. Birds could be valuable indicators of ecosystem contamination as they occupy a wide range of trophic levels in different food chains and are thereby exposed to different concentrations of heavy metals in their food. Burger *et al.* (1984) found evidence for bioamplification of mercury, cadmium and lead in waterbirds from Raritan Bay, U.S.A., in species of increasing trophic status. Comparisons between species have found differences in metal levels and attributed them to diet and habitat (Hutton 1981, Gochfeld and Burger 1982, 1987a). Hutton (1981) attributed differences in metal burdens in oystercatchers, herring gull and great skua to both the diet and habitat contamination of the study species. Di Giulio and Scanlon (1984b) also found trophic level differences; carnivorous seaducks contained higher levels of cadmium than herbivorous species. Maegden *et al.* (1982) found adult royal and sandwich terns had higher cadmium levels than other species (laughing gulls, herons and egrets) considered to be feeding at the same trophic level. This was attributed to metal accumulated while over-wintering in different locations. Care must be taken when making interspecific comparisons as species have amongst other differences, different seasonal patterns of migration and comparing metal levels in one species with another is virtually impossible. However using different species from different habitats can allow general points to be made about the metals available in a particular place (Gochfeld and Burger 1982). Some studies compare two or more populations of one species, (Osborn 1979b, Howarth *et al.* 1981, Stoneburner and Harrison 1981a, Renzoni *et al.* 1986) to try and enable a more direct comparison. Obviously the choice of species must try to establish the most suitable for the situation to be monitored. Studies often merely sample a species and record metal levels at one moment in time, without reflection on the monitoring suitability. Nevertheless the data

can be used to gain an overview, and establish background levels in areas far from anthropogenic outputs which are valuable for comparative purposes. Perhaps some sort of standardised protocol could be developed in order to make use of this large data bank.

3.0 Studies in monitoring

Table 2.4. summarises and illustrates some monitoring studies which have analysed cadmium concentrations in birds

Table 2.4. Monitoring studies of cadmium in birds.

Area	Species	Tissues sampled Kidney (μg/g)	Liver (μg/g)	Reason for study	Conclusions	Reference
Corpus Christi, Texas, U.S.A.	Avocet Dunlin Greater yellowlegs Least sandpiper Lesser yellowlegs Sanderling Western sandpiper	1.82 1.94 1.47 4.31 1.33 1.38 2.74 (wwt)	N.A.	South shore of the Nueces bay has an industrial complex: zinc smelting plant, chemical plants etc. Sediments from parts of the bay are heavily contaminated with zinc and cadmium. Study to determine levels of contaminants and evaluate this in relation to population survival.	Cadmium levels very variable within species, but low. Residues of organochlorines and heavy metals generally low, below levels which can cause toxic effects. Selenium residues were high, and could cause toxic effects.	White *et al* 1980
Port Kembla harbour, Australia.	Crested tern. pre- post- (dumping)	 2.45 2.04 (wwt)	 0.77 1.04	Port receives heavy metal contamination from effluent discharge from industries and dredge spoil has been dumped. Study carried out pre and post dumping	No conclusive evidence was found of increased assimilation of heavy metals into the trophic structure of communities after dredging and dumping operations.	Howarth *et al* 1981
Denmark	Common eider	38 (dwt)	13	As part of a monitoring programme	Liver cadmium seemed high from a consumers point of view, as these birds are commonly shot for consumption.	Karlog *et al* 1983

Table 2.4 continued

Area	Species	Tissues sampled Kidney (µg/g)	Liver (µg/g)	Reason for study	Conclusions	Reference
Italy	Black-headed gull Herring gull	12.88(c) 17.28(i) 3.95-4.5(c) 3.75-8.26(i) (dwt)	3.62(c) 4.20(i) 0.85-1.91(c) 0.73-3.54(i)	Coastal feeding gulls were compared with gulls feeding at inland dump sites. coastal sites(c) inland dumps(i)	No real differences in cadmium levels were found between the sites	Leonzio *et al* 1986
Atlantic coast colonies, U.S.A.	Black-crowned night-herons	N.A. (dwt)	1. n.d.-0.64 2. n.d.-0.33 3. n.d.-0.38	1. Narragan bay, Rhode island: major centre for electroplating, receives industrial waste and sludge from large metropolitan area. 2. Newport bay, North Carolina 3. Duxbury bay, Massachusetts. Both 2 & 3 receive chronic low level effluents and sludge from small communities.	Sample sizes were very low (n= 7, 3, and 12). Metal levels low but as birds found dead this may affect the levels.	Custer and Mulhern 1983

Table 2.4 Continued

Area	Species	Tissues samples (μg/g) Kidney	Liver	Reason for study	Conclusions	Reference
S.E. Tasmania	Fairy Prion	N.A. (wwt)	4.51 (br. p) 3.10 (nbr. up.) 8.84 (br. p) 9.76 (nb. up)	Ocean dumping of jarosite from 1973- 1986 (composed of zinc, cadmium, lead, iron, copper, mercury, arsenic). br. = breeding birds nb. = non breeding birds p. = polluted area up. = unpolluted area	Fairy prions would provide a useful means of long term monitoring. Levels from polluted dumping sites were almost double those of the unpolluted site.	Brothers and Brown 1987
Shetland, UK.	Great skua	n.d.-336.0 (dwt)	n.d.-31.4	Unpolluted area, birds range widely throughout the north Atlantic.	Could potentially be an indicator of offshore pollution in north-east Atlantic. Showed large variation in metal levels and other pollutants	Furness and Hutton 1979
Gough island, South Atlantic	Rockhopper penguin Wandering albatross Atlantic petrels	> 100 in some samples (wwt)		Clean unpolluted environment, far from any source of pollution.	Very high levels far exceed those found in other birds, but probably levels are natural.	Muirhead and Furness 1986

Table 2.4 continued

Area	Species	Tissues sampled Kidney (μg/g)	Liver (μg/g)	Reason for study	Conclusion	Reference
London, UK.	Pigeon	1. 12.3 2. 1.52 3. 50.57 4. 1.75 (dwt)	2.45 0.40 9.48 0.54	To monitor pollution with distance out of London 1. Chelsea, central London. 2. Mortlake, suburban area 3. Heathrow, outer urban area 4. Cambridge, rural control	. Pigeon reflected pollution well - elevated cadmium levels at Heathrow were from wear of aeroplane tyres.	Hutton and Goodman 1980
Rhode island, U.S.A.	Common tern	N.A.	N.D.	Discharge from electroplating industry.	Zinc, nickel and copper in the diet elevated levels in tern livers, but no effects on breeding success were found.	Custer *et al* 1986
Great lakes. Canada	Herring gull	1. 1.69 2. 0.069 (wwt)	0.429 0.052	Gulls analysed as part of the Canadian wildlife service monitoring programme. 1. adults, 2. prefledgings.	Levels lower than those associated with metal toxicosis. Spatial differences appeared to relate to site exposure and differences in feeding behaviour. Levels in birds from some lakes suggested that more research into metal exposure and uptake is required.	Struger *et al* 1983

Table 2.4 continued

Chile	Kelp gull Grey gull Franklin's gull Whimbrel Sanderling Oystercatcher	N.A. (wwt)	2.8-13.7 12.41 1-2 1-89.7 1-7.6 8.4-19.9	Copper mine discharging waste.	Elevated levels thought to be due to mine discharge but could be from upwelling: requiring further investigation into the role and uptake of cadmium in the food chain in this area.	Vermeer and Castilla 1991
Sweden 1973-76	Eider duck juvenile			As part of a search for biomonitors in Sweden	The juvenile eider duck should be used as a biomonitor: renal cadmium accumulation is rapid and birds can be sampled 10 weeks after hatching, before they leave the breeding ground	Frank 1986
Svalbard and Antarctic	Fulmar Macaroni penguin	55 49 (wwt)	17 9	Clean unpolluted environment.		Norheim 1987
San Francisco and Chesapeake bay, U.S.A.	Various seabirds and wildfowl			Review paper on the work done on levels of pollutants(heavy metals, organochlorines) in birds using these bays. Polluted bays which are important for over wintering birds	Major contaminants identified as selenium, cadmium, mercury, PDB and DDE. Conclusion was that there was a considerable amount of research still needed.	Ohelendorf and Fleming 1988

wwt = wet weight. dwt = dry weight.

Chapter Three Materials and methods

3.1 Introduction

Each chapter describes the materials and methods specific to that study. There are various methods used in more than one chapter which are described here for easy reference.

3.2 Methods used in this thesis

1. Atomic absorption spectrophotometry using a Phillips PU 2000 flame spectrophotometer to measure cadmium, zinc, copper and silver concentrations.
2. A silver saturation technique to measure metallothionein content of bird tissues.
3. Cold vapour atomic absorption spectrophotometry using a Data Acquisition Ltd. DA 1500-DP6 Mercury Vapour Detector to analyse for mercury concentrations.
4. Gel filtration chromatography using Sephadex G-75 gel to separate bird kidney proteins.

3.2.1 Sample preparation and analysis

Birds were dissected fresh or after defrosting, depending on the study. All tissues (kidney, liver, muscle, stomach and contents, gizzard, duodenum) were dissected out using stainless steel blades and instruments. Wet samples were weighed to the nearest 0.001g using a Precisa top-pan balance (Metagram Instruments, Aspley Guise, Buckinghamshire). These were minced using a scalpel. Sub-samples were taken for the subsequent analyses and treated according to requirements.

3.2.2 Glassware laundering

All glassware was cleaned by soaking in Decon 90 (Decon Laboratories Ltd., Hove, West Sussex) detergent for 24 hours, followed by repeated rinsings with distilled water. Specific labelled items of glassware were used for only one particular solution or acid to reduce the possibility of contamination.

3.3 Flame atomic absorption spectrophotometry

3.3.1 Sample preparation for cadmium, zinc and copper analysis.

1. Samples were dried to constant mass, determined by repeated weighing, in an oven at 50°C. The water content was calculated and used to convert concentrations in dry weight terms to wet weight equivalents, or *vice versa*, whenever necessary.

2. Samples of 0.5-1.0g of dried tissue were weighed out accurately (to 0.001g) using a Precisa 300 MC top-pan balance, and placed in 50ml glass round-bottomed flasks.

4. Samples were digested by adding 15ml concentrated. nitric acid and placed on a hot plate (Porcelain B290 J. Bibby Science products Ltd., Stone Staffordshire) in a fume cupboard at 100°C for three hours. Then the temperature was increased to 120°C and samples boiled until digested. Samples appear clear when digested.

5. After samples had cooled completely they were diluted to 15ml using distilled water. The digestion flasks were rinsed with distilled water and the rinsings made up part of the 15ml. Samples were stored in individual universal tubes until analysis.

3.3.2 Analysis

The atomic absorption spectrophotometer

Concentrations of the metals in the tissues were measured by flame atomic absorption spectrophotometry (Phillips PU 9200 Spectrophotometer, Pye Unicam Ltd., Cambridge, Cambridgeshire). The instrument is an automated analytical machine which incorporates advanced processing facilities and operates under a central control microprocessor. An array of sensors provides input and output data to the processing system which constantly monitors the instrumental conditions and provides analytical results. The instrument burns a mixture of air and acetylene gases and uses one hollow cathode lamp and one (deuterium) background correction lamp. Sampling time for each sample was set at four seconds. Lamp current and wavelength were changed automatically depending on the chosen metal. Zinc, cadmium, copper and silver were read by the instrument at wavelengths of 213.9nm, 228.8nm, 324.8nm and 328nm, respectively. Standard solutions of the metals were prepared by diluting standard solutions for atomic absorption with distilled water, or in the case of Ag^{+} analysis, glycine buffer (BDH Chemicals Ltd., Poole, Dorset). When standards did not give a linear calibration, the instrument emitted a warning sound. Standards were then repeated or re-prepared. The following concentrations of metals were prepared for standard calibration. These levels show a linear relationship with absorbance values.

Cd = 25, 50, 100, 500, 1000µg/l

Cu = 500, 1000, 5000, 10,000μg/l

Zn = 250, 500, 1000, 2500μg/l.

Ag = 250, 500, 1000, 2000, 4000, 6000μg/l.

After calibration of the instrument using standards, several standards were repeated throughout each set of analyses. Blanks of distilled water were also run every ten samples or so to check the instrument. The concentration of metal in each tissue was calculated as follows. Absorbance values and concentrations of the metal in aspirated solution were obtained from the instrument. The concentration was multiplied by the dilution factor (15ml) and divided by the dry weight of tissue sample.

Method accuracy and detection limits

Accuracy of analysis was checked using International Atomic Energy Agency horse kidney Reference Material H-8; results are shown in Table 3.1.

3.4 Metallothionein analysis

The metallothionein concentrations in the tissues were measured by using a silver saturation technique developed by Scheuhammer and Cherian (1986, 1991). The only further modification was the use of sheep blood (Advanced Protein Products) to prepare the red blood haemolysate.

3.4.1 Sample Preparation

1. A sample of 0.5-1.0g fresh or frozen tissue was minced using a scalpel and put into a test tube with an added four volumes of 0.25M sucrose solution. This was homogenised at high speed, using an Ultraturrax (T25 Janke and Kunkel, IKA Labortechnik) for 60 seconds. For very fibrous tissues this took up to 120 seconds.
2. The homogenate was centrifuged in a refrigerated centrifuge (Europa centrifuge) at 4 °C at 20,000g for 20 minutes.
3. The resulting supernatant fluid was transferred into a universal tube, sealed and stored frozen in an ultracold freezer at -70°C (Ultrafreezer, New Brunswick Scientific). The samples could then be stored until analysis without degradation (even for months), but were routinely analysed within a couple of days.

3.4.2 Metallothionein analysis

1. 0.5ml of each sample, and two blanks of sucrose solution were placed in a test tube. Glycine buffer (0.5M pH 8.5) was added to make the sample up to 0.8ml.

2. 0.5ml of 20ml/L Ag^+ solution ($AgNO_3$ in glycine buffer) was added and the preparation incubated at room temperature for five minutes, to ensure complete binding of Ag^+ to the metallothionein.

3. 0.1ml of sheep red blood cell haemolysate was added to each sample, and shaken to mix thoroughly.

4. Samples were heated in a boiling water bath for 1.5 minutes (100°C), until the blood proteins denatured, shown by turning brown.

5. Samples were then centrifuged for 5 minutes. at 1200g using a Minifuge RF (Heraeus, Sepatech) centrifuge at room temperature.

6. Another 0.1ml haemolysate was added to each sample and the heating/centrifuging steps were repeated. This process was repeated once more.

The final volume of the supernatant sample was 1.6ml.

7. The supernatant sample was transferred into Eppendorf tubes. To remove any remaining denatured blood this was centrifuged for five minutes at 15,000g in a Biofuge A. (Heraeus, Sepatech).

8. The silver concentration of the final supernatant was measured by A.A.S., as described before.

9. The A.A.S. standard curve for silver is generated using known amounts (0.25, 0.5, 1.0, 2.0, 4.0 6.0μg/l) in glycine buffer. Each sample was measured in a sampling time of one second, due to the small quantity of sample available. In addition, in each run of samples a number (around ten) were duplicated to check the consistency of measurement.

3.4.3 Calculation of Metallothionein concentration

1mg Ag represents 3.55mg MT. This is calculated from the assumtion that 1 mole metallothionein weighs 5,500g and will bind to 14g/atoms silver, following the method of Scheuhammer and Cherian (1986).

The amount of MT in the sample is calculated with the following equation:

$$\text{mgMT/kg tissue} = \frac{(C_{Ag} - C_{BK})\,3.55\,V_T\,SDF}{S_V}$$

C_{Ag} = concentration of silver in final supernatant.

C_{BK} = background reading in the supernatant of blank.

V_T = total volume in the assay sample (usually 1.6ml).

SDF = sample dilution factor, which depends on the weight of the sample and the volume of the homogenising media.

S_V = sample volume used in the assay (usually 0.5ml).

This method gives a detection limit of around 5mg/kg in wet weight of whole tissue (Scheuhammer and Cherian 1986).

3.4.4. Method accuracy and detection limits

The method accuracy was checked using purified horse kidney metallothionein (Sigma chemicals), and the results are shown in Table 3.1.

3.4.5. Solutions for Metallothionein analysis.

1. Silver nitrate solution was prepared by adding 2ml $AgNO_3$ (1000ml/L standard) to 98ml glycine buffer. This stock solution was stored in a dark bottle covered with aluminium foil to protect it from direct light because of the photosensitivity of Ag^+. Working solutions of Ag^+ in glycine buffer were made up for each set of analyses and stored in dark bottles.

2. 0.25M sucrose solution was prepared by dissolving 42.78g sucrose in 500ml of distilled water.

3. Glycine buffer was prepared by dissolving 75.07g of sucrose in two litres distilled water. The pH was adjusted to 8.5 with NaOH, added dropwise.

4. Preparation of sheep RBC haemolysate.

Solutions were made up as follows: 1.15% KCl was prepared by dissolving 2.3g KCl in 197.7ml distilled water.

30mM Tris buffer. 0.946g Tris was dissolved in 200ml distilled water. The pH was adjusted with HCl, added dropwise.

Preparation

1. 10ml of blood was added to 20ml KCl solution (1.15% w/v).
2. This was centrifuged at 3000g for five minutes.
3. The pellet was resuspended in 30ml 1.15% KCl and centrifuged again at 3000g for five minutes.
4. The washing procedure and centrifugation steps were repeated a third time.
5. The red blood cell pellet was then lysed in 15ml Tris buffer pH8 at room temperature for ten minutes.
6. The lysate was centrifuged at 8000g for ten minutes.
7. The pellet was discarded and the supernatant (red blood cell haemolysate) divided into aliquots and stored frozen at -70°C until needed.

The haemolysate was discarded when oxidised as indicated by a dark brown colour.

3.5 Total Mercury analysis

The total mercury concentrations were analysed, the analysis of each batch of samples being spread over two days.

Day one

1. Samples of 0.050-0.250g were weighed out accurately (to 0.001g) using a Precisa 300 MC top-pan balance and placed in numbered Kjeldahl flasks. Two samples of horse kidney standard (International Atomic Energy Agency horse kidney Reference Material H-8) were also weighed out and processed in the same way. Samples were digested using a 4ml:1ml mixture of concentrated sulphuric acid and concentrated nitric acid in a water bath at 50°C for two hours. The flasks were shaken occasionally to aid sample digestion.

2. On complete digestion the flasks were placed in a refrigerator (at 4°C) to cool for 30 minutes.

3. A 5% potassium permanganate solution (25g $KMnO_4$ added to 500ml distilled water) was made up in a dark bottle using a magnetic stirrer for at least three hours. The solution was cooled in the refrigerator for 30 minutes.

4. The cooled 5% $KMnO_4$ solution was added to the cooled digested samples in 2ml aliquots. Flasks were placed back in the refrigerator between additions for around ten minutes to prevent the mixture becoming too hot and developing froth. A total of 10ml of the $KMnO_4$ solution was added to each sample to completely oxidise the tissue. Samples were left in the refrigerator overnight.

5. A reducing agent was prepared by adding 85g of tin (II) chloride to 250ml distilled water in a conical flask, to which was added 250ml concentrated hydrochloric acid. This mixture was aerated overnight to drive off any mercury impurities which may have been present.

Day two

1. The excess 5% $KMnO_4$ solution was decolourised using 30 % hydrogen peroxide solution added dropwise.

2. Each sample was made up to 36ml with distilled water.

3. Standard solutions of mercury (II) nitrate were prepared by adding 100µl mercury (II) nitrate and 100µl 5% $KMnO_4$ to a 100ml volumetric flask, then made up to volume with water. Three replicate standard solutions were made up each time. These were thoroughly mixed and poured into beakers for analysis.

Mercury analysis of the sample was done by adding 10ml of sample with 25ml distilled water to a Dreshel flask. This mixture was reduced with 10ml of the reducing agent and the free mercury produced was drawn through the drying agent (magnesium perchlorate) and into the analyser as vapour. 'Background' mercury levels in chemicals used were analysed by this method, but obviously omitting the 10ml of sample. All readings were corrected using the mean of several blank values. Calibration of the analysis was performed with replicate analyses of the standard mercury (II) nitrate

solutions. The relationship between the reading obtained and the amount of mercury in the standard solution has been shown to be linear (Muirhead 1986) and therefore only one concentration of the mercury (II) nitrate solution was analysed. Thirty samples were analysed each run, blank and standard readings were checked during the course of sample analysis. The concentration of mercury in the samples was calculated as follows:

$$\text{mg/kg Hg} = \frac{0.36 \times (\text{reading-blank})}{\text{mean standard} \times \text{mass digested}}$$

(0.36 = dilution of samples).

All chemicals used were 'Spectrosol', 'Analar' or 'Puranal' analytical grades throughout. The mercury vapour detector was allowed to equilibrate to its working temperature for at least two hours before each set of analyses. All methods follow those described in Furness *et al.* (1986)

3.5.1 Method accuracy and detection limits

Method accuracy was checked using International Atomic Energy Agency horse kidney Reference Material H-8, results are shown in Table 3.1.

3.6 Gel filtration

A gel filtration column was prepared using Sephadex G-75, and Tris buffer (10mM pH 7.4). This was calibrated using several proteins of known molecular weight. Figure 3.1 shows the calibration curve obtained. Lysozyme (14,6k) characteristically comes through the column slower than lower molecular weight proteins, such as cytochrome (12.4k).

3.6.1 Sample preparation

1. Tissues, after dissection (see above) were placed in five volumes (w/v) of 0.25M sucrose containing 10mM Tris buffer pH 7.4 and homogenised, in an Ultra turrax. T25, for up to 120 seconds to ensure complete homogenisation.

2. The supernatant fraction was obtained by centrifugation at 20,000g for 30 minutes in an Europa centrifuge.

3. The supernatant was decanted and stored at -60°C until analysis.

3.6.2 Gel filtration

4ml of the prepared sample was thawed and then loaded onto an 86×1.6cm column of Sephadex G-75 gel (Sigma Chemicals). Fractions of 5ml were collected at a flow rate of 2ml/min, with 10mM Tris buffer pH 7.4 containing 3.1mM sodium azide, as an antibacterial agent.

The fractions were measured for the absorption at 250nM and 280nM using a Philips PU8700 UV/Visible spectrophotometer. On addition of 1ml concentrated hydrochloric acid fractions were measured for cadmium, zinc and copper on the A.A.S, as before. The sampling time for each sample set on the .A.A S. was reduced to one second, due to the small volume of sample available.

3.6.3 Solutions for gel filtration

1. Homogenising sucrose solution (0.25M sucrose with 10mM Tris) was made up by adding 85.57g sucrose (Sigma Chemicals) and 1.57g Tris (Sigma Chemicals) to one litre of distilled water and stirring with the magnetic stirrer, until fully dissolved. This was stored at 4°C until used.

2. Tris buffer solution (10mM Tris with 3.1mM sodium azide) was made up using 7.88g Tris and 1.00g sodium azide, dissolved in five litres of distilled water, using a magnetic stirrer. This was made pH 7.4 with HCl. All solutions were made up freshly for each set of analyses.

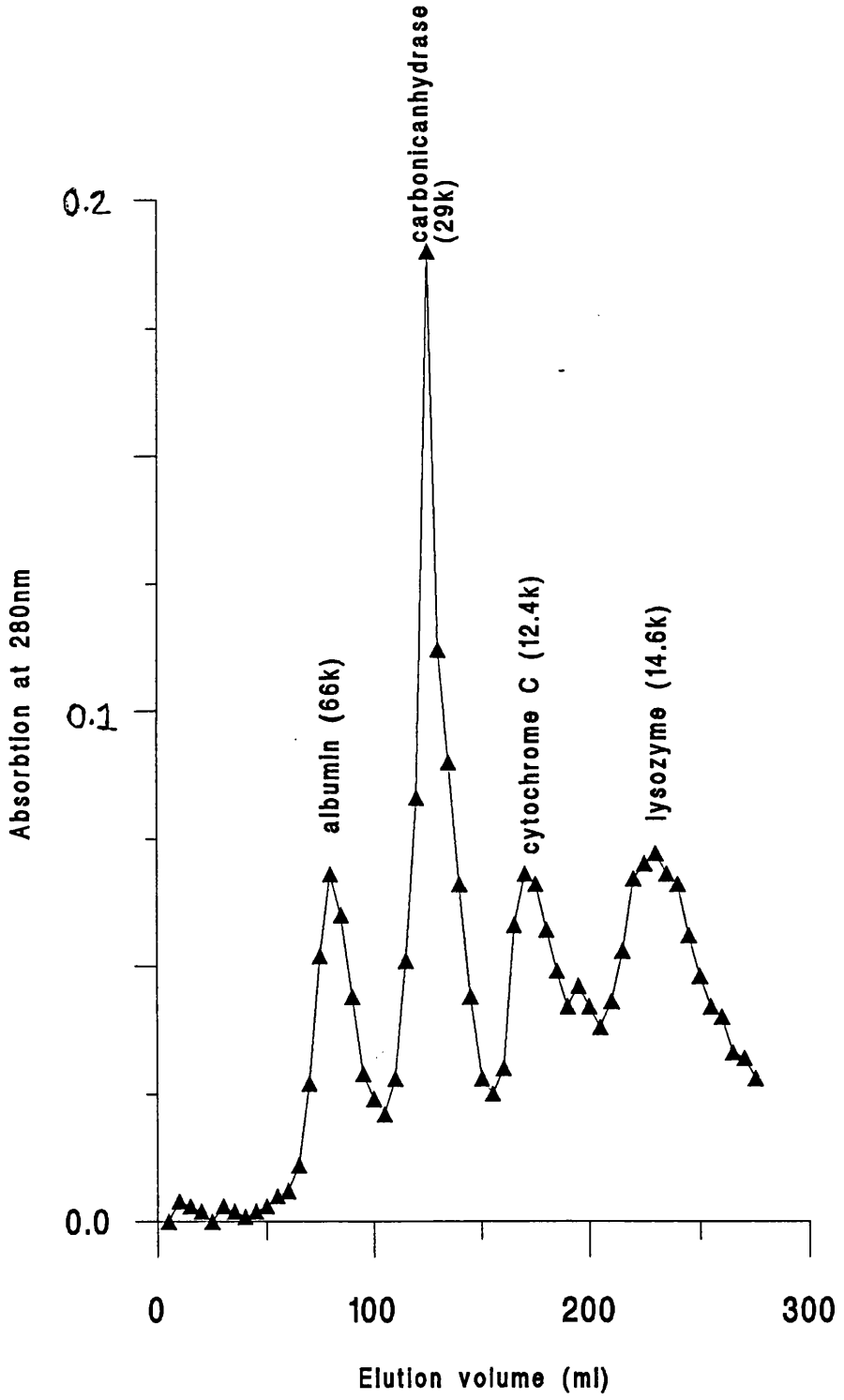
Gel filtration follows the procedure set out in Osborn (1978), with modifications from Scheuhammer (1989).

Table 3.1 Concentrations of metal in International Atomic Energy Agency horse kidney Reference Material H-8, and concentrations of metallothionein in purified horse kidney metallothionein (Sigma Chemicals). Reference concentrations are given, with the 95% confidence interval in concentration units below. Concentrations are given as μg/g dry weight. Measured concentrations are the levels I have measured by the methods and instruments used in this thesis, with standard deviation and range below.

	Mercury	Cadmium	Zinc	Copper	Metallothionein*
Reference concentration	0.91 0.83-0.98	189 184-193	193 187-199	31.3 29.5-33.0	6.00 (actual value of diluted sample)
Measured concentration n = 10	0.94 0.02 0.97-0.92	187.27 7.1 182-192	192.08 11.85 184.4-209.7	30.44 1.95 29.1-33.0	5.19 0.05 5.1-5.2

* Metallothionein- the measured value is slightly lower than the actual value. This could be due to two possibilities. 1. There is some controversy as to whether silver in this assay binds to copper-bound metallothionein, and if it does not, this could account for the reduction in the value measured. 2. The molecular weight of metallothionein in the assay method was taken as 5,500g, and not 6000-7000g, thus making the value 1mg Ag representing 3.55mg metallothionein slightly low. However this value given by Scheuhammer and Cherian (1986) was used throughout the thesis.

Figure 3.1 Gel filtration calibration curve



Chapter Four Aspects of metal dynamics in lesser black-backed gull *Larus fuscus,* and great skua *Catharacta skua,* with reference to the use of seabird tissues as monitors of heavy metals.

4.1 Introduction

Seabirds have been used extensively as monitors of heavy metals, and there is a large amount of published material on metal concentrations in seabirds (see Chapter 2, and Thompson (1990) for a comprehensive review). However, in order to use them to monitor trends in an effective way, the physiology and dynamics of metals in seabirds must also be understood.

One aspect of cadmium accumulation in birds which remains poorly understood is the effects of age. Cadmium has a tendency to increase with age in mammals, including humans. Friberg *et al.* (1974) calculated the biological half-life of cadmium in the human kidney to be twenty years or more, and marine fish have also shown an increase in kidney cadmium concentrations with increasing age and size (Scheuhammer 1987). It is generally thought that the kidney is the main storage organ for cadmium, accumulating throughout the whole life-span (Nordberg 1974). The effect of increasing age on cadmium concentrations in birds is less clear. Birds are almost impossible to age. Seabirds can usually be aged only as chicks, juveniles and adults. Juveniles can show immature plumage which distinguishes them from fully adult birds. However unless birds are ringed as chicks, they cannot be aged once they are fully mature. As populations of known-age birds are very rare and valuable for other ecological studies, few have been available for metal studies, especially as this involves killing the birds. An increase in cadmium concentrations in kidney tissues between chicks, juveniles and adults is often found (Cheney *et al.* 1981, Nicholson 1981, Maegden *et al* 1982, Reid and Hacker 1982, Karlog *et al.* 1983, Blomqvist *et al.* 1987) Very few studies have been able to use known-age birds to measure internal organ metal concentrations. Furness and Hutton (1979) found increasing levels of cadmium with increasing age in a small sample of great skuas, but no age accumulation was found in two studies of known age herring gulls (Hutton 1981, Nicholson 1981).

Feathers have been used successfully to monitor mercury levels in birds. Mercury is excreted into the feathers during moult, where it is bound into a stable complex (Applequist *et al.* 1984). The levels of mercury found in the feather reflect the levels of

mercury in the internal tissues (Thompson *et al.* 1991). Other metals however, will behave differently. Cadmium has also been measured in bird feathers (e.g. Osborn *et al.* 1979, Stoneburner *et al.* 1980, Howarth *et al.* 1981, Cheng *et al.* 1984, Honda *et al.* 1986a, see Chapter 2), but concentrations found are very low. Few studies have looked into the relationship between feathers and internal tissues, and there is no study which demonstrates a clear relationship. Hahn *et al.* (1989a, b) concluded that cadmium levels in feathers were a result of external contamination onto the surface of the feathers. However, the gross contamination from the atmospheric pollution they studied could have masked the cadmium bound into the feather matrix, and any relationship it may have with the internal tissues.

This chapter presents data on the lesser black-backed gull, to investigate cadmium relationships between plumage and internal organs, relationships with essential metals, and age-related trends in birds grouped into age categories by plumage. It also presents further data on cadmium in 25 known-age skuas which is added to and compared with data from Furness and Hutton (1979).

