Studies of Mercury Dynamics in Birds

Sharon Alison Lewis

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CANDIDATE'S DECLARATION

I declare that the work recored 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.

Sharon Lewis

August, 1991

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So we beat on, boats against the current,
bourne back ceaselessly into the past.

F. Scott. Fitzgerald (1925)

CONTENTS

SUMMARY

1. A review is presented of the use of seabirds as biomonitors of heavy metals and factors influencing mercury dynamics in birds.

2. The effect of atmospheric deposition on mercury levels found in the plumage of seabirds was assessed and found to be negligible. No difference was found between feathers exposed for ten months to the atmosphere and feathers sealed over the same period.

3. Significant differences were found between the tip, middle and bottom portion of the feather but this was concluded to be due to natural depletion of the body pool of mercury as the feathers grew and not due to the effect of exogenous contamination.

4. The effect of age and sex upon mercury concentrations in feathers of red-billed gulls was investigated. Feather concentrations were not influenced by age nor sex in adults nor chicks. Dietary specialisation was assumed to be an important determinant of mercury levels in this species.

5. The effect of growth development on tissue distribution and excretion in kittiwake chicks was examined. No age-related changes in the retention of mercury by any of the tissues analysed were found nor were any differences in mercury excretion apparent. There was a positive correlation between the total amount of mercury going into the plumage and age which was suggested to reflect an increase in the amount of mercury circulating in the blood of older birds.

6. No difference in the mercury distribution between the liver, kidney and pectoral muscle was found which differed markedly from results of other studies. It was suggested that this may be an indication that the levels in chicks were approaching a

toxic threshold.

7. Mercury accumulation and excretion were investigated in laboratory-reared black-headed gull chicks in relation to mercury intake. There was an increase in the proportion of mercury deposited in the kidney as the dose increased. The proportional accumulation of mercury in the carcass was also dose-dependent. The amount of mercury administered had no effect on the total excretion rate.

8. Mercury accumulated differentially in the internal tissues, concentrations in the kidney were greater than in the liver which in turn exceeded concentrations found in the pectoral muscle. All feather types contained much higher mercury concentrations than internal tissues and there was a consistent difference between different feather types in relation to mercury content. There was a pronounced reduction in the concentration of mercury in the primary feathers as the growth sequence progressed.

9. An average of 71% of the dose administered was excreted over the fledging period. Of this, 22% of the dose was excreted into the feathers. Sixty five percent of the total body burden of mercury was present in the plumage after the completed moult. Of administered mercury, 49% was contained in the plumage.

10. The retention and excretion of mercury after a single dose of methyl mercury was investigated in male and female quail. The role of eggs in mercury excretion was also assessed. The birds accumulated mercury rapidly in their internal tissues and lost it relatively slowly. Mercury accumulated differentially in the internal tissues. Kidney concentrations exceeded liver concentrations which exceeded pectoral muscle concentrations.

11. For up to eight weeks after mercury administration there was

a difference between the mercury levels in the internal tissues of male and female birds, with less mercury in the female tissues. Twelve weeks after administration differences in the mercury levels of the kidney and pectoral muscle were still found.

12. There was no difference in mercury levels of the male and female plumage, although total mercury excretion did differ between the sexes with females losing up to 80% of their mercury intake in the first four weeks after mercury administration compared to 40% lost by male birds. Male birds lost over 80% of the mercury intake via the faeces compared to only 44% lost this way by the females. Initial mercury concentration in the eggs was over 3.5 µg/g. Over 40% of the females' intake was lost by this route supporting the use of bird eggs to sample for environmental contamination.

13. The effect of sex and species on mercury retention was examined in two species of seabird (shags and kittiwakes) after a single dose of methyl mercury. The excretion of mercury into the eggs and chicks was also assessed. Mercury accumulated differentially in the internal tissues. In both species there was no difference between the concentration of mercury found in the liver and kidney although levels in both these tissues were greater than levels found in the pectoral muscle.

14. Mercury concentrations in both species of seabird were similar. There were differences in the total amount of mercury in the tissues analysed, shag tissues having up to four times the amount of mercury in their tissues as those of the kittiwakes.

15. Mercury as a percentage of the body burden in the kidney, liver and pectoral muscle approached a 1:3:4 ratio for both

species of seabird. It was estimated that shags excreted 5% of the dose administered over a 25 day period compared with 38% excreted over a 47 day period by the kittiwakes.

16. Administered mercury was not excreted into the eggs of shags although a portion of the dosage did reach kittiwake chicks of experimental birds.

17. The levels of mercury in eggs, internal tissues and feathers from herring gulls from the German Wadden Sea coast were measured. Mercury levels in the tissues analysed showed no difference between male and female birds. No gender difference was found in the concentration of mercury in the body feathers although levels in the primary feathers of male and female birds did differ.

18. Egg mercury levels were correlated to liver levels only. Liver levels were correlated with the levels found in the ovary and a strong relationship was found between levels in the body and primary feathers.

19. The validity and use of mercury concentration conversion ratios for tissue comparisons was briefly examined. The ratio of feather concentration: egg concentration ranged from 3.7 to 5.5 according to feather type. Mercury intake and excretion via the eggs in one breeding season was assessed. It was estimated that herring gulls in the Wadden Sea consume 1000 µg of mercury in one year. Females could excrete over 20% more mercury in one breeding season than excreted by male birds.

20. A discussion presenting possible factors to be taken into consideration when designing programmes to monitor marine levels of mercury using seabirds was made. These factors included variations in the age structure of a population, time of moult and sex-related differences. The use of feathers and

eggs to monitor and quantify mercury pollution was also briefly discussed

CHAPTER 1

General Introduction

1.1 INTRODUCTION

The accumulation of abnormal concentrations of metals by the tissues of marine organisms is the basis for much concern about metal contamination particularly in the northern hemisphere where metal pollution is common. This concern lies mainly with the fact that accumulation of high concentrations in commercial species may prove harmful to man and other higher vertebrates and also high metal levels may affect the productivity of marine or estuarine organisms.

Only in the most heavily contaminated areas have effects been observed which can be attributed to metallic contamination (Bryan, 1984). Among the reasons for this scarcity of evidence are the interaction of metals with other contaminants, the lack of sensitivity in monitoring methods based on population and species distribution, and adaptations of some populations to metal pollution (Langston, 1990).

In recent years concern regarding the long-term effects of environmental contamination has increased especially for those metals considered to be most significant from the point of input and toxicology. Of all the metals that are thought to be a pollution threat mercury is generally considered to be one of the most damaging in aquatic environments (Bryan, 1984).

Recently, mercury has attracted more attention than most any other trace element. It can be considered a non-essential element, having no known biological function, yet it is the heavy metal most readily absorbed from solution (Bryan, 1979). Mercury is also the most toxic metal in virtually all taxonomic groups. Next to lead, it has the highest value for the ratio of anthropogenic/natural inputs into the environment (Lanzy & Mackenzie, 1979) and of all the heavy metals only mercury or

more specifically methyl mercury, shows appreciable signs of being biologically magnified as a result of food chain transfer (Gardiner, 1978).

The most important example of contamination by mercury was that at Minamata Bay, Japan (Tsubaki & Irukayama, 1977). The metal was used as a catalyst in the production of acetaldehyde and it is estimated that over 80 tonnes were discharged to the Bay between 1932 and 1968. By 1960, Minamata disease was recognised to be similar to organochlorine poisoning. Analysis of tissues of various fish species gave values in the range of 7-23 µg/g in 1961. A study on mercury by the World Health Organisation (1976) has reported that lowest levels likely to produce effects in humans are in the range of 0.2-0.5 µg/g in blood and 50-125 µg/g in hair. A more generalised threat to public health came in the 1960's in Sweden due to the increasing agricultural use of mercurial fungicides which resulted in high amounts of mercury being found in fish, birds and people (Borg et al., 1966).

There are two main sources of mercury contamination to the worlds' ecosystem. Firstly, it is a naturally-occurring trace element which is cycled in the environment. It is estimated to be present in the earths' crust at concentrations between 50 to 80 ng/g, existing in the form of various sulphides, especially red sulphide (cinnabar)(Harhung & Dinman, 1972), and flowing into the oceans as a natural result of land erosion. Due to its high vapour pressure metallic mercury is also evaporated from the worlds soils into the atmosphere.

The second source of mercury to the environment is anthropogenic emissions. The consumption of mercury and its components has, since World War Two, increased severely,

especially in such uses that empirically lead to considerable losses of mercury to the environment. Figures for the U.S.A. in 1968 show that America consumed 5.7 million pounds of mercury (West, 1969), furthermore the U.S.A. has increased its mercury consumption by 50% during the last ten years. The main consumers of mercury compounds include the electrical apparatus industry, the chlor-alkali industry, the paint industry, agricultural pesticides, the paper and pulp industry, fossil fuels, and catalyst and extraction needs (including gold ore) (Harhung & Dinman, 1972).

The main compounds of mercury that are commonly released into the aquatic environment are bivalent inorganic mercury, elemental mercury and phenyl mercury. The biokenetics and toxicology of organomercurials, particularly the most stable form, methyl mercury, have been much more extensively studied than that of the inorganic forms of the metal. A major reason for this bias is the fact that, whereas the intestinal absorption of inorganic mercury (Hg^{2+}) is only a few percent of the ingested mercury, absorption of methyl mercury approaches 100% (Berglund & Berlin, 1969). The different compounds of mercury may be converted into methyl mercury. This conversion starts with the oxidation of elemental mercury and the breakdown of phenyl mercury. The mercuric ion can then be methylated to monomethyl- or dimethylmercury by microorganisms and some macroorganisms (e.g. Anodonta sp). The monomethylmercury leaches into the water and accumulates in algae, invertebrates and fish by uptake through the skin surface and gills and thus up the food chain into other aquatic organisms such as birds and mammals. This effect is well demonstrated in a study by Gardiner et al. (1978). Here total mercury in sediment samples ranged

from 0.06-1.7 µg/g dry weight and none of the samples contained measurable concentrations of methyl mercury. Plant tissues contained measurable concentrations of methyl mercury and various invertebrates had levels ranging from 0.3-9.4 µg/g with a significant proportion in the methyl form. However in several fish species total mercury ranged from 0.3-2.4 µg/g of which over 90% was methyl mercury.

Methyl mercury is more slowly metabolised than other organomercurials and it has a lower excretion rate. Thus its biological half life is relatively long (2-3 months in a variety of bird species) (Swensson & Ulfvarson, 1968; Odsjo & Edelstam, 1975; Stickel et al., 1977). The other organomercurials decompose rapidly to inorganic mercury, thus their biological half lives are more similar to that of inorganic mercury (Millar et al, 1960; Swensson & Ulfvarson, 1968).

Methyl mercury is chemically very stable and is readily soluble in lipids. Thus it readily penetrates the blood brain barrier and therefore the major toxic effects of methyl mercury are central nervous system (CNS) dysfunction and spinal cord degeneration (Fimreite, 1971; Pass et al., 1975).

There is a large and varied literature on the effects of methyl mercury in birds. Briefly, the clinical symptoms of methyl mercury poisoning are similar for many bird species (Borg et al., 1966, 1970; Tejning, 1967; Fimreite & Karstad, 1971) and are characterised by reduced food intake leading to weight loss, progressive weakness in wings and legs with difficulty in flying, walking and standing, and an inability to coordinate muscle movements. Once such symptoms occur, death is a virtual certainty even if the source of exposure is removed (Fimreite &

Karstad, 1971).

Toxic effects may occur in birds at relatively low dietary levels of methyl mercury. Reproductive effects occur at even lower doses than required to produce other pathological effects. Fimreite (1971) found that at levels of 2-3 µg methyl mercury per gram of food for a period of 12 weeks there were no neurological defects in pheasants Phasianus colchicus. However there was a significant increase in the proportion of shell-less eggs laid and a significant decrease in the egg weight compared to the controls. The major effect was a decrease in hatchability due to early embryonic mortality and an increase in the number of unfertilised eggs. Similarly, in breeding mallards Anas platyrhynchos dosed with 3 µg/g dietary methyl mercury general health seemed unaffected over a 21 week exposure but there were significant reproductive effects (Heinz, 1974).

Reproductive impairments similar to those reported under controlled exposure conditions also occur in wild populations. A strong negative relationship was noted between the successful territories held by breeding divers Gavia immer and the degree of environmental contamination. Egg laying and territorial fidelity were also impaired at mercury concentrations of 0.3-0.4 µg/g wet weight in these birds (Barr, 1986).

1.2 Monitoring of mercury pollution

Mercury is toxic even at low levels to many marine and estuarine organisms. This gives rise to concerns both with respect to the possible detrimental effect of mercury on coastal resources, and in terms of the impact of metals on human health. Monitoring programmes are therefore required to establish both spatial and temporal trends in marine abundance and bioavailibility in estuaries and other coastal waters. Such

monitoring relies upon the quantification of mercury in the waters, in the underlying sediments, or in the organisms themselves (Phillips, 1977, 1980).

It is now generally felt that analysis of metals in water has several disadvantages. Metal concentrations are very low and thus difficult to quantify while avoiding extraneous contamination (Bruland et al., 1978). Temporal variability is also a problem. There is no definite relationship between metals in soluble and particulate fractions of natural waters and their availability to organisms (Phillips, 1980). Analysis of sediments is difficult due to the effect of particulate size and organic carbon content on metal levels (Phillips, 1977, 1980). In addition, no simple method exists to determine bioavailiablity of metals in sediments (Ayling, 1974; Kriezovich et al., 1987).

Therefore, the use of organisms is now the most widely employed method to monitor trace elements in coastal waters (Phillips, 1977, 1980). Organisms employed to quantify pollutant abundance or bioavailibilty by the use of their tissue concentrations of contaminants are referred to as bioindicators or biomonitors.

1.2.1 The use of seabirds as biomonitors of heavy metals in the marine environment.

Seabirds as monitors of marine pollution offer specific advantages over other marine biological indicators.

1. They are usually at the top of the marine food chain and therefore in some cases (notably organochlorines and mercury) their tissues accumulate higher concentrations than that found

in their food items.