4.2 Materials and methods

Lesser black-backed gulls were obtained from a colony nesting on moor land near Abbeystead, Lancashire which were culled by the landowners, under licence from the Nature Conservancy Council on 24 May 1991. Gulls were culled by feeding poisoned baits. This colony has shown recent rapid expansion; the main food source for these gulls is from local rubbish tips and from fisheries waste. The great skuas were shot under licence by the local inhabitants on Foula, Shetland, in 1988, and the tissues stored dried. Any tissues containing lead shot were not used due to the possibility of contamination. The ages of the skuas were determined from individual leg bands the birds had been ringed with as chicks.

All lesser black-backed gulls were frozen at -20°C as soon as possible and stored frozen, until analysis. The gulls were defrosted overnight, then weighed, aged by plumage, and samples of feather, whole kidney, whole liver and one whole pectoral

muscle (pectoralis major plus pectoralis minor) were dissected out. These were then placed in an oven at 50°C and dried to constant mass. Analyses for cadmium, zinc and copper were carried out as described in Chapter 3. For the analysis of feathers 1g-1.5g of feathers were weighed out and samples only diluted to 5ml. This was to create a concentrated sample for analysis of cadmium. The detection limit for cadmium in the digested sample was 0.014μg/g. The feathers were analysed for cadmium only.

The lesser black-backed gulls were assigned to one of two age categories, adult or immature, on the basis of plumage. Birds with brown primaries, secondaries or tail feathers were classed as immature. Birds with no brown mottling on these areas were classed as adult. Of 91 birds, 58 were classed as adults, 18 as immature and 15 birds were not classified into an age group. The great skuas were aged by their individual leg rings.

4.2.1 Statistical analysis

Preliminary tests were performed of the goodness of fit to Normal distribution (Kolmogorov-Smirnov one sample tests). Where fit was good, further analyses were made using parametric statistics. Where data were significantly different from normal (defined as $p < 0.05$), nonparametric tests were used. All statistical analyses were performed using the SPSS-PC+ package (Norusis 1986, 1988).

4.3 Results

The levels of metals measured in tissues of lesser black-backed gulls and great skuas are given in Table 4.1. Cadmium was not reliably detected in any of the 91 feather or muscle samples in the lesser black-backed gulls. Great skua did contain detectable levels of cadmium, mean value = 1.00μg/g.

Great skua cadmium levels in the kidney were higher than the gulls (skua mean = 40.90μg/g, gull mean = 25.95μg/g), and skua concentrations showed a wider range of values (see Table 4.1). In the liver tissue of the two species the mean concentrations were similar, with lesser black-backed gulls having slightly higher concentrations (gulls mean = 5.59μg/g, skuas mean = 5.30μg/g).

Table 4.1. Metal levels in tissues of lesser black backed gulls *Larus fuscus* and great skua *Catharacta skua.* Mean values are given, with standard deviation in parentheses, and range underneath.

		Cd in kidney	Cd in liver	Cd in muscle	Zn in kidney	Zn in liver	Zn in muscle	Cu in kidney	Cu in liver	Cu in muscle
Lesser black-backed gulls	Adults n = 58	24.48 (15.06) 4.87-89.26	5.60 (3.01) 12.12-18.32	N.D.	88.08 (17.19) 38.35-148.55	79.37 (28.26) 50.86-224.64	56.84 (16.89) 6.04-126.87	12.54 (3.20) 5.58-18.88	20.00 (5.97) 8.54-42.40	19.76 (3.84) 14.37-44.00
	Juveniles n = 18	18.92 (10.76) 4.01-48.31	4.47 (2.93) 0.22-11.69	N.D.	83.26 (11.87) 61.74-100.67	73.74 (14.94) 52.08-118.22	67.81 (23.84) 39.97-120.94	12.15 (3.05) 5.00-17.62	15.99 (4.50) 6.99-23.66	22.69 (10.79) 15.91-63.16
	All birds n = 91	25.95 (14.39) 4.01-89.26	5.59 (3.01) 0.22-18.32	N.D.	87.99 (17.29) 38.35-148.55	79.37 (28.26) 50.86-224.64	59.22 (18.53) 6.04-126.87	12.05 (3.35) 1.72-18.88	20.20 (7.47) 6.99-52.63	20.32 (5.80) 14.37-63.16
Great skuas	Adults n = 27	40.90 (35.05) 6.80-129.80	5.30 (3.90) 0.50-17.50	1.00 (1.00) N.D.-4.10	173.70 (17.70) 91.00-216.84	102.70 (48.30) 69.90-317.40	69.70 (26.70) 44.82-159.70	20.10 (5.40) 11.90-38.30	18.70 (4.50)14.30-30.00	18.00 (2.07) 13.90-24.40

Zinc concentrations in the great skua liver and kidney were much higher than the lesser black-backed gulls (skua mean value, liver = 102.70μg/g, kidney = 173.70μg/g, gull mean values, liver = 79.37μg/g, kidney = 87.99μg/g), but zinc concentrations in muscle tissues were similar (skua mean = 69.70μg/g, gull mean value = 59.22μg/g). Zinc concentrations in both the gull samples and the skua samples showed a wide range of values, with gull concentrations ranging from 50.86 - 224.64μg/g, and skua concentrations ranging from 69.90 - 317.40μg/g

Copper concentrations in the kidney of the lesser black-backed gulls were lower than great skua kidney concentrations (mean value in gull = 12.05, mean value in skuas = 20.00μg/g). In the liver tissue levels were higher and gull copper concentrations were similar to great skua concentrations (gull mean value = 20.20, skua mean value = 18.70). Copper concentrations in muscle tissues throughout all samples analysed were very similar (see Table 4.1).

4.3.1 Age effects

In the gulls, the group classed as adults had significantly higher cadmium concentrations in their kidney than the immatures (t-test, t = 2.35, n = 76, D.F. = 69, P <0.05), but not in the liver (t = 1.21, n = 76, D.F. = 69, N.S.). Figure 4.1. shows the relationship between cadmium in the liver and kidney in the adults and immatures. There was a significant relationship between cadmium in the kidney and liver in both adult and immature birds; birds with higher levels of cadmium in kidney had higher levels of cadmium in liver. However, the two regression lines did not differ significantly in slope, (Manova test, $F_{1,69} = 1.25$, n = 76, N.S.), or elevation (Manova test, $F_{1,69} = 0.78$, n = 76, N.S.). Therefore cadmium was accumulated by adults and immatures in the same way. No other age differences were found in the sample. Zinc concentrations in both adult and juvenile gulls were similar in liver and kidney, but adult concentrations in muscle tissue were lower in adults,(adult mean value = 56.84μg/g, juvenile mean value = 67.81μg/g). Copper values in the kidney of the adult and juvenile gulls were very similar (adult mean value = 12.54μg/g, juvenile mean value

= 12.15μg/g) In liver tissue levels were higher than kidney and mean concentrations measured in adult gulls were slightly higher than juveniles (adults mean = 20.00μg/g, juveniles mean = 15.99μg/g),

In great skuas the relationship between cadmium in the kidney and age is illustrated in Figure 4.2. There was no significant relationship when current data are considered: $r = 0.11$, $n = 27$, N.S., or when combined with data from Furness and Hutton (1979), $r = 0.16$, $n = 40$, N.S., despite the highly significant results of Furness and Hutton data when considered alone ($r = 0.71$, $n = 13$, $P < 0.01$).

4.3.2 Inter-organ and inter-metal relationships

Inter-organ and inter-metal correlations are shown in Table 4.2.

In the lesser black-backed gulls, the essential metals copper and zinc concentrations correlate between the liver and kidney tissue. Within each organ copper and zinc concentrations correlate together in the kidney but not in the liver.

Cadmium concentrations also correlate between the liver and kidney tissues. Cadmium concentrations have a strong relationship in the kidney with zinc concentrations, but has no significant relationship with zinc concentrations in the liver tissue. Cadmium concentrations in liver tissue however, correlates with copper concentrations in both the liver and kidney tissues.

In the great skua the essential metal zinc correlates between liver and kidney, but copper concentrations shows no relationship between the two organs. Zinc and copper concentrations in the liver correlate well, but there is no relationship in the kideny.

Cadmium concentrations in the kidney and liver correlate well, and liver and muscle cadmium concentrations correlate, but there is no relationship between kidney and muscle cadmium concentrations.

Cadmium concentrations in kidney tissue correlate with the zinc concentrations and the copper concentrations but cadmium in liver correlate only with zinc, and not copper concentrations.

In general the concentrations of metals measured in the muscle show little relationship with the concentrations of metals in the liver and kidney, although zinc concentrations

Table 4.2 Correlation matrix of inter-metal and inter-organ relationships in lesser black-backed gull and great skua. (l) = liver, (k) = kidney, (m) = muscle.

a. Lesser black-backed gull (n = 91)

	Cd (k)					
Cd (l)	0.72 ***	Cd (l)				
Zn (l)	0.133 N.S.	0.08 N.S.	Zn (l)			
Zn (k)	0.50 ***	0.32 **	0.28 **	Zn (k)		
Cu (k)	-0.07 N.S.	-0.26 *	0.17 N.S.	0.29 ** -	Cu (k)	
Cu (l)	-0.07 N.S.	0.22 *	0.19 N.S.	-0.02 N.S.	-0.23 *	Cu (l)
Zn (m)	-0.21 *	-0.12 N.S.	-0.04 N.S.	-0.18 N.S.	0.09 N.S.	-0.05 N.S.
Cu (m)	0.08 N.S.	0.04 N.S.	-0.03 N.S.	-0.20 N.S.	0.05 N.S.	-0.10 N.S.

b. Great skua (n = 27)

	Cd (m)						
Cd (K)	-0.00 N.S.	Cd (k)					
Cd (l)	0.48 *	0.73 ***	Cd (l)				
Zn (l)	0.68 ***	0.36 N.S.	0.65 ***	Zn (l)			
Zn(k)	0.26 N.S.	0.84 ***	0.47 *	0.50 *	Zn (k)		
Cu (k)	-0.07 N.S.	0.74 ***	0.28 N.S.	0.28 N.S.	0.78 ***	Cu (k)	
Cu (l)	0.21 N.S.	0.16 N.S.	0.16 N.S.	0.00 N.S.	0.00 N.S.	0.17 N.S.	Cu (l)
Zn (m)	0.25 N.S.	-0.06 N.S.	0.54 **	0.54 **	0.11 N.S.	0.00 N.S.	0.17 N.S.
Cu (m)	037 N.S.	-0.26 N.S.	0.17 N.S.	0.17 N.S.	-0.00 N.S.	0.04 N.S.	-0.04 N.S.

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Figure 4.1. The accumulation of cadmium in adult and immature lesser black-backed gulls

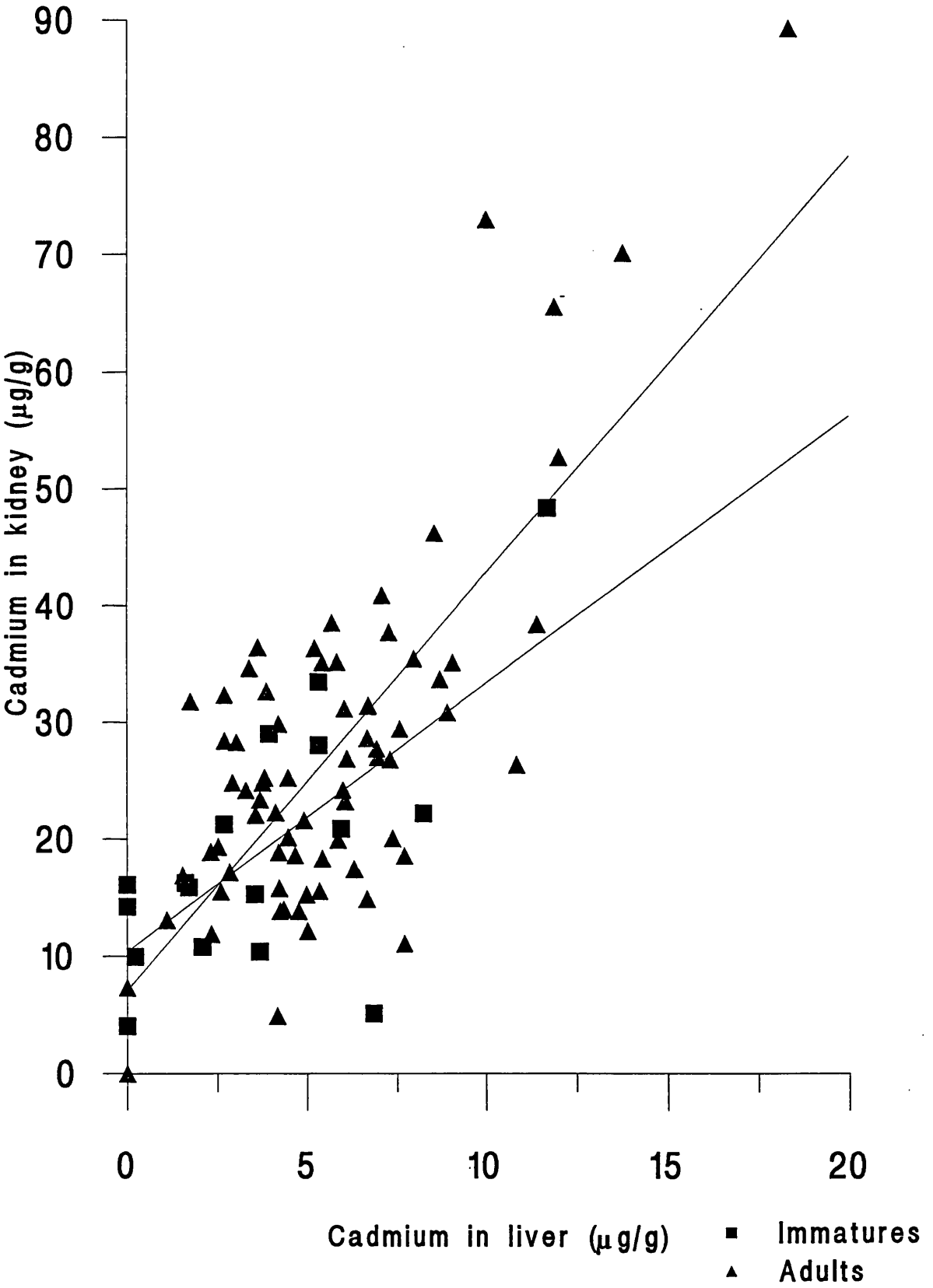
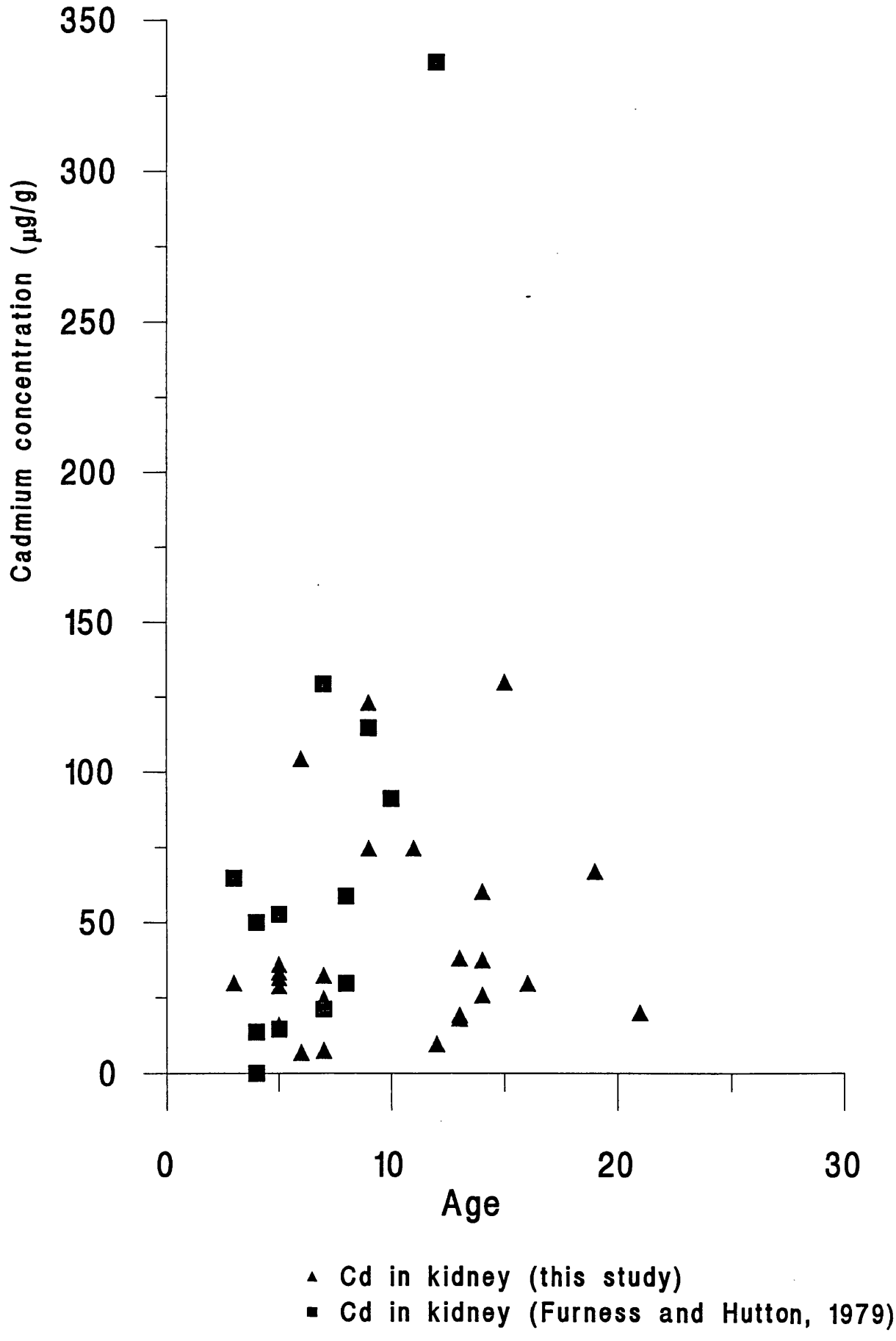


Figure 4.2 Age and cadmium concentrations in great skua



in the muscle of gulls show a weak correlation with cadmium in the kidney, and zinc in the muscle of great skua correlates with zinc and cadmium concentrations in the liver.

In gulls over the whole sample, cadmium and zinc show a strong relationship ($r = 0.56$, $n = 91$ $P < 0.001$, Figure 4.3). If they are separated into adults and immatures, adults show a significant correlation, ($r = 0.59$, $n = 58$, $P < 0.001$), but immatures do not ($r = 0.28$, $n = 18$, N.S.). Data were combined (adult, immature, and non-aged birds), to look at the relationships between metals and between tissues, and investigate the possibility of a threshold level. This is illustrated in Figures 4.4 and 4.5. Values of cadmium below 25 μg/g, do not correlate with zinc in the birds' kidney ($r = 0.22$, $n = 50$, N.S.), but levels above 25 μg/g do correlate ($r = 0.43$, $n = 35$, $P < 0.05$), although not so strongly as the relationship over the whole sample. This was not tested using the skua data set, due to the small sample sizes.

Figure 4.3 The relationship between cadmium and zinc concentration in Lesser black-backed gulls

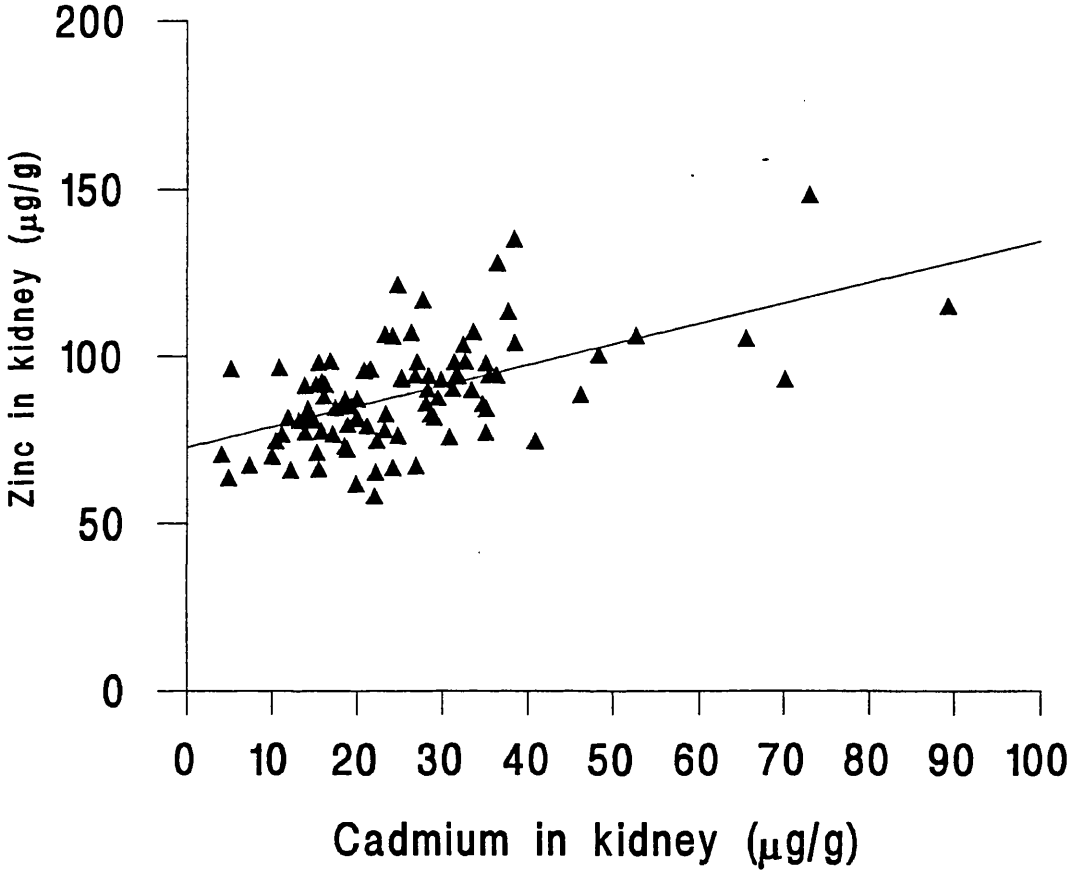


Figure 4.4 The relationship between cadmium and zinc in the kidney of Lesser black-backed gulls, when cadmium concentrations < 25 μg/g

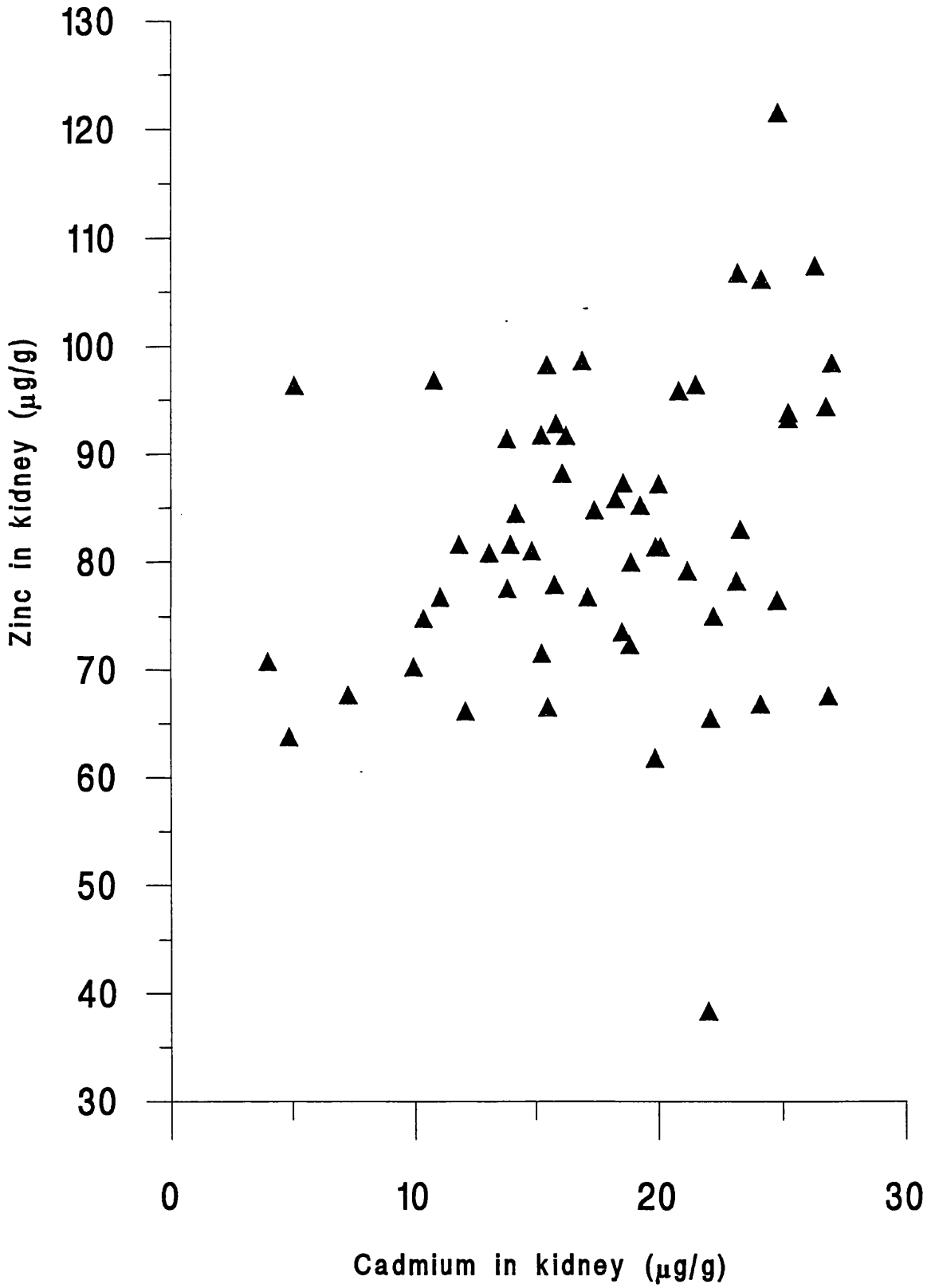
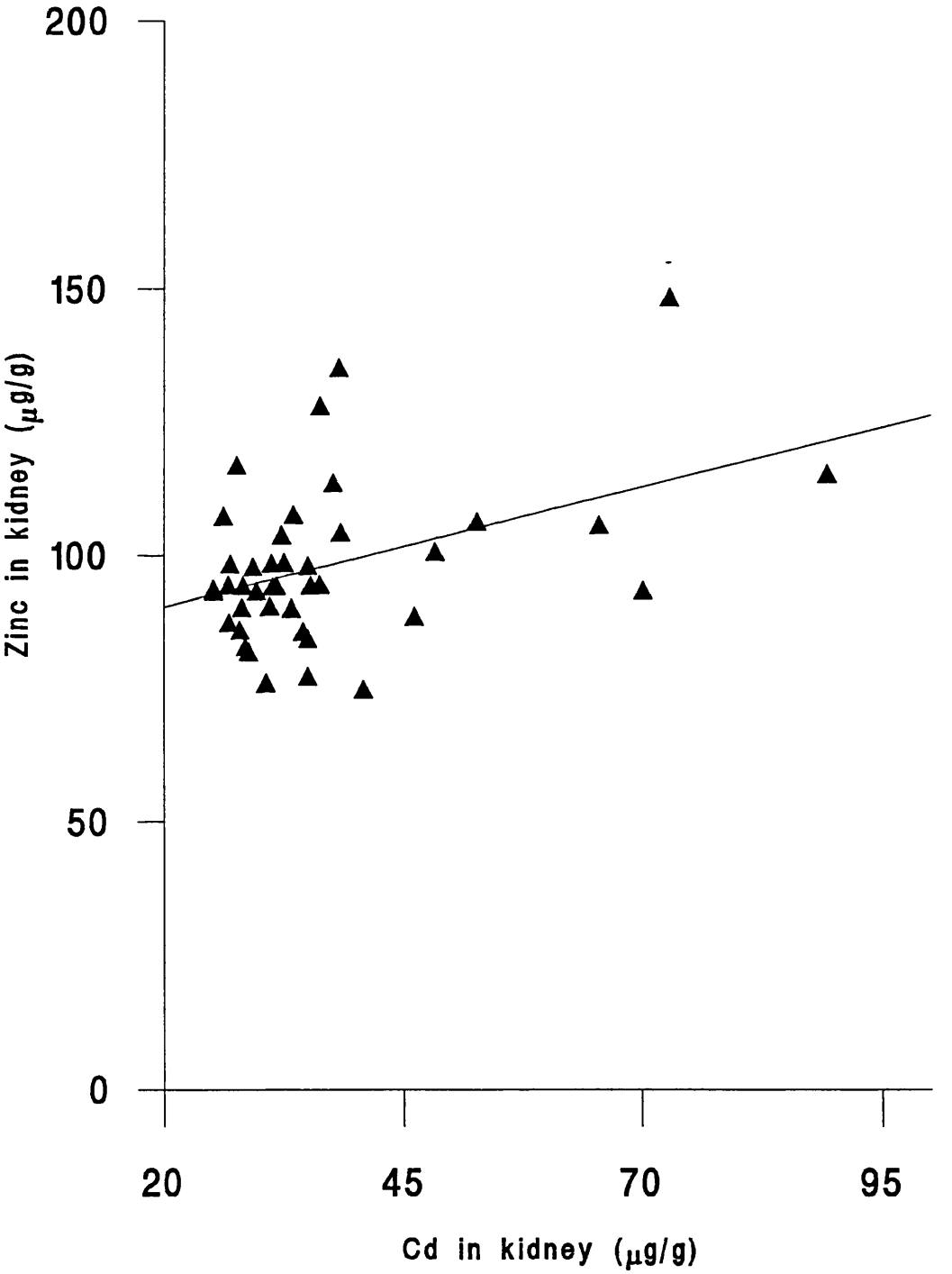


Figure 4.5 The relationship between cadmium and zinc in the kidney of Lesser black-backed gulls, when cadmium concentration >25μg/g



4.4 Discussion

The levels of cadmium measured in the great skua tissues compare well with those reported by Muirhead (1986), but are lower than reported by Furness and Hutton (1979). Furness and Hutton (1979) found a mean value of 81.33μg/g in kidney, with a range of levels from below the detection limit up to 336.0μg/g. However, in all three studies there is a wide and overlapping range of cadmium concentrations in both liver and kidney tissue. Concentrations of cadmium in the gulls are lower than in the skuas, probably due to their differences in diet and ecology. Cadmium concentrations in these lesser black-backed gulls are however higher than those measured in the herring gull. Herring gulls from the Isle of May, Scotland had mean kidney cadmium values of 13.0μg/g and 13.7μg/g respectively (Hutton 1981 Nicholson 1981,) and liver values of 2.01μg/g and < 1.86μg/g, respectively. Herring gulls sampled in northern and central Italy had mean concentrations of 3.75-8.26μg/g in kidney tissue, depending on location (Leonzio *et al.* 1986). Laughing gulls (Hulse *et al.* 1980) had lower concentrations of 4.3μg/g in kidney and 0.46μg/g in liver. Southern black-backed gulls, had comparable liver and kidney cadmium concentrations (mean kidney cadmium value = 20.8μg/g, liver = 2.8μg/g) and red-billed gulls had higher values (42.4μg/g in kidney, and 6.8μg/g in liver) (Lock *et al.* 1992). Glaucous gulls had much higher mean levels of 92.0μg/g (range 16-232μg/g) (Schneider *et al.* 1985).

Gulls in general show a wide range of levels, both inter- and intra-specifically. Ranges from 0-100μg/g (dry weight) were reported in a review, (Walsh 1990). The large differences in levels between the herring gulls and the lesser black-backed gulls is quite surprising, but could be attributed to dietary differences. The Isle of May herring gulls may well have a more marine diet, and therefore a lower cadmium intake than the lesser black-backed gulls. The lesser black-backed gulls in this study are known to scavenge on human refuse tips, which may have cadmium containing batteries and other toxic refuse, but it is also likely that gulls feeding in the Irish Sea are exposed to higher environmental levels of cadmium than are birds in the east of Scotland.

Zinc and copper are essential metals and as such their tissue concentrations are thought to be closely regulated, reflected by low inter and intra-specific variation. However within both the skua and gull data, there is still a range of concentrations recorded in the zinc concentrations, and this has also been found in the literature. Copper concentrations show less variation; concentrations of 29.2μg/g in liver and 20.8μg/g in kidney were measured in glaucous gull and in the Brown skua 18.4μg/g in liver and 18.0μg/g in kidney were reported (Norheim 1987). Herring gulls from the Great lakes in North America had 19.56μg/g in liver and 16.00μg/g in kidney, all of which are similar to those measured in this study. A review by Thompson (1990) presents data on copper concentrations of many seabird species. Mean copper concentrations in liver tissue tend to be around 24μg/g (dry weight), with few values greater than 40μg/g. Kidney concentrations are generally lower, and muscle concentrations lower still. This low variation which is aparent both inter-specifically and geographically is most likely to reflect the close metabolic regulation of the metal (Thompson 1990). The reason for analysing copper concentrations here are to elucidate any relationship concentrations may have with other metals, especially cadmium. Zinc concentrations show a greater amount of variation. Herring gulls from the Isle of May had a mean zinc value in kidney of 97.6μg/g, with values ranging from 78.7-148.2μg/g (Hutton 1981), and Nicholson (1981) recorded mean zinc concentrations in herring gulls of 93.0μg/g, with a range of 75.5-126.0μg/g, also from the Isle of May. Birds had mean concentrations of 91.6μg/g, ranging from 55.8-135.2μg/g (Hutton 1981), and mean concentration of 66.6μg/g, ranging from 46.8-77.9μg/g (Nicholson 1981). Sampling birds at one point in time may result in a range of values. Birds' nutrient intakes and requirements will fluctuate on a seasonal basis, and will be reflected by changes in kidney and liver concentrations of essential metals. The large range in zinc concentrations in the liver of the gulls may be due to collection date. These birds were collected in the breeding season, during which time zinc requirements are high, and both male and female birds undergo large physiological changes (Lofts and Murton 1973). This will also affect the relationship between cadmium and zinc in this organ.

The inter-tissue and inter-metal correlations found in this study show an interesting pattern of relationships. As copper, zinc and cadmium are all bound onto metallothionein (Scheuhammer 1987), and as these metals are distributed throughout the liver, kidney and muscle tissues they may be expected to show several inter-relationships (Scheuhammer 1987, Walsh 1990). However, as previously discussed, the birds physiological requirements will fluctuate, and this may create different relationships between the metals within them. In particular, for example cadmium in the lesser black-backed gull shows no correlation with zinc concentrations in the liver, although this may be expected (Walsh 1990). This is most likely because these birds are breeding, and the large amount of zinc stored in the liver (see above, Lofts and Murton 1973), could in effect, overwhelm any zinc-cadmium relationships. Alternatively there could be some kind of threshold effect. Cadmium, zinc and copper in the liver and kidney is bound to metallothionein. Metallothionein production can be experimentally induced by dosing with heavy metals, and increased levels of metallothionein result in increasing the number of binding sites available to zinc, thus accounting for the parallel accumulation of cadmium and zinc (Scheuhammer 1987). In this present study, concentrations of less than 25μg/g cadmium do not correlate significantly with the zinc levels, and concentrations above 25μg/g do correlate significantly. It is possible that there is some threshold value, above which sufficient metallothionein is synthesised to produce the parallel accumulation of zinc and cadmium, and below which, there is no parallel accumulation. If there were a threshold level, this could also, in part, account for the different inter-relationships between metals seen in lesser black-backed gulls and skuas,whereby relationships will change if metal levels are above or below a given threshold value. Bremner and Davies (1975) found concentrations of 30μg/g zinc in the kidney of rats induced metallothionein synthesis, but concentrations below this did not, and Jeffery *et al.* (1989) reported a threshold concentration of 40μg/g zinc in horses, above which metallothionein was accumulated in parallel with zinc. However there is no real evidence in this study for a threshold level, especially as the relationship between cadmium and zinc in the kidney is strongest over the whole range of values.

Further chapters in this thesis focus on particular aspects of metal dynamics and will not present every metal interaction.

Adults had significantly higher concentrations of cadmium in the kidney tissue, and higher concentrations in the liver, although not significantly, than immature birds. This agrees with many studies in which there is strong evidence for accumulation of cadmium in the first few months or years after hatching (Cheney *et al.* 1981, Nicholson 1981, Maegden *et al.* 1982, Reid and Hacker 1982, Karlog *et al.* 1983, Blomqvist *et al.* 1987). Stock *et al.* (1989) proposed that adult oystercatchers accumulated cadmium in a different way from the immatures from the relationship between cadmium in kidney and liver. In gulls, although immatures have lower levels, their pattern of accumulation is the same. The immature birds in this study were attending or breeding at the colony, and would be at least 3-4 years old, which may account for the overlap in concentrations and the similar accumulation patterns. A sample of younger birds would be needed to test this hypothesis further. Alternatively this could merely reflect a physiological difference between the two species in their accumulation of cadmium. Unfortunately as the two samples will be a mixture of different ages of birds, they are not directly comparable.

Evidence for continued accumulation throughout the birds' life-span is scarce. Furness and Hutton (1979) found significant positive correlations of cadmium with age in kidney, liver and pectoral muscle in 13 known-age skuas. When current data are combined (see Figure 4.2) this relationship becomes non-significant, the cadmium in skuas does not seem to increase with increasing age. It is the very high value of 336μg/g in the oldest bird which actually creates the significant relationship in the previous study (Furness and Hutton 1979). In addition, the analysis by Muirhead (1986) of 27 known-age skuas found only a weak relationship between cadmium in the muscle and age and none in other tissues. In conclusion there is no evidence of continuing accumulation with increasing age. However the wide range of concentrations found in great skuas of all ages could be due to dietary differences. Great skuas feed on a wide range of prey species and some individuals within a colony specialise on one particular

prey type (Furness 1979). Dietary specialisation of individuals who consistently feed on different prey types with a range of several trophic levels will have associated differences in cadmium concentrations. This may reduce effects of age on cadmium accumulation in internal tissues. A skua which specialises in eating seabirds will have much higher cadmium concentrations in internal organs than one which feeds on whitefish discards. For example, skuas will prey on puffins, which have been reported to have cadmium concentrations of 114μg/g in kidney and 5.02μg/g in liver tissues (Osborn *et al.* 1979). Skuas who show the same dietary preferences may show accumulation with age, but detailed dietary data for individuals are not available. The great skua is not the best species to look for cadmium accumulation with age in. Ideally a population of birds ringed as chicks which feed uniformly on the same prey species all year round would be required to solve this problem. The only other studies to look at cadmium accumulation in known-age birds found no evidence of increasing cadmium concentrations with increasing age. Hutton (1981) and Nicholson (1981) sampled herring gulls of known age and found no increase in cadmium concentrations with increasing age in any tissue sampled. Nicholson (1981) showed a steady renal and hepatic increase up to three years of age, thereafter birds age four to eleven did not show any age-related increase.

The theory that seabirds could have evolved some mechanism of regulation of cadmium has been proposed by several authors (Stock *et al.* 1989, Schneider *et al.* 1985, Muirhead and Furness 1986). This contrasts with mammals, where research shows that the kidney accumulates cadmium for the whole life-span (Nordberg 1974) and has a tendency to accumulate with age, even at quite low exposure levels (Scheuhammer 1987). To date, there is no conclusive evidence for the continued accumulation of cadmium throughout the adult bird's life. Seabirds have been exposed to natural levels of cadmium throughout their evolution and they may have developed a mechanism to cope with and/or regulate the tissue concentrations of heavy metals.

In the gulls kidney, levels of cadmium up to 89.26μg/g produced no reliably measurable amount of cadmium in the feather. Chapter 2 explains fully the research

done on cadmium in feathers and I can only conclude they are of no value in monitoring cadmium levels in seabirds.

Chapter Five The metal and metallothionein relationships in adult lesser black-backed gulls, *Larus fuscus*

5.1 Introduction

Cadmium and mercury are two heavy metals naturally present in the marine environment. These are toxic even at relatively low levels and are known to accumulate in many marine animals. It is well established that seabirds can accumulate high concentrations of these (see Chapter 2 and Thompson (1990) for a review of concentrations), although they have no known biological function. There is some evidence that the concentrations found in free-living birds can cause kidney damage. Nicholson and Osborn (1983) found evidence of damage in seabirds with mean concentrations ranging from 94.5-228 μg/g cadmium and 5.02-13.4 μg/g mercury, but Elliot *et al.* (1992) found no damage in seabird kidneys, with comparable cadmium concentrations.

Cadmium and inorganic mercury are bound up within the liver and kidney to a low molecular weight protein called metallothionein. This metal binding protein was first discovered in 1957 by Margoshes and Vallee (1957) in horse kidney and later isolated and characterised by Kägi and Vallee (1960). Metallothionein is thought to play a key role in normal metabolism of essential metals and also in the storage of non-essential metals (Karin 1985). The natural ability to sequester and store heavy metals could be seabirds' physiological adaptations to non-essential metals.

A metal binding protein thought to be metallothionein was first described in seabirds by Osborn (1978). Since then few studies have investigated this. Hutton (1981) described a metal-binding protein from the great skua kidney cytosol, which bound the greater part of the cadmium in the kidney. Elliot *et al.* (1992) measured the protein levels as well as the metal levels in free-living Canadian seabirds. Cosson (1989) measured the metal and metallothionein-like proteins in flamingos and little egrets over-wintering in the Camargue, collected after they had starved and frozen to death.

Experimental studies have induced the synthesis of metallothionein by injecting or dosing animals with various heavy metals and found that some metals are more effective than others in inducing the synthesis and increase in metallothionein, and the kidney is

less responsive than the liver in producing metallothionein (Olafson 1981, Sendlebach and Klassen 1988). However, Scheuhammer and Templeton (1990) used ring doves as a bird model to look at metallothionein induction after feeding dietary cadmium, and demonstrated that differences in liver and kidney metallothionein synthesis could be explained by simultaneous changes in the zinc and copper concentrations, and these should be considered as well as the cadmium concentrations.

This study was designed to investigate the role of metallothioneins in the storage and dynamics of both the essential metals zinc and copper, and the non-essential metals cadmium and mercury. The aims were to use adult birds which had naturally accumulated heavy metals, and which would allow these relationships to be followed through a range of cadmium concentrations. I was able to obtain a large sample of adult lesser black-backed gulls from a cull which presented an outstanding opportunity to investigate these aspects of metal dynamics in healthy free-living seabirds sampled at one time in the breeding season.

5.2 Materials and methods

Gulls were obtained from two sources; a cull at Abbeystead, Lancashire and a cull by the Royal Society for the Protection of Birds, on the Firth of Forth, both in June 1992. Birds were killed using poisoned bait and a total of 58 gulls were obtained. Corpses were dissected fresh using stainless steel instruments, and whole kidney and liver tissues were weighed and then frozen at -20°C before further analyses. Frozen sub-samples were taken for gel filtration and metallothionein analysis. Other sub-samples were taken and dried in an oven at 50°C, to constant mass, prior to metal analyses. Whole liver and kidney tissues were dissected out using stainless steel instruments, and samples were transported frozen to Glasgow. Whole organs were weighed, and sub-samples taken for both metallothionein and metal analyses. Sub-samples were dried in an oven at 50°C to constant mass prior to metal analyses.

5.2.1 Metal analysis

Analyses were performed as described in Chapter 3 for cadmium, zinc, copper and mercury.

5.2.2 Metallothionein analysis

Metallothionein in the kidney and liver tissues were analysed as described in Chapter 3.

5.2.3 Gel filtration

This was performed on kidney samples, and the methods are described in Chapter 3.

5.2.4 Statistical analyses

Preliminary tests were performed of the goodness of fit of data to Normal distributions (Kolmogorov-Smirnov one-sample tests). As fit was good for all gull data, all subsequent analyses were made using parametric statistics. No differences were detected between the Isle of May and the Abbeystead collections of birds. No sex differences were found in the sample of gulls for any of the variables, using t-tests, therefore data were pooled for all analyses.

5.3 Results

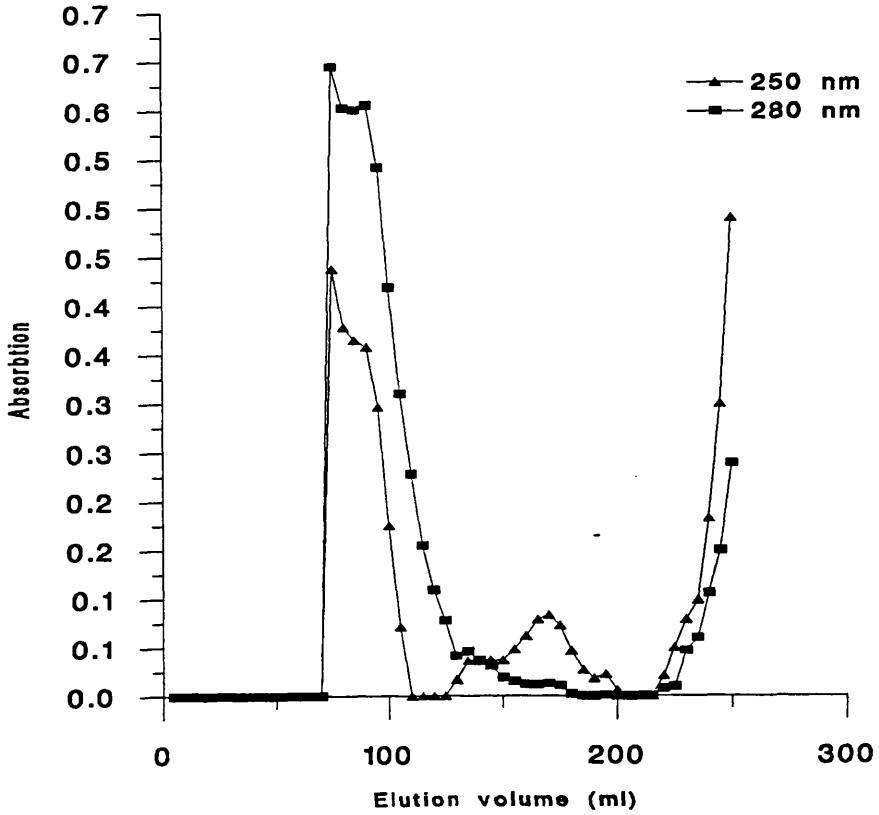
5.3.1 Gel filtration

The Sephadex gel metal profile in kidney is shown in Figure 5.1. In the kidney most of the cadmium from the cystolic region was bound onto the low molecular weight proteins, and only a small amount onto higher molecular weight proteins. The molecular weight of these proteins could not be assessed accurately, as they were run on the column after it had dried out and been re-poured, thus the original calibration (Figure 3.3), was not applicable. However as these were heat resistant and bound onto the Ag^{+} in the silver saturation assay, and bound onto cadmium, zinc and copper in the organs, they were almost certainly metallothionein and shall be referred to as such from now on.

5.3.2 Metal and metallothionein concentrations

These are given in Table 5.1. There was considerable variation in heavy metal and metallothionein concentrations in adult gulls, although they did conform to a Normal distribution. This variation allows the trends in metallothionein and essential metals with changing cadmium concentrations to be followed. Concentrations of cadmium, zinc and metallothionein are much higher in kidney tissue than liver tissue of gulls. The

Figure 5.1 Sephadex gel profile of lesser black -back gull kidney cytosol



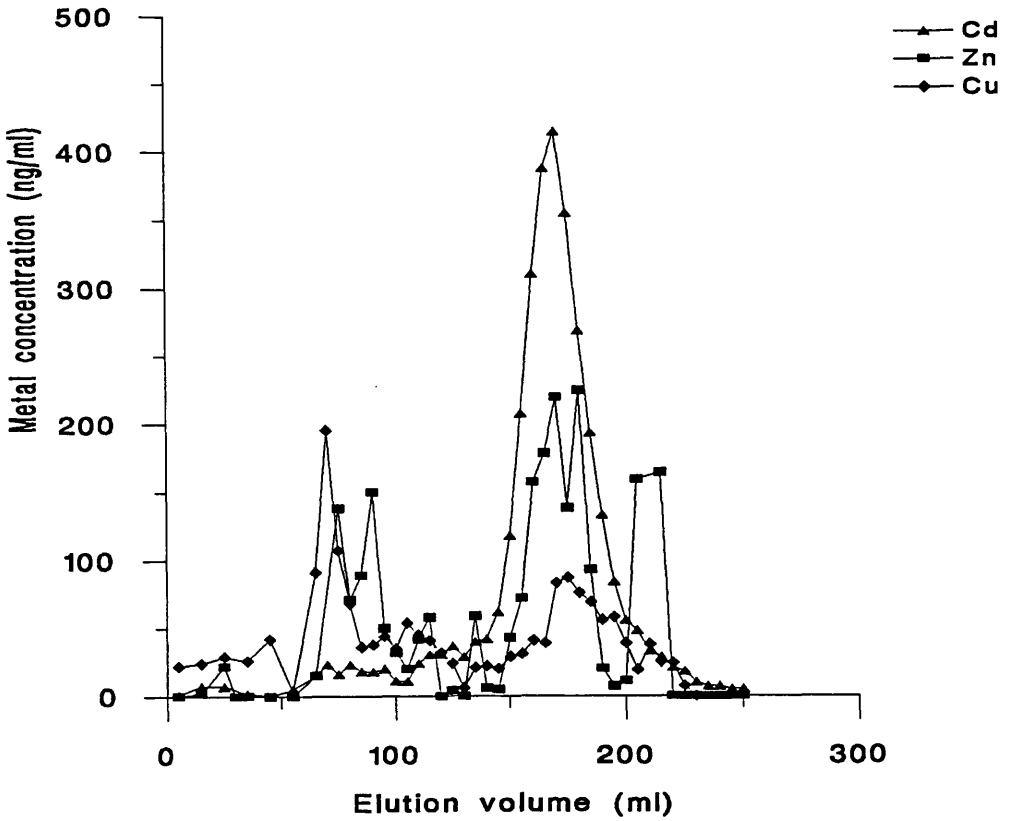


Table 5.1 Metal and metallothionein concentrations in lesser black backed gulls. Concentrations are given as μg/g dry weight for metals and μg/g wet weight for metallothionein, n = 58 for all samples except Hg (n = 21 for kidney, n = 28 for liver).

Organ	MT	Cadmium	Zinc	Copper	Mercury
Kidney					
Mean	137.60	28.86	118.98	13.51	1.84
S.D.	67.76	18.57	33.41	3.95	1.19
S.E.	8.89	2.43	4.39	0.52	0.26
C.V.					
Range	39-360	9-106	75-208	8-28	1-4
Liver					
Mean	27.17	2.83	61.21	12.56	1.55
S.D.	19.51	1.71	19.65	3.43	1.09
S.E.	2.56	0.22	2.58	0.45	0.20
C.V.					
Range	7-110	1-9	22-136	7-23	0-5

copper and mercury concentrations are similar in the two organs.

5.3.3 Inter-organ and inter-metal relationships in lesser black-backed gulls

In order to compare the metal and metallothionein accumulation patterns, all data is expressesd as μmol/g. Most metals (cadmium, copper and mercury), and metallothionein show a strong positive relationship between liver and kidney; if levels are high in one organ, they will be high in the other (Table 5.2). Mercury concentrations are quite low (Table 5.1) and accumulate at the same rate in both liver and kidney tissues (Table 5.2). Mercury concentrations show no significant relationship with any of the other metals. This contrasts with the other metals and metallothionein, which do.

Using multiple regression analyses, cadmium is calculated as the single most important factor in determining the metallothionein concentrations in both the kidney and liver tissues. The multiple regression equations for cadmium and metallothionein are given in Table 5.3. The best-fit linear regression equations for each metal and metallothionein were also calculated individually and are given in Table 5.3, along with the correlation coefficients. The value of B in these equations can be used to describe the relative rate of accumulation of a particular metal between liver and kidney, of different metals in the same organ, or to compare rates of accumulation of metallothionein and metals. Cadmium accumulates some seven times faster in the kidney than the liver, but metallothionein accumulates at equal rates in both organs. Metallothionein accumulates at a much lower rate than cadmium in both the kidney (around one third as fast), and in the liver (one half as fast). This is because for each mole of metallothionein, seven moles of excess metal can be bound onto the metallothionein. Metallothionein accumulated in the liver and kidney at a much slower rate than both zinc and copper (see Table 5.3).

The single most important influence on metallothionein concentrations in kidney tissue is cadmium, as revealed by the multiple regression analyses (cadmium-metallothionein r = 0.88). However, using single regression analyses zinc is highly correlated with metallothionein (zinc-metallothionein r = 0.46), and copper is also correlated

Table 5.2. Best-fit Linear regression equations to describe the inter-organ metal and metallothionein relationships (k = kidney tissue , l = liver tissues), * = P<0.05, ** = P<0.01, *** = P<0.001

Inter-organ relationships	
Equation	r value
Cd(k) = 7.34 Cd(l) + 0.053	0.68***
MT(k) = 1.19 MT(l) + 0.072	0.34**
Cu(k) = 0.33 Cu(l) + 0.146	0.30*
Hg(k) = 0.95 Hg(l) + 0.001	0.75***

Table 5.3 Multiple regression equation to describe the relationship between cadmium and metallothionein in the tissues, and Best-fit Linear regression equations to describe the other inter-metal and metal-metallothionein relationships (k = kidney tissue, l = liver tissue), * = P<0.05, ** = P<0.01, *** = P<0.001

Multiple regression	
Equation	r value
MT(k) = 0.24 Cd(k) + 0.038	0.83***
MT(l) = 0.41 Cd(l) + 0.008	0.46***
Simple regression	
Equation	r value
MT(k) = 0.05 Zn(k) + 0.006	0.53***
MT(l) = 0.02 Zn(l) + 0.003	0.37**
MT(k) = 0.21 Cu(k) + 0.049	0.28*
MT(l) = 0.08 Cu(l) + 0.002	0.34**
Zn(k) = 1.66 Cd(k) + 1.453	0.53***
Zn(l) = 9.77 Cd(l) + 0.688	0.49**
Cu(k) = 0.15 Cd(k) + 0.176	0.40***
Cu(l) = 1.31 Cd(l) + 0.164	0.37**
Zn(k) = 4.23 Cu(k) + 0.947	0.51***
Zn(l) = 2.49 Cu(l) + 0.441	0.45***

Figure 5.2 Cadmium and metallothionein in the kidney of lesser black-backed gulls

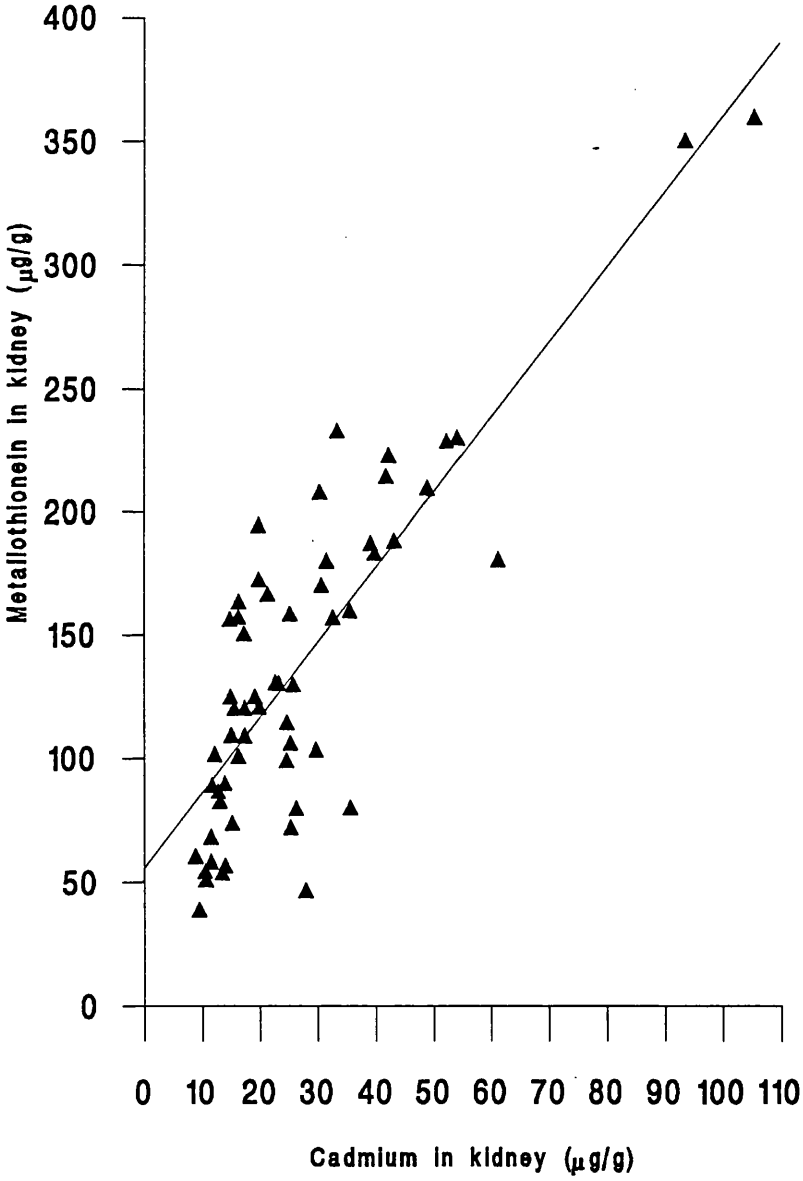
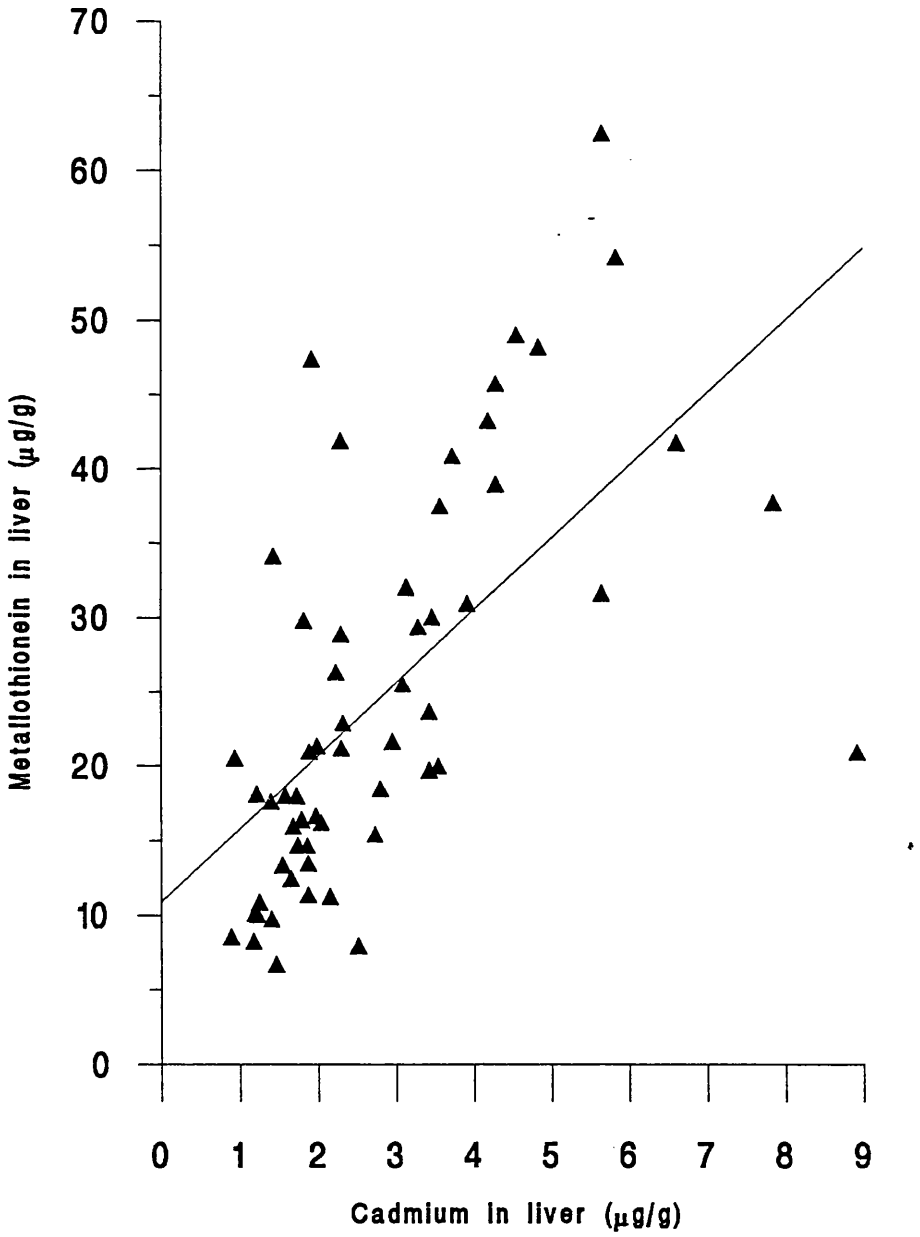


Figure 5.3 Cadmium and metallothionein in the liver of lesser black-backed gulls



significantly, but more weakly (copper-metallothionein r = 0.28). An identical pattern is seen in the liver. Cadmium is the most important factor in influencing metallothionein, (cadmium-metallothionein r = 0.46), and zinc is also correlated with metallothionein (zinc-metallothionein, r = 0.37), and copper is significantly correlated but the relationship is weaker, (copper-metallothionein r = 0.34). The relationship between cadmium and metallothionein in the kidney and liver is illustrated in Figure 5.2 and 5.3.

5.4 Discussion

A metal-binding protein thought to be metallothionein was first described in seabirds by Osborn (1978), and Hutton (1981) described a metal binding protein from the great skua kidney cytosol, which bound the greater part of cadmium in the kidney. In both these studies concentrations of cadmium were very high, Atlantic fulmar kidney and liver contained 240μg/g and 49.4μg/g respectively (Osborn 1978), and great skua kidney contained 81.4μg/g (mean value) cadmium (Hutton 1981). The metal binding protein is also detected in the lesser black-backed gull, although kidney cadmium concentrations were much lower (mean value 28.86μg/g). Cadmium in the kidney was bound onto a protein of low molecular weight, which was heat resistant and becomes bound onto the Ag^{+} in the assay, and is therefore almost certainly a metallothionein.