2. Because seabird movements are relatively well known the more sedentary seabirds can be used to indicate geographical variation (Howarth et al., 1981; Reid & Hacker, 1982; King et al., 1983) which would be more difficult using marine mammals and fish due to the vast areas such species cover.

3. The use of seabird feathers as indicators of mercury contamination means that seabirds may be used for assessment of historical variations in mercury inputs to the sea by direct comparison of contemporary and museum feathers samples (Thompson et al., in press).

4. Species can be selected to represent metal levels in either inshore or offshore waters.

An ideal seabird monitor should be resident within a restricted radius of the breeding ground throughout the year. Its diet should be well known and should show no systematic variation between sampling locations or over decades. Species feeding on a few prey items are particularly suitable. The seabird species should also be readily available and relatively common, as well as being sufficiently widespread to provide good coverage of the proposed region being monitored for geographical variation. Most seabirds meet only a few of these criteria.

It is, however, the choice of target organ with respect to mercury monitoring which gives birds a distinct advantage over other vertebrate groups. Although some pelagic fish and marine mammals have been shown to accumulate mercury to relatively high levels (Shomura & Craig, 1974; Honda, et al., 1983a,b) the choice of organ to be analysed is restricted to the liver, kidney or muscle, which has the unavoidable consequence of killing the animal sampled. With birds it is possible to use

feathers and eggs to monitor for some marine pollutants.

1.2.2 Mercury in feathers.

It is the use of feathers that has provided a means by which large numbers of individuals can be assessed readily in a manner that avoids kìlling the specimens. Mercury is deposited in growing feathers and is strongly bound to the disulphide linkages within the keratin molecule (Crewther et al., 1965). Levels of mercury in the feather are unaffected by various vigorous treatments (Appelquist et al., 1984). Hence, once fully grown, feathers are both chemically stable and effectively isolated from processes occurring in the bird. In addition, mercury does not concentrate in the uropygial gland or in the secreted oils, thus contamination by this route after their formation is minimal (Frank et al., 1983).

In wild birds it has been estimated that up to 70% of the total body burden of mercury may be present in the plumage (Honda et al., 1986a). Although leaching out of mercury from the feathers is not a problem, the possibility that feather mercury levels may become artificially elevated due to external contamination after feather formation must be carefully considered. Such contamination has been reported to be a significant factor influencing the metal content of feathers under certain conditions such as airborne deposition from industrial sources (Rose & Parker, 1982; Hahn et al., 1989a,b).

Furness et al. (1986) indicated that body feathers would be the most appropriate feather to use in mercury analysis. This would overcome the problems associated with great inter-primary mercury concentration variation, inconsistent numbering for the primaries and thus allow greater compatibility between studies.

However, relationships between levels of mercury in

feathers and other tissues and relationships between dietary intake, age, sex, moult, toxicology and physiology need to be established before feathers can be used to study mercury pollution with confidence.

1.2.3 Mercury in eggs

The egg has been used in several studies to monitor mercury levels in both the terrestrial and the marine environment (Parslow & Jefferies, 1975; Ohlendorf et al., 1985, 1986; Becker, 1989; Barrett et al., 1985). Mercury accumulates particularly in the albumen portion of the egg after exposure to methyl mercury (Backstrom, 1969). Eggs have several advantages as a monitoring tool over body tissues. They have a highly consistent composition and fat content and are produced by the same segment of the population and therefore reflect pollutant levels in the adult female. They can be readily sampled from the same locations each year, and their collection places less of a drain on the population than the sampling of adults. Sampling one egg from the clutch means that breeding is only marginally reduced (intraclutch variation versus interclutch variation is low (Potts, 1968; Becker, 1989)). They have also been shown to reflect metal uptake from local foraging more closely than do the tissues from adult birds (Parslow & Jefferies, 1975, 1977; Barrett et al., 1985).

Eggs, however, do have some drawbacks. They are representative of only part of the population (the breeding female) and for only part of the year and they do not adequately indicate some of the heavy metals such as cadmium and lead (Becker et al., 1985, 1989), although mercury is certainly transferred to the eggs (Heinz, 1974; Vermeer & Peakall, 1977,

Stoneburner et al., 1980). It is also not clear whether the contamination of the female originates from the wintering area and migration routes or from the actual breeding ground. There is also conflicting evidence as to how important eggs are in the process of mercury excretion (Helander et al., 1982; Becker et al., 1985; Honda et al., 1986b).

1.3 Mercury contamination in birds

Total mercury levels vary enormously both within and between species. In addition to greater variation in mercury levels compared to variation in essential metals, the distribution of mercury concentrations within tissue samples from seabird populations tend to be skewed with several high mercury values, and therefore differ markedly from a Gaussian distribution (Muirhead & Furness, 1988). This finding is consistent with mercury accumulation rather than metabolic regulation.

Liver and kidney of birds raised in captivity and exposed to only "normal background levels" of mercury generally contain <0.2 µg/g mercury wet weight (Pass et al., 1975; Heinz, 1976; Stickel et al., 1977). In wild bird species living in environments receiving little or no industrial contamination, levels of mercury in the liver range from 1-10 µg/g, the highest values being associated with scavengers and piscivores (Fimreite et al., 1974). Maximum liver mercury concentrations from other studies rarely exceed 20 µg/g (wet weight) and, even on a dry weight basis, are usually less than 10 µg/g (Fimreite et al., 1970; Furness & Hutton, 1979; Holt et al., 1979; Hutton, 1981; Delbeke et al., 1984; Norhiem & Kjos-Hanssen, 1984; Honda et al., 1986b; Braune, 1987).

Seabird populations usually have high and varied levels of

mercury in their tissues. In a study examining mercury levels of seabirds from the southern and northern hemispheres total mercury levels varied enormously both within and between species. Low levels were reported in adelie penguins Pygoscelis adeliae(<0.1-0.2 µg/g) and broad-billed prions Pachyptila vittata (0.1-1.1 µg/g wet weight) whereas mercury levels were as high as 227 µg/g for the sooty albatross Phoebetria fusca and up to 271 µg/g in the wandering albatross Diomedea exulans (Honda et al., 1986b; Muirhead & Furness, 1988).

In wild populations in general, feather levels tend to exceed those of the liver, which in turn exceed those found in the kidney (Anderlini et al., 1972; Connors et al., 1975; Hutton, 1981; Nicholson, 1981; Ohlendorf et al., 1985; Honda et al., 1986a; Noble & Elliot, 1986; Muirhead & Furness, 1988). Mercury levels in eggs tends to be low, rarely exceeding 0.5 µg/g wet weight (Jensen et al., 1972; Fimreite et al., 1974; Dyck & Kraul, 1984; Barrett et al., 1985; Noble & Elliot, 1986; Ohlendorf & Harrison, 1986), although elevated levels have been reported in royal terns Sterna maxima eggs from the Texas coast (King et al., 1983) and in gannet Sula bassana eggs from Norway (Fimreite et al., 1974, 1980).

1.4 Factors influencing mercury dynamics in birds

Variations in heavy metal concentrations between bird species potentially reflect many factors, including feeding and migratory habits, body size, life span, moult strategy and taxonomic influences on physiology. Therefore, inferences about variation in environmental metal levels using different species are open to question, though interspecific comparisons may shed light on the relative metal loads in different foodchains. There

are many factors to be taken into account when examining mercury levels in birds.

1.4.1. Age-related variation

Variations in age composition of a sample of seabirds could potentially contribute to differences in tissues concentration between species, populations or years. Unlike other marine vertebrates for which length, weight or otolith characteristics (fish) or tooth structure (seals and odentate whales) can be related to age, birds can only be aged accurately by means of a unique and durable identification marker. There are relatively few bird populations with a large number of individuals marked in this way and investigations into age-related changes in mercury concentrations in birds have been few in number.

Age has been found to effect bioaccumulation of some metals in a variety of bird species (Hoffman & Curnow, 1979; Cheney et al., 1981; Hutton, 1981; Lambertini, 1982; Maedgen et al., 1982), although evidence for age accumulation with mercury is lacking.

It is thought that elimination of mercury into feathers during the moult is usually efficient enough to prevent age accumulation. However there is also evidence for demethylation by seabirds and subsequent storage of inorganic mercury in the liver (Thompson & Furness 1989a). This suggests that age accumulation of mercury is likely to occur in species having particularly high concentrations of inorganic mercury (for example certain albatross species with slow moult patterns) (Muirhead & Furness, 1988; Thompson & Furness, 1989a,b).

1.4.2 Sex related variation

Reproductive processes could lead to differences in mercury dynamics between males and females. In higher vertebrates it has

been shown that mercury can cross the placental barrier (Nordberg & Skerfving, 1972; Reijnders, 1980; Ronald et al., 1984) and methyl mercury has been reported to be excreted in the milk of lactating female marine mammals (Kim et al, 1974; Born et al., 1981). With losses of mercury via these routes one might predict mercury levels might fluctuate over the breeding season in females and tend to be higher in males.

Egg production offers a route by which female birds eliminate mercury from the body. However, evidence on the quantity eliminated is conflicting. Honda et al.,(1986b) concluded that mercury loss via this route was negligible compared to the mercury burden of the female Adelie penguin whereas Becker et al., (1989) reported low mercury concentrations in herring gulls Larus argentatus but suggested that the loss of mercury via this route acted as an important method of depollution for the female.

Since many bird species undergo a complete post-breeding moult, there are likely to be pronounced changes in the mercury accumulation of internal tissues of such birds during the course of the breeding season and subsequent moult, although evidence for seabirds is lacking as few authors have examined sex as a variable. Peterson & Ellarston (1976) found no sex differences in mercury levels of long-tailed ducks Clangula hyemalis and Fleming (1981) found no differences between male and female canvasbacksAythya valisineria with respect to either mercury or nickel. Gochfeld & Burger (1987) found significant differences in several metals between male and female ducks, although mercury levels were the same for both sexes.

1.4.3 Dose response relationships

The use of seabirds as metal monitors depends on the assumption that environmental variation will be reflected by levels of heavy metals in tissues. At the very least, tissue concentrations should reflect dietary uptake of the particular metal being monitored.

Exposure of experimental mallards and black ducks Anas rubripes to low dietary levels of methyl mercury (0.5-3.0 µg/g) resulted in a significant dose-dependent deposition of mercury in the primary feathers (Heinz, 1976). For waders, Goede (1985) reported a significant positive correlation between mercury levels in the shafts of the feather and mercury in the liver tissue. In some experimental studies, tissue levels have increased in direct proportion to dietary levels over a particular dose range (Scheuhammer, 1987) but dose responses are often non-linear with a lower ratio of tissue to dietary concentration at high doses. Mercury also accumulates in eggs in a dose-dependent fashion in response to increasing dietary methyl mercury (Tejning, 1967; Heinz, 1976; March et al., 1983), although there is evidence for a decrease in dose response of up to 20% in eggs (Tejning, 1967).

However it must be noted that chronic exposure of seabirds to heavy metals over a period of years or even decades with associated seasonal variations in uptake and elimination may invalidate comparisons with acute experimental dosing over weeks or months using species with physiology considerably removed from that of a seabird species.

1.4.4 Physiological and seasonal variation

Traditionally the liver has been used for mercury analyses when examining metal contamination. This organ was initially

chosen because it was easy to identify and of a consistent structure throughout. However its physiological function is such that it is continually changing in size and composition during both the day and the year (N.E.R.C., 1983). Also it has been suggested that heavy metal concentrations may increase due to stress and/or starvation (Osborn, 1979) and therefore the use of feathers or eggs may give a better indication of contamination.

Seasonal variation in metal concentrations in seabirds in respect to moult cycles, however, can be considerable. Seabirds at the start of a moult will contain relatively high concentrations of mercury in the internal tissues but as the body pool of this metal diminishes with the formation of new feathers, birds sampled at the later stage of the moult cycle will contain relatively less mercury in the internal tissues. This variation is a well known process but one which is not always taken into account when soft tissue levels are presented. The body burden of mercury (excluding the plumage) in Bonapartes gulls Larus philadelphia declined by 93% during the autumn moult in south-east Canada (Braune & Gaskin, 1987). In the black-eared kite Milvus migrans lineatus body burden declined by 70% during the moult and concentrations in the liver by up to 64%, kidney, 59% and other tissues 28-78% (Honda et al., 1986a).

Mercury concentrations tend to be higher in feathers grown early in the moult sequence (Furness & Hutton, 1980; Furness et al., 1986; Braune & Gaskin, 1987; Lewis & Furness, in press). Therefore the average mercury concentration of the plumage can be altered at different times of the year due to stages in the moult effecting the overall body concentration. Feather mercury concentration will therefore be most consistent in samples taken outside the moult period, while soft tissue levels should always

be considered in relation to timing of the moult.

Seasonal changes in tissue concentrations of essential elements, notably zinc, have been documented in the house sparrow Passer domesticus (Ojanen et al., 1975), starling Sturnus vulgaris (Osborn, 1979) and waders (N.E.R.C., 1983). Some such changes are associated with moult and can be accompanied by elevated concentrations of non-essential elements (e.g. cadmium and mercury). Seasonal variation in fat or protein content of tissues may also be important (Osborn et al, 1979, Osborn, 1979). For example an increase in metallothionein content may increase the number of binding sites available to essential elements (e.g. zinc) and their toxic competitors (e.g cadmium and mercury) (Osborn, 1978).

Gilbertson (1974) found no significant correlations between mercury concentrations and season date in common terns Sterna hirundo. However, Becker (1988) did find that the laying sequence of herring gulls effected mercury concentration, with the first laid eggs holding significantly more mercury than subsequent eggs.

1.4.5 Species variation

Species differences exist with regard to sensitivity of the toxic effects of methyl mercury. Gardiner (1972) found that feeding a diet containing 33 µg/g of mercury for 35 days to pheasants, ducks and chickens resulted in 90%, 85% and 7.5% mortality, respectively. Koeman et al. (1971) reported lethal levels in kestrelsFalco tinnunculus at around 13 µg/g but Fimreite (1971) found no deaths or wet loss in pheasants at 10 µg/g.