Quantitative studies of metallothionein in bird tissues are rare, but Elliot *et al.* (1992) measured metal and metallothionein concentrations in free-living Canadian seabirds. Their data for herring gulls show comparable concentrations to those found here in lesser black-backed gulls. Herring gull kidney cadmium concentrations ranged from 11-69μg/g and liver concentrations from 1.0-6.3μg/g. Metallothionein values in kidney (liver levels were not analysed) ranged from 33.9-377μg/g, which compare well with concentrations reported here. Concentrations measured in both these studies are much lower than those measured in little egret and greater flamingo (Cosson 1989). Flamingo and egret concentrations of both metals and metallothionein-like proteins were extremely high (metallothionein-like protein levels in liver of flamingo were from 2090-4840μg/g, kidney from 530-1070μg/g, and little egret liver concentrations from 720-1560μg/g wet weight). Zinc concentrations in these birds were also extremely high,

zinc in flamingo liver reaching 600μg/g (wet weight, Cosson *et al.*1988). These birds had starved and frozen to death, and were collected subsequently, which may account for the extraordinary levels. Cosson (1989) measured metallothionein-like proteins, using a differential pulse polarographic analysis, and perhaps this differs from metallothionein measured using the silver saturation technique.

In this study I found a strong relationship between cadmium and metallothionein concentrations in both the liver and kidney in the adult gulls. This was also found in Canadian seabirds in kidney tissues, in Leach's storm-petrel ($r = 0.692$), Atlantic puffin ($r = 0.845$) and herring gull ($r = 0.866$), but not in the double-crested cormorant (Elliot *et al.* 1992). Metallothionein concentrations were much lower in liver tissue of the gulls, but there were still strong correlations with the cadmium, zinc and copper concentrations. Multiple regression analyses demonstrated that among all the metals analysed, cadmium was the most important factor in determining metallothionein concentrations in both organs. This is evidence that in this case metallothionein is acting mainly in a detoxifing role, binding and storing the cadmium on the protein where it will not have any toxic effects.

Significant positive correlations between zinc and cadmium have been reported in many seabird species, particularly in kidney tissue (Scheuhammer 1987, Walsh 1990). In lesser black-backed gulls (this study) and Cory's shearwaters (see Chapter 6) and in great skua kidney (data from Chapter 4), there is also a strong positive relationship between zinc and cadmium in the liver and kidney. This makes physiological sense as both these metals (and copper) are bound onto metallothionein, and are illustrated by the inter-relationships found in this study. Cadmium-copper correlations are also significant in this study and in great skua tissues (Chapter 4). Cadmium-copper correlations are rare in the seabird literature, zinc-copper and cadmium-copper correlations were found in brown pelicans (Ohlendorf *et al.* 1985). In several species of Gough Island seabird there was no clear evidence of any such relationship (Muirhead and Furness 1986), despite very high cadmium concentrations. The absence of inter-metal relationships in the literature, and the lack of cadmium-metallothionein and

copper-metallothionein correlations in the immature shearwaters (Chapter 6) could be as a consequence of several factors. The time of year birds are sampled may be important. The lesser black-backed gulls sampled in Chapter 4 did not show any zinc-cadmium relationships in the liver, as these were sampled in the early breeding season, when zinc requirements are high (Lofts and Murton 1973). A similar pattern may be expected during moult, when zinc requirements are also high (Supplee *et al.* 1958, Sunde 1972). Sample sizes may be too small, as birds do have a high degree of variation in metal concentrations (see Chapter 4, Table 5.1). For some species the concentrations of metal may be too low to induce an increased synthesis of metallothionein, and therefore there will be no parallel increase in the other metals. Some studies have detected a threshold effect between zinc and metallothionein. Bremner and Davies (1975) also found a threshold concentration of 30μg/g zinc in liver, in experimentally dosed rats, and Jeffery *et al.* (1989) a threshold concentration of 40μg/g zinc in horses, above which metallothionein was accumulated in parallel with zinc.

Karin (1985) stated that metallothioneins are naturally present in animal liver and kidney and serve as the major storage form for the essential metals zinc and copper and will also function as a protective mechanism against the toxicity of non-essential metals. In adult lesser black-backed gulls cadmium has the most important influence on the metallothionein levels in both the kidney and liver, indicating that metallothionein is functioning as a detoxification mechanism. Unfortunately there is very little information on metallothionein concentrations in birds. It has been proposed that zinc may have a protective effect against the effects of cadmium (Hutton and Goodman 1980, Walsh 1990), but there is no real evidence of this, the theory seems based on the fact that zinc increases in parallel with cadmium. Zinc and copper are needed in many other cellular functions, and the parallel increase in these metals with cadmium may only be incidental with the induction of metallothionein by cadmium, or *vice versa.* Mercury in lesser black-backed gull kidney and liver is found in approximately equal quantities in the organic (methyl) form, and in the inorganic form (Thompson *et al.* 1990). Inorganic mercury can induce metallothionein synthesis and will readily bind

with the protein (Piotrowski *et al.* 1974). However mercury concentrations in this study did not show any relationship with any other metals, or with metallothionein. This is probably due to the rather low levels these gulls had accumulated. Elliot *et al.*(1992) found mercury and metallothionein correlations only in Atlantic puffin kidney, and not in other species studied. Osborn (1978) also did not find that mercury bound onto metallothionein in fulmar kidney and liver. In fulmar tissues there is a larger proportion of mercury in the methyl form (Thompson *et al.* 1990), and so this may be expected.

Experimental studies have looked at the differential accumulation of heavy metals and induction of metallothionein by injecting or dosing animals with varying amounts of cadmium. On sub-cutaneous injection of cadmium chloride the kidney of rodents is less responsive than the liver and will produce less metallothionein (Olafson 1981, Onosaka and Cherian 1981, Sendelbach and Klassen 1988). These studies did not consider zinc and copper concentrations, and these may have an important role in the induction of metallothioneins also. Scheuhammer and Templeton (1990) used the ring dove as a bird model. Birds were fed three levels of dietary cadmium. Feeding birds dietary cadmium at low doses is far superior to injecting cadmium as it mimics far better the natural absorption and uptake processes. They concluded that differences in liver and kidney metallothionein synthesis in response to dietary cadmium could be accounted for by changes in zinc and copper concentrations, as cadmium in the diet caused an increase of zinc and copper in experimental birds compared to control birds. For each μmol of metallothionein in the liver 7.6μmol of excess metal was accumulated, and for each μmol metallothionein in the kidney 8.8μmol excess metal was accumulated. These calculations were not possible for the lesser black-backed gull data, as these birds had accumulated cadmium, zinc and copper naturally. However for every 1μmol metallothionein in kidney, 4μmols cadmium were bound, and for every 1μmol metallothionein in the liver, 2μmols cadmium were bound. This means that in the kidney and liver zinc and copper will be bound onto the remaining metallothionein-binding sites. However there is a great excess of copper and zinc which is not metallothionein bound (see Table 5.3). This is due to the many other functions of

copper and zinc, metallothionein-binding forms only a small part of copper and zinc metabolism in liver and kidney.

Lesser black-backed gulls have accumulated cadmium naturally through their diet from hatching to adulthood. Cadmium, zinc and copper all have strong relationships with each other, and with metallothionein, as the metals are all bound onto the protein, to a greater or lesser extent, with the remaining zinc and copper being closely regulated.

This study used a unique opportunity to study the interactions of metallothionein and metals in free-living birds with a naturally occurring range of cadmium levels. It seems that the cadmium concentrations are the most important in determining tissue metallothionein in adult lesser black-backed gulls. Further insight into the role of metallothionein in metal dynamics is beyond the scope of this study, but these are certainly worthy of further investigation. The difficulty with studies of this nature is that samples can only be taken at one point in time, and the metal and protein dynamic processes cannot be followed through time. Chapter 6 uses the opportunity to sample young fledgling Cory's shearwaters, to attempt to quantify and compare metal dynamics in birds at a different point in their life-cycle.

Chapter Six. The natural accumulation of cadmium in Cory's shearwater *Calonectris diomedea* fledglings from the Azores, Portugal

6.1 Introduction

Bioaccumulation of heavy metals in birds is a well documented phenomenon, and pelagic seabirds often accumulate high levels of heavy metals naturally from the marine ecosystem. Concentrations of heavy metals are often reported for adult birds but less often for chicks or fledglings. However, chicks have been proposed as particularly useful pollution monitors, as they concentrate heavy metals during a specific period of time (i.e. hatching to fledging), and from a local and definable foraging area (Frank 1986, Walsh 1990, Furness 1993). This can be much more valuable than measurements of adult tissue levels where it is rarely possible to evaluate the accumulation period or define the feeding area from which metals are accumulated.

The Cory's shearwater is a long-lived pelagic seabird found in warm marine waters from temperate to sub-tropical zones of the North Atlantic and the Mediterranean (Cramp and Simmons 1980). High concentrations of heavy metals have been reported in tissues of adult Cory's shearwater (Renzoni *et al.* 1986) and attributed to natural accumulation from prey items. There is little information on the diet of Cory's shearwater but it is thought that squid form a significant proportion (around 26%) of their prey (Furness 1992). There is some evidence to suggest that squid can accumulate unusually high metal levels (Martin and Flegal 1975).

Squid beaks can remain undigested in predators' stomachs and gizzards for some time after being eaten, being considerably more resistant to digestion than fish otoliths and crustacean carapace. They have previously been used successfully to look at the diets of whales, seals, fish and birds (Clarke and MacLeod 1976, Clarke 1980, Clarke and Kristensen 1980, Clarke and Trillmich 1980, Clarke and Prince 1981, Clarke *et al.* 1981, Clarke and MacLeod 1982, Clarke 1986). Beak morphology can be used to identify the species of squid and the mass can be calculated by measuring part of the beak and using a conversion equation for the genus or family (Clarke 1986).

This chapter includes two quite distinct studies on heavy metal dynamics, and they were conducted on two collections of shearwaters, in 1991 and 1992. The aims of the first study were to assess the amount of cadmium accumulated by fledglings in the three months from hatching and to test the hypotheses that variations in concentrations of

cadmium measured were due to high levels of squid in individual chick diets. Although gizzards contained few hard parts from fish or crustaceans, I hoped to use the squid beak remains to gain an insight into the species the young shearwaters were being fed, and attempt to estimate the amount of squid each individual had consumed in its lifetime. The aims of the second study were to investigate heavy metal and metallothionein dynamics in fledglings. The sample of young shearwaters provided an excellent opportunity to study this at a distinct point in the life cycle of a seabird.

In the Azores there are many shearwater colonies situated close to towns. When chicks fledge (always at night) at around twelve weeks old, a considerable number of them are confused by the bright town lights and crash into street lamps or car headlights, and they are killed. These birds were collected for these studies.

6.2 Materials and methods

6.2.1 Sample collection and preparation

Freshly dead shearwaters were collected in October and November 1991 and 1992 on the Azores islands of Horta and Pico. An advertisement was placed in the local paper by Luis Monteiro of the University of the Azores, and a total of 39 birds in 1991 and 35 in 1992 were handed in or collected by scouring the streets early in the morning. Confused fledglings handed in alive were released somewhere dark that evening. All dead specimens were weighed and then stored frozen at -20°C before dissection. For the first study, in 1991, liver, kidney and gizzard were dissected out using stainless steel instruments. Gizzards were stored whole in 70% alcohol for transport back to Glasgow. All other tissues were stored and transported frozen. In Glasgow tissues were weighed and samples dried in an oven at 50°C to constant mass, prior to metal analysis. Gizzards were cut open and turned inside out to remove all fragments of squid beaks and other contents. Beaks were sorted into different types and counted. Upper and lower beaks were separated as only the lower beak is used for identification and measurement. Samples of the most intact specimens were sent to experts for identification, to Dr K. Thompson, Ms B. Santos, Dr N. Klages and Dr G. Pierce. An attempt was made to measure the most complete beaks of Ommastrephidae. The mass of the squid was

estimated by measuring the lower rostral length (LRL) and using the equation lnW = 2.714 + 2.93 ln LRL (Clarke 1986). The minimum LRL was measured, as beaks were badly eroded. Consequently sizes of squid are only a rough estimate. An independent assessment of the accuracy of measurement of beak LRL and size calculation was carried out using fresh whole squid, *Loligo sp.*, by comparing the wet weight and the estimate from the relevant regression equation.

For the second study in 1992, whole liver and kidney tissues were dissected out using stainless steel instruments, weighed, and sub-samples were prepared for metal and metallothionein analysis.

6.2.2 Metal analysis

Analyses for cadmium zinc, and copper were carried out for all the samples. Methods are described in Chapter 3.

6.2.3 Metallothionein analysis

This was performed on the liver and kidney tissues of birds collected in 1992 only. Methods are described in Chapter 3.

6.2.3 Statistical analyses

Preliminary tests were performed of the goodness of fit of data to Normal distributions (Kolmogorov-Smirnov one-sample tests). Fit was good for all data in birds from 1991 and all subsequent analyses were made using parametric statistics. Pearson product moment correlations were used to investigate the relationships between the metal concentrations in the liver and kidney tissues. In the sample of birds collected in 1992 cadmium concentrations in the kidney and liver tissues were not normally distributed. This was due to two individuals having much higher values than the rest of the sample. These variables were logarithmically transformed and analysed with the rest of the data cn copper, zinc and metallothionein concentrations.

6.3 Results

6.3.1 Metal and metallothionein levels

Fledgling Cory's shearwaters of age 12 weeks had accumulated measurable amounts of cadmium in both kidney and liver tissue. The metal concentrations measured in both samples of birds were very similar. Because the two samples were collected to look at

two distinct aspects of metal accumulations they were analysed separately. Concentrations are given in Table 6.1 for birds from 1991 and Table 6.2 gives metal and metallothionein concentration in birds collected in 1992.

Birds collected in 1991 conformed to a Normal distribution but with a wide range of concentrations (kidney from 1μg/g to 40μg/g, liver concentrations from 1μg/g to 9μg/g). The range of concentrations are illustrated in Figure 6.1. This could be associated with variations between individuals in diet.

Birds collected in 1992 also showed a range of cadmium concentration, with two individuals accumulating 40.64 and 40.47μg/g in their kidney, and 11.59 and 13.29μg/g in their liver, respectively.

6.3.2 Inter-organ and inter-metal relationships in Cory's shearwater

In the 1991 sample the concentrations of metals accumulated showed few inter-metal and inter-organ correlations. Only cadmium correlations in the liver and kidney were significantly correlated ($r = 0.42$, $n = 39$, $P < 0.05$), and only cadmium and zinc concentrations were significantly correlated in the kidney ($r = 0.56$, $n = 39$, $P < 0.001$).

Inter-metal and inter-organ relationships in the 1992 sample are given in Table 6.3. In this sample cadmium, zinc and copper show strong positive correlations between liver and kidney, but the concentrations of metallothionein in the kidney had no relationship with the concentrations of metallothionein in the liver.

In the kidney there are strong correlations between concentrations of metallothionein and all the metals measured, and between the different metals. Metallothionein concentration correlates most strongly with copper followed by zinc and then cadmium. The accumulation pattern in the liver is quite different. Only the concentrations of zinc in the liver shows a significant correlation with the concentrations of metallothionein, although cadmium and zinc concentrations, and cadmium and copper concentrations, accumulate together. The metallothionein-zinc relationship in the liver is illustrated in Figure 6.2.

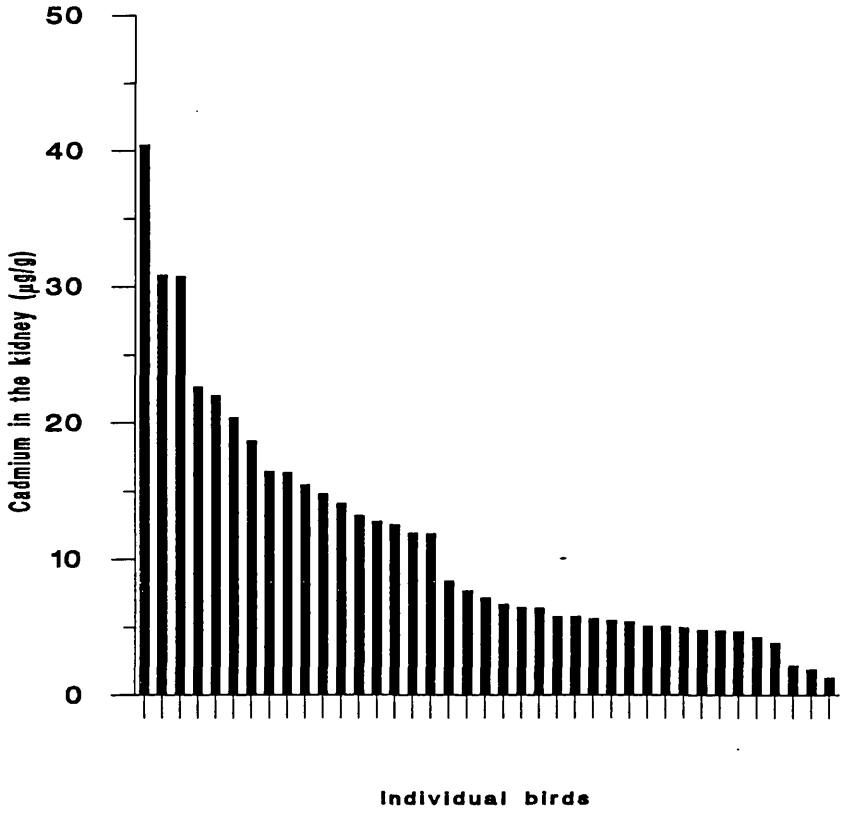
Table 6.1 Concentrations of cadmium, zinc and copper in Cory's shearwater fledglings collected in 1991. Concentrations are given as μg/g dry weight, n = 39

	Cadmium	Zinc	Copper
Kidney			
mean	11.28	110.68	21.16
S.D.	8.74	32.35	7.99
S.E.	1.38	5.05	1.28
range	1-40	40-194	3-45
Liver			
mean	3.03	198.86	18.86
S.D.	1.72	80.44	5.29
S.E.	0.28	12.88	0.86
Range	1-9	39-389	12-30

Table 6.2 Metal and metallothionein concentrations in Corys' shearwater fledglings collected in 1992. Concentrations are given as μg/g dry weight for metals and μg/g wet weight for metallothionein. n= 35 for cadmium, zinc and copper in kidney, n= 34 for metallothionein, zinc and copper in liver, n=33 for metallothionein in kidney)

Organ	MT	Cadmium	Zinc	Copper
Kidney				
mean	24.76	9.31	114.94	12.59
S.D.	16.58	10.07	24.39	3.54
S.E.	2.89	1.70	4.12	0.59
Range	3.08-65.60	2.27-40.64	62.53-172.93	6.78-22.64
Liver	106.05	2.03	176.43	13.29
mean	40.59	2.78	48.61	7.38
S.D.	6.96	0.47	8.34	1.26
S.E.	16.54-195.92	0.39-13.29	28.28-91.96	5.85-43.77
Range				

Figure 6.1 Individual concentrations of cadmium in Cory's shearwater collected in 1991



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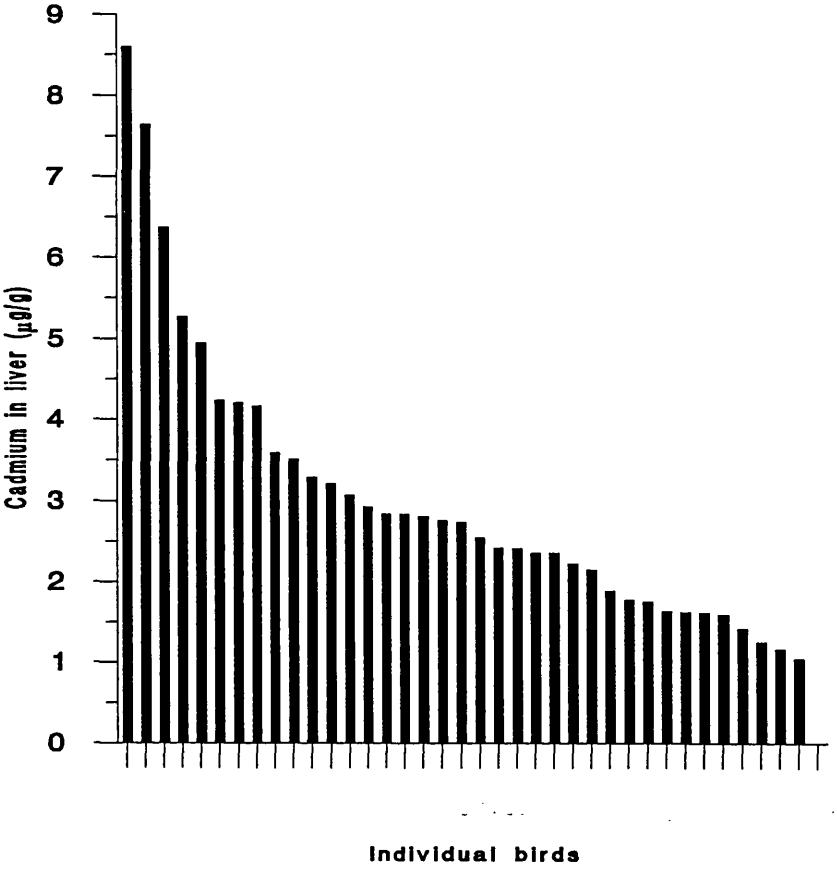


Table 6.3 Pearson correlation coefficients to describe the inter-organ and inter-metal relationships in fledgling Corys' shearwater, collected in 1992. (k= kidney, l= liver, MT = metallothionein * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$)

Kidney

Zn(k)	0.37*		
Cd(k)	0.36*	0.40**	
Cu(k)	0.48**	0.57***	0.56***
	MT(k)	Zn(k)	Cd(k)

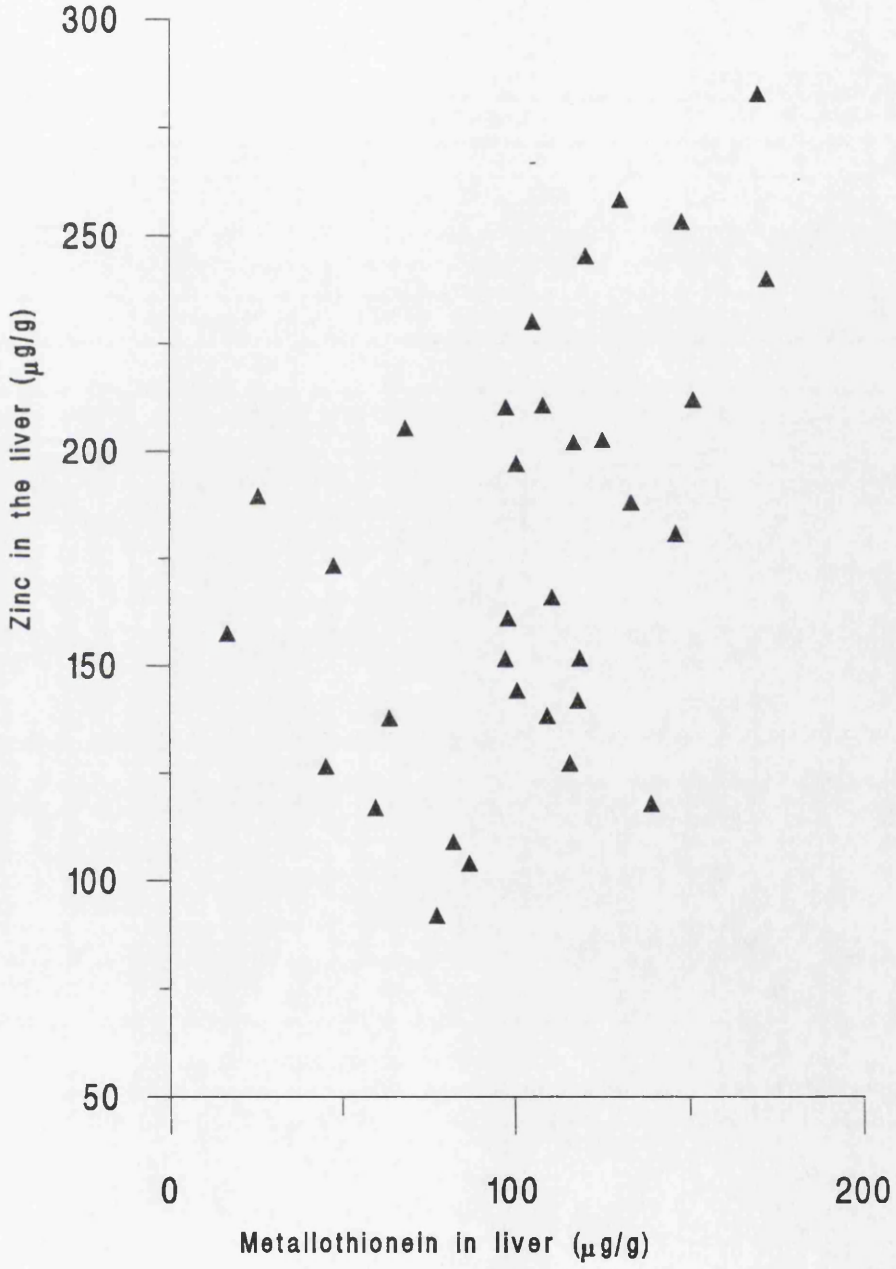
Liver

Zn(l)	0.49**		
Cd(l)	0.07 N.S.	0.35*	
Cu(l)	-0.11 N.S.	0.08 N.S.	0.69***
	MT(l)	Zn(l)	Cd(l)

Inter-organ

MT	Cd	Zn	Cu
-0.06 N.S.	0.73***	0.40*	0.43*

Figure 6.2 The relationship between zinc and metallothionein in Cory's shearwater fledglings



6.3.2 Squid beaks

Of the 39 fledglings obtained in the study, all had at least one squid beak in the gizzard. The number of beaks ranged from one to nineteen, with the mean number being six (s.d. = 4.09, n = 39). Squid beaks were badly eroded and each gizzard contained a number of large fragments and many small fragments. Many of the gizzards also contained small plastic pellets. The beaks were tentatively identified as mainly being Family Ommastrephidae, but some were thought to be Cranchiidae, *Taonis sp.*, an Octopoteuthidae, and *Gonatus sp.* Expert opinion was that beaks were too badly eroded for definite identification or detailed analysis. From the measurements of the most intact Ommastrephids, squid had a wet mass of from 40-180g, with the exception of the largest which was estimated at 370g. There was no direct relationship between the number of squid individuals had eaten, and the concentration of cadmium accumulated, (kidney r = 0.15, n = 39, N.S., liver r = 0.07, n = 39, N.S). Unfortunately because of the degree of erosion of the beaks making accurate identification and measurement was impossible. It was therefore not possible to look at any direct relationship between individual cadmium concentration variations, and the total amount of squid ingested.

6.4 Discussion

Cory's shearwater fledglings accumulated a mean cadmium concentration of 11.28μg/g (1991), and 9.31μg/g (1992) in kidney tissues and 3.03μg/g (1991), and 2.03μg/g (1992) in liver tissues in the three months from hatching to fledging. It should be noted that eggs contain very little cadmium and less than the 0.03μg/g detection limit were reported for Cory's shearwater by Renzoni *et al.* (1986). Fledglings' cadmium concentrations are lower than values measured in adults sampled at the Selvage Islands in the Atlantic, and Majorca, Linosa and Crete in the Mediterranean (Renzoni *et al.* 1986). Mean adult concentrations were 214.17μg/g, 42.82μg/g, 106.21μg/g and 187.32μg/g in kidney tissue and 26.48μg/g, 7.52μg/g, 13.91μg/g and 55.92μg/g in liver tissue, respectively. The mean concentrations of cadmium in the liver and kidney in adults from Majorca were comparable to some individual concentrations in the Azorian fledglings. Unfortunately there are no data on cadmium concentrations in adult Cory's shearwater from the Azores for comparison. Copper concentrations were not analysed

in adult shearwaters, but the levels here are comparable to others reported in the literature for seabirds (Thompson 1990). Zinc concentrations in the kidney are similar to adult values recorded in Majorca and Linosa, but lower than in the Selvages and Crete. In comparison zinc concentrations in the liver of Cory's shearwater fledglings are higher than zinc concentrations in adult Cory's shearwater from Selvages, Majorca and Linosa, but similar to five adults sampled in Crete. These had high zinc concentrations (mean values of 181.33μg/g), probably in association with high concentrations of cadmium (mean value 55.92μg/g), although correlation coefficients were not calculated. This is quite different from the immature shearwaters. Concentrations of zinc in the liver are high (mean value 176.43μg/g in 1992, 198.86μg/g in 1991), but cadmium concentrations are low (mean value 2.03μg/g in 1992, 3.02μg/g in 1991) with no significant correlations between the metals. The high zinc concentrations in the liver of fledgling shearwaters are associated with high metallothionein concentrations. Indeed, zinc is the only metal in the liver to show a significant correlation with metallothionein. Cadmium and zinc concentrations and cadmium and copper concentrations correlate significantly but neither has any relationship with metallothionein. Therefore in these birds the major role of metallothionein at fledging is clearly as a zinc store although other proteins in the liver will also store zinc (Lofts and Murton 1973). High concentrations of zinc are required during the fledglings' development. Birds had just grown full plumage, although some birds still had small amounts of down on their bodies. Experimental work on poultry has shown high levels of zinc are needed for feather growth, and zinc deficiency results in a frayed feather condition (Supplee *et al.* 1958, Sunde 1972). Several workers have also reported high concentrations of zinc in the liver prior to moulting in sparrows (Haarakangas *et al.* 1974) and starlings (Osborn 1979b). Honda *et al.* (1986b) found that concentrations of metals fluctuated during the different phases of chick growth, in eastern great white egret. Egret chicks showed a steady increase from hatching to around day 40, then a decrease between days 55-70 (and, as expected much higher levels in adult birds). The fluctuations in cadmium mirrored the fluctuations in zinc and copper. This was attributed to the growth of flight feathers in the latter sample of chicks requiring high zinc concentrations.

Cory's shearwater fledglings accumulate a higher concentration of cadmium in three months than is found in some studies of either young or adults of many seabirds including pelicans, gannets, cormorants, eider ducks, gulls, terns and auks (Walsh 1990, and see review Chapter 2). Shearwaters, in common with other petrels, albatrosses, penguins and skuas tend to have high natural concentrations of cadmium in their tissues (see Chapter 2). This is usually attributed to their diet, which consists of prey items which concentrate cadmium, and will be accumulated by the birds.

Squid form a major portion of the diet of albatrosses and some petrels, but there is little information on the concentrations of heavy metals in squid. Values of cadmium in the digestive gland of the squid *Nototodarus gouldi* ranged from 19-110μg/g (Smith *et al.* 1984), and Martin and Flegal (1975) measured concentrations of cadmium of 71-694μg/g in *Ommastrephes bartrami*, 42-1106μg/g in *Symplectoteuthis oulananiensis* and 33.0-233.1μg/g in *Loligo opalescens*. These rather limited data indicate squid can certainly concentrate large amounts of cadmium which would account for the concentrations measured in their predators.