Such differences may in part be a result of the differences

in the ability of various species to decompose methyl mercury to the less toxic inorganic form. The form of mercury in birds has received little attention. Osborn et al. (1979) reported that mercury is predominantly organic in the liver and kidney of three species of seabird from the north east Atlantic, while Norhiem et al. (1982) found that the proportion of organic mercury in the liver tissues of south polar skuas Catharacta maccormicki varied from 20% to almost 100%.

Thompson & Furness (1989a) found that in some bird species the organic fraction can be as little as 3% of the total mercury, although the prey items contained mainly methyl mercury. It appears that in some species, eliminatory mechanisms and detoxifying processes may be important in determining the mercury levels of the internal organs.

Mercury transfer to eggs may also be species specific (Scheuhammer, 1987). Eggs from fish-eating birds may be more tolerant than those of non piscivorous species, since mallards have been reported to have reproductive dysfunctions at mercury levels of 6-9 µg/g, whereas levels of 2-16 µg/g have been reported to have no apparent effect on hatching and fledging in herring gulls (Vermeer & Peakall, 1977).

The aims of this study were to shed some light onto these aspects of mercury accumulation, storage and elimination in birds. In particular, patterns of mercury accumulation with age, sex and species were examined. The way in which feather mercury levels related to those of the internal tissues, eggs, and dietary mercury intake was also studied as well as an assessment of the usefulness of feathers and eggs to monitor mercury contamination.

The reader's attention is drawn to the format of this thesis, in which each chapter has been treated as a separate section, with its own introduction, methods and result section, discussion and reference list. This will obviously mean that some works will be cited repeatedly, but many are relevant only to the chapter in which they appear, and as such are more appropriately placed there than as part of one large reference list at the end of the work. Similarly, introduction and method sections overlap to some extent because the aim has been to prepare most chapters in a form appropriate for publication as independent papers.

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CHAPTER 2

General materials and methods: sample collection, preparation and mercury analysis

Each chapter gives materials and methods specific to that study. However there are various methods used throughout this work which will be included for easy reference in this chapter.

2.1.Sample preparation.

2.1.1 Feathers

Gross surface contamination of feathers was removed by a feather laundering process as outlined below.

1.Feathers were placed in clean boiling tubes and covered with chloroform. The tubes were then placed in the tank unit of an ultrasonic cleaner (Burndept Ltd., Erith, Kent) for 5 minutes.

2.The chloroform was then decanted off and the above process repeated.

3.Samples were then dried for one hour at ca. 50°C to 'drive off' excess chloroform.

4.Feathers were then covered with acetone and washed in the ultrasonic bath for 10 minutes. The used acetone was decanted off.

5.Feathers were covered with distilled water and washed in the ultrasonic bath for 5 minutes. Used water was then decanted off and this step repeated 3 times.

6.Samples were dried at 50°C for 24 hours, and allowed to equilibrate with ambient laboratory temperature (ca. 22°C) prior to weighing.

Bound mercury is not removed from the feather by this procedure (Furness, unpublished data). Feather weight was taken as that obtained for a given sample dried at ambient laboratory temperature. This was due to feather weight increasing quickly once out of the oven, presumably due to the reabsorption of water vapour. It was therefore felt that an accurate dry feather weight could not be obtained with any consistency and the 'dried

at ambient laboratory temperature' weight was used.

2.1.2 Internal tissues

Internal tissues (liver, kidney and pectoral muscle) were dissected out using stainless steel blades and instruments. Wet samples were weighed and dried, in an oven at 50°C, to a constant weight as determined by repeated weighing. The remaining body of the bird (the total body minus the liver, kidney and pectoral muscle) known as 'carcass' was also treated this way. All tissues were ground using a pestle and mortar. The carcasses were ground using an electric food processor. Dried samples were stored in air-tight glass vials prior to analysis.

2.1.3 Eggs

Egg samples were usually frozen prior to treatment. These were then thawed, opened and the contents removed. The entire contents were then homogenised using a food processor and dried to a constant weight in an oven at 50°C and stored in an air-tight glass vial until analysis.

All internal tissues and eggs were dried to a dry weight and all subsequent calculations are given on the dry weight basis. This was to reduce variation caused when using a wet weight (samples can vary up to 78% due to dehydration effects when using a wet weight. (Adrian & Stevens, 1979)). Eggs are especially susceptible to variation as water is lost during incubation and due to processes such as freezing (Potts, 1968).

2.2 Mercury analysis

Total mercury concentrations were measured using a cold vapour, atomic absorption spectrophotometry technique, incorporating a Data Acquisition Ltd. DA 1500-DP6 Mercury Vapour Detector. For reasons of logistics and relative convenience,

mercury analysis was spread over 2 days. Samples (both feathers and internal tissues) were subjected to the following procedure prior to mercury measurement:-

Day 1

1. Samples of ca. 0.05-0.25 g were weighed out accurately (to 0.001 g) using a Precisa 300MC (Metragram Instruments Ltd., Aspley Guise, Buckinghamshire) top-pan balance and placed in Kjeldahl flasks. Samples were digested using a 4 ml: 1 ml mixture of concentrated sulphuric and concentrated nitric acids (analytical grade) in a water bath at 50^{o}C for ca. 2 hours. Flasks were shaken occasionally to aid sample digestion.

2. On complete tissue digestion, the flasks were placed in a refrigerator (ca. 4^{o}C) to cool for 30 minutes.

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

4. The cooled 5% potassium permanganate solution was added to the cooled, digested samples in 2 ml aliquots using a graduated syringe. Flasks were placed back in the refrigerator between additions for ca. 10 minutes to prevent the reaction mixture becoming too hot and developing froth. A total of 14 ml of the 5% potassium permanganate solution was added to each sample which was considerably more than required to effectively oxidised the tissue present. The flasks were kept in the refrigerator overnight.

5. A 2% potassium permanganate solution (12 g potassium permanganate added to 600 ml distilled water) was made up in another dark glass bottle and left on a magnetic stirrer over night.

6. A 50% sulphuric acid solution was prepared by carefully adding 300 ml of concentrated sulphuric acid to an equal volume of distilled water in a conical flask placed in a cold water bath. The flask was covered and left over-night.

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

Day 2

1. Any precipitate in the Kjeldahl flasks was dissolved using 30% hydrogen peroxide solution added drop-wise.

2. Each sample was poured into a 25 ml volumetric flask and made up to volume with distilled water. The Kjeldahl flasks were rinsed with distilled water and these rinsings made up part of the 25 ml. The volumetric flasks were inverted repeatedly to ensure complete mixing and part of each sample was poured into a 10 ml beaker to await analysis.

3. Standard solutions of mercury (II) nitrate were prepared by adding 100 µl of the 2% potassium permanganate solution and 100 µl mercury (II) nitrate to a 100 ml volumetric flask, made up to volume with distilled water. Replicate standard solutions, usually three, were made up in this way, inverted repeatedly to ensure complete mixing and poured into beakers to await analysis.

4. The remaining 2% potassium permanganate solution was mixed with the 50% sulphuric acid in a dark glass bottle and cooled in the refrigerator for ca. 15 minutes.

Mercury analysis of samples was performed by adding 1 ml of

sample, 20 ml of the acidified potassium permanganate solution and 25 ml distilled water to a Dreshel flask; this mixture was reduced with 10 ml of the reducing agent (see step 7, Day 1) and free mercury so produced drawn through magnesium perchlorate drying agent and into the analyser as a vapour. 'Background' mercury levels in chemicals used were accounted for by repeating the above procedure, but omitting the 1 ml of sample. All readings were subsequently corrected for the 'blank' reading which was taken as the average of at least twelve blanks performed during the run. Calibration of the analysis was performed with replicate analyses of standard mercury (II) nitrate solutions; 100 µl (equivalent to 100 ng of mercury) of the standard solution was analysed as above. 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 mercury (II) nitrate solution was analysed. Between six and nine measurements of standards were made to give a mean reading for 100 ng of mercury. The above recipe allowed for up to 32 samples to be analysed; blank and standard readings were checked during the course of sample analysis. All chemicals used were of 'Spectrosol', 'Analar' or 'Puranal' analytical grades throughout. The mercury vapour detector was allowed to equilibrate to its working temperature for at least 2 hours prior to every set of analyses.

To determine the mercury concentration in a particular sample, the following steps were followed:-

1. Blank readings were subtracted from standard readings, and the mean 100 ng standard reading obtained.

2. The blank reading was subtracted from the sample reading and the amount of mercury (in ng) determined by...

$$\frac{\text{Reading}}{\text{Mean Standard}/100}$$

3. The mercury concentration (ppm or µg/g) is given by...

$$\frac{\text{ng Mercury X } 25^{*}}{\text{Sample Weight X } 1000^{**}}$$

2.2.1 Method accuracy and detection limits

The accuracy and reproducibility of the mercury determination method were tested by analysing International Atomic Energy Agency horse kidney Reference Material H-8. The results obtained from replicate analyses of mercury concentration in this material are presented in Table 2.1 (see also Thompson & Furness, 1989). It can be seen that the results obtained agree closely with those presented from other laboratories, indicating that the technique employed is both accurate and reproducible, especially since the mercury levels in the H-8 material was compared with most of the samples presented in this thesis. The lower limit of detection was 0.01 µg/g fresh weight of tissue, based on a reading of 1 unit on the digital scale of the analyser. Given that the average 100 ng standard reading was likely to be 200 units and up to 0.25 g of dry tissue could have been analysed, the reading of 1 unit would result in a concentration of ca. 0.05 µg/g dry weight or 0.01-0.02 µg/g wet weight, depending upon the water content of the tissue analysed.

* Multiple by 25 due to only 1ml in a 25ml Sample (see Step 2, Day 2) analysed

** Multiple by 1000 to give concentration in µg/g rather than ng/µg.

Table 2. 1. Total mercury concentration (µg/g dry weight) of International Atomic Energy Agency horse kidney Reference Material H-8.

Replicates	x	95% Conf. limits	sd	se	Range	Source
12	0.88	0.86-0.90	0.02	0.01	0.84-0.91	This study
19	0.91	0.83-0.98	0.16	0.04	0.52-1.13	IAEA Data

IAEA data based on accepted laboratory averages combining 85 individual determinations.

2.3 Glassware laundering

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

2.4 Statistical analysis

Since mercury distribution patterns have been shown to deviate significantly from a Gaussian distribution (Muirhead & Furness, 1988) all data were initially tested for normality using Kolmogrov-Smirnov one sample tests. Any data found to deviate from a Gaussian distribution were tested using non-parametric techniques, to assess trends and differences within and between sets of data in the study. Data that were normally distributed were analysed using parametric techniques. Specific details relating to tests used and distribution patterns of particular data are presented in the individual chapters.

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CHAPTER 3

The effect of atmospheric deposition on levels of mercury found in the plumage of seabirds.

This study may be considered to be supplementary to Chapter Two.

3.1 INTRODUCTION

The level of mercury in bird feathers has been used by numerous authors to indicate the degree of mercury contamination in the environment but specifically with regard to contamination within the food-web (e.g. Berg et al., 1966; Furness & Hutton, 1979, 1980; Appelquist et al., 1985). The rationale for this is that biomagnification of mercury may occur through food webs, and birds on the higher trophic levels accumulate greater concentrations of mercury than found in animals at lower levels. Feathers as an indicator tissue have several advantages over internal tissues as it is known that important heavy metals such as mercury can be present in the plumage in higher concentrations than found in organs, which allows a more reliable analysis (Westermark et al., 1975), and a large number of individuals can readily be assessed in a manner that avoids killing the specimens. Historical changes may also be examined by analysis of museum specimens (Appelquist et al., 1985; Thompson et al., in press).

If bird feathers are to be used to elucidate trends in the marine environment where the variation in mercury concentration of feathers from unpolluted to polluted areas is often less than one order of magnitude, a knowledge of the routes by which mercury becomes attached to feathers becomes extremely important.

Uptake of mercury from the environment on or into the plumage can occur via three routes. Firstly, it can be incorporated during feather growth. Mercury contained in the diet is deposited into the growing feathers during a moult and is bound strongly to disulphide linkages within the keratin molecule (Crewther et al., 1965). Secondly, contamination of the

feathers can occur during preening as mercury may be present in secretion products of the salt and/or the preen glands. Finally, the metal may be deposited onto the feathers through contact with the habitat, for example from the water or from atmospheric deposition of mercury from the air.

Many authors have assumed that the concentration of mercury found in the feathers reflects levels circulating in the blood at the time of feather growth (e.g Johnels & Westermark, 1969; Westermark et al., 1975; Linderberg & Odsjo, 1983; Solonen & Lodenius, 1984) and have ignored or dismissed the possibility that exogenous sources could contribute to the metal levels found during routine analysis of plumage.

Goede & de Bruin (1984) showed by exposing juvenile feathers to water and mud taken from the natural habitat that mercury is not taken up from seawater by the feathers. Appelquist et al., (1984) showed that the influence of U.V. light, heating and freezing on mercury levels in primary feathers was also negligible.

However, Ranta et al. (1978) suggested that certain metals in feathers might be of exogenous origin and Kelsall & Burton (1979) noted that visibly soiled feathers showed elevated levels of iron and silica. Other evidence was said to be seen in the work undertaken by Hanson & Jones (1976) and Kelsall & Burton (1979) where differing mineral content was seen between half grown feathers and feathers that had been fully emerged for a year. Mammalian hair has also been shown to change in mineral content due to exogenous contamination (Van den Berg et al., 1967).

Rose & Parker (1982) reported that ruffed grouse Bonasa umbellus feathers take up nickel, copper and iron from the

environment near a smelter emissions factory in relation to exposure time and in proportion to the metal concentration. The mechanism by which these gains occur was not investigated but physiochemical surface deposition as described by Nieboer et al. (1976) for lichens was thought to be involved.

More recently Hahn et al. (1988a, b) reported that cadmium, lead, copper, nickel and cobalt found on feathers of terrestrial birds in a polluted area of Germany was mostly due to external build up on the feather. By demonstrating a correlation between the heavy metal content in feathers and the deposition obtained from wet deposition samples they suggested that the plumage integrates heavy metals from the atmosphere in proportion to time and distance between a birds' home range and emission sources, although no attempt was made to distinguish between metal on the feather surface and metal chemically bound in the feather structure. They supported this idea by examining metal concentrations in newly hatched chicks (not exposed to the atmosphere) with levels in feathers from one year old birds, which were found to be higher in cadmium, copper, nickel and cobalt. They also examined different parts of the feather and concluded that higher heavy metal concentrations were seen on the more exposed feather parts such as the tip of the primaries.

When the feather is used as an indicating or monitoring tissue it is essential to know the under-lying mechanisms which determine the final feather concentration otherwise problems or errors will arise in the interpretation of results. Most studies examining external deposition of metals have not looked in detail at mercury levels and it has been generally assumed by other authors who have studied mercury levels that mercury concentrations found in feathers are due to incorporation from

the diet and not from exogenous sources. If it is indeed the case that mercury is substantially deposited onto the feathers by external sources many past studies will have to be viewed in a new light.

In this present study it was hoped to determine if mercury is deposited onto bird feathers during the course of a ten month exposure time. It was also hoped to determine if the more exposed parts of the plumage such as the tips of the feathers are naturally higher in mercury content due to the physiology of moult and metal incorporation from the blood or whether this phenomenon is indeed due to external deposition.

3.2 MATERIALS AND METHODS

3.2.1 Experimental procedures

Between May and June 1990 fourteen pairs of bird wings from a variety of species found dead on Foula, Shetland were collected. The species sampled were as follows; puffin Fratercula arctica, pigeon Columba livia, black guillemot Cepphus grylle, great skua Catharacta skua, herring gull Larus argentatus, great black-backed gull Larus marinus, gannet Sula bassana and shag Phalacrocorax aristotelis. Nine pairs of wings were randomly selected to act as the experimental group and these were separated. The left wing from each pair was secured to a roof top of one of the houses on Foula and exposed to the atmosphere for 10 months. The right wings were placed in mercury-free polythene bags and frozen for 10 months at ca.-20°C to prevent mercury gain during storage, as were five pairs of wings which were to act as a control group. After ten months the left wings were collected and stored as the controls prior to analysis. All samples were thawed and dried to ambient room

temperature. No washing procedure was performed.

There is a characteristic mercury distribution pattern to be found between primaries (see Chapter 6 and Furness et al., 1986). In order to account for this natural variation in mercury concentration, primary feathers numbers 1, 5 and 10 of each wing were taken (primary 1 is defined as the innermost primary feather, the first moulted).

To examine mercury concentrations in different parts of the feather a bird not yet exposed to the outside atmosphere was used. Primary feathers 1-10 from a laboratory-reared black-headed gull chick (used in a previous experiment, see Chapter 6) were removed. The feathers were measured and split into equal thirds, an upper, middle and bottom section. These sections were then analysed separately.

Mercury analysis was carried out as described in Chapter 2. Any feather too large to be analysed whole was split longitudinally using a scalpel blade and the left portion of the feather analysed.

3.2.2 Statistical analysis and calculations

All data were tested for normality using a Kolmogorov-Smirnov one sample test. Differences between exposed (left) and unexposed (right) wings were tested using a paired t-test. Differences between the top, middle and bottom sections of the feather were tested using one-way analysis of variance. Absolute percentage variation was used to examine the difference between mercury concentrations in the left and right wing. This was calculated by subtracting the lowest concentration from the highest concentration and the difference was divided by the smaller figure, to give the largest possible percentage

variation.

3.3 RESULTS

Mercury concentration in all the feathers analysed are presented in Table 3.1. Mean mercury concentrations for the different bird species analysed are summarised in Table 3.2. There was no significant difference between the exposed left wing and the unexposed right wing (paired t-test, t= -1.92, n=9, N.S.), although the unexposed feathers showed a tendency to have higher mercury concentrations than those of the exposed feathers. It is noted that variation between the primary feathers of the control group was a mean of 8.4% compared to the experimental group where the mean difference between the left and right wing was 7.4%

Table 3.3 Mercury concentration in different portions of the primary feathers of a black-headed gull fledgling

Primary	Upper	Middle	Bottom
1	20.68	16.74	7.53
2	20.37	16.86	6.94
3	19.84	17.11	6.09
4	19.34	14.22	6.10
5	18.99	14.51	5.63
6	18.04	12.90	5.02
7	16.11	13.30	4.84
8	18.25	11.85	4.50
9	18.49	11.63	4.51
10	17.47	10.12	3.38
Mean	18.76	13.92	5.45
SD	1.39	2.42	1.25

Mercury concentrations in the different parts of the primary feathers of the black-headed gull fledgling can be seen in Table 3.3. There is a significant difference between levels of mercury in the top portion of the shaft to the levels in lower portion. (One-way analysis of variance $F_{2,29}$= 145.7, P<0.001) (—Tukey's range test P<0.05)

Table 3.1 Mercury concentrations in primary feathers of exposed and unexposed wings of a variety of bird species.

Bird species	Primary No	Exposed wing Hg(µg/g)	Unexposed wing Hg(µg/g)
Experimental			
Pigeon	1	0.42	0.44
	5	0.05	0.04
	10	0.06	0.04
Black guillimot	1	2.55	2.47
	5	2.14	2.03
	10	1.16	0.96
Puffin	1	7.79	7.81
	5	7.81	7.93
	10	7.10	7.44
Great skua(A)	1	5.52	5.49
	5	2.96	3.01
	10	2.92	3.12
Great skua(B)	1	4.51	5.74
	5	3.01	3.94
	10	2.97	2.80
Great skua(C)	1	7.21	7.34
	5	7.20	6.95
	10	6.01	6.35
Herring gull	1	1.98	1.87
	5	1.24	1.37
	10	1.01	1.24
Great black-backed gull	1	2.31	2.25
	5	1.55	1.77
	10	1.49	1.50
Gannet	1	2.49	2.65
	5	1.41	1.69
	10	1.03	1.54
Controls		Left wing	Right wing
Puffin	1	9.34	10.23
	5	9.01	9.25
	10	6.41	5.59
Puffin	1	8.84	7.34
	5	7.01	7.01
	10	6.98	5.28
Puffin	1	5.01	5.00
	5	4.87	4.91
	10	4.79	4.60
Black guillimot	1	4.41	4.01
	5	3.56	3.79
	10	2.51	2.89
Shag	1	4.39	2.90
	5	3.87	3.42
	10	3.06	2.99

Table 3.2 Mean mercury concentrations (µg/g) in the exposed and unexposed wings of a variety of bird species

Bird species	Exposed Wing Hg(µg/g)		Unexposed wing Hg(µg/g)		% Variation
	x	sd	x	sd	
Experimental					
Puffin	7.56	0.40	7.72	0.25	2.1
Pigeon	0.18	0.40	0.17	0.23	5.8
Black guillemot	1.95	0.71	1.82	0.78	7.1
Great skua	3.80	1.49	3.87	1.40	1.8
Great skua	3.50	0.88	4.16	1.48	18.9
Great skua	6.81	0.69	6.88	0.49	1.0
Herring gull	1.41	0.51	1.49	0.33	5.6
Great black-backed gull	1.78	0.46	1.70	0.47	4.7
Gannet	1.64	0.76	1.96	0.60	19.5
Controls	Left wing Hg(µg/g)		Right wing Hg(µg/g)		% Variation
	x	sd	x	sd	
Puffin	8.25	1.60	8.36	2.45	1.3
Puffin	9.22	0.45	6.54	1.11	16.4
Puffin	4.89	0.11	4.84	0.21	1.0
Black guillemot	3.49	0.95	3.55	0.61	1.7
Shag	3.77	0.67	3.10	0.28	21.6

3.4 DISCUSSION

Comparatively little research has been carried out to determine whether the metal composition of a sample of feather material reflects the metal concentrations at the time of

feather formation. The assumption has been made that no changes occur in concentration after the formation of the feather has been completed. Hahn et al. (1989a,b) reported that levels of cadmium, lead, copper, nickel and cobalt found on bird feathers were due primarily to atmospheric deposition rather than to excretion from internal tissues. Rose & Parker (1982) found that the plumage of ruffed grouse took up metals from the environment near a smelter emissions factory. However, none of these studies looked at mercury levels.

In the present study no evidence was found that the relatively high mercury levels detected in feathers was external in origin although there was some suggestion of a reduction in mercury concentration during the ten month exposure time. This, however, was not sufficient for there to be any statistically significant difference between exposed and unexposed wings. It seemed unlikely that mercury would be deposited externally onto feathers, particularly seabird feathers, for several reasons.

The major source of mercury to the open ocean although from atmospheric transport, is through introduction by rainfall (Fogg & Fitzgerald, 1979; Gill & fitzgerald, 1987). The sparse geochemical data which are available indicate that there is a high transfer of mercury from the continents via rivers and into the oceans at a rate of about 5000 tonnes per year (Gill & Fitzgerald, 1987) and it is this mercury which is assumed to be reflected in the feathers of seabirds via the mercury taken up by birds from the foodchain.

Very little satisfactory information exists on the amount of mercury accumulated and distributed by atmospheric air masses but Berglund et al. (1971) found the levels of mercury in rainfall to be around 0.1 ng Hg/g and contributions by rainfall

to mercury in the soil to be around 0.06 mg/m^2. This is a small amount when compared to the amount of mercury seabirds consume in their food. Concentrations of mercury over the oceans are also significantly lower than over the land (Williston, 1968) so this figure would be further diminished for seabirds and it seems unlikely that this mercury would contribute significantly to the mercury found in seabird feathers, although the picture may be different in the case of terrestrial birds near highly industrialised areas and this requires further research.

The other main reason it was though unlikely that atmospheric mercury played a significant part in the total mercury found in the plumage is that the prevailing form of mercury reaching water bodies through the atmosphere and run off is inorganic and phenyl mercury (Jensen & Jernelov, 1969, Jernelov, 1969a,b). Virtually all mercury in feathers of birds is in the methyl form (Thompson & Furness, 1989). This would suggest that mercury is incorporated into feathers from the diet alone and not from atmospheric deposition.

All individuals of the nine species of bird showed the same basic pattern of high levels of mercury in the first primary feathers moulted, with decreasing mercury levels in later-grown feathers (Table 3.1). This is a general pattern and one resulting from the physiology of moult and amount of mercury stored (see Chapter 5). The body pool of mercury is reduced during moult so that amounts entering growing feathers decline through the moult (Furness et al., 1986; Braune & Gaskin, 1987; Lewis & Furness, in press).

When Hahn et al. (1989a,b) examined different parts of the feather they found that the more exposed parts, i.e, the tips of the feathers contained higher metal levels than the parts of the

feathers near to the base. From this study it is seen that, at least for mercury, this is a purely physiological phenomenon resulting from a decline in mercury content in the body as the feathers are grown. It is worth noting therefore that when feathers are too large to be analysed whole, they should be split longitudinally otherwise elevated or depressed data will result according to which part of the feather is used.

It appears that external mercury contamination is a negligible factor when considering results gained from feather analysis for seabirds. Contamination due to dirt may be a problem as this would increase the mass of the feather and could give a deflated mercury concentration. This may have been the case in the present study where washing the feathers was not feasible due to the nature of the experiment. It is therefore preferable to wash feathers vigorously prior to analysis. While this may be advocated to remove or reduce extrinsic metal contamination by atmospheric sources (Gochfeld, 1980), it is also necessary to reduce deposited non-metal material that will otherwise reduce measured levels of metal.

Mercury monitoring using feathers appears to be a valuable technique providing that assumptions concerning the relationships between mercury levels in feathers and the uptake and accumulation by birds are valid. It is with this in mind that some of the following studies were undertaken, having demonstrated that atmospheric deposition of mercury onto feathers does not bias results.

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CHAPTER 4

Mercury levels in the plumage of red-billed gulls *larus novaehollandiae scopulinus* of known age and sex

This Chapter is based on a paper published in *Environ. Pollut.*, (1990), 63, 33-39, but with a considerable amount of further data added.

4.1 INTRODUCTION

Mercury has been studied extensively from the viewpoint of terrestrial and marine environmental pollution (Bagley & Lockey, 1967; Martin & Nickerson, 1973; Munoz et al., 1976; Hulse et al., 1980), mainly because it is a non-essential metal and is highly toxic even at low levels. Unlike other heavy metals, mercury also shows biomagnification up food chains, due to the organic form (methyl mercury) being lipid soluble (Bryan, 1979).

Metal concentrations in animal tissues are generally assumed to reflect the extent of habitat contamination (Phillips, 1990). Such assessments often fail to consider possible confounding effects of other factors such as sex, age or intraspecific variations in the diet. It is well known that mercury and cadmium levels in fish increase with fish size and/or age. These metals also show age-related increases in levels in livers and kidneys of marine mammals (Caputi, 1979; Schultz & Ito, 1979; Watling et al., 1981; Ronald, 1984). Contrary examples seem to be rare, at least for mercury.

Birds have been used extensively to study heavy metal exposure and toxicity and are widely used as monitor organisms (Anderlini et al., 1972; Martin & Coughtrey, 1975; Simpson et al., 1979; Muirhead & Furness, 1988; Thompson et al., in press). They are ubiquitous, occupy high positions in the food web and in particular, analysis of feathers has been used to provide a method for examining mercury levels without killing the birds. This also allows comparisons between contemporary and historical samples to be made (Borg et al., 1966; Lindberg & Odsjo, 1983; Appelquist, 1985; Thompson et al., in press).

Influences of age on metal levels in seabirds are not clear, largely because it is impossible determine accurately

the age of adult birds, especially seabirds, except when these have been ringed as chicks. A number of authors have found higher metal levels in adult birds than in juveniles or chicks (Hoffman & Curnow, 1979, Cheney et al., 1981; Hutton, 1981; Lambertini, 1982, Maedgen et al., 1982; Lindberg & Odsjo, 1983; Honda et al., 1986). Furness and Hutton (1979) found statistically significant increases with age in the levels of cadmium in the kidney and liver of great skuas Catharacta skua. Hutton (1981) found no difference in the levels of cadmium or mercury between small samples of herring gulls Larus argentatus aged 2-4 years compared to birds over 9 years old.