Dietary data for Cory's shearwater are scarce. Squid beaks from the gizzards could only be tentatively identified and measured due to their poor condition. Further studies would be necessary to sample crop contents to obtain squid beaks before they are worn down by the action of the gizzard. It would seem from the size estimation made here that fledglings are being fed reasonably large squid. Furness (1992) sampled voluntary regurgitations and also used a warm saline off-loading technique to collect food samples. Some 195 food samples were collected from Cory's shearwater at colonies in the Azores in 1989-91, 26% of these contained squid of around 40-140g. A diet including one quarter squid of this size could feasibly account for the high concentration of cadmium in fledglings. If squid fed to shearwater chicks are assumed to be mainly, or wholly Ommastrephids (this study, Furness 1992) some calculations can be made.

Cory's shearwater chicks reach a mass of around 1100g. The estimated daily intake of Cory's shearwater chicks is 59g/night, and if chicks take 75 days to reach maximum weight this requires a total of 4400g of food to be ingested (Klomp and Furness 1992, Hamer and Hill 1993). This can be estimated in another way. Montevecchi *et al.*

(1984) gave a gross growth efficiency for seabird chicks as 25-33%: therefore a 1100g chick would need 4000-5000g food to reach maximum weight. If 26% of this diet were squid, and using the value of 4400g for total food intake this means chicks eat 1144g of squid. This corresponds to at least ten squid of around 140g. This is not unrealistic given the number of beaks in this study ranged from 1-19, and the estimated size of squid ranged from 40g-180g. In squid cadmium is concentrated almost exclusively in the digestive gland (liver), and this is estimated at around 3% of squid body weight giving a value of 34.3g for the amount of digestive gland ingested by the chicks, corresponding to a dry weight of 6.3g (wet:dry ratio = 5.44, Martin and Flegal (1975)). Martin and Flegal (1975) measured cadmium concentrations in Ommastrephid squid and found a wide range of concentrations from 71μg/g to 694μg/g, with a mean value of 287μg/g. These values can be used for three separate estimates of the total amount of cadmium likely to be accumulated by a Cory's shearwater chick in twelve weeks, shown in Table 6.5. The mean dry weight of kidney and liver tissues in the Cory's shearwater sample were calculated, and it was assumed that half the cadmium burden would be accumulated by each organ (see Chapter 2).

Table 6.5 Estimation of cadmium concentrations in fledgling Cory's shearwaters accumulated from squid in their diet

	Minimum	Mean	Maximum
Squid cadmium concentrations =	71 μg/g	287 μg/g	694 μg/g
Total metal ingested by shearwater =	447.3μg	1808.1μg	4372.2μg
(digestive gland x metal concentration)			
Amount absorbed = 0.5 % =	2.23μg	9.04μg	21.86μg
(data from Chapter 8)			
Concentration in kidney =	1.27μg/g	5.16μg/g	12.49μg/g
liver =	0.49μg/g	2.00μg/g	4.85μg/g
(dry kidney wt = 0.875g, liver = 2.25g)			

These figures are only rough estimates, assuming shearwaters ingest squid with a uniform concentration of cadmium. This is unrealistic as birds would most likely ingest squid with a range of metal concentrations. It also assumes that the fish making up the remaining 75% or so of the shearwaters' diet would contain very low concentrations of cadmium. Cadmium concentrations in fish muscle are usually low and tend to be higher in liver and kidney (Thompson 1990). The species of fish eaten by Cory's shearwater are not yet known, making their contribution to the metal burdens of fledglings impossible to assess. Further work on Cory's diet would be required for a more detailed analysis. However these calculations illustrate that a diet of around 26% squid could result in the accumulation of cadmium concentrations actually measured in the fledgling shearwaters in this study. Indeed the highest estimate agrees well with the actual mean cadmium concentrations.

It is interesting that young shearwaters accumulate such high concentrations of cadmium in their three month growth period, higher than those measured in several adult seabirds. This can be attributed to the considerable amount of squid in their diet. It would be interesting to compare these data with adult concentrations from the same area. Further studies on chick diet, and perhaps metal analysis of prey items could give

a much greater insight into the dynamics and accumulation of cadmium in these shearwaters.

Metallothionein is thought to have several functions, including the storage of essential metals and the detoxification of non-essential metals. Sampling the shearwaters at fledging has demonstrated that metallothionein can play a role in the storage of zinc, and at this time i.e. during the latter phase of chick development its role in binding cadmium may be unimportant or indeed masked by the large amount of zinc-bound metallothionein. This is quite in sharp contrast to the dynamics of cadmium and metallothionein found in the adult lesser black-backed gulls in Chapter 5. This will be discussed further in Chapter 9.

Chapter Seven Seasonal variation in heavy metal levels in tissues of Common Guillemots *Uria aalge* from North-west Scotland

This chapter is in press in Archives of Environmental Contamination and Toxicology as follows:

Seasonal Variation in Heavy Metal Levels in Tissues of Common Guillemots, *Uria aalge* from North-west Scotland

F. M. Stewart*, D. R. Thompson*, R. W. Furness*, and N. Harrison**.

*Applied Ornithology Unit, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom

**R.S.P.B., The Lodge, Sandy, Bedfordshire SG19 2DL, England, United Kingdom

Mercury, cadmium, zinc and copper concentrations were analyzed in three samples of common guillemot (in April, June and November). Levels measured were uniformly low, and not thought high enough to have any toxic effects. Adult guillemots had significantly more cadmium in their livers and kidneys than juveniles, with juvenile levels ranging from 25-89% of adult levels. Mercury concentrations in liver and kidney were also higher in adults. Juvenile levels represented from 80-94% of adults, but there were no age differences in feather and muscle mercury. Mercury levels declined throughout the year in internal tissues from April through June to November. There was a strong seasonal fluctuation in cadmium levels in liver and kidney, rising significantly between April and June and declining again from June to November. These changes were apparent in both adult and juvenile birds. The influences of seasonal processes (namely breeding and moult) and seasonal dietary differences as causative factors in the changes in metal burdens are discussed. These findings have implications for the use of seabirds as monitors of heavy metals in the marine environment.

7.1 Introduction

Seabirds have been advocated as useful monitors of heavy metal pollutants in the marine environment (Walsh 1990, Thompson *et al.* 1992). Although there is an extensive literature describing heavy metal levels in seabirds, few papers have examined seasonal variation in metal burdens or concentrations within a population. One of the most important features of a biomonitor is that measurements adequately reflect the bioavailability of the contaminant (Phillips 1990). Therefore it is important to have knowledge of the kinetics of the contaminant in the species used, considering such factors as metabolic, physiological and dietary specialisation. Only then can seabirds be used as effective indicators of environmental quality (Walsh 1990, Furness 1993). In particular, variation in metal concentrations in soft tissues has to be considered as mercury excretion occurs in eggs (Becker *et al.* 1989) and growing feathers (Honda *et al.* 1986a), and cadmium burdens may also be affected by physiological processes.

Problems common to studies of seasonal variations in cadmium concentrations in birds have been low sample sizes and high individual variation in metal levels, and often seasonal changes in fat or protein and content of organs have not been monitored.

It has been suggested that the common guillemot *Uria aalge* is particularly suitable as an indicator of pollution in European coastal areas, as it is a common bird in most areas, is a year-round resident, and thus could reflect local bioavailability of heavy metals (Tasker and Becker 1992). In making comparisons between seasons it is essential that birds from only one, discrete, population are being sampled at each time of year. Analysis of ring recovery data and of biometrics has shown that this was the case for these guillemots, and results are published in Furness *et al.* (in press a).

In this paper we present mercury, cadmium, zinc and copper concentrations in common guillemots from the population in north-west Scotland sampled in spring, summer and winter, and examine season, sex and age effects. The north-west of Scotland is thought to be a clean environment, especially in comparison to some European coastal areas.

7.2 Materials and Methods

Sample Collection and Preparation

Guillemots were sampled on three occasions, on 27 April, 25 June and 1-2 November 1988, from the waters surrounding the Summer Isles at the mouth of Loch Broom, north-west Scotland. These samples were taken for dietary studies by the Joint Nature Conservation Committee Seabirds-at-Sea Team, and were subsequently made available to us. All birds were shot at sea, under licence, from an inflatable boat using a 12-bore shotgun. Guillemots were returned to shore within ca. five minutes and were weighed, measured and plumage and moult status recorded.

The body cavity was opened, the liver carefully removed and the crop and gizzard were removed for dietary analysis. Birds were sexed by internal examination, and aged by presence or absence of bursa noted. The composition of the three samples was as follows: 26 April = 24 adults, 6 immatures (23 males and 7 females), 25 June = 21 adults, 6 immatures (16 males, 11 females) and 1-2 November = 20 adults, 5 immatures (17 males, 7 females, and 1 which could not be sexed due to gun-shot damage).

After transport to the laboratory, kidney and muscle tissues were dissected out and fresh masses of tissues were determined (to 0.001g). All tissues were stored deep frozen at ca.-20°C prior to further treatments. Liver, kidney and muscle tissues were defrosted, homogenized and dried to constant mass in an oven at 50°C prior to metal analysis. In addition, four to ten body feathers were sampled from the back (dorsal) region of each bird, surface contamination removed using an acetone/chloroform washing regime (Muirhead 1986) and feathers dried at ambient laboratory temperature (ca. 22°C) prior to analysis. In order to determine whether changes in concentration of metals in tissues reflected changes in tissue burdens of metal the entire liver and the pectoral muscle from the left side of the keel were dissected and dried to constant mass so that metal burdens could be determined. Because of the difficulty in dissecting complete kidney tissues these were taken from a subsample of six birds.

Mercury Analysis

Liver, kidney and pectoral muscle samples from birds from the first (April) collection initially underwent a fractionation in order to extract methyl mercury. The method used was based on that of Uthe *et al.* (1972) and is described in Thompson and Furness

(1989). Extracted methyl mercury concentrations were compared to corresponding total mercury concentrations for each bird and for all three tissues. The two measurements were found not to differ significantly from each other (paired t-tests for liver, kidney and pectoral muscle; P = 0.13, 0.47 and 0.21, respectively), indicating that virtually all the mercury present in the three tissues was methyl mercury. Consequently, all feather samples and internal tissues from June and November samples were analysed for total mercury only.

Total mercury in acid-digested samples was determined by a cold vapor technique using a Data Acquisition Ltd. DA 1500-DP6 Mercury Vapor Detector (Furness *et al.* 1986). Accuracy and reproducibility of mercury determination were tested by analysing International Atomic Energy Agency horse kidney Reference Material H-8.

Cadmium, Zinc and Copper Analyses

Samples of 0.5-1.0g dried tissue (kidney, liver and muscle) were acid digested in 10 ml concentrated nitric acid on a hot plate, by first soaking at 100°C for two hours, then boiling at 120°C for 20 minutes. Samples were diluted to 15ml, using distilled water. Metal concentrations were analysed by Atomic Absorption Spectrophotometry using a Phillips PU 2000 AAS. Accuracy and reproducibility of metal determinations were tested by analyzing horse kidney Reference Material H-8. Detection limits were 0.014 μg/g for Cd, 0.01 μg/g for Zn and 0.035 μg/g for Cu, in the digested sample. All metal concentrations are expressed on a dry weight basis.

Statistical Analyses

Preliminary tests were performed of the goodness of fit of data to Normal distributions (Kolmogorov-Smirnov one-sample tests). Where fit was good, subsequent analyses were made using parametric statistics. Where data deviated significantly from normality, defined throughout this paper as $P < 0.05$, we used nonparametric statistics. Statistical tests were performed using the SPSS-PC+ package (Norusis 1986, 1988).

7.3 Results

Organ Weights and Metal Content

A two-way ANOVA revealed that neither sex nor sampling date were significant factors with respect to liver dry mass (sex: $F_{1,63} = 1.50$, N.S.: collection date: $F_{2,63} = 1.2$, N.S.). Similarly, sample date did not affect pectoral muscle mass significantly ($F_{2,63} = 1.8$, N.S.), although a significant difference was found between the sexes ($F_{1,63} = 4.9$, $P < 0.05$). Thus significant changes in metal burden (μg), in liver and muscle tissues over the sampling period were likely to have been a function of metal concentration, rather than tissue mass. Kidney masses averaged 15.01g (fresh) and 3.56g (dry) for a sample of six birds.

Tissue masses and total metal contents of the birds are shown in Table 7.1. As organ weights did not change seasonally, all data analyses were performed using metal concentrations (μg/g) in tissues.

Metal concentrations in tissues and feathers of adult and juvenile birds are shown in Table 7.2. Results of two-way ANOVA tests of metal level by age (juvenile/adult) and collection date, are shown in Table 7.3. Cadmium was not detected in any of the muscle samples (< 0.015 μg / g dry tissue mass).

Sex Effects, Age Effects and Seasonal Effects

Due to the small sample size for juveniles, differences in metal concentrations between the sexes were examined only for adults (Table 7.3). Mercury in feathers showed a significant difference, males tending to have higher levels than females (males n = 56, mean = 2.00, s.d. = 0.85, females n = 25, mean = 1.77, s.d. = 0.66). Copper concentrations were significantly higher in the kidney of females (females n = 25, mean = 14.44, s.d. = 2.29, males n = 56, mean = 13.27, s.d. = 1.82).

Mercury concentrations were significantly higher in adult liver and kidney, but not in feathers or muscle (Table 7.3). Juvenile mercury levels represented from 80 to 94% of adult mercury concentrations. Cadmium concentrations were also significantly higher in liver and kidney of adults. Juvenile cadmium

Table 7.1. Tissue dry masses (g), and mercury and cadmium content (μg) of liver, kidney and one pectoral muscle of adults, and juveniles (J) from the three collections. All values are means (μg) with standard deviations in parentheses.

Group	Coll.	Liver			Kidney (mass 3.56 g)		Muscle	
		Mass	Hg cont.	Cd cont.	Hg cont.	Cd cont	Mass	Hg cont.
Adults.	April	10.82 (1.99)	39.16 (12.05)	17.36 (7.38)	13.98 (3.77)	32.04 (18.67)	28.56 (2.33)	49.80 (16.31)
Juveniles		12.97 (0.84)	31.36 (5.95)	17.64 (4.24)	9.44 (1.05)	14.43 (4.33)	26.97 (3.72)	33.72 (6.82)
Adults	June	10.47 (1.99)	26.30 (11.38)	26.35 (15.06)	9.04 (3.16)	41.74 (18.57)	28.34 (2.77)	23.67 (10.71)
Juveniles		11.02 (2.32)	17.01 (5.38)	21.29 (5.63)	6.81 (2.13)	37.38 (17.63)	28.50 (1.48)	18.63 (8.13)
Adults	Nov.	11.34 (1.44)	9.73 (3.12)	18.27 (5.83)	2.97 (0.86)	21.87 (7.28)	29.41 (2.36)	13.63 (6.64)
Juveniles.		12.80 (1.63)	13.28 (5.26)	14.06 (3.69)	3.63 (0.88)	5.87 (2.53)	29.37 (2.55)	14.94 (4.19)

Table 7.2. Mercury, cadmium, copper and zinc in tissues collected in April (coll. 1), June (coll. 2), and November (coll. 3).

Group	Coll.	Cadmium		Zinc			Copper.			Mercury.			
		Liver Conc. (s.d.) c.v.	Kidney Conc. (s.d.) c.v.	Liver Conc. (s.d.) c.v.	Kidney Conc (sd.) c.v.	Muscle Conc. (s.d.) c.v.	Liver Conc. (s.d.) c.v.	Kidney Conc. (s.d.) c.v.	Muscle Conc. (s.d.) c.v.	Liver Conc. (s.d.) c.v.	Kidney Conc. (s.d.) c.v.	Muscle Conc. (s.d.) c.v.	Feather Conc. (s.d.) c.v.
Adults n=24	April	1.56 (0.49) 31.41	9.00 (5.25) 58.22	58.42 (7.72) 12.36	72.22 (9.90) 13.71	25.17 (3.23) 12.83	15.02 (1.76) 11.72	13.82 (2.01) 14.54	11.52 (1.41) 12.24	3.66 (1.05) 28.69	3.93 (1.06) 26.97	1.76 (0.62) 35.23	2.15 (0.52) 24.19
Adults n=21	June.	2.49 (1.33) 53.41	11.72 (5.21) 44.45	68.89 (7.41) 10.75	74.13 (11.90) 16.05	25.98 (7.28) 28.02	16.09 (3.10) 19.27	13.69 (2.09) 15.27	13.96 (1.32) 9.46	2.52 (0.99) 39.28	2.54 (0.89) 35.03	0.84 (0.38) 45.24	2.09 (0.78) 35.88
Adults n=20	Nov.	1.66 (0.52) 31.32	6.14 (2.04) 33.22	69.70 (11.58) 16.61	72.31 (6.98) 9.65	20.89 (1.80) 8.62	15.48 (2.29) 14.79	13.00 (1.70) 13.08	10.68 (1.20) 11.23	0.87 (0.28) 32.18	0.84 (0.24) 9.45	0.47 (0.26) 55.32	1.71 (0.57) 33.33
Juv. n=6	April.	1.35 (0.30) 22.22	4.05 (1.21) 29.87)	58.56 (7.62) 13.01	67.62 (8.28) 12.24	23.29 (2.71) 11.63	12.92 (0.86) 6.66	13.96 (1.72) 12.32	12.03 (1.77) 14.71	2.40 (0.34) 14.17	3.43 (0.58) 16.91	1.27 (0.30) 23.62	1.26 (0.33) 26.19
Juv. n=6	June.	1.98 (0.56) 28.28	10.50 (4.95) 47.14	67.77 (8.15) 12.02	74.03 (13.11) 17.71	24.11 (2.34) 9.70	15.62 (1.65) 10.56	15.24 (2.13) 13.98	13.50 (1.48) 10.96	1.57 (0.53) 33.76	1.91 (0.60) 31.41	0.65 (0.27) 41.38	2.68 (1.64) 61.91
Juv. n=6	Nov.	1.09 (0.22)	1.56 (0.71)	60.78 (7.65)	59.30 (14.79)	22.54 (3.61)	13.34 (2.64)	12.26 (2.66)	10.21 (1.11)	1.06 (0.44)	1.02 (0.25)	0.52 (0.18)	0.87 (0.42)

Table 7.3. Two way ANOVA analyses of metal concentrations in Guillemots, *uria aalge,* to show age, sex and seasonal differences.

Metal.	Organ	Age and season.		Sex and season	
		Age ($F_{1,81}$)	Season ($F_{2,81}$)	Sex ($F_{1,63}$)	Season ($F_{2,63}$)
Cadmium	Liver	5.26 *	13.62 ***	0.44 N.S.	8.54 **
	Kidney	9.73 **	15.14 ***	3.63 N.S.	8.11 **
Mercury	Liver	10.86 **	66.04 ***	2.2 N.S.	57.2 ***
	Kidney	8.60 *	89.73 ***	1.5 N.S.	73.2 ***
	Muscle	3.89 N.S.	57.34 ***	0.0 N.S.	43.8 ***
	Feather	3.7 N.S.	6.26 *	4.9 *	3.0 N.S.
Zinc	Liver	1.64 N.S.	12.60 ***	0.59 N.S.	14.15 ***
	Kidney	3.87. N.S.	1.29 N.S.	0.23 N.S.	0.22 N.S.
	Muscle	0.48 N.S.	7.17 *	1.52 N.S.	7.18 *
Copper	Liver	6.34 *	2.92 N.S.	0.03 N.S.	1.12 N.S.
	Kidney	0.54 N.S.	2.58 N.S.	6.27 *	0.99 N.S.
	Muscle	0.10 N.S.	40.00 ***	0.67 N.S.	32.77 ***

N.S.= not significant at $p< 0.05$, *= $p<0.05$,**= $p<0.01$,***= $p<0.001$.

levels ranged from 25 to 89% of adult levels. Zinc concentration showed no age differences. Copper concentration in adult liver was significantly higher than in juveniles.

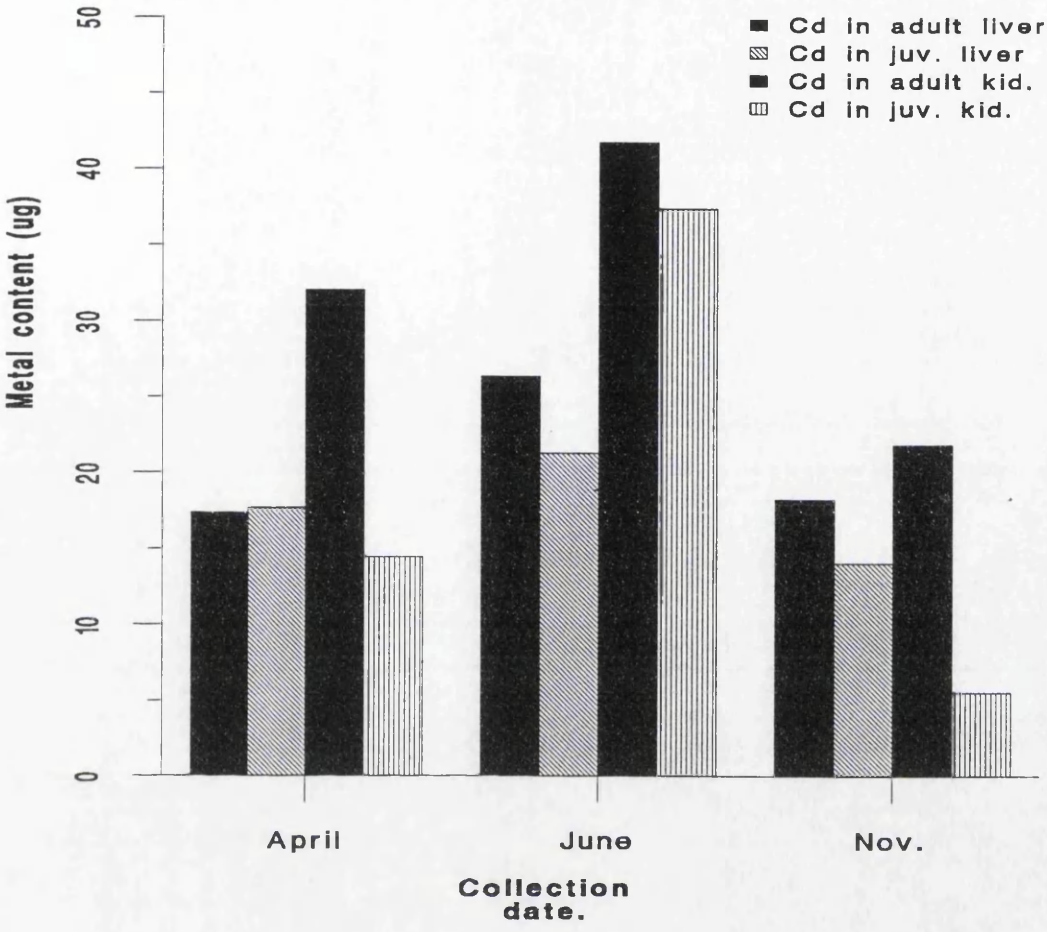
Both mercury and cadmium concentrations in the liver and kidney showed strong seasonal trends. This is seen in both adult and juvenile birds. These are illustrated in Figure 7.1. Mercury concentrations in internal tissues showed a general decline from April through to November. Cadmium concentrations however, increased significantly between April and June, then decreased to almost half the June level by November. Mercury levels in the muscle of the juvenile birds drops by almost half between April and June and is even lower by November. The feather level also varies in juveniles but the small sample sizes (n = 6, in each collection), makes further analysis difficult. Zinc levels in both adult and juvenile liver show a peak in the June collection, and muscle zinc levels also show some seasonal variation (Table 7.3).

Inter-Tissue and Inter-Metal Correlations

There were several inter-tissue and inter-metal relationships in the sample. Mercury levels in liver and kidney tissues were found to be significantly positively correlated in birds from all three collections (Figure 7.2), (April, $r = 0.77$, $P < 0.05$; June, $r = 0.81$, $P < 0.001$; November; $r = 0.48$, $P < 0.05$). Mercury levels in liver and muscle tissues were significantly and positively correlated in birds from the first and second collections, (April; $r = 0.59$, $P < 0.01$: June; $r = 0.51$, $P < 0.05$). Feather mercury concentrations were found to be positively and significantly correlated with liver mercury levels in birds from the third collection ($r = 0.47$, $P < 0.05$).

Cadmium in the kidney was significantly positively correlated with zinc in the kidney in April and June, but not in the November collection (April, $r = 0.43$, $P< 0.05$; June, $r = 0.52$, $P < 0.05$; November, $r = 0.12$, N.S.). Cadmium in the liver was significantly negatively correlated with zinc in the liver in the first collection (April; $r = -0.46$, $P <0.05$), but significantly positively

Figure 7.1 Seasonal variation in common guillemot metal loads.



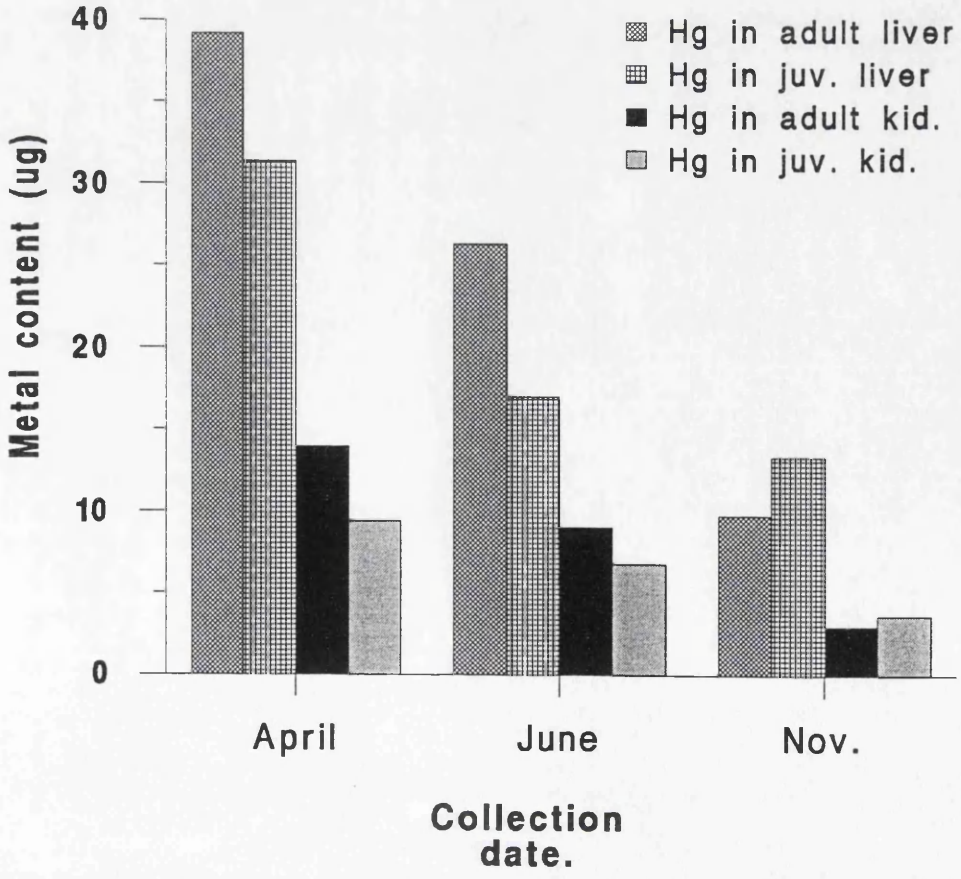
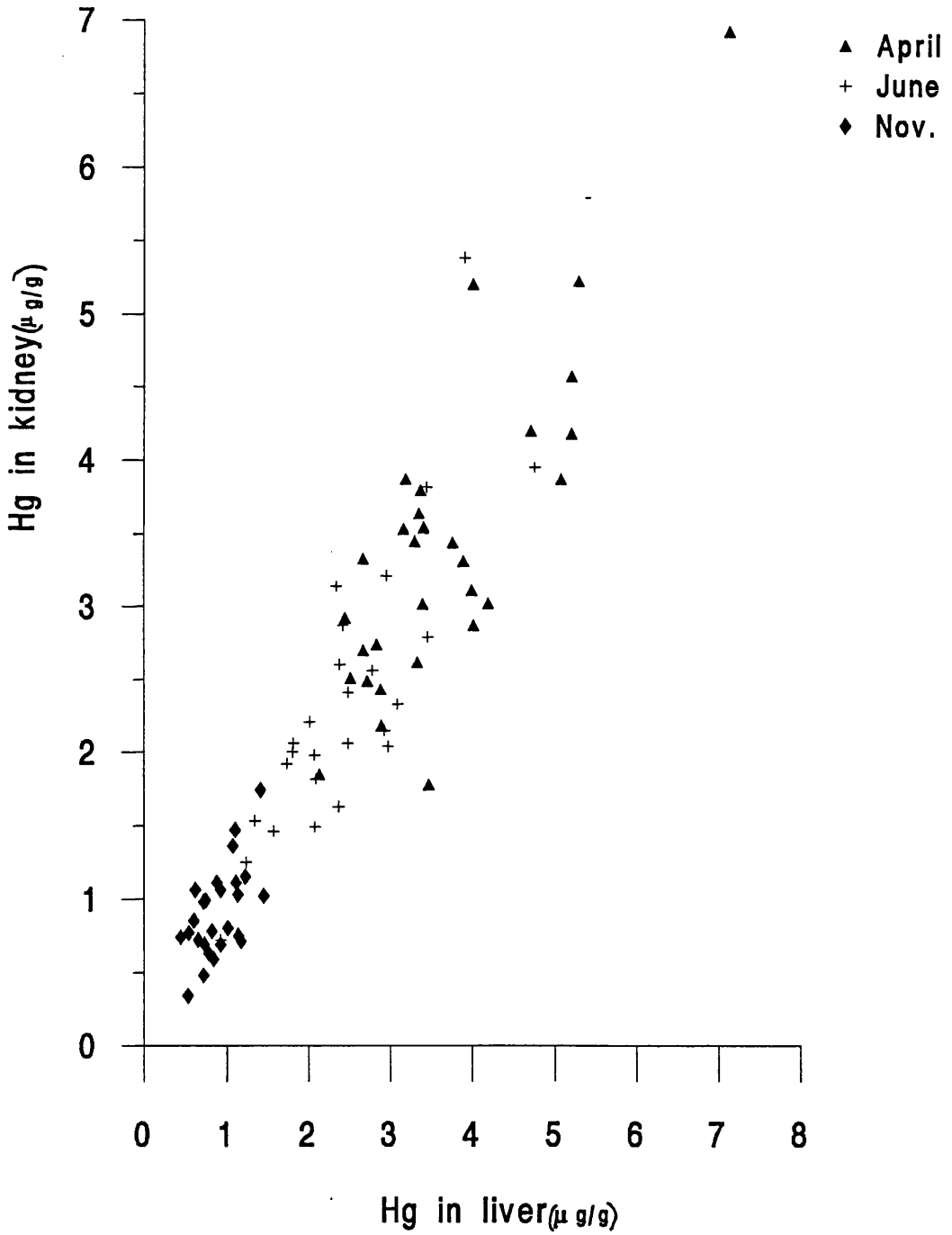


Figure 7.2 Mercury distribution in liver and kidney in the three collections



correlated in the second and third collections, (June; $r = 0.82$, $P < 0.001$: November; $r = 0.53$, $P < 0.05$).