Further information on age-related variations in metal levels in birds is required as considerable use has been made in recent years of birds as indicators. If mercury levels increased with age the use of feathers as a monitoring tool would be hindered as comparisons between populations differing in age structure could give misleading results.

Mercury also accumulates in bird eggs (Tejning, 1967; Backstrom, 1969; Heinz, 1974; March et al., 1983) and these have been used extensively in monitoring for mercury pollution (Barrett et al., 1985, Becker et al. , 1985; Newton et al., 1989). If mercury is excreted into eggs in large quantities it may be expected that differences in male and female mercury levels would have been observed for birds with high mercury burdens. Few authors have examined sex as a variable when considering metal accumulation (Evans & Moon, 1981; Hutton, 1981; Custer & Mulhern, 1983) and of those authors that have, most have found no significant difference between male and female mercury levels (Peterson & Ellarston, 1976; Fleming, 1981; Gochfeld & Burger, 1987). However, Braune & Gaskin (1987)

found that adult female Bonaparte's gulls Larus philadelphia had significantly lower mercury concentrations in their primary feathers than the male birds, suggesting that the females reduce their body pool of mercury through egg laying. If this were the case then once again the use of feathers as indicators would be impaired as comparisons between samples with differing sex ratios would give misleading results.

Many red-billed gulls Larus novaehollandiae scopulinus nesting in colonies on the Kaikoura Peninsula, New Zealand, had been ringed there as chicks for studies of the biology of known-age individuals. This study reports levels of mercury in samples of body feathers from some of these adults, aged 2-30 years old, and from chicks. Mercury levels will be related to diet and will be affected by features such as individual dietary specialisations. During the breeding season, extending from October to January, red-billed gulls feed principally in the inshore region on surface swarming crustaceans and fish larvae. Regurgitations of 517 adults captured during the 1986-1987 breeding season revealed a diet consisting of 75% euphausiids (mostly Nyctiphanes australis), 6% fish larvae, 1.5% decapod larvae and amphipods, 0.6% small fish, 3% fish offal, 3% garbage from rubbish dumps and 9% earthworms (Lumbricus sp) from surrounding pasture farmlands (J.A.Mills unpublished). Outside the breeding season, some red-billed gulls remain in the Kaikora area and continue to feed inshore, but the majority of the population disperses, feeding at a range of sites such as mudflats, rubbish dumps and tidal beaches. Levels of mercury in foods taken at the terrestrial sites are likely to be lower than in the marine foods (Hutton, 1981; Thompson, 1990).

4.2 MATERIALS AND METHODS

4.2.1 Sample collection

From 27 chicks (16-26 days old) and 147 adult (known-age) red-billed gulls captured randomly with nest funnel-traps at Kaikoura in December 1988 and December 1990, samples of 4-8 small body feathers, chosen at random, were removed from the mantle. The feathers were stored in labelled polythene bags. Once in the laboratory the feathers were washed using procedures outlined in Chapter 2 and dried at 20°C. They were then weighed and analysed. Mercury analysis was carried out as described in Chapter 2.

The age and sex of each adult bird was determined from the ringing records, after mercury levels had been measured.

4.2.2 Statistical analysis

All data were tested for normality using Kolmogorov-Smirnov one sample tests. Comparisons between the two collections were made by a Mann-Whitney U-test as were comparisons between the levels in male and females, since the mercury levels differed from a Gaussian distribution. The relationship between age and mercury levels was examined using Spearman Rank Correlation.

4.3 RESULTS

There was no difference between the two collections (1988 vs 1990) of feather samples (Mann-Whitney U-test, $n_1=114$, $n_2=33$, $U_1=1775$, , $P>0.1$). There was no difference between male and female mercury levels in body feather samples (Mann-Whitney U-test, $n_1=89$, $n_2=58$, $U_1=2215$, , $P>0.1$)

The wide range of mercury concentrations within the sample from one breeding population and the skewed distribution of the

data should also be noted (Table 4.1).

Table 4.1 Mercury levels (µg/g) fresh weight in body feathers collected from red-billed gulls at colonies on the Kaikoura Peninsula, New Zealand, in relation to sex and age category.

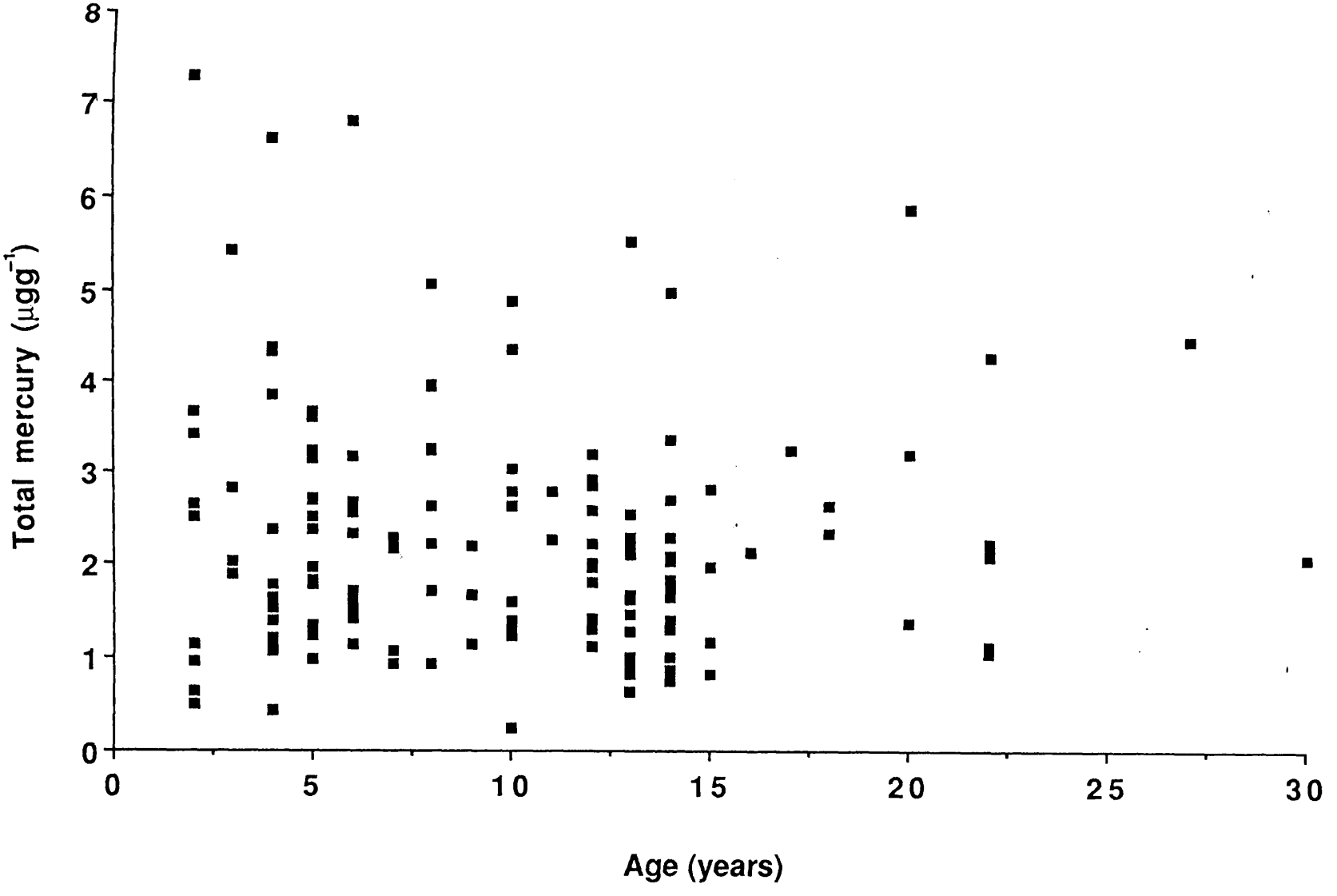
	Hg in feathers (fresh weight)				
	n	mean	sd	Median	CV
Adults	147	2.25	1.31	1.96	58
Adult males	58	2.39	1.33	2.15	56
Adult females	89	2.15	1.29	1.73	60
Chicks	27	2.02	1.16	1.63	57

Where CV = coeffiecient of variation

Mercury levels showed no trend with adult age (Spearman Rank Correlation Coefficient, r_s=-0.069, n=147, P>0.1), and visual inspection of the data indicated that the high level of individual variation in feather mercury concentrations is not associated with age (Fig. 4.1).

There was no difference between adult mercury levels and those found in chick feathers (Mann-Whitney U-test, N_1=147, n_2=27, U_1=1760, , P>0.1). However, the average concentration of mercury in chick feather samples was 83% of that in the adult feathers indicating a slightly lower level of mercury in chick plumage compared to adults.

Figure 4.1 Mercury levels in the plumage of adult red-billed gulls in relation to age.


Total mercury (μgg⁻¹)
8
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
Age (years)

4.4 DISCUSSION

Custer & Mulhern (1983) found significantly higher levels of cobalt in female black-crowned night herons Nycticorax nyticorax and Evans & Moon (1981) found higher cadmium levels in females of the bar-tailed godwit Limosa lapponica. Gochfeld & Burger (1987) found significant differences in levels of several metals between male and female ducks, although mercury levels were the same in both sexes. Hutton (1981) found differences in the levels of cadmium and zinc between male and female oystercatchers Haematopus ostralegus and Hoffman & Curnow (1979) found higher levels of mercury in male herons. Only one other study (Braune & Gaskin, 1987) found significant differences between male and female mercury levels. However, in most studies the levels of metals have not differed between the sexes, as found in the red-billed gulls.

The slightly lower levels of mercury found in chicks compared to adults is probably due to the short exposure time the chicks are subjected to between hatching and growing their feathers. Adults presumably accumulate mercury during the whole year whereas chicks accumulate it over a period of only a few weeks of development. In this respect the difference between chicks and adults is rather smaller than might be expected.

The lack of an age-related increase in the mercury levels in the plumage of adult red-billed gulls contrasts with the situation found in fish and marine mammal soft tissues. However, it is in accord with other recent studies of mercury dynamics in birds. Mercury levels in feathers appear to reflect the levels circulating in the blood at the time of feather growth. All mercury in the feathers is in the methyl form (Thompson & Furness, 1989) but inorganic mercury may form a large part of

the total mercury found in the liver of some birds. Therefore the amount of mercury going into the feathers depends on the amount of methyl mercury in the diet and on amounts stored in the soft tissues between moults (Furness et al., 1986; Honda et al., 1986).

Feathers are the major excretory pathway for mercury (Furness et al., 1986; Honda et al., 1986; Braune, 1987) and the amount in the plumage would suggest that age-dependent accumulation of mercury would not occur. While red-billed gulls appear to be able to eliminate mercury into the plumage each autumn, when they moult and regrow new feathers, it is not clear whether this is the case for all bird species. Some species, for example albatrosses, that have a high mercury intake and/or more limited opportunities to eliminate it (i.e. that moult less of the plumage or moult less often) may be forced to demethylate organic mercury to the inorganic form and so avoid the accumulation of the more toxic form of the metal. This may lead to the accumulation of inorganic mercury with age, but it seems unlikely to result in the accumulation of the organic form. Thus it appears that feathers taken from adult birds will reflect the intake of organic mercury over the period of less than one year previous to the growth of the feather. Lack of any age-accumulation effect on mercury levels in feathers of adult birds enhances their use as tools for monitoring of mercury levels in the environment.

Variability in metal levels within samples can be compared by calculating the coefficient of variation for each sample. Muirhead & Furness (1988) found that coefficients of variation of essential elements (zinc and copper) in liver and kidney samples from some seabird species were between 18 and 27, while

coefficients for non-essential elements (mercury and cadmium) were 30 to 57. This suggested that the levels of regulated essential elements showed less variability than levels of cadmium and mercury, with mercury being the most variable. In this study the coefficients of variation of mercury levels in the red-billed gull feather samples were 57 for chicks and 58 for adults (Table 4.1). The wide range of mercury levels, and the skewed distribution in the sample of gulls suggest that some specific factors that vary widely between birds are important in determining levels in individual birds. Hutton (1981) considered dietary specialisations or differences in the wintering areas or habitats frequented by different individuals to be the most important factors in determining individual heavy metal levels in populations of other seabirds and it is these factors that may lead to differences in mercury concentrations between individuals here. Of the two it would appear that dietary specialisation may be the more important factor since the coefficient of variation in chick feather mercury level was almost equal to that for adults, yet the food fed to chicks must all have been gathered from an area quite close to the Kaikoura colony.

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CHAPTER 5

Age-related changes and tissue distribution of mercury in kittiwake *Rissa tridactyla* chicks

5.1 INTRODUCTION

Contamination of marine ecosystems by mercurial compounds is a widespread and frequently documented problem. Analysis of mercury concentration in the feathers and internal tissues of birds have often been used for the study of mercury hazards to wildlife (Berg et al., 1966; Fimreite, 1974; Odsjo & Lindberg, 1977; Goede, 1985; Thompson et al., in press) as well as measuring the degree of mercury contamination in the environment (e.g. Borg, 1966; Furness & Hutton, 1979; Appelquist et al., 1985).

Mercury levels in birds result from a dynamic balance between rates of intake and elimination (Miller, 1960; Swensson & Ulfvarson, 1968; Becker et al., 1985). There are various difficulties in elucidating the factors concerning the bioaccumulation of mercury. Metal concentration and distribution in birds may be influenced by several physiological and biological processes, such as feeding habits, reproduction, moulting and migration as well as the metal species, route of exposure and antagonistic effects of other metals (Bagley & Locke, 1967; Martin & Nickerson, 1973; Martin & Coughtrey, 1975; Munoz et al., 1976; Sell, 1977; White et al., 1977; Simpson et al., 1979; Hulse et al., 1980, Honda et al., 1986; Blomqvist et al., 1987). In particular, the growth stage or age of the bird may be one of the causes of variation.

Osborn (1978) suggested that interpreting data gathered in metal pollution studies was more complicated than first assumed since variations in both essential metals and protein metabolism, which occur both seasonally and with age, might have considerable influence upon both the amount of toxic metal present in an animal and the site to which it is bound

(Haarakangas et al., 1974; Osborn 1979; Min et al., 1984). There may be differences due to age in the protein content of internal tissues such as the liver and kidney and also changes in the proportion of different proteins present. If such changes were to occur in the proteins with which toxic metals such as mercury are likely to interact (for example metallothioneins (Kagi & Valee, 1961)), then the toxic significance of the metal residue is likely to vary with those changes.

Age differences in heavy metals levels have been reported for a wide variety of bird species (Hoffman & Curnow, 1979; Hulse et al., 1980; Hutton, 1981; Maedgen et al., 1982; Gochfeld & Burger, 1987). However most studies have only investigated two or three age categories (i.e adults versus fledglings, chicks or eggs). Several authors have shown that some metals are accumulated in certain tissues of birds with growth (Haarakangas et al., 1974; Ojanen et al., 1975; Furness & Hutton, 1979; Osborn, 1979; Hulse et al., 1980; Cheney et al., 1981) and a number of authors have found higher metal levels in adult birds than in juveniles (Hoffman & Curnow, 1979; Hutton, 1981; Lambertini, 1982; Maedgen et al, 1982; Lindberg & Odsjo, 1983; Honda et al., 1986; Furness et al., 1990) although it has been found that mercury does not accumulate with age in some adult birds (see Chapter four; Thompson et al., in press). Any differences arising in metal levels due to age when examining adults and juveniles may be due to individual processes and physiological differences in how the different age categories store metals or, and more likely, the levels reflect the past exposure to the metals and the fact that adults will presumably have had a much longer exposure time to the metal than the

younger birds.

A detailed analysis of organ and tissue distribution of mercury and its variations with age in relation to developmental processes of chicks has not yet been presented. This chapter attempts to evaluate if mercury is accumulated differentially at successive growth stages in kittiwake chicks and the distribution of the metal after a single dose of methyl mercury is also reported.

5.2 MATERIALS AND METHODS

5.2.1 Sample collection and experimental procedure

In June 1990, 24 kittiwake Rissa tridactyla chicks were removed from the nest site (under licence) from a colony on Foula, Shetland (60° 08'N 2° 05'W). Each bird was weighed and wing length and head+bill length were measured. Each experimental bird was given 1000 µg of methyl mercury in the form of analytical grade methyl mercuric chloride solution. This was administered orally via a gelatin capsule. Each bird was ringed and the chicks returned to their nests which were subsequently marked by painting a number on the cliff next to the nest. Because kittiwake chick survival rate on Foula has been relatively low since 1988 many of the study group would be likely to die due poor food availability (Walsh, 1990) and a decrease in the experimental group was therefore expected. After 10 days all surviving experimental birds were sacrificed by cervical dislocation and stored intact at -20°C prior to further treatment. A sample of nine control birds of various ages were also taken to measure background mercury levels obtained from the egg and their food during early growth.

5.2.2 Sample preparation

On thawing, the birds were measured and wing length, head+bill length and weight recorded. The birds were plucked; primaries, secondaries, wing coverts, body feathers and tail feathers were removed, weighed and stored separately. In some cases the chicks' plumage had not yet developed and therefore the whole down was removed and stored intact. The chicks were dissected and kidney, liver and left pectoral muscle (pectoralis and supracoracoideus) removed. These along with the remainder of the body (referred to as "carcass") were dried to a constant weight in an oven at 50°C. Once dried the samples were ground using a pestle and mortar and a sub-sample removed for mercury analysis. A sample of all feather types was also analysed.

Mercury analysis was carried out as described in Chapter 2.

5.2.3 Calculations and statistical analysis

Calculations were made in both concentrations and in absolute terms because in the case of growing tissue or tissues with a fast turnover, concentrations would be declining as a result of dilution as new tissue was added. Mercury in the tissues is given on a dry weight basis to avoid errors associated with varying amounts of moisture (Adrian & Stevens, 1979). Mercury is also expressed as a percentage of the mercury intake and as a percentage of the body burden which refers to the amount of mercury in specific tissues as a percentage of the total mercury present in the whole body including plumage.

Wing length and head+bill length were used to indicate the age of the bird (Furness, 1983).

Because older chicks would have eaten more fish and therefore potentially taken in more mercury, controls were

separated into two groups according to age as defined by the wing length. This allowed an approximate measure of their natural mercury accumulation. Control group A consisted of birds with a wing length above 100 mm. Control group B consisted of birds with a wing length below 100 mm. All mercury levels expressed as a percentage of the mercury intake were corrected by subtracting the mean mercury levels in the appropriate control group.

Faecal excretion rates were gained indirectly. The total excretion value was calculated by adding the corrected total amounts of mercury in all the tissues analysed (including the carcass) for each individual bird. This was subtracted from the amount of mercury administered. Faecal excretion was calculated by subtracting the amount of mercury present in the entire plumage from the total excretion value on the assumption that the only routes of mercury elimination were into the plumage or in faeces.

Data were tested for normality as determined by Kolmogorov-Smirnov one sample tests. Relationships between mercury levels in the liver, kidney, pectoral muscle, carcass, plumage and age were tested using Pearson Product Moment Correlations. Relationships between mercury excretion, mercury levels expressed as a percentage of the body burden or mercury intake, with age were also tested using Pearson Product Moment Correlations. Differences between mercury levels in the internal tissue levels and feather types were tested using a One-way analysis of variance.

5.3 RESULTS

5.3.1 Mercury accumulation in internal tissues

Table 5.1 shows mercury concentrations and total amounts of

mercury in the tissues of experimental and control birds in relation to age as indicated by wing and head measurements. Table 5.2 shows the coefficients and associated probabilities for Pearson Product Moment Correlations between mercury concentration in the tissues analysed and age for experimental birds only and after subtracting for background (control) levels.

Table 5.2 Pearson product moment correlation coefficients for tissue concentration and total mercury with age as measured by wing length (Wgl) and head+bill measurements (H+B).

Tissue		Measurement of age	
		Wgl	H+B
Liver Hg concentration	r=	-0.640	-0.672
	P=	0.004	0.002
Liver Hg total	r=	0.185	0.245
	P=	0.463	0.349
Kidney Hg concentration	r=	-0.605	-0.466
	P=	0.008	0.041
Kidney Hg total	r=	-0.376	-0.289
	P=	0.125	0.245
Pectoral Hg Concentration muscle	r=	-0.405	-0.444
	P=	0.095	0.065
Pectoral Hg total muscle	r=	0.008	0.103
	P=	0.974	0.684
Carcass Hg concentration	r=	-0.508	-0.515
	P=	0.031	0.029
Carcass Hg total	r=	-0.132	-0.061
	P=	0.602	0.809
Plumage Hg concentration	r=	-0.689	-0.715
	P=	0.002	0.001
Plumage Hg total	r=	0.724	0.657
	P=	0.001	0.003

Table 5.2 Mercury concentration and total mercury in tissues of kittiwake chicks ten days after administration of 1000 µg of methyl-mercury.

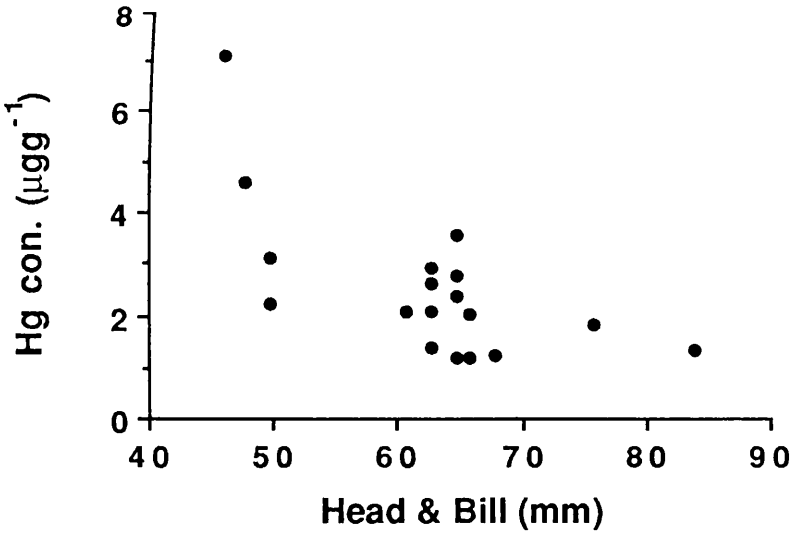
Wing length	Head+ Bill length	Liver		Kidney		Pectoral Muscle		Carcass		Plumage		Total Mercury
		ppm	µg	ppm	µg	ppm	µg	ppm	µg	ppm	µg	µg
180	83	1.16	4.18	2.90	1.83	0.99	0.73	2.05	123.63	15.64	206.63	337.25
146	65	1.01	3.75	1.20	1.20	1.40	1.93	0.40	21.34	12.55	299.16	327.38
132	67	1.05	3.31	2.40	1.46	0.89	0.63	1.11	70.62	16.23	236.61	312.63
143	65	1.85	5.57	1.27	0.66	1.43	0.60	1.54	55.04	21.97	234.04	295.91
122	62	1.20	3.00	2.40	1.39	1.33	0.99	0.78	36.22	17.42	217.99	259.59
40	49	2.89	0.92	3.04	0.33	3.40	0.17	1.38	16.13	21.00	22.47	40.02
91	62	2.72	6.31	2.32	1.14	2.35	0.66	1.60	45.63	19.37	155.96	209.70
114	62	2.40	5.33	2.60	1.33	1.58	0.73	0.60	21.04	22.37	179.78	208.21
100	64	3.36	7.29	1.83	0.79	2.51	4.13	1.86	63.78	23.56	252.35	328.34
126	62	1.88	5.41	0.83	0.51	2.29	1.05	1.10	42.77	22.51	232.58	282.32
41	47	4.40	0.62	4.59	1.74	0.77	1.22	3.84	72.96	31.13	45.14	123.68
42	45	6.89	5.79	2.68	1.94	7.74	0.62	6.47	118.98	31.52	83.84	211.12
120	64	2.58	6.68	2.32	1.30	4.63	1.71	1.08	33.73	18.36	223.18	266.78
172	75	1.65	5.41	1.82	0.98	1.18	0.83	0.58	25.40	17.11	141.86	177.13
93	60	1.90	4.00	2.12	0.93	2.20	0.64	1.18	48.30	22.07	188.79	242.66
103	64	1.00	4.86	1.36	0.14	2.48	3.70	1.01	66.79	9.49	217.77	293.26
135	64	2.17	4.51	0.62	0.30	1.70	0.73	0.66	28.04	18.50	188.50	222.08
30	49	2.03	6.52	9.45	4.25	1.20	1.24	1.09	39.86	28.11	104.29	156.16
Control groups.												
A.>100 mm		0.05	3.33	0.02	0.01	0.01	0.01	0.03	1.31	1.10	13.23	17.89
		0.04	0.06	0.02	0.02	0.02	0.02	0.02	1.23	1.03	10.78	12.11
		0.06	0.08	0.01	0.01	0.04	0.07	0.04	1.90	1.29	12.75	14.91
		0.03	0.07	0.03	0.01	0.01	0.01	0.03	1.56	1.09	13.11	14.76
B.<100 mm		0.03	0.01	0.01	0.00	0.01	0.01	0.02	0.23	0.90	1.31	1.56
		0.06	0.23	0.04	0.09	0.03	0.01	0.05	0.99	0.76	1.89	3.09
		0.02	0.02	0.01	0.03	0.03	0.02	0.02	0.50	0.61	3.67	4.24
		0.08	0.54	0.02	0.01	0.01	0.03	0.05	1.20	0.67	1.18	2.96
		0.03	0.01	0.01	0.02	0.04	0.03	0.03	0.25	0.67	1.89	2.20

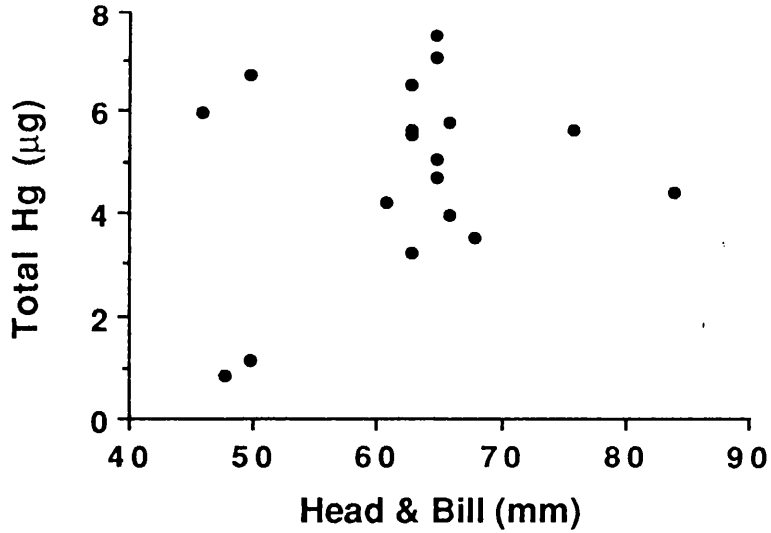
Concentrations in the liver, kidney and carcass decreased significantly with increasing age (see Fig. 5.1-5.3) ($P < 0.01$ in all cases). Pectoral muscle concentrations showed no relationship with age over the narrow range of chick sizes in this study ($P > 0.05$) (Fig. 5.4).