Copper and zinc concentrations in the kidney were significantly positively correlated in all three collections grouped, ($r = 0.63$, $P < 0.001$). Copper and zinc concentrations in the liver were significantly positively correlated in all collections grouped ($r = 0.55$, $P < 0.001$).

Mercury concentration in the kidney was significantly positively correlated with copper and zinc concentrations in the kidney in the first collection (April, $r = 0.66$, $P < 0.05$, $r = 0.63$, $P < 0.05$, respectively), but not in the other two collections. There was no relationship between cadmium and mercury levels in any of the collections.

Coefficients of Variation

Coefficients of variation (CV) are shown in Table 7.2. The CV can be a useful tool in looking at the regulation of metals in birds (Walsh 1990). The CV of the essential metals, copper and zinc are generally low and quite uniform between age classes and seasons, usually under 20, whereas the CV of the non-essential metals are much higher and fluctuate more through the two age classes and three collection dates.

7.4 Discussion

Few studies have analysed metal levels in common guillemots. Most studies sampled birds during the summer months and so are comparable with the June collection in this study (e.g. Norheim 1987, Honda *et al.* 1990, Furness *et al.* in press b). Where necessary, we have converted concentrations expressed in relation to wet weights to a dry weight equivalent, using mean water content of common guillemot tissues determined in this study.

Mean liver cadmium levels were 2.49 μg/g and kidney levels of 11.72 μg/g (dry weight) in birds from Loch Broom. These are slightly higher than in common guillemots sampled in the North Pacific which had mean liver levels 1.4 μg/g and kidney levels 9.0 μg/g (Honda *et al.* 1990), but lower than in

common and Brünnich's guillemots *Uria lomvia* from Iceland which had mean liver levels of 5.58 and 9.84 μg/g, respectively (Furness *et al.* in press b). Brünnich's guillemots from Spitsbergen (Norheim 1987) also had considerably higher levels, with mean liver levels of 15.6 μg/g and kidney 64 μg/g.

Mean liver and kidney mercury levels in this study (2.52 μg/g and 2.54 μg/g, dry weight) are slightly higher than in North Pacific common guillemots (0.88 μg/g in liver and 0.72 μg/g in kidney), and also higher than mercury in common and Brünnich's guillemots from Iceland (1.32 and 1.20 μg/g dry weight in livers, respectively) but similar to the Brünnich's guillemot from Spitsbergen (2.4 μg/g in liver),(Norheim 1987, Honda *et al.* 1990, Furness *et al.* in press b).

These differences both within a species and between two closely related species probably reflect geographical variations in the cadmium and mercury in the oceans, and thus in the foodweb, though dietary differences may also be important. Levels were uniformly low in all studies, and would not cause any toxic effects.

Inter-Organ and Inter-Metal Correlations

There was a correlation of mercury concentrations between internal tissues (particularly liver and kidney tissues), birds with relatively high mercury levels in one tissue tending to have high levels in other tissues. Such inter-tissue correlations have been found in a variety of species (Fimreite 1974, Furness and Hutton 1979, Hutton 1981, Ohlendorf *et al.* 1985, Thompson *et al.* 1991), and indicate that mercury is able to accumulate in a range of tissues, although the highest levels have invariably been found in the liver and kidney (Table 7.1). The single weak correlation between liver and body feather mercury concentrations in birds from the third collection may be a result of body feathers being relatively unimportant in terms of mercury loss. It may be that internal tissue mercury concentrations would correlate more strongly with the mercury levels in first moulted feathers (flight feathers).

The relationship between cadmium and zinc concentrations in kidney and liver tissues is well established (Scheuhammer 1987). They both tend to bind onto the same low molecular weight protein (metallothionein). The correlation between cadmium and zinc concentrations in the kidney in June ($r = 0.52$) was stronger than in April ($r = 0.43$). It is possible that because of the rise in cadmium, more zinc has become bound onto the metalloprotein compared to the other zinc proteins. The November collection shows no significant relationship between cadmium in the kidney and zinc in the kidney tissue. Cadmium levels were almost half that of the June value in November. This may mean that cadmium levels are below the threshold limit for inducing increased metallothionein synthesis and therefore do not cause a corresponding increase in zinc levels. The relationship in the liver was somewhat different, probably due to the liver function as a zinc store and therefore likely to have high levels of endogenous metallothionein.

Mercury and cadmium in the birds' tissues showed rather different patterns throughout the year, and there were no relationships between cadmium and mercury concentrations in any of the collections. This is probably because all the mercury was in the methyl form and so would be lipid-soluble and also not be bound to metallothionein for storage.

Age Differences

Significantly higher cadmium levels in adults than in juveniles have been found in several other species (e.g. Maegden *et al.* 1982, Reid and Hacker 1982, Honda *et al.* 1986b, Blomquist *et al* 1987). Comparisons of adult and juvenile mercury levels in internal tissues are less well documented. Pectoral muscle levels of mercury in adult Baltic cormorants, *Phalacrocorax carbo*, common guillemots and black guillemots, C*epphus grylle,* were higher than in juveniles (Jensen *et al.* 1972). In the present study muscle levels were not significantly higher in adults, though levels in liver and kidney levels were. Several studies have found an increase in cadmium with increasing age (e.g.

Furness and Hutton 1979, Hutton 1981, Maegden *et al.* 1982, Reid and Hacker 1982, Blomquist *et al.* 1987), suggesting that the metal remains bound in the kidney, with a long biological half-life.

The evidence for bioaccumulation of both mercury and cadmium between hatching and reaching maturity is strong (see Walsh 1990 for more examples), but the continuing accumulation with increasing adult age is less clear.

Seasonal Variations in Metal Levels

There were clear differences in mercury, cadmium and zinc levels through the year in both adult and juvenile birds.

There are two hypotheses to consider: 1. The seasonal changes observed are due to physiological processes especially related to breeding and moult. 2. The fluctuations are a result of seasonal dietary changes.

1. Physiological Processes

The dates of the three collections spanned two major processes which could affect metal levels in internal tissues and feathers; namely reproduction and moult. Precise data for the timing of egg laying were not obtained for the birds sampled but most eggs would almost certainly have been laid in May (Birkhead 1980, Harris and Wanless 1985), between the first (April 26th) and second (June 25th) collections.

The seasonal fluctuations shown by metal concentrations and burdens between the first and second collections could be associated with lipid and protein mobilisation during reproduction. The peak concentrations of both cadmium and zinc in the internal tissues appear in the June collection, just after egg laying. During reproduction both male and female birds undergo large physiological changes which involve the uptake of nutrients and essential metals, including zinc and copper (Lofts and Murton 1973). Cadmium is known to associate with these metals, binding onto the same metalloprotein, and it is possible that more protein binding sites are available at this time and so more cadmium could be bound in the liver and kidney. Between the first

and second collections the mercury contents of tissues were reduced significantly. As the mercury in guillemot tissues was in the methyl form and not bound to metalloprotein, it would not be affected by changes in zinc and copper concentrations. The reduction in mercury in the birds at this time could be due to transport into the eggs (Lewis *et al.* 1993). Using data provided by Ratcliffe (1970) and Birkhead and Harris (1985), the fresh mass of egg contents in a guillemot egg from north-west Scotland would be approximately 100g. Parslow and Jefferies (1975) reported mean mercury concentrations in guillemot eggs from north and north-west Scotland of 1.2 μg/g dry weight (ca. 0.24 μg/g fresh weight). These values would lead to a theoretical figure of 100 x 0.24 = 24 μg mercury in a guillemot egg from north-west Scotland. The reduction in mercury contents in liver and muscle tissues in female guillemots in this study over the egg-laying period (17 μg and 42 μg respectively) exceed this value. The egg can act as an excretory route for mercury but mercury concentrations in eggs are generally low when compared to mercury burdens of females (Honda *et al.* 1986c). In addition the reduction in mercury content of the male birds and the lack of sex differences in metal loads, suggest that the egg is not being used as a major mercury sink.

Guillemots undergo a complete post-nuptial moult which generally commences in July in British birds (Harris and Wanless 1990). The primaries, secondaries and tail feathers are dropped simultaneously, though at slightly different times depending on the feather type, once the adults leave the nesting ledges. Moult of body and head feathers may commence before the birds depart for the sea (Birkhead and Taylor 1977, Ginn and Melville 1983, Harris and Wanless 1990). Therefore, birds will have undergone, and largely completed , post nuptial moult between the second (June 25th) and third (November 1st-2nd) collections. Two juveniles birds, collected in June (that is, one year old), had already dropped, and were regrowing their primary feathers.

Between the second and third collections, internal tissue mercury levels dropped still further as moult and feather growth was underway. These results clearly point towards the feathers being a major eliminatory pathway for mercury. Such a finding agrees with the well documented examples for other species. A decline in tissue mercury concentrations associated with feather growth has been demonstrated in black-eared kites *Milvus migrans lineatus* (Honda *et al.* 1986a) and Bonaparte's gull *Larus philadelphia* (Braune and Gaskin 1987), whilst mercury concentrations in primary feathers have been shown to correspond to the relative position of a feather in the moult sequence (Furness *et al.* 1986, Honda *et al.* 1986a, Braune 1987, Braune and Gaskin 1987). The mercury levels in guillemot body feather samples exhibited no significant seasonal trend over the three collection dates, as would be expected since these are renewed once per year. The significant drop in cadmium concentrations in liver and kidney between the second and third collections could also be due to loss into growing feathers. Although concentrations of cadmium in feathers are usually extremely low and often below detection limits, the amount of cadmium lost from kidney and liver is small and would be difficult to detect in feathers.

2. Dietary Changes

The changes in metal levels during the breeding season could be explained if birds switch to a prey item which is naturally higher in cadmium and zinc but lower in mercury. The birds sampled were found to show seasonal differences in diet. In April, 97% of the diet consisted of sandeel, *Ammodytes marinus*, but by June this was reduced to around 50%, with whiting, *Merlangius merlangus*, making up the largest part of the diet. By November no sandeel and few whiting were found, and the diet was mainly made up of poor cod, *Trisopterus minutus* (34.4%), sprats, *Sprattus sprattus* (25.8%), cod, *Gadus morhua* (23.4%) and herring, *Clupea harengus* (16.4%), (Halley *et al.* in press). Although no data are available on metal levels in internal organs of

these prey specimens, they are likely to have differing metal burdens, between age/size and species, and birds will concentrate the heavy metals (Thompson 1990, Burger and Gochfeld 1993). Therefore the diet alone could create the changes in metal levels if turnover of cadmium and mercury is faster than previously estimated. Blomquist *et al.* (1987), calculated a half-life of 1.5-2.0 years for cadmium in the kidney of the dunlin, *Calidris alpina,* and some authors suggest cadmium concentrations in kidney increase throughout life (Hutton 1981). However Nicholson (1981) Stock *et al.* (1989) and Muirhead and Furness (1988), suggest that some seabirds could regulate levels of cadmium.

The active excretion of cadmium has been suggested by Stock *et al.* (1989), from their study of oystercatchers, *Haematopus ostralegus*. They found birds showed an increase in renal and hepatic cadmium levels with increasing age up to maturity but that adult birds did not have increasing cadmium burdens. Many other studies have found significant age differences between adult and juvenile birds, but there is little evidence for an increase with increasing age continuing throughout adulthood. Stock *et al.* (1989) proposed birds such as the oystercatcher which would have been exposed to natural sources of cadmium could balance cadmium intake with excretion, agreeing with the findings of Nicholson (1981). If guillemots could partially regulate cadmium concentrations in the kidney and liver, this may explain the seasonal variations in metal levels, caused by seasonal changes in their diet. This would explain the parallel changes in metal levels in juvenile birds through the season. Guillemots do not start breeding until 4-5 years old (Cramp and Simmons 1985). The young birds in this study therefore would not be breeding and so would not undergo the physiological changes which may influence the uptake and excretion of metals. However they were foraging along with the adult birds. In addition the birds in the study were shot at sea and not at the colony, so not all the birds classed as adults would be breeding.

The ability to regulate concentrations of some non-essential metals could also explain the lack of seasonal variation in cadmium and mercury in dunlin (Goede *et al.* 1989). However levels of metals in that study were very low (often below detection levels) and the samples sizes were very small. Osborn (1979) found fat and protein content of the liver varied throughout the year as much as metal concentrations and concluded that organ loads of metals should be used in preference to concentrations. In this study we used dry weight, and organs did not vary in mean dry weight through the year. Therefore changes in concentrations of metals were equivalent to changes in organ loads.

Birds which undergo a complete annual moult and which are exposed to relatively low levels of mercury, feather growth following moult constitutes the major eliminatory pathway for mercury. The single egg, although likely to contain mercury at levels comparable to those found in other studies of this species (Parslow and Jefferies 1975, Barrett *et al.* 1985), is unlikely to represent an important excretory route for mercury in this species.

This study has suggested that cadmium has a shorter biological half-life than previously thought, in contrast with cadmium in humans and other mammals (Scheuhammer 1987). Further studies would be needed to establish if this is due to an active regulatory mechanism, or merely a product of normal protein and cell turnover. Seabirds have been exposed to cadmium in the marine environment throughout their evolution and it is possible they have evolved stronger regulatory mechanisms than found in terrestrial mammals or birds.

7.5 **References**

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Chapter Eight An Experimental study of the uptake and assimilation of cadmium, and its effects on metal-binding proteins and essential metals in great skua *Catharacta skua* chicks

8.1 Introduction

The usefulness of seabirds as monitors of heavy metals is based on the assumption that the concentrations recorded in seabird tissues reflect environmental variation. The assimilation and tissue accumulation of metals by seabirds must therefore reflect the amount in the seabirds' diet. Diet dependency in seabirds is usually inferred from knowledge of prey items, or proximity to known areas of pollution (Hulse *et al.* 1980, Hutton 1981, Howarth *et al.* 1981, Maegden *et al.* 1982, Reid and Hacker 1982, Ohlendorf and Harrison 1986, Brothers and Brown 1987, Muirhead and Furness 1988). Experimental work has addressed this assumption for mercury (Lewis 1991), but there has been no study to assess the degree of dietary uptake of cadmium by seabirds. This is essential if seabirds are to be effective monitors of cadmium in the marine environment.

The intestinal absorption of cadmium in mammals is relatively low and dose dependent, and the percentage of the dose retained increases as the dose increases (Engstrom and Nordberg 1979, Lehman and Klassen 1986). Scheuhammer (1988) demonstrated this experimentally in quail, the only study to date which has investigated the dose response in birds.

The absorption of cadmium is also affected by other factors. Dietary deficiencies of calcium, zinc or iron, dietary fibre and copper content and damage to the intestinal epithelium can all cause increased cadmium uptake (Scheuhammer 1987, Rambeck and Kollmer 1990). Nicholson and Osborn (1983) also suggested that there may be interactions between cadmium and mercury, resulting in co-accumulation of high concentrations in seabirds.

Seabirds are a highly specialised group which have been exposed to naturally occurring heavy metals throughout their evolution. As a consequence they are likely to show specialised physiological responses in their absorption and assimilation of heavy metals. This will be affected by several biological processes i.e. feeding specialisation, reproduction, moult, migration, and there may also be antagonistic effects of other

metals. Seabirds may exhibit quite different responses in heavy metal uptake in comparison with mammals and terrestrial birds.

Experiments on the uptake of cadmium using adult seabirds would be almost impossible logistically, and confounded by the amount of cadmium already accumulated which could not easily be assessed. Nestlings and fledglings have been advocated as useful metal biomonitors, as chick metal burdens should reflect bioavailability from the foraging area around the breeding grounds, and metals are accumulated over a known time period after hatching (Frank 1986, Walsh 1990, Furness 1993). Free-living great skua chicks were therefore used as a seabird model in an experimental study to quantify the dietary uptake of cadmium in their natural environment at the colony. Growth of chicks during the course of the experiment may have an effect on the metal uptake, although Thompson *et al.* (1991) found no differences in mercury concentrations with age in great skua chick feathers.

Two Experiments were designed to test the responses of seabirds to elevated dietary cadmium levels. The objectives of this study were;

1. To measure the uptake from a range of cadmium doses in seabirds living in natural conditions.
2. To compare the distribution of cadmium in the kidney, liver and duodenum over the dose range.
3. To investigate whether higher concentrations of the metal-binding protein metallothionein are induced with increased dietary levels of cadmium and the consequences of this on concentrations of the essential metals zinc and copper.
4. To investigate whether higher cadmium levels in the diet result in any synergistic increase in mercury concentrations
5. To look at the effects of age on the natural uptake of cadmium and mercury in great skua chicks.

8.2 Materials and methods

These Experiments were carried out under licence from Scottish Natural Heritage and the Home Office in late June and early July 1992. Great skua nests were located and

marked with a bamboo cane placed 10 metres north of the nest. A single chick from each brood was ringed and assigned to an Experimental group. Five birds were assigned to each group. Chicks were weighed to the nearest 5g and wing length measured to the nearest mm. Chicks can be aged accurately from wing measurement (Furness 1977). Chicks of roughly the same age were selected, to correct for any age related differences and dosed orally using a gelatine capsule containing cadmium chloride. It has been shown that handling chicks and dosing them orally via a gelatine capsule will not affect them in any adverse way (Lewis 1991). Cadmium was administered as cadmium chloride as it has been shown that oral dosing either as cadmium chloride or cadmium metallothionein results in similar uptake by the kidney and liver tissues (Maitani *et al.* 1984, Scheuhammer 1988), and the latter appears to be more toxic (Nordberg *et al.* 1975).

In Experiment one, single doses of 0.1, 1.0, 10.0, 50.0 and 100mg/kg cadmium chloride were administered (dose groups 1-5). Three days were allowed for the uptake and assimilation of the single dose, then birds were sacrificed by cervical dislocation. In Experiment two, three groups were given two doses of 1.0, 5.0 and 20.0mg/kg, and three groups were given three doses of 1.0, 5.0 and 20.0mg/kg (dose groups 6-11), with three days between each dose. Again, three days were allowed after the last dose before the birds were sacrificed. Lower doses were administered in the multiple dose groups to avoid the accumulation of concentrations high enough to be toxic.

A control group of chicks of varying ages was collected by walking around the colony and retrieving any freshly killed chicks. Unattended chicks are quite frequently killed by adults from adjacent territories. A total sample of 22 controls were collected. Wing measurements were taken for these birds, but they could not be weighed accurately as often the body cavity had been opened and the viscera partially eaten. All birds were aged from their wing length measurements (Furness 1977). The chick survival rate was generally low, food availability deteriorated as the season advanced and some Experimental birds were also eaten, or recovered with organs missing. Consequently

sample sizes in each group were smaller than expected, and some analyses are missing kidney, liver or duodenum data.

All birds were frozen at -20°C after collection, prior to further treatment and transported frozen back to Glasgow. After thawing, chicks were dissected using stainless steel instruments. Whole liver and kidney and an 8cm portion of the intestine just posterior to the stomach, the duodenum (after Scheuhammer 1988), were removed. The duodenum was cut open, the contents removed by washing with distilled water, before blotting on filter paper and weighing. Whole liver and kidney were weighed. Portions of around 1.0g were placed in 4 volumes (w/v) 0.25M sucrose solution and prepared for metallothionein analysis. Portions of liver, kidney and duodenum were weighed and dried in an oven at 50°C to constant mass for metal analysis. The ratio of wet weight/dry weight was calculated for use as a conversion factor.

8.2.1 Metallothionein analysis

Methods as described in Chapter 3.

8.2.2 Metal analysis

Analyses were performed as described in Chapter 3 for cadmium, zinc, copper and mercury. Mercury concentrations in the duodenum were not analysed.

8.2.3 Statistical analysis

Due to small sample sizes non-parametric statistics were used in the initial analyses. For further analyses the different treatment groups were pooled and the data were tested for normality using Kolmogorov-Smirnov one-sample tests. As these data were normally distributed, parametric correlation analyses were performed.

8.3 Results

There were no differences in the weight of Experimental chicks when compared with the other chicks of the same age in the colony, (data from Ratcliffe 1993), Mann-Whitney U-test, U =0.29, W = 0.32, Z = -0.57, n = 276, N.S. Cadmium concentrations in the kidney and liver of control birds showed no significant relationship with age (kidney: r = 0.20 n = 20, N.S., liver: r = -0.43, n = 13, N.S.). Mercury concentrations in soft tissues of control birds appeared to decrease with age, but this trend was not

significant (kidney, r= -0.40, n = 20, N.S., liver: r = -0.35, n = 13, N.S.). These results are illustrated in Figure 8.1 and 8.2.

8.3.1 Dose response

The dose response was calculated as the percentage of the total dose given to each chick recovered in the whole kidney plus whole liver plus duodenum. The total amount of cadmium recovered was subtracted from the mean amount of cadmium accumulated in all intact control birds. The administration of oral cadmium resulted in an increase in the cadmium burden of chicks in all but the lowest dose group. At a single dose of 0.1 mg/kg chick metal burdens were not higher than control birds. The dose responses of all the other treatments are illustrated in Figure 8.3. In Experiment one the percentage of the dose recovered did not vary significantly with the cadmium dose administered. At 1.0 mg/kg the dose response was 0.89% (n = 4), at 10 mg/kg it was 0.37% (n = 5), at 50 mg/kg 0.42 %, (n = 5) and at the highest dose, 100 mg/kg, 0.33% (n = 4), Kruskal-Wallis 1-way ANOVA, $\chi^2 = 3.44$, n = 22, N.S.

In Experiment two the results were similar. Two doses of 1mg/kg gave a dose response of 0.83% (n = 5), three doses of 1mg/kg, 0.56 % (n = 4). Two doses of 5mg/kg gave a dose response of 0.54% (n = 5), and three doses, 0.50 (n = 3). Two doses of 20mg/kg, 0.23% (n = 4), with three doses giving a response of 0.50% (n = 2). Again, there were no significant differences in these responses, Kruskal-Wallis 1-way ANOVA, $\chi^2 = 3.7$,n = 23, N.S.

When doses were compared over all the treatments there were no significant differences in the dose response (groups 2-11), Kruskal-Wallis 1-way ANOVA, $\chi^2 =$ 10.40, n = 40, N.S. The overall average dose response was 0.51%.

8.3.2 Tissue distribution of cadmium

In general, the cadmium given as an oral dose is distributed throughout the duodenum, liver and kidney. Tissue distribution into liver, kidney and duodenum from a single dose is shown in Figure 8.4.

Mean tissue concentrations of cadmium, zinc, copper and metallothionein in control birds and birds given a single dose are given in Table 8.1. for Experiment one, Table 8.2

for Experiment two. Mean concentrations of mercury for all treatments are given in Table 8.3.

In Experiment one, at the lower doses (0.1, 1.0 and 10.0mg/kg), there was a tendency for concentrations of cadmium in the kidney to be higher than the concentrations of cadmium in the liver, and at the higher doses (50 and 100mg/kg), the concentrations in the liver exceed those in the kidney. Duodenum concentrations are generally slightly higher than the kidney concentrations, except at 50mg/kg where cadmium in the kidney is higher.

In Experiment two, cadmium concentrations in the kidney exceed those in the liver in all treatments. Cadmium concentrations in the duodenum are generally higher than cadmium concentrations in the kidney, with the exception of group 10 and 11, where mean duodenum concentrations are lower than those accumulated either by the liver or kidney. Tissue distribution is illustrated in Figure 8.5 for Experiment one. and Figure 8.6 for Experiment two.

8.3.3 Effects of different doses

In Experiment one the concentrations of cadmium in the kidney, liver and duodenum were all significantly affected by the different dose levels. There were no significant differences in zinc, copper, mercury or metallothionein concentrations between the dose groups. The results of the Kruskal-Wallis one-way ANOVA, of metal concentrations by dose group are shown in Table 8.4.

For Experiment two the results are similar, cadmium concentrations were affected by dose group, but concentrations of the other metals and metallothionein were not (Table 8.5).

8.3.4 The effects of increased cadmium concentrations on metals and metallothionein concentrations

As there were no significant differences in the dose response throughout the dose groups, and as the sample sizes were small in each group, all groups in each Experiment were pooled to look at correlations among metals and metallothionein concentrations both within and between tissues. The results of these are given in Tables 8.6 and 8.7 for

Experiment one, and Tables 8.8 and 8.9 for Experiment 2. It is clear that although the different dose levels had no significant effect on the zinc and metallothionein concentrations, the elevated cadmium concentrations resulting from the doses did show some significant positive correlations between the metal and metallothionein concentrations.

There were few correlations between metals and metal-metallothionein concentrations in the control birds. Sample sizes were however small (n = 20 for kidney, n = 13 for liver and n = 15 for duodenum), with many control chicks missing organs. In control birds metallothionein concentrations in liver and kidney correlated ($r = 0.68$, $n = 13$, $P = 0.06$).

In Experiment one cadmium showed a strong relationship throughout the kidney, liver and duodenum (Figure 8.4). Zinc concentration in the kidney was weakly, but non-significantly correlated with zinc concentration in the liver. Metallothionein concentrations in the liver and kidney showed a strong positive correlation. Copper concentrations showed no relationships between any tissues.

Within each organ the cadmium concentration showed a significant positive correlation with both zinc and metallothionein concentrations, with the exception of cadmium and metallothionein in the duodenum. The copper concentrations did not correlate with any other metal or metallothionein, apart from with zinc concentrations in the liver. Some relationships are illustrated in Figures 8.7, 8.8, and 8.9.

In Experiment two the results are rather different. Again cadmium in the liver and kidney are correlated, and metallothionein in liver and kidney are correlated.

However within each organ there are fewer significant correlations. Metallothionein concentration in kidney and duodenum correlate with copper concentrations in kidney and duodenum, and the metallothionein concentrations in the liver and duodenum correlate with zinc concentrations in these two organs. However metallothionein shows no significant relationship with cadmium concentration in any of the organs.

8.3.5 The effect of increased cadmium concentrations on the mercury concentrations

There were no significant relationships between the concentrations of mercury and those of any other metal or the metallothionein in the tissues analysed in either the control group or Experiment one or two.

8.4 Discussion

Thompson *et al.* (1991) found no increase with age in the mercury concentrations of great skua chick feathers sampled from age 20 to 45 days, and found that the concentrations were low compared to adult levels. The lack of an age-related trend was attributed to the dilution effect on metal concentrations caused by the growth of chicks. This also appears to be the case in kittiwake chicks dosed with mercuric chloride (Lewis 1991). This present study found no significant age-related changes in either mercury or cadmium concentrations in liver or kidney (Figure 8.1 and 8.2), although mercury concentration did appear to decrease with age (although not significantly), which would again be due to the dilution effect on mercury concentrations caused by growth of chicks' tissues. The lack of any significant effects of age on metal levels means that results from the Experiments would not be biased by chick age.

Other studies have found that chicks can accumulate metals with age (Cheney *et al.* 1981, Frank 1986, Honda *et al.* 1986b,). In these studies chicks were sampled over a much longer period (up to twelve weeks), which would account for the higher concentrations of metal accumulated.

Tissue concentrations of cadmium are dependent on the dose given (Table 8.1 and 8.2). Interestingly two doses of 5mg/kg result in the accumulation of higher concentrations of cadmium in tissues than a single dose of 10mg/kg. The administration of multiple doses may result in enhanced uptake of later doses due to the increase in metallothionein synthesis following the initial dose. This seems to be the case here; the dose response of the two 5mg/kg doses is a cumulative total of 0.52% in comparison with the single 10mg/kg dose which has a dose response of 0.37%.

Overall tissue concentrations accumulated in all the Experimental group are low, well below levels thought to cause toxic effects (see Chapter 2 for details).

These Experiments measured the uptake of cadmium in great skua chicks from a range of single oral dose concentrations of cadmium, in their natural environment. At the lowest dose administered (0.1mg/kg), the cadmium absorbed into the internal organs did not exceed the mean value accumulated by control birds. At 1.0mg/kg for both single and multiple doses the responses of 0.89% and 0.83%, respectively, are higher than the dose response from the higher doses, although not significantly so (see Results section). This is most likely because the dose response calculation uses the mean cadmium concentration of the control birds as a baseline, and as the actual cadmium content of individual birds varies considerably, this will affect the calculated result.

The dose response of all the other dose groups is very similar in both Experiments one and two, the percentage uptake of cadmium does not increase with increasing dose. These dose responses are lower than those found in laboratory dosed quail (Scheuhammer 1988) and in mammals (Decker *et al.* 1957, Kotsonis and Klassen 1977, Lehman and Klassen 1986). In quails at comparable doses of 0.5-5.0mg/kg the dose response was 0.6-0.7%, and at 50 mg/kg it rose to 2%. However these quail were dosed for four consecutive days, effectively quadrupling the amount administered. An initial increase in metallothionein concentrations may also have created more binding sites for the cadmium in the subsequent doses, although in this particular Experiment metallothionein concentrations did not appear to be elevated above basal concentrations (Scheuhammer 1988). A single oral dose of 6.6mg/kg given to laboratory rats resulted in an uptake of 1-2% into liver and kidney tissue (Decker *et al.* 1957), and rats given a range of doses (25, 50, 100, 150mg/kg) absorbed 1.5% of the dose (Kotsonis and Klassen 1977). Kotsonis and Klassen (1977) also found that the percentage absorbed was independent of the dose. However Lehman and Klassen (1986) orally dosed rats (doses of 1.0, 10.0, 1000, and 10,000μg/kg) and found that the concentration of cadmium in the tissues increased at a greater rate than the increase in dose; 0.4% was retained at the 1μg/kg dose and 1.65% was retained at 100μg/kg and higher doses. Scheuhammer (1988) also found that uptake was dose dependent in quail, rising with increasing dose. Great skua chicks seem to have a rather different response to the

administration of dietary cadmium. The percentage absorption of cadmium from a given dose is lower than in these studies and does not increase with increasing dose, though in my Experiment no birds were given very large doses.

Tissue distribution following the oral administration of cadmium in the great skua chick is comparable with studies on rats and quail. At the lowest doses the cadmium accumulates preferentially in the kidney (as it does under natural conditions), but at higher doses the liver accumulates more cadmium (Engstrom and Nordberg 1979, Lehman and Klassen 1986, Scheuhammer 1988).

There is little known about the mechanism of transfer of cadmium from the intestinal mucosa into the general circulation (Foulkes and McMullen 1986, Scheuhammer 1988). Cadmium absorbed in the intestinal epithelium is bound mainly to metallothionein (Lehman and Klassen 1986, Scheuhammer 1988), and may move into the blood as cadmium-metallothionein or a similar cadmium polypeptide complex. Once cadmium is in this form it will preferentially accumulate in the renal tissue.

In great skua chicks cadmium distributes preferentially to the kidney at both low single doses (0.1, 1.0, and 10.0mg/kg) and all multiple dose levels (1.0, 5.0, and 20.0mg/kg). Only at the highest single doses (50.0 and 100mg/kg) does the distribution change and more cadmium accumulates in the liver in comparison with the kidney. At these higher doses the mechanism of uptake is thought to be different. Cadmium may be absorbed as a free metal and become bound to albumen and other high molecular weight proteins which are then initially deposited into the liver (Lehman and Klassen 1986, Scheuhammer 1988).

The preferential accumulation of cadmium by the liver compared to the kidney, coupled with the increase in the dose response at the highest dose was thought to indicate a toxic response in quail, by the direct cytotoxicity of cadmium on the intestinal epithelium (Scheuhammer 1988). Quail were given 50mg/kg/day, a total dose of 200mg/kg. The preferential accumulation of cadmium in the liver of the great skua chicks occurred at single doses of 50mg/kg and 100mg/kg. At these dose levels, although liver concentrations did exceed kidney concentrations the overall percentage

accumulated was no greater than from the lower doses. This indicates that at higher doses cadmium distributes differently even in the absence of a toxic response. It is possible however that in the highest treatment groups, cadmium concentrations could be approaching a level where there would be such a toxic response.

Cadmium concentrations in the duodenum accumulated in association with cadmium in the liver and kidney (Figure 8.6). These did however vary between the groups. Initial cadmium uptake by the intestine can be up to 60% of the dose, but most of this will be excreted by the rapid desquamation of the intestinal cells (Scheuhammer 1987). Therefore duodenal concentrations vary according to the exact delay since the initial dose, and possibly also individual differences in food uptake, which affect digestion rates. Although in this study, birds were collected as close as possible to three days after dosing, there was some variation. Birds dosed early on the first day may have been collected late on the third day.

The different dose levels did not have an effect on essential metals or metallothionein concentrations, but the resulting cadmium concentrations showed significant correlations with zinc and metallothionein (see Results section). In control birds there were few inter-organ and inter-metal correlations. This was probably due to the fact that they were growing, had only accumulated low concentrations of cadmium, and also the sample sizes were small. Zinc, copper and metallothionein concentrations in control birds would be in a state of constant flux, as growth and development create changing requirements for these metals (Honda *et al.* 1986 b). Gochfeld and Burger (1987b) found distinct differences in inter-metal correlations between adult and young terns, and attributed this 'correlation chaos' to the young terns inability to regulate metals at a young age.

Metallothionein can be rapidly synthesised and degraded and therefore will show a rapid response to the presence or absence of metalls (Karin 1985). However, in Experiment two the concentrations of cadmium administered were not high enough to cause a significant change in metallothionein homeostasis and so resulted in few inter-metal correlations (Tables 8.7 and 8.6). Cadmium administered in quails did not elevate

the metallothionein concentration in the kidney, liver or duodenum (Scheuhammer 1988) and this was attributed to high basal metallothionein levels because of a high zinc content in the birds diet. This is also seen in Experiment one.

Results from Experiment one show there were several strong correlations both inter-organ, inter-metal, and between metals and metallothionein. However, there seems to be no consistent increase in the concentrations of metallothionein and zinc in the experimental birds when compared to the control birds. This is most likely because the cadmium taken up into the birds became bound onto metallothionein which was already synthesised, and did not induce an increase in metallothionein production. There does appear to be an increase in zinc and metallothionein at the highest dose given (100 mg/kg), whereby the metallothionein would have increased first, followed by an increase in the zinc concentrations, because of the increased availability of binding sites. Scheuhammer (1987), in an Experimental study of ring doves found where both zinc and copper concentrations were affected by the changes in cadmium concentrations. Di Guilio and Scanlon (1984 a) found significant effects of dietary cadmium on the concentrations of zinc and copper in the kidney, but only on zinc in the liver, with copper concentrations remaining unaffected. However both these studies were conducted over a much longer period of time, and resulted in a higher uptake of cadmium. In great skua chicks the metallothionein induced at the highest dose could be acting in a detoxifying role by storing the cadmium, which also occurs in adult lesser black-backed gulls, which have naturally accumulated higher concentrations of cadmium bound onto metallothionein (see Chapter 5).

Nicholson *et al.* (1983) hypothesised that mercury and cadmium may have some synergistic effect, as birds which have high natural cadmium burdens often also have high mercury burdens (Osborn *et al.* 1979, Nicholson and Osborn 1983, Thompson 1990). The synthesis of metallothionein can be induced by several metals including zinc, copper, cadmium and mercury (Scheuhammer 1987), and it is known that inorganic mercury binds to metallothionein (Piotrowski 1974). In Chapter 5 where metals had accumulated naturally there were no relationships between concentrations of

cadmium and mercury but levels in these birds were low. In these Experiments on great skua chicks dosing created cadmium concentrations which were much higher than the naturally accumulated levels but which did not result in synthesising an increase from endogenous levels of metallothionein. Only one bird, from the highest single dose group, showed an elevated mercury concentration in liver, (2.3μg/g) corresponding with a high cadmium concentration (34.0μg/g), but there was no evidence of this in Experiment two. The possibility of synergistic accumulations at very high levels of both metals cannot be ruled out as in this Experiment metallothionein levels were not increased and birds did not have particularly high cadmium concentrations. The Experiments also were conducted over a short period of time. The implications of this will be discussed in Chapter 9.

In conclusion, cadmium administered to seabirds at the lower doses in the form of cadmium chloride successfully mimics natural accumulation, whereby at the lower doses the kidney is the preferential accumulation site. Increased cadmium in birds' tissues only appeared to affect the natural balance of metallothionein and zinc at the highest concentrations and there were no interactions with either copper or mercury. Great skua chicks seem to absorb a lower percentage of cadmium from a given dose in comparison to mammals and terrestrial birds, and this does not increase with increasing dose.

This provides preliminary evidence that seabirds are perhaps relatively resistant to the presence and effects of cadmium. Seabirds have been exposed to heavy metals throughout their evolution and could have evolved mechanisms to limit absorption of this metal.

This study has provided a baseline for use in future monitoring research. The dose response figure of 0.5% uptake from diet can now be used in future work on the relationships between the cadmium content of seabird diets and the cadmium concentrations in internal tissues, enabling more accurate calculations of the metal bioavailability to the seabirds.

Figure 8.1 Cadmium in the liver and kidney of control birds

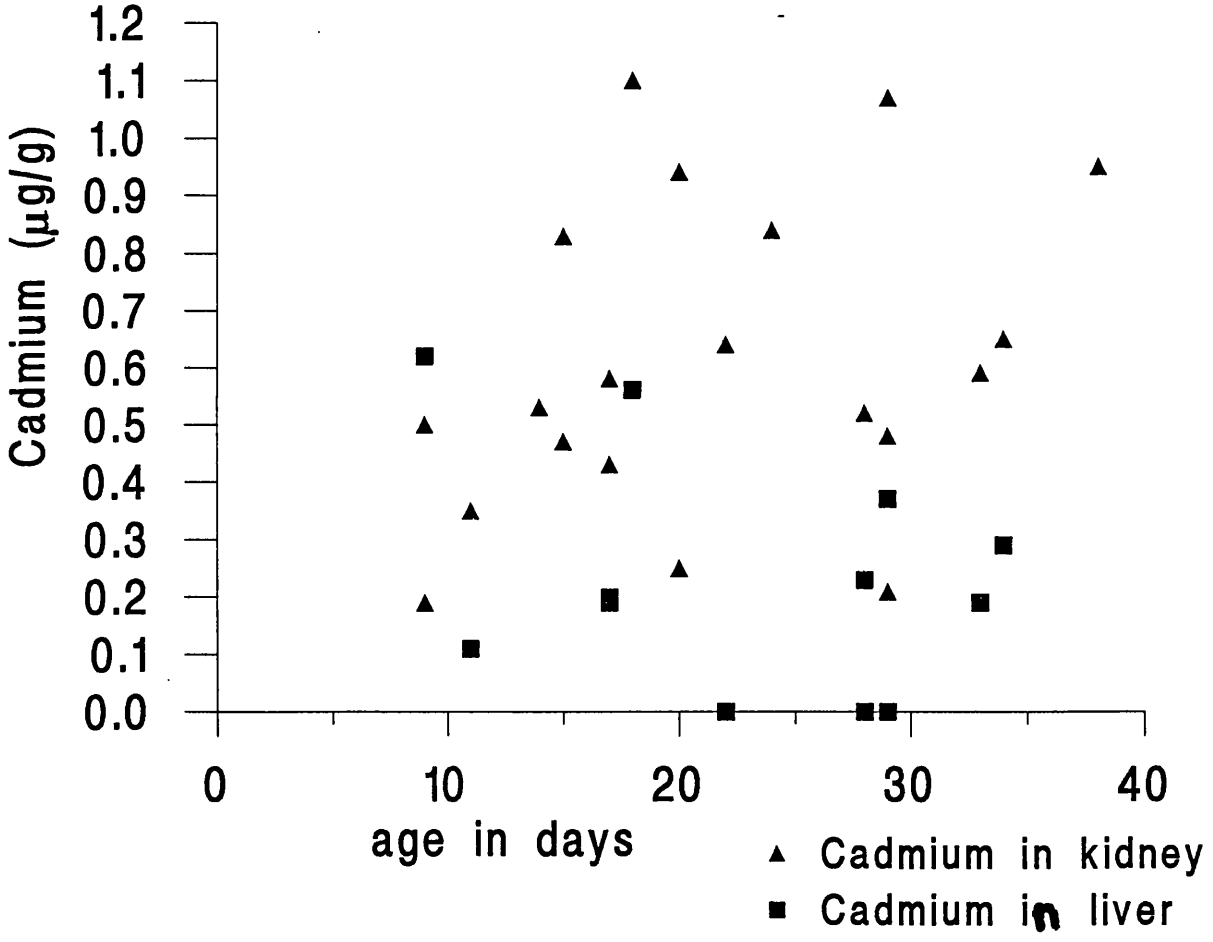


Figure 8.2 Mercury in the liver and kidney of control birds

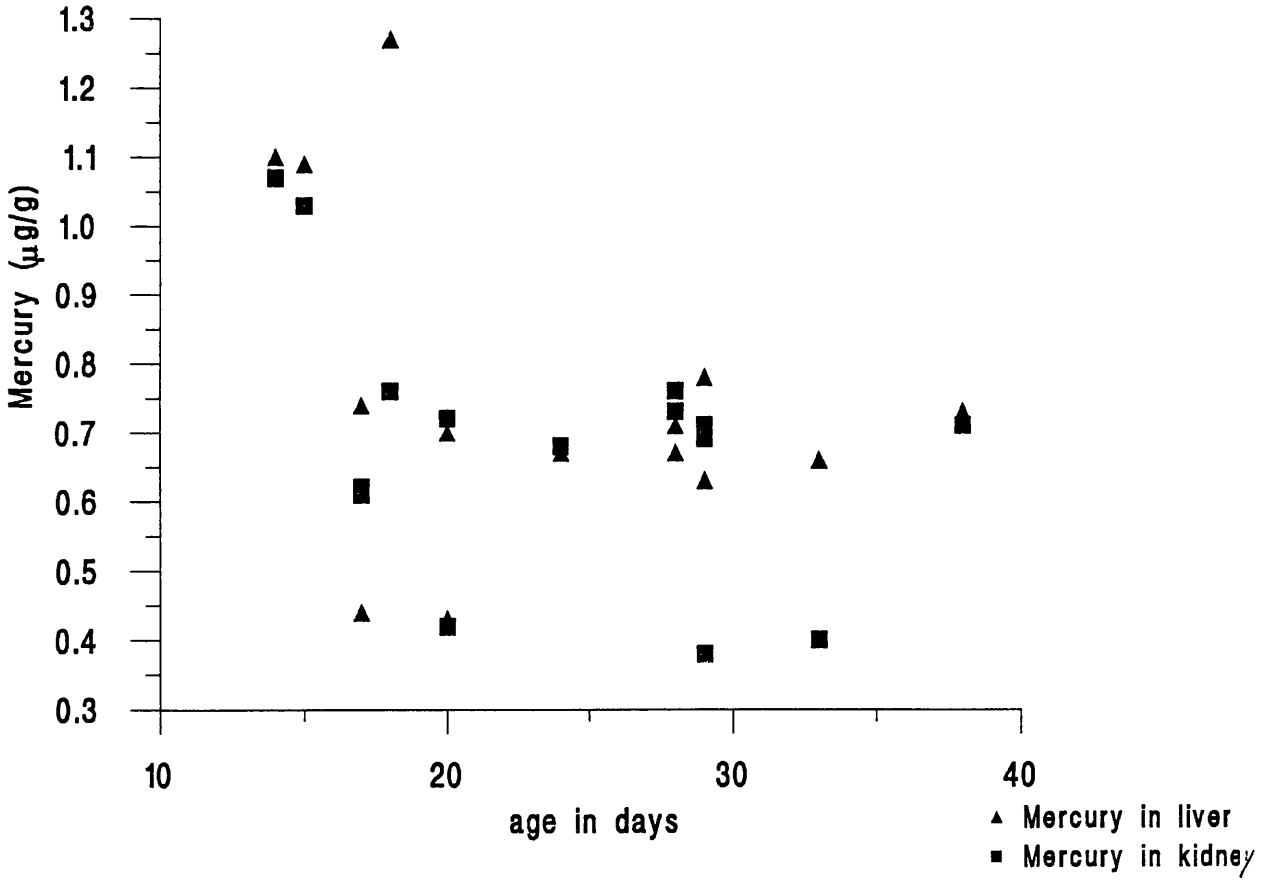


Figure 8.3 % of total dose recovered in the kidney+liver+duodenum from a single dose (values are means with S.E. bars)

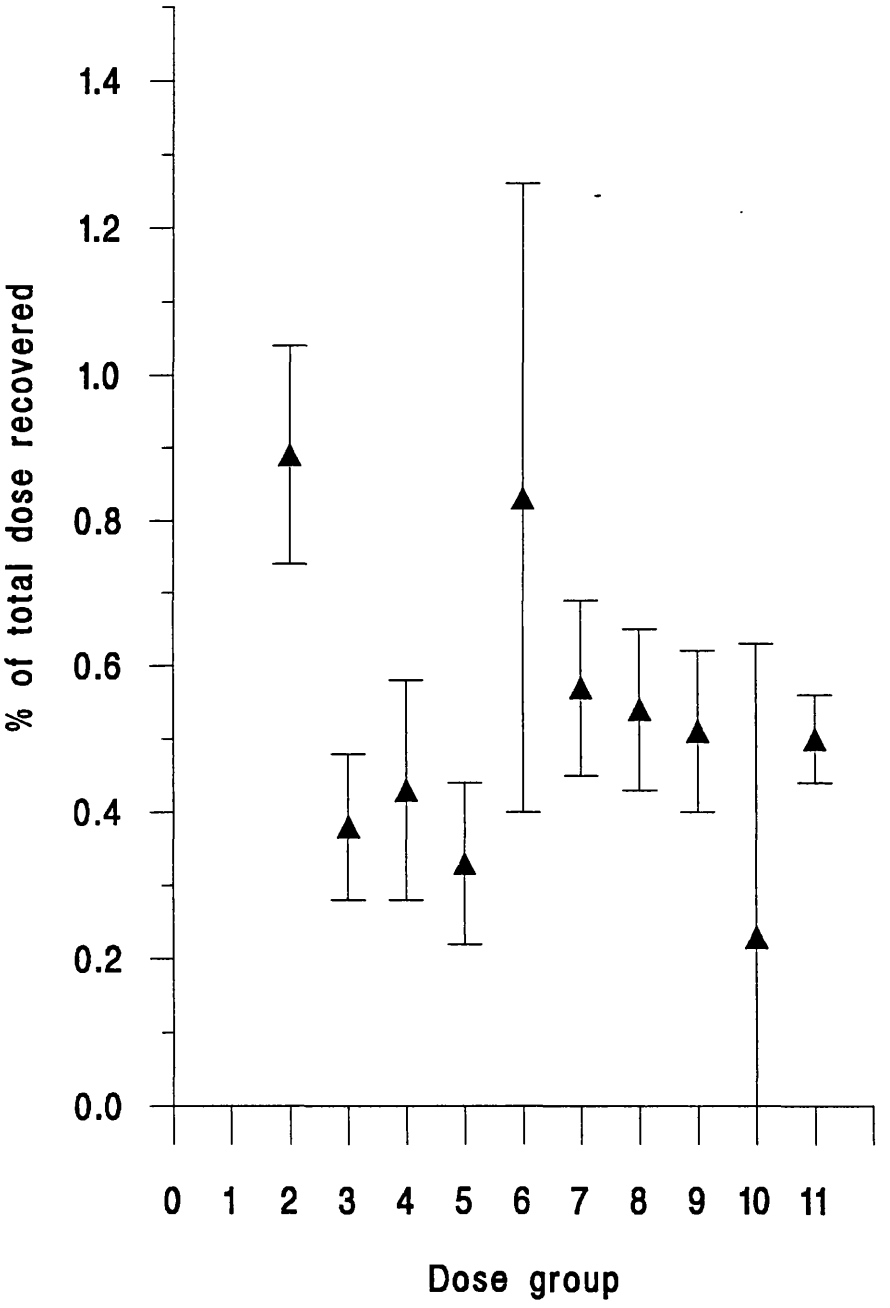


Figure 8.4 The distribution of cadmium in bird tissues from a single dose

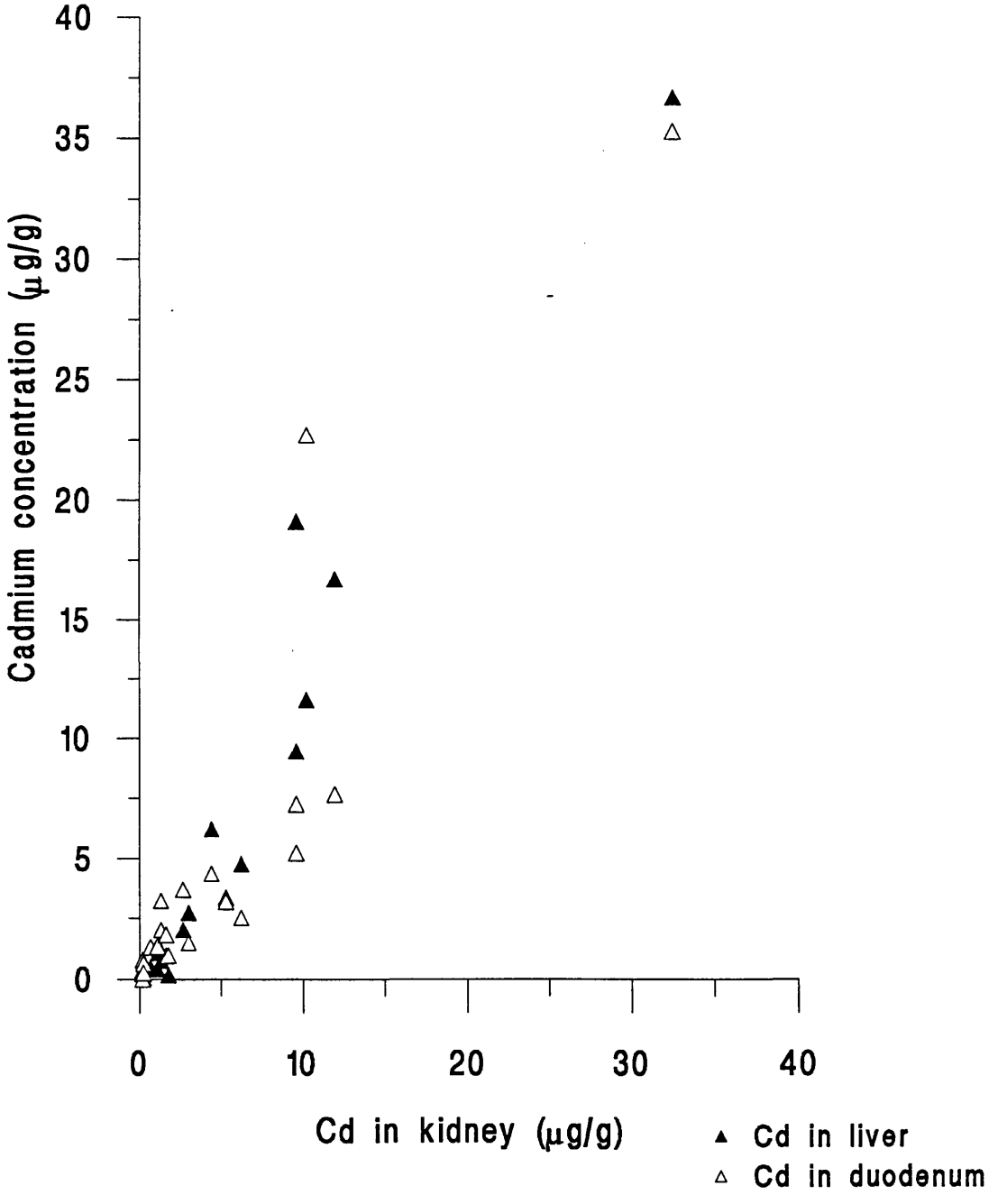


Figure 8.5 The accumulation of cadmium concentrations in kidney, liver and duodenum after a single dose.

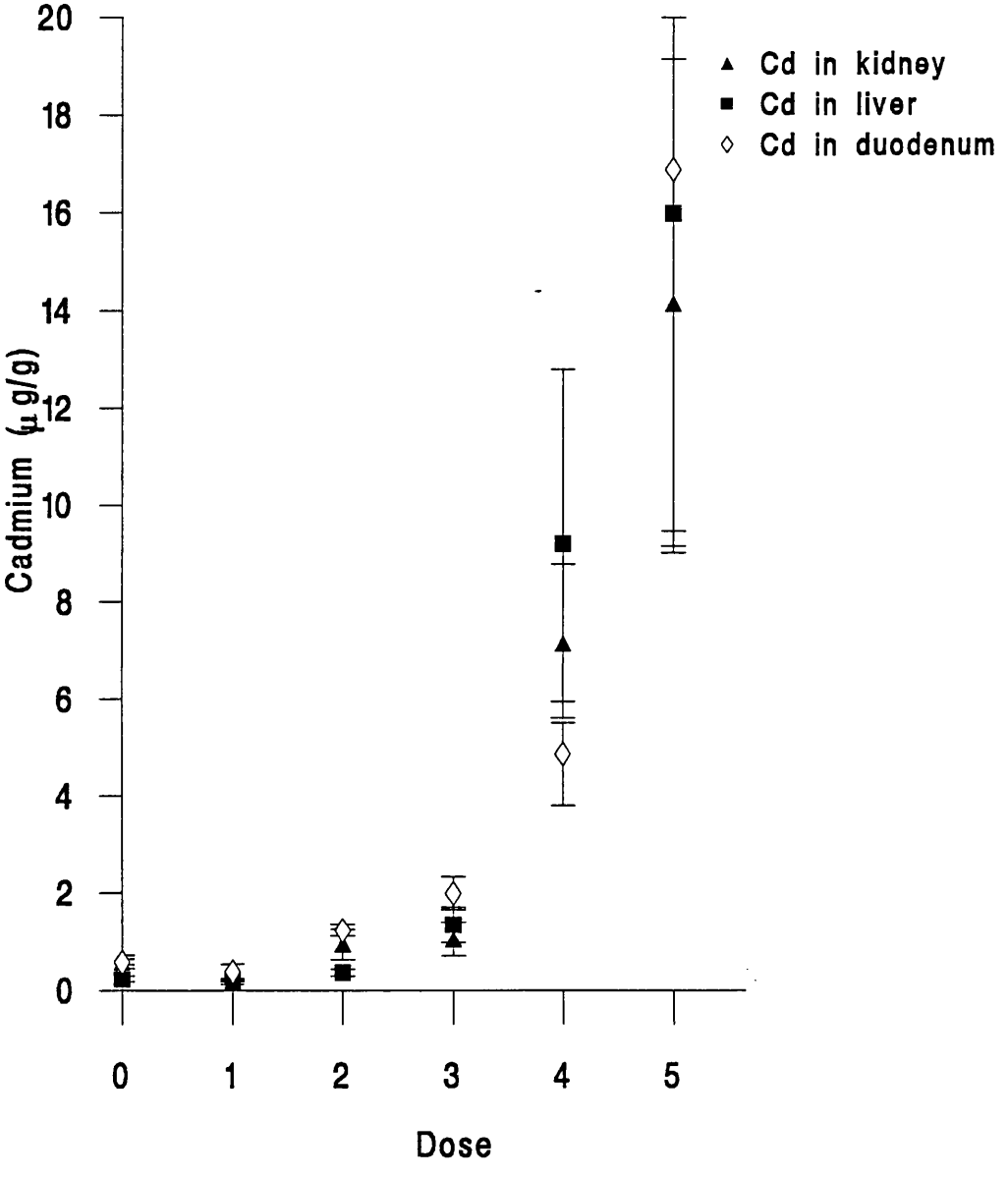


Figure 8.6 Cadmium concentration in tissues in birds from experiment two

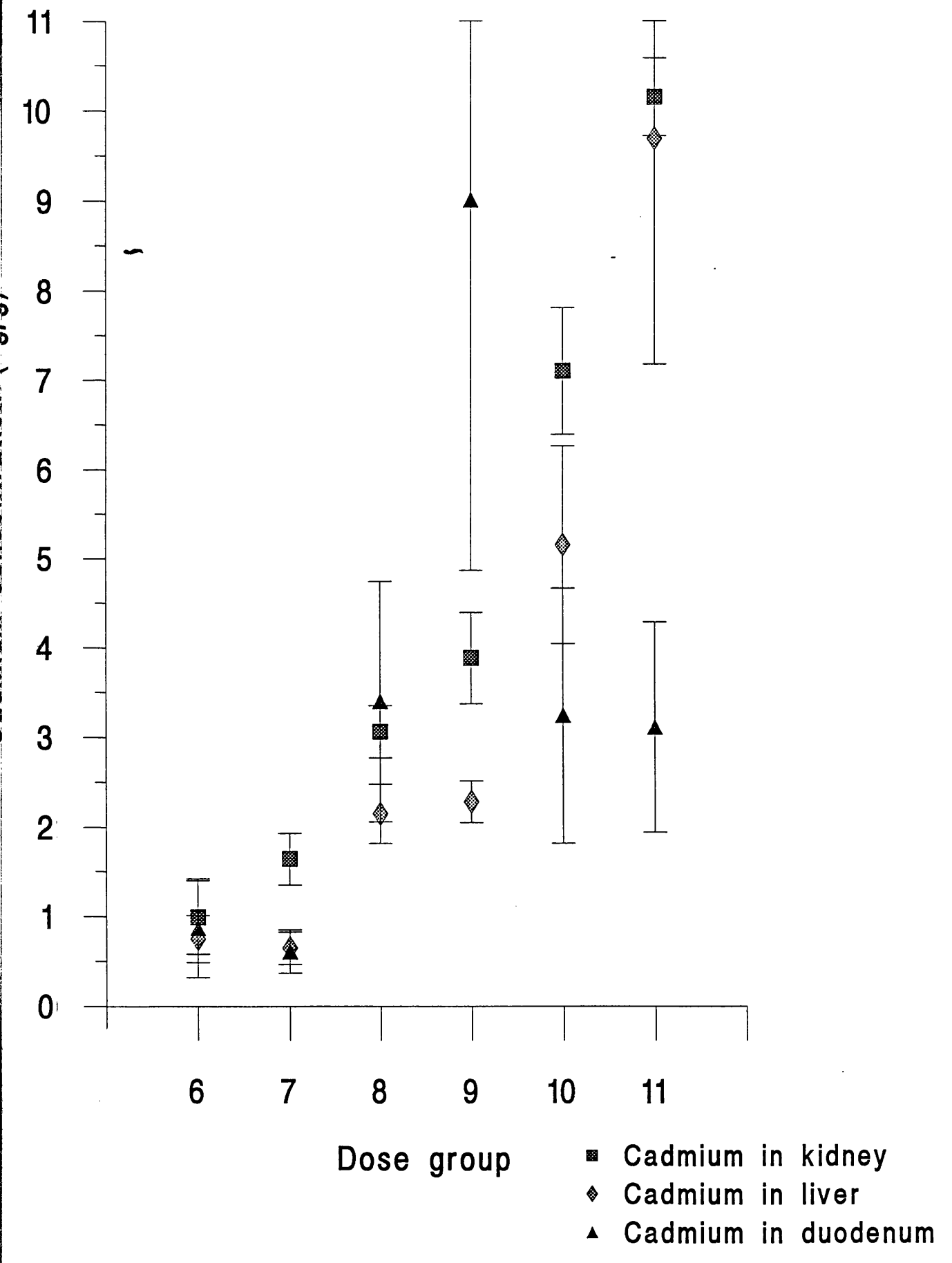


Table 8.1 Mean concentrations of cadmium, zinc, copper and metallothionein in great skua chicks given a single dose of cadmium, and control chicks. Concentrations are given in μg/g dry weight for metals and μg/g wet weight for metallothionein, with standard deviations in parentheses, control group n = 22, group 1 n = 5, group 2 n = 4, group 3 n = 5, group 4 n = 5, group 5 n = 4.

Dose group	Cd	Zn	Cu	MT
	Kidney			
Control	0.58 (0.38)	107.83 (23.05)	24.37 (3.02)	20.52 (26.30)
1 (0.1mg/kg)	0.19 (0.04)	86.35 (12.06)	17.36 (3.67)	9.27 (8.11)
2 (1.0mg/kg)	0.94 (0.62)	85.72 (6.98)	24.24 (2.53)	21.92 (11.84)
3 (10mg/kg)	1.65 (0.76)	165.72 (70.49)	21.51 (1.28)	8.24 (4.74)
4 (50mg/kg)	7.13 (3.65)	110.66 (26.84)	21.19 (0.68)	20.66 (7.27)
5 (100mg/kg)	14.14 (12.46)	184.69 (158.92)	21.90 (2.27)	37.11 (36.56)
	Liver			
Control	0.23 (0.20)	147.28 (62.56)	25.82 (10.53)	70.24 (59.77)
1 (0.1mg/kg)	0.18 (0.14)	117.73 (30.33)	20.60 (3.96)	25.57 (21.97)
2 (1.0mg/kg)	0.36 (0.14)	104.39 (11.04)	27.78 (15.17)	20.97 (7.86)
3 (10mg/kg)	1.35 (0.89)	97.52 (8.30)	19.24 (1.53)	7.88 (8.66)
4 (50mg/kg)	9.19 (8.04)	120.76 (8.58)	17.93 (1.84)	37.19 (43.53)
5 (100mg/kg)	15.99 (13.97)	126.48 (22.32)	20.91 (2.53)	66.30 (68.45)
	Duodenum			
Control	0.58 (0.54)	130.69 (20.36)	16.88 (11.73)	17.35 (15.61)
1 (0.1mg/kg)	0.40 (0.33)	164.63 (70.99)	20.91 (15.39)	36.70 (12.83)
2 (1.0mg/kg)	1.23 (0.20)	105.59 (15.79)	13.01 (2.33)	28.81 (19.54)
3 (10mg/kg)	1.99 (0.75)	135.92 (2.05)	18.29 (0.19)	89.55 (52.42)
4 (50mg/kg)	4.86 (2.39)	171.14 (33.97)	18.77 (2.46)	47.69 (13.08)
5 (100mg/kg)	16.89 (14.87)	222.02 (139.78)	21.10 (3.73)	56.23 (38.03)

Table 8.2 Mean concnetrations of cadmium, zinc, copper and metallothionein in great skua chicks from experiment two. Concentrations are given in μ/g/ dry weight for metals and μg/g wet weight for metallothionein, with standard deviations in parentheses, group 6 n = 5, group 7 n = 4, group 8 n = 5, group 9 n = .3, group 10 n = 4, group 11 n = 2.