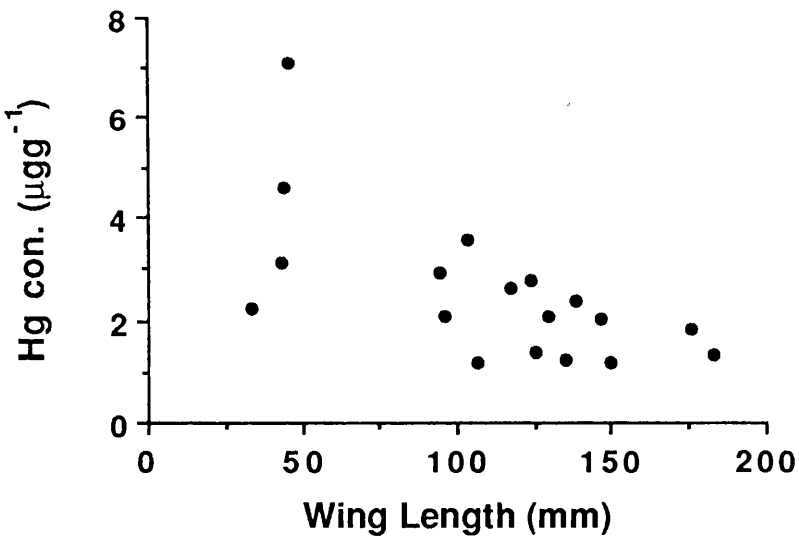
Table 5.2 also shows coefficients and associated probabilities for Pearson Product Moment Correlations between total amounts of mercury in the tissues analysed and age. Total amounts of mercury in the liver, kidney, pectoral muscle and carcass showed no correlation with age ($P > 0.1$ in all cases; see Fig. 5.1-5.4).

Table 5.3 shows the mean concentration of mercury in the tissues analysed. Mercury did not accumulate differentially between the tissues. Liver, kidney and pectoral muscle showed no significant difference from each other (One-way analysis of variance $F_{2,53}=0.157$, $P>0.1$).

Figure 5.1 Concentrations and total amounts of mercury found in the liver of kittiwake chicks fed 1000 µg of methyl mercury, in relation to age as defined by wing length and head+bill measurements.


Hg con. (µgg-1)
0
2
4
6
8
40
50
60
70
80
90
Head & Bill (mm)


Total Hg (µg)
0
2
4
6
8
40
50
60
70
80
90
Head & Bill (mm)


Hg con. (µgg-1)
0
2
4
6
8
0
50
100
150
200
Wing Length (mm)

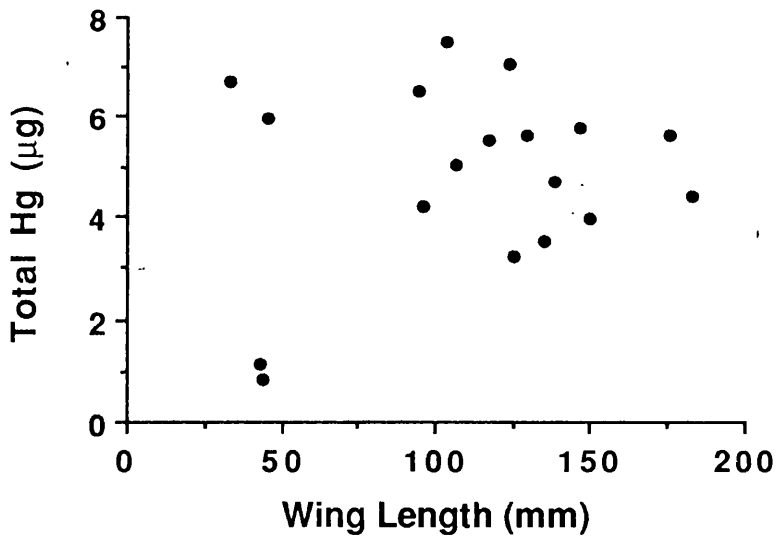
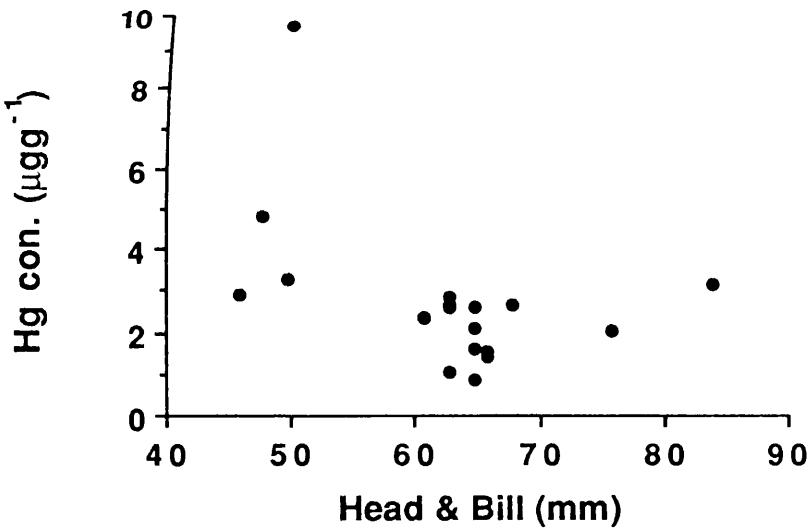
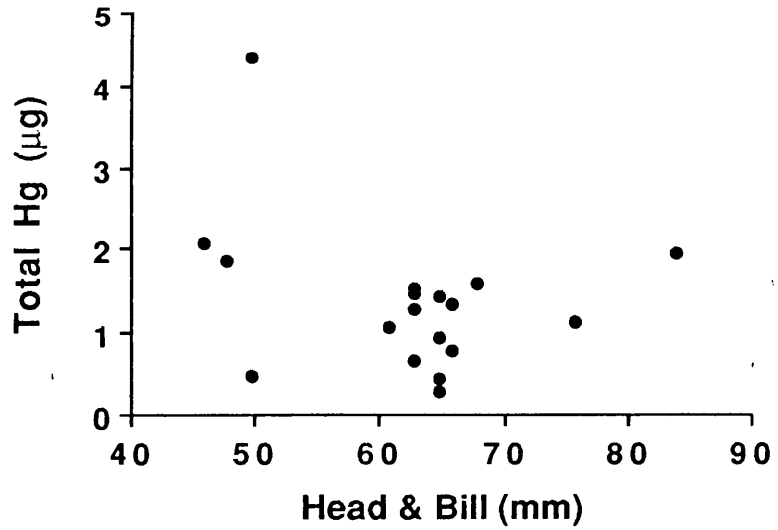
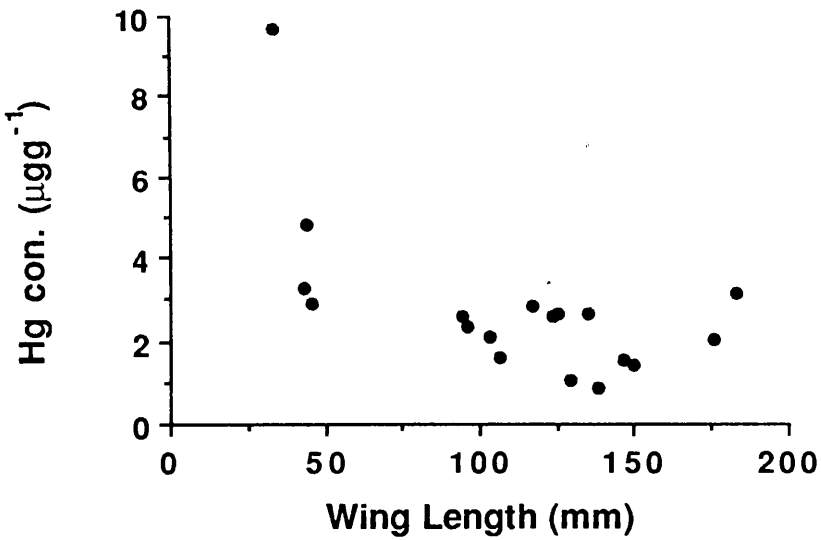

Total Hg (µg)
0
2
4
6
8
0
50
100
150
200
Wing Length (mm)

Figure 5.2 Concentrations and total amounts of mercury found in the kidney of kittiwake chicks fed 1000 µg of methyl mercury, in relation to age as defined by wing length and head+bill measurements.


Hg con. (µgg-1)
10
8
6
4
2
0
40
50
60
70
80
90
Head & Bill (mm)


Total Hg (µg)
5
4
3
2
1
0
40
50
60
70
80
90
Head & Bill (mm)


Hg con. (µgg-1)
10
8
6
4
2
0
0
50
100
150
200
Wing Length (mm)

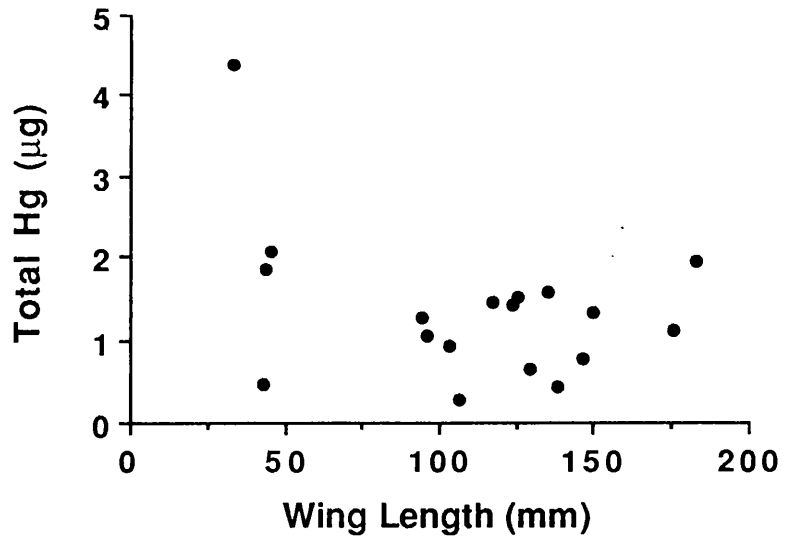
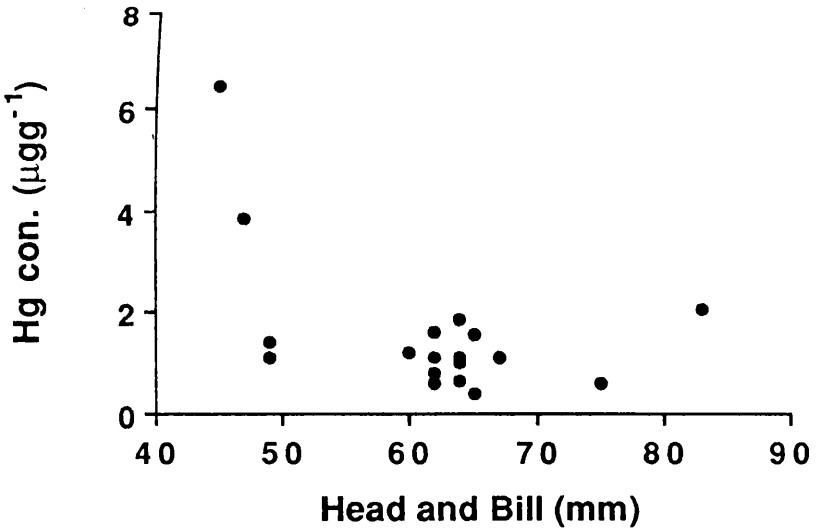
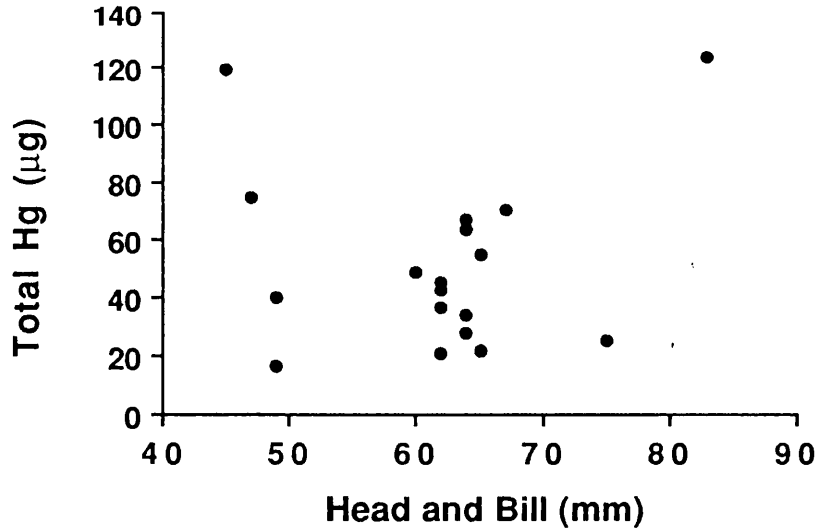
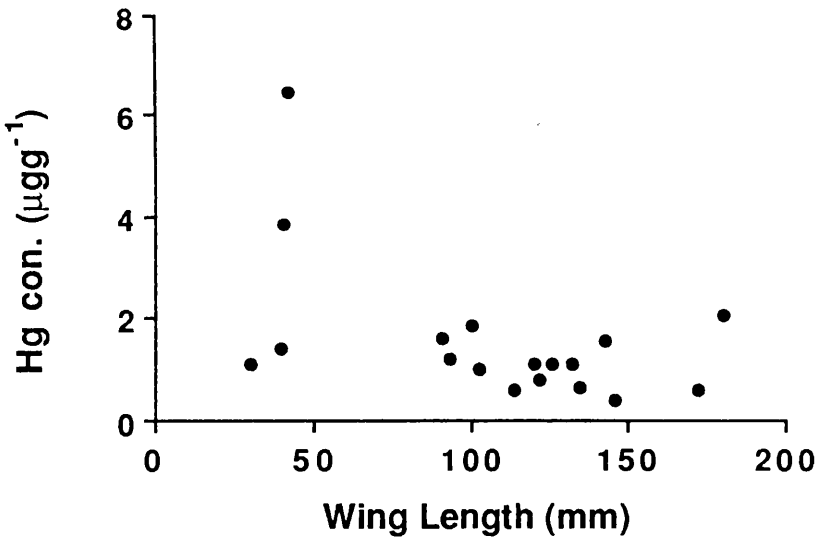

Total Hg (µg)
5
4
3
2
1
0
0
50
100
150
200
Wing Length (mm)

Figure 5.3 Concentrations and total amounts of mercury found in the carcass of kittiwake chicks fed 1000µg of methyl mercury, in relation to age as defined by wing length and head+bill measurements.