Dose group	Cd	Zn	Cu	MT
	Kidney			
6 (1.0mg/kg)	0.99 (0.92)	95.36 (9.06)	25.54 (7.23)	24.24 (27.95)
7 (5.0mg/kg)	1.64 (0.59)	80.53 (15.66)	25.07 (3.28)	19.56 (10.73)
8 (20mg/kg)	3.06 (0.66)	95.45 (35.06)	21.41 (0.59)	12.36 (14.33)
9 (1.0mg/kg)	3.88 (0.90)	87.73 (10.85)	21.01 (0.86)	0.29 (0.13)
10 (5.0mg/kg)	8.63 (3.10)	105.65 (30.42)	21.77 (2.48)	17.01 (12.77)
11 (20mg/kg)	7.09 (1.58)	115.53 (27.91)	22.51 (1.99)	9.23 (6.23)
	Liver			
6 (1.0mg/kg)	0.75 (0.58)	144.86 (61.11)	21.62 (3.66)	146.47 (164.58)
7 (5.0mg/lg)	0.65 (0.37)	108.98 (27.63)	17.50 (2.45)	42.29 (55.33)
8 (20mg/kg)	2.15 (0.74)	108.98 (27.63)	17.50 (2.45)	42.29 (55.33)
9 (1mg/kg)	2.28 (0.41)	94.88 (22.82)	21.97 (1.29)	1.92 (1.17)
10 (5mg/kg)	7.44 (4.65)	107.46 (12.07)	19.67 (0.91)	30.19 (13.85)
11 (20mg/kg)	5.89 (2.06)	101.66 (7.98)	21.81 (1.24)	14.12 (10.28)
	Duodenum			
6 (1mg/kg)	0.87 (1.24)	141.73 (25.15)	15.23 (2.71)	71.10 (59.74)_
7 (5mg/kg)	3.40 (2.99)	113.25 (23.16)	18.31 (2.26)	18.35 (15.40)
8 (20mg/kg)	3.40 (2.99)	113.25 (23.16)	18.31 (2.26)	18.35 (15.40)
9 (1mg/kg)	9.01 (7.20)	86.23 (70.59)	16.16 (0.53)	17.17 (20.12)
10 (5mg/kg)	2.27 (1.88)	101.20 (8.58)	15.76 (0.25)	13.13 (0.65)
11 (20mg/kg)	3.90 (3.25)	97.16 (23.39)	17.25 (8.21)	19.90 (3.55)

Table 8.3. Mean concentrations of mercury in great skua chicks from Experiments one and two and control groups. Concentrations are given in μg/g dry weight, with standard deviations in parentheses

Dose group	Kidney	Liver
Control		
1 (0.1mg/kg)	0.57 (0.16)	0.52 (0.10)
2 (1.0mg/kg)	0.63 (0.19)	0.64 (0.27)
3 (10mg/kg)	0.67 (0.24)	0.76 (0.15)
4 (50mg/kg)	0.63 (0.18)	0.75 (0.38)
5 (100mg/kg)	1.34 (0.52)	0.97 (0.36)
6 (1mg/kg)	1.34 (0.52)	0.97 (0.36)
7 (5mg/kg)	1.19 (0.39)	0.89 (0.20)
8 (20mg/kg)	0.48 (0.04)	0.74 (0.15)
9 (1mg/kg)	0.79 (0.04)	0.63 (0.88)
10 (5mg/kg)	0.64 (0.22)	1.07 (0.52)
11 (20mg/kg)	0.71 (0.32)	0.77 (0.39)

Table 8.4 Effects of different single doses on metal and protein concentrations in birds from experiment one. Kruskal-Wallis one-way ANOVA analyses.

Organ and metal	Kruskal-Wallis results		
Kidney	Sample size	Chisquared value	Significance
Cd	23	19.31	$p < 0.001$
Zn	23	5.98	$p = 0.20$
Cu	23	8.95	$p = 0.06$
MT	20	8.57	$p = 0.07$
Hg	17	1.49	$p = 0.83$
Liver			
Cd	23	19.55	$p < 0.001$
Zn	23	9.01	$p = 0.06$
Cu	23	6.41	$p = 0.17$
MT	20	7.29	$p = 0.12$
Hg	22	4.68	$p = 0.32$
Duodenum			
Cd	22	18.73	$p < 0.001$
Zn	22	9.66	$p = 0.04$
Cu	23	5.44	$P = 0.20$
MT	18	6.85	$p = 0.14$

Table 8.5 Effects of two and three doses of cadmium on metal and metallothionein concentrations in birds from experiment two.

Organ and metal	Kruskal-Wallis results		
Kidney	Sample size	Chi-squared value	Significance
Cd	26	22.08	$P < 0.001$
Zn	26	5.42	$P = 0.36$
Cu	25	3.44	$P = 0.63$
MT	24	8.02	$P = 0.15$
Hg	23	9.85	$P = 0.17$
Liver			
Cd	23	18.76	$P < 0.01$
Zn	24	2.40	$P = 0.79$
Cu	24	9.75	$P = 0.08$
MT	23	24.47	$P = 0.03$
Hg	23	9.85	$P = 0.21$
Duodenum			
Cd	24	12.87	$P < 0.05$
Zn	24	7.36	$P = 0.19$
Cu	24	6.65	$P = 0.25$
MT	24	7.56	$P = 0.21$

Table 8.6. The relationship of individual metals between the organs analysed, and the relationship of metallothionein between the organs analysed, in birds given a single dose of cadmium (groups 1-5 combined)

	Duodenum	Liver
Cadmium in kidney	0.92 ***	0.97 ***
Cadmium in liver	0.88 ***	
Zinc in kidney	0.04 N.S.	0.36 p= 0.09
Zinc in liver	0.14 N.S.	
Copper in kidney	0.27 N.S.	0.18 N.S.
Copper in liver	-0.26 N.S.	
Metallothionein in kidney	0.12 N.S.	0.82 ***
Metallothionein in liver	0.14 N.S.	

* P <0.05, ** P <0.01, *** P <0.001, the actual P value is also given if the results approach significance

Table 8.7 Inter-metal and metal-metallothionein concentration correlations in birds given a single dose of cadmium (groups 1-5)

Kidney			
	Cadmium		
Zinc	0.73 ***	Zinc	
Copper	0.28 N.S.	0.33 N.S.	Copper
Metallothionein	0.90 ***	0.63 **	0.34 N.S.
Liver			
	Cadmium		
Zinc	0.57 **	Zinc	
Copper	-0.06 N.S.	0.03 N.S.	Copper
Metallothionein	0.92 ***	0.72 ***	0.003 N.S.
Duodenum			
	Cadmium		
Zinc	0.55 **	Zinc	
Copper	0.06 N.S.	0.44 *	Copper
Metallothionein	0.25 N.S.	00.08 N.S.	-0.07 N.S.

* P <0.05, ** P <0.01, *** P <0.001, the actual P value is also given if the results approach significance

Table 8.8 The relationship of individual metals between the organs analysed, and the relationship of metallothionein between the organs analysed, in birds given two and three doses of cadmium (groups 6-11 combined).

	Duodenum	Liver
Cadmium in kidney	0.22 N.S.	0.88 **
Cadmium in liver	0.11 N.S.	
Zinc in kidney	-0.07 N.S.	0.13 N.S.
Zinc in liver	0.38 P=0.07.	
Copper in kidney	0.05 N.S.	0.15 N.S.
Copper in liver	0.12 N.S.	
Metallothionein in kidney	0.00 N.S.	0.67 ***
Metallothionein in liver	0.06 N.S.	

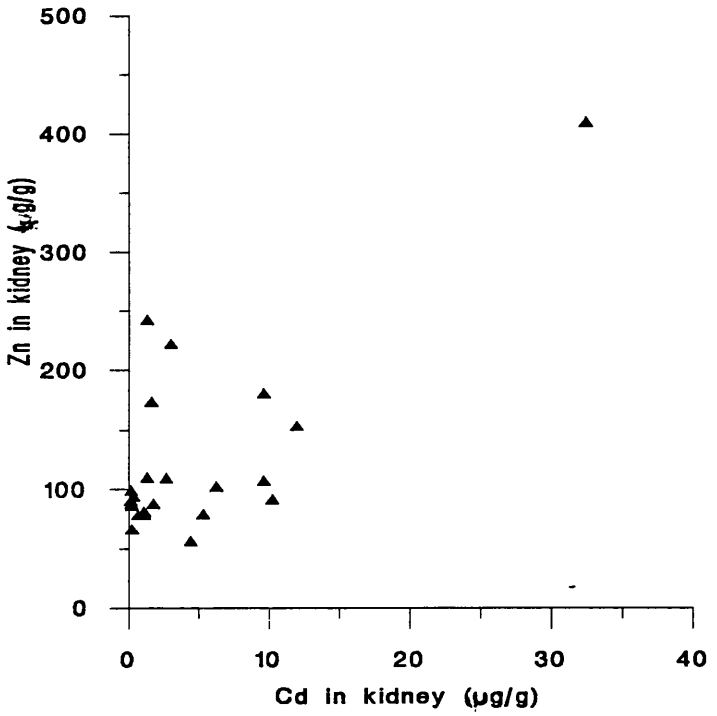
* P <0.05, ** P <0.01, *** P <0.001, the actual P value is also given if the results approach significance

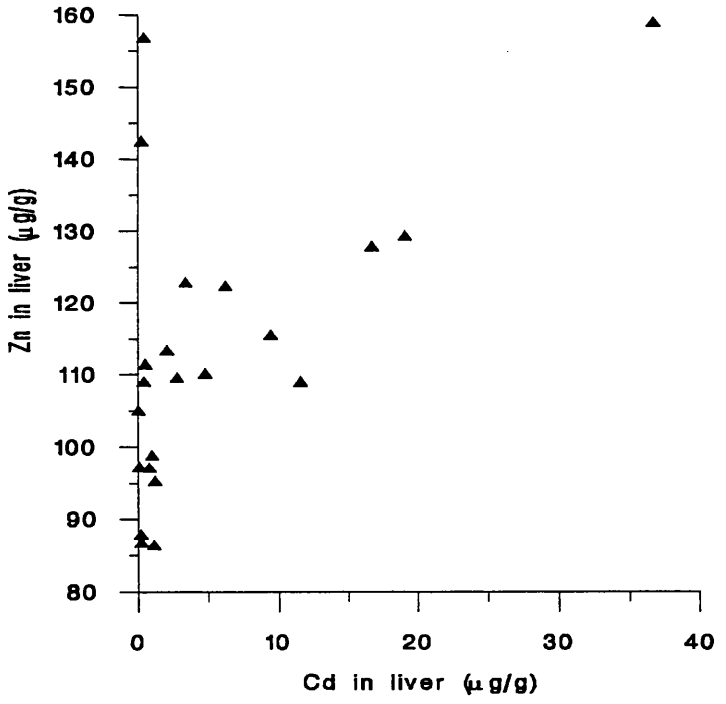
Table 8.9 Inter- metal and metal-metallothionein concentraton correlations in birds given two and three doses of cadmium (groups 6-11)

Kidney			
	Cadmium		
Zinc	0.29 N.S.	Zinc	
Copper	-0.13 N.S.	-0.09 N.S.	Copper
Metallothionein	0.00 N.S.	0.16 N.S.	0.63 - **
Liver			
	Cadmium		
Zinc	-0.04 N.S.	Zinc	
Copper	-0.22 N.S.	-0.25 N.S.	Copper
Metallothionein	-0.18 N.S.	0.91 ***	00.22 N.S.
Duodenum			
	Cadmium		
Zinc	-0.29 N.S.	Zinc	
Copper	-0.37 P=0.08	0.48 *	Copper
Metallothionein	-0.16 N.S.	0.23 N.S.	0.62 **

* P <0.05, ** P <0.01, *** P <0.001, the actual P value is also given if the results approach significance

Figure 8.7 The relationship between cadmium and zinc in the tissues, after a single dose of cadmium





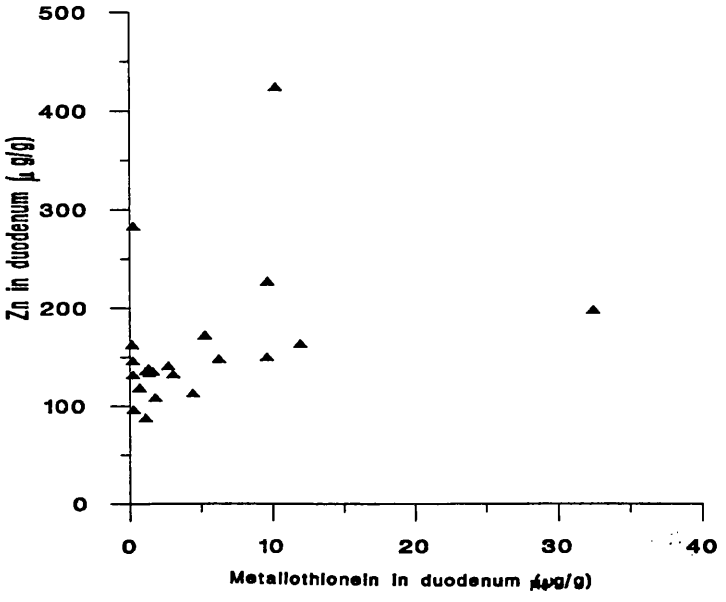
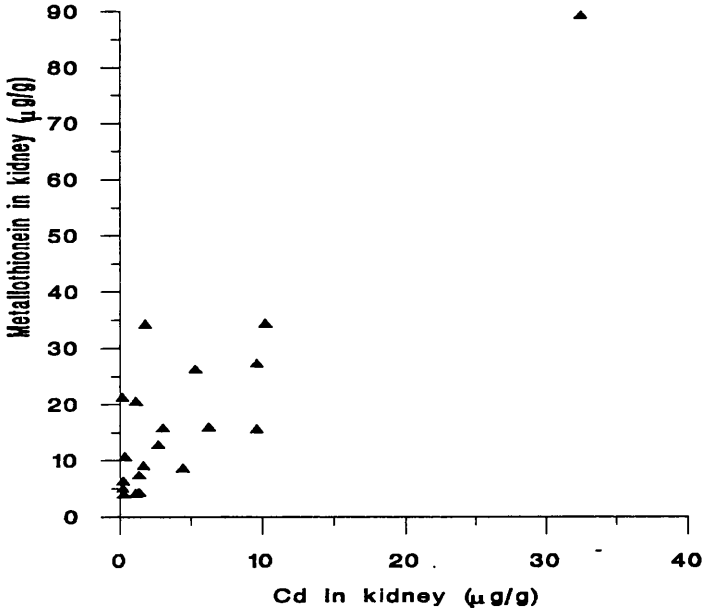
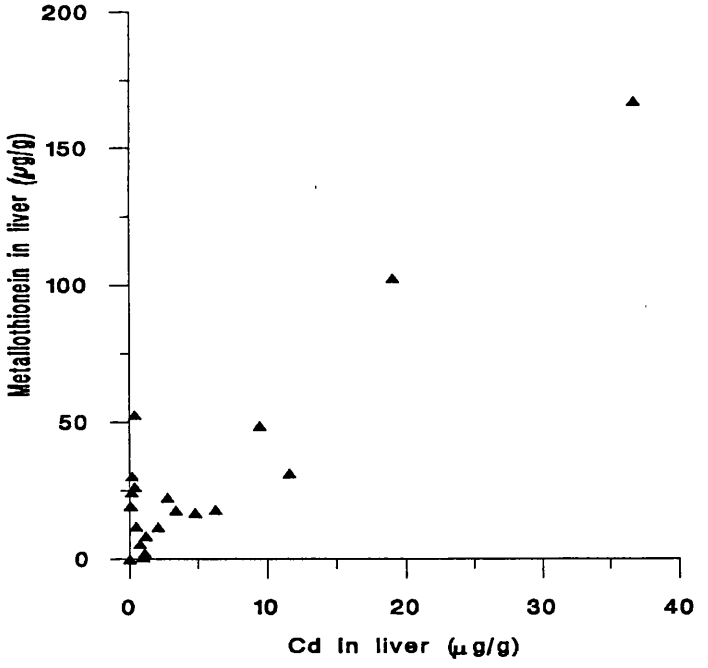


Figure 8.8 The relationship between cadmium and metallothionein concentrations in tissues after a single dose of cadmium





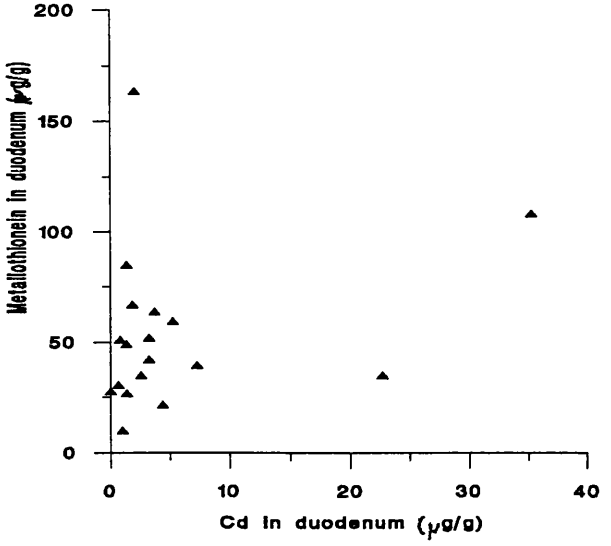
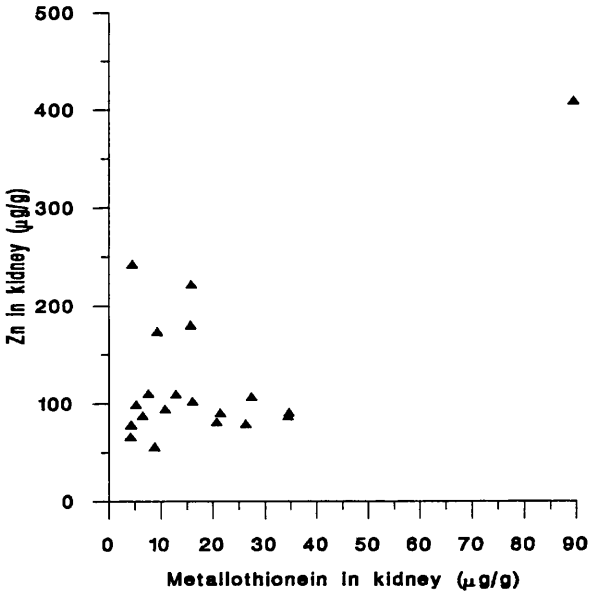
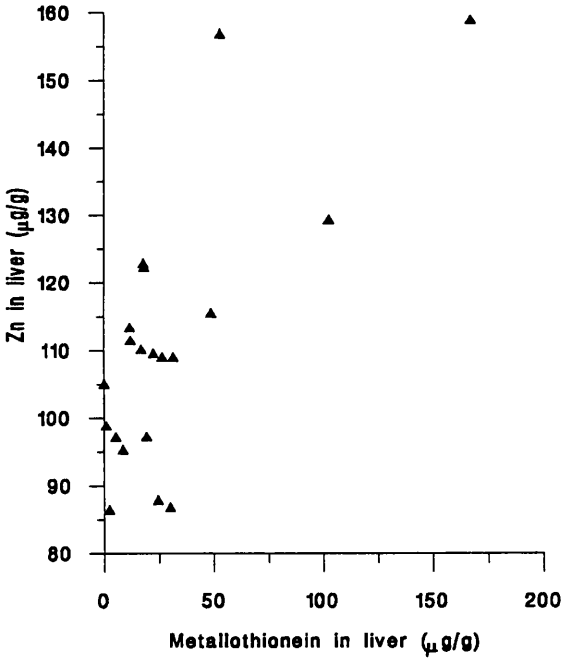
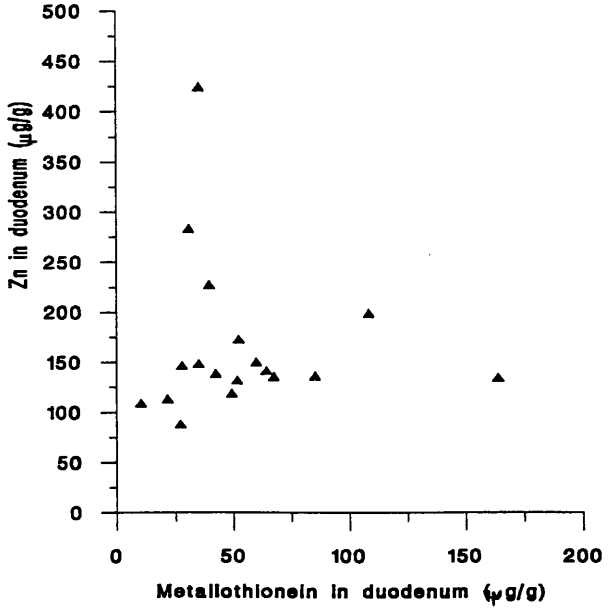


Figure 8.9 The relationship between zinc and metallothionein concentrations in tissues after a single dose of cadmium







Chapter Nine Discussion

The analysis of seabird tissues for heavy metals clearly has advantages over other biomonitors for certain types of studies. Interesting trends have been shown by the analysis of mercury levels in seabird feathers, but this approach is not possible for cadmium. Cadmium and mercury are often found in high concentrations together in seabirds, and some researchers have proposed there may be some sort of interaction between the two. However no such relationship was found between them in either lesser black-backed gulls with naturally accumulated concentrations (Chapter 5) or great skua chicks with experimentally elevated levels (Chapter 8). This is unfortunate as theoretically, if there were any interactions then levels of mercury in feathers could be used as an approximate indicator of cadmium concentrations in soft tissues. This is not the case. Both inorganic mercury and cadmium bind to metallothionein, and other workers have shown that there is a relationship between both inorganic and methyl (organic) mercury in the liver, and between total mercury in the liver and in the feathers of great skuas. However cadmium and inorganic mercury do not appear to accumulate in parallel, despite both binding onto metallothionein in liver and kidney tissue. Perhaps there would be a relationship in species, such as some albatrosses and petrels, with higher concentrations of mercury and cadmium than those measured in this thesis, and this may be worth further study.

Age accumulation of cadmium occurs in many animals, including humans and other mammals and marine fish can accumulate increasing amounts with increasing age/size. This was generally thought to be the case for seabirds, and is widely quoted. It is clear that seabirds accumulate cadmium from hatching to adulthood, and the results from Chapter 4 show there can be a difference between fully adult birds and those with some immature plumage, although there was quite an overlap in concentrations. Few studies have looked at continuing accumulation with age in adult birds. The results from Chapters 4, 5, 6 and 7, demonstrate that age accumulation of cadmium most likely does not occur, and there could be some form of excretion and regulation. There was no accumulation with increasing age in adult great skuas of age 3-21 years, and the common guillemots showed that cadmium burdens can fluctuate throughout the year in

both adult and immature birds. These findings tend to suggest that the biological half-life of cadmium in seabirds is shorter than was previously thought. In addition metallothionein can be rapidly synthesised or degraded, and will probably play different roles at various stages in the birds' life, (Chapters 5 and 6). Since metallothionein levels can fluctuate, the metals bound onto the protein could potentially be mobilised and excreted during the process. Further work is needed to clarify this important aspect of seabird metal dynamics.

The roles of metallothionein in the regulation of metal concentrations appear complex and are not yet fully understood. In the adult gulls in Chapter 5 metallothionein appears to act in a detoxifying role, i.e. is induced by cadmium and binds and stores it where it would cause no damage. In contrast, metallothionein was much more important as a zinc store in the fledgling shearwaters at a time when zinc was required for feather formation, and any relationship with cadmium concentrations may have been overwhelmed. It would be difficult to try and tease apart these different functions. Metallothionein may have many roles and it is not impossible that cadmium and inorganic mercury binding onto the protein may be fortuitous and that once bound both metals are in a non-toxic form. Other studies have suggested that zinc may protect tissues from the toxic effects of cadmium. This would be manifested by the induction of metallothionein synthesis by zinc increasing the binding sites available for cadmium, as shown by an experimental study on rats. There is however, as yet no study demonstrating an active role of protection of zinc upon cadmium in the natural situation. Free-living birds could not 'predict' a high cadmium intake and actively increase their zinc uptake. Chapter 5 showed that metallothionein production was mainly determined by cadmium concentrations in birds' liver and kidney, but zinc and copper will also bind onto metallothionein. This study also uses two quite different seabird species of two age classes collected at different stages in the life-cycle, and as such are not directly comparable. However it is interesting that they appear to show metallothionein can function in different ways. There are very few studies on metallothionein in seabirds and there is considerable scope for further studies, both experimental and on free-living

animals. The gulls and shearwaters had relatively low levels of cadmium when compared with some albatrosses and petrels which can concentrate extremely high cadmium (and mercury) levels. There is also the potential for a more biochemical approach to investigate the structure and variability of metallothionein in seabirds, about which practically nothing is known.

The probability that seabirds may be more resistant to the presence and effects of cadmium has yet to be investigated. The uptake of cadmium from a given dose is low and the value of 0.5% measured in skua chicks (Chapter 8) is lower than comparable studies in mammals and terrestrial birds. This value can now be used in future studies to interpret the concentrations found in seabirds, especially in those as described below.

Seabirds can be useful indicators of cadmium in the marine environment, but care must be taken to make full use of their potential, and many things must be considered. Variation in metal levels through the year must be taken into account. This is not such a problem for the monitoring of mercury as it is clear that excretion occurs into the plumage, and this is an indicator of birds' mercury intake from the previous moult (Chapter 7). The seasonal fluctuation in cadmium burdens and the controversy as to whether birds accumulate cadmium continuously or are capable of its excretion limits the present nature of cadmium monitoring studies using birds, at least until these problems are investigated more fully. The use of nestlings or fledglings to monitor cadmium in the marine environment would circumvent these problems.

Cory's shearwater fledglings accumulated high levels of cadmium in three months and from knowledge of their diet and ecology, their cadmium intake, uptake and accumulation could be modelled (Chapter 8). There is great potential for future studies of this type. These would require detailed knowledge and analysis of the metal burdens and dynamics of prey items, including fish, crustaceans and cephalopods. For monitoring purposes analyses of top predators with a good knowledge of their diet and ecology means that a clearer picture of the bioavailability of metals and flow of heavy metals through the ecosystem could be obtained.

The large amount of literature on seabirds could be used and integrated into some kind of global monitoring scheme. In Chapter one I mention that this would have to be standardised in order to be useful. In addition to the points made above, that is taking into account seasonal variation, age of birds in the sample (data on known age chicks would be particularly valuable), physiological processes etc., there would have to be other critetria. The data should be expressed as μg/g dry weight of tissue (those measured as wet weight could be converted), and the choice of monitoring should be selected according to physiological properties of each metal. For mercury monitoring and comparison, data on eggs, feathers, kidney and liver tissues would be suitable. Data on cadmium would use levels in liver and kidney. This could be collated into a baseline study of worldwide temporal and geographical trends in metals in seabirds, using data from the last twenty years or so which already exists. The pulling together of all this existing data would allow a general picture of metals in seabirds to be formed, and would be valuable in assessing further monitoring and research needs and possibilities.

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Appendix 1 Species names

Adelie pengiun - *Pygoscelis adeliae*
Atlantic petrel - *Pterodroma incerta*
Atlantic puffin - *Fratercula arctica*
Avocet - *Recurvirostra avosetta*
Bar-tailed godwit - *Limosa lapponica*
Barn owl - *Tyto alba guttatus*
Blackbird - *Turdus merula*
Black-crowned night-heron - *Nycticorax nycticorax*
Black-eared kite - *Milvus migrans lineatus*
Black guillemot - *Cepphus grylle*
Black-headed gull - *Larus ridibundus*
Black skimmer - *Rhynchops niger*
Blue-grey / Grey noddy - *Procelsterna cerulea*
Bonaparte's gull - *Larus philadelphia*
Brown booby - *Sula leucogaster*
Brown noddy - *Anous stolidus*
Brown pelican - *Pelecanus occidentalis*
Brünnich's guillemot - *Uria lomvia*
Cattle egret - *Bulbulcus ibis*
Cod - *Gadus morhua*
Common guillemot - *Uria aalge*
Common tern - *Sterna hirundo*
Cormorant - *Phlacrocrorax carbo*
Cory's shearwater - *Calonectris diomedea*
Crested tern - *Sterna bergii*
Curlew sandpiper - *Calidris ferruginea*
Domestic fowl - *Gallus gallus*
Domestic sheep - *Ovis sp.*
Double-crested cormorant - *Phalacrocorax auritus*
Dunlin - *Calidris alpina*
Eastern great white egret - *Egretta alba modesta*
Eider duck - *Somateria mollissima*
Fairy prion - *Pachyptilla turtur*
Feral pigeon - *Columba livia*
Forster's tern - *Sterna forsteri*
Franklin's gull - *Larus pipixcan*

Fulmar - *Fulmarus glacialis*
Gannet - *Morus bassanus*
Glaucous gull - *Larus hyperboreus*
Goshawk - *Accipiter gentilis*
Great skua - *Catharacta skua*
Greater flamingo - *Phaenicopteris ruber*
Greater yellowlegs - *Tringa melanoleuca*
Grey gull - *Larus modesta*
Grey heron - *Ardea cinerea*
Herring - *Clupea harengus*
Herring gull - *Larus argentatus*
Horse - *Equus sp.*
House sparrow - *Passer domesticus*
Human - *Homo sapiens sapiens*
Icelandic redshank - *Tringa totanus robusta*
Japanese quail - *Cotonurix cotonurix japonica*
Kelp / Southern black-backed gull - *Larus dominicanus*
Kittiwake - *Rissa tridactyla*
Knot - *Calidris canutus*
Laughing gull - *Larus atricilla*
Laysan albatross - *Diomedea immutablis*
Laysan duck - *Anas laysaneis*
Leach's petrel - *Oceanodroma leucorhoa*
Least sandpiper - *Calidris minutilla*
Lesser black-backed gull - *Larus fuscus*
Lesser yellowlegs - *Tringa flavipes*
Little egret - *Egretta garzetta*
Louisiana heron - *Hydrasana tricolor*
Macaroni penguin - *Eudyptes chrysolophus*
Magpie - *Pica pica*
Mallard duck - *Anas platyrhynchos*
Manx shearwater - *Puffinus puffinus*
Oystercatcher - *Haematopus ostralegus*
Poor cod - *Trisopterus minutus*
Rat - *Rattus norvegicus*
Red-billed gull - *Larus novaehollandiae scopulinus*
Red-footed booby - *Sula sula*
Rhinoceros auklet - *Cerorhina monocerata*

Ring dove - *Streptopelia risoria*
Rockhopper penguin - *Eudyptes chrysocome*
Roseate tern - *Sterna dougallii*
Royal tern - *Sterna maxima*
Sandeel - *Ammodytes marinus*
Sanderling - *Calidris alba*
Sandwich tern - *Sterna sandvicensis*
Sooty tern - *Sterna fuscata*
Sprat - *Sprattus sprattus*
Starling - *Sturnus vulgaris*
Turtle dove - *Streptopelia risoria*
Wandering albatross - *Diomedea exulans*
Wedge-tailed shearwater - *Puffinus pacificus*
Western sandpiper - *Calidris mauri*
Whimbrel - *Numenius phaeopus*
Whiting - *Merlangius merlangus*
Wild turkey - *Meleagris gallopava*
Wood duck - *Aix sponsa*
Wood stork - *Mycteria americana*