Hg con. (µgg-1)
8
6
4
2
0
40
50
60
70
80
90
Head and Bill (mm)


Total Hg (µg)
140
120
100
80
60
40
20
0
40
50
60
70
80
90
Head and Bill (mm)


Hg con. (µgg-1)
8
6
4
2
0
0
50
100
150
200
Wing Length (mm)

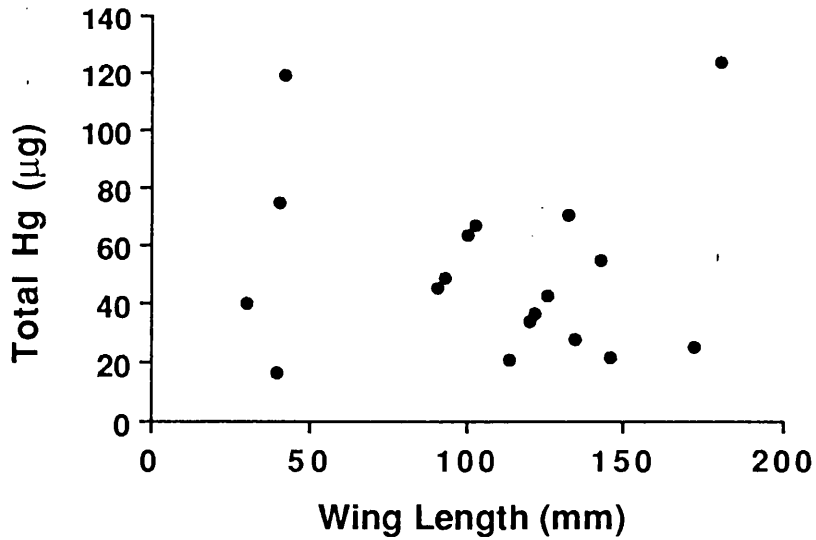
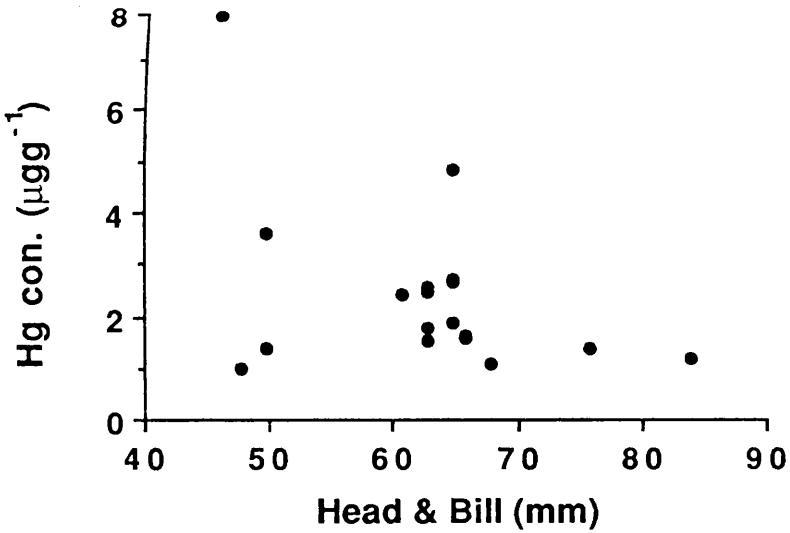
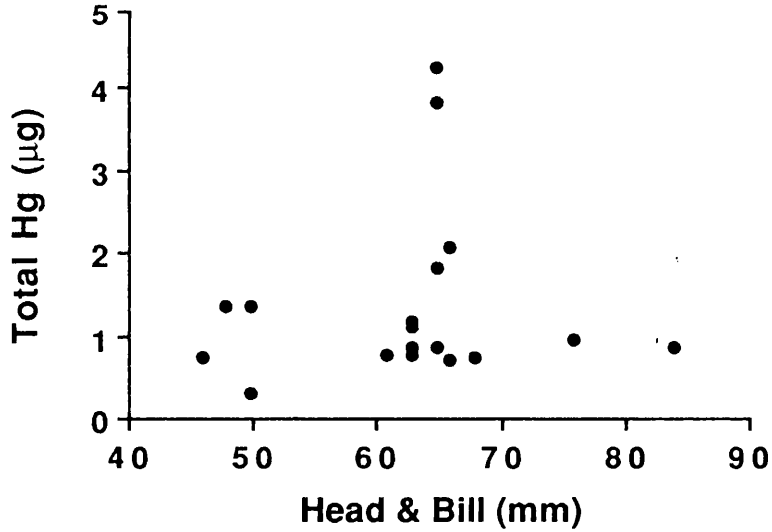
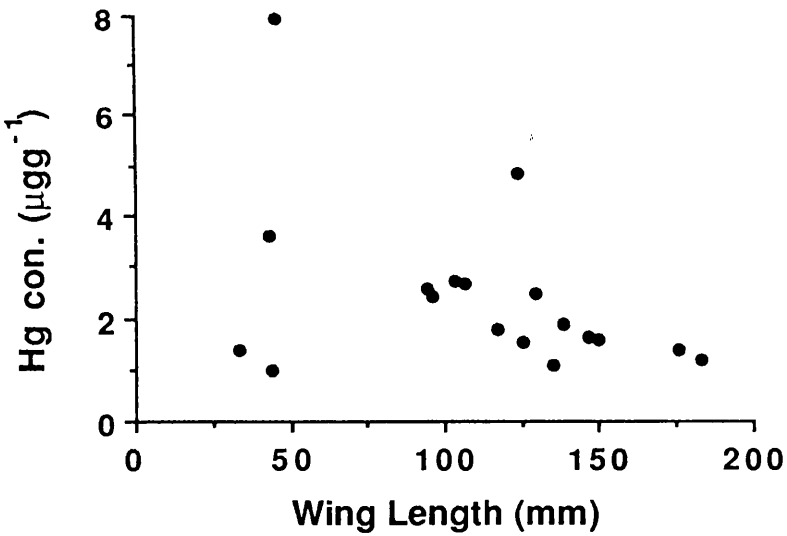

Total Hg (µg)
140
120
100
80
60
40
20
0
0
50
100
150
200
Wing Length (mm)

Figure 5.4 Concentrations and total amounts of mercury found in the pectoral muscle of kittiwake chicks fed 1000 µg of methyl mercury, in relation to age as defined by wing length and head+bill measurements.


Hg con. (µgg-1)
0
2
4
6
8
40
50
60
70
80
90
Head & Bill (mm)


Total Hg (µg)
0
1
2
3
4
5
40
50
60
70
80
90
Head & Bill (mm)


Hg con. (µgg-1)
0
2
4
6
8
0
50
100
150
200
Wing Length (mm)

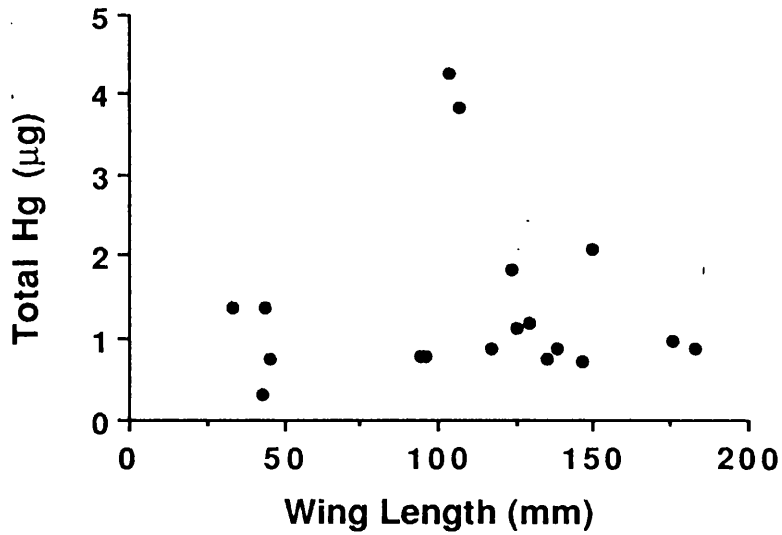

Total Hg (µg)
0
1
2
3
4
5
0
50
100
150
200
Wing Length (mm)

Table 5.3 Mean mercury concentration (µg/g) and total mercury (µg) in analysed tissues of kittiwake chicks after administration of 1000 µg methyl-mercury (n=18). (n=14 for feather types)

Tissue	Hg Conc(µgg/g) x	sd	Total Hg(µg) x	sd
Liver	2.34	1.44	4.64	1.83
Kidney	2.54	1.96	1.23	0.92
Pectoral muscle	2.23	1.68	1.24	1.06
Carcass	1.57	1.45	51.68	30.78
Primaries	15.70	4.30	20.85	9.74
Secondaries	19.97	4.04	11.90	5.60
Coverts	20.26	5.17	69.82	13.15
Body feathers	20.52	5.11	98.37	35.38
Tail feathers	19.47	5.80	9.16	3.65

Table 5.4 shows the mean amount of mercury in the tissues analysed as a percentage of the mercury intake and as a percentage of the body burden. The carcass was the major storage site for mercury, accumulating up to 12% of the mercury intake compared to 0.8% and 0.4% of the mercury intake found in the liver and kidney, respectively.

Table 5.4 Mean amounts of mercury in various tissues of kittiwake chicks administered 1000 µg of methyl-mercury expressed as a percentage of the mercury intake and as a percentage of the body burden.

Tissue	Hg as % of intake			Hg as % of body burden		
	x	sd	Range	x	sd	Range
Liver	0.46	0.19	0.06-00.77	2.17	0.20	0.50-02.56
Kidney	0.12	0.09	0.01-00.43	0.54	0.07	0.04-00.09
Pectoral mus.	0.12	0.11	0.01-00.83	0.06	0.09	0.03-01.26
carcass	5.17	9.91	2.13-12.43	17.45	29.60	10.10-40.30
Body feathers	9.84	10.10	0.75-17.01	35.86	10.84	4.41-51.95
Primaries	2.09	2.73	1.01-03.83	7.71	3.44	3.51-14.74
Secondaries	1.19	1.04	0.81-02.85	4.41	1.66	2.47-08.69
Coverts	6.98	8.94	4.76-09.09	27.06	8.56	14.88-51.29
Tail feathers	0.92	1.87	0.41-01.79	3.56	1.59	1.92-06.93
Total plumage	17.96	7.77	4.51-29.92	80.27	17.20	32.08-91.38

5.3.2 Mercury excretion

Table 5.1 shows mercury concentration and total amounts of mercury in the plumage of kittiwake chicks in the experimental group. All feather types contained higher mercury concentrations than the internal tissues (Table 5.3). There was a significant difference between mercury concentration in different feather types (One-way analysis of variance $F_{4,69}$= 3.181, P>0.01). Body and covert feathers were the major elimination routes for mercury in the plumage accumulating up to 10% and 7% of the mercury intake, respectively (Table 5.4). In kittiwake chicks, over 80% of the mercury burden in the whole body was present in the plumage which comprised only 4.7% of the body weight (range

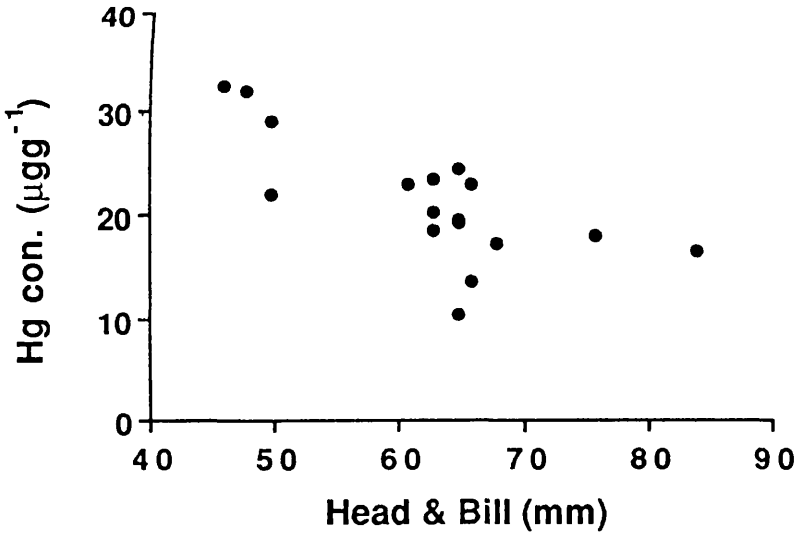
0.7-10.8%) and over 21% of the mercury dose was present in the plumage of chicks not yet fledged. The concentration of mercury in the feathers decreased significantly with increasing age (Table 5.2; $P<0.01$; see fig.5.5). The total amount of mercury in the plumage increased with age (Table 5.5; $P<0.01$; see Fig. 5.5).

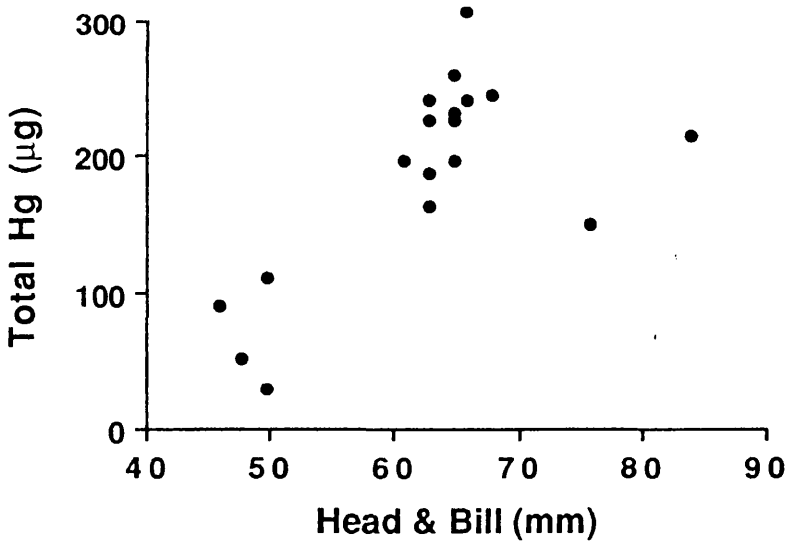
The amount of mercury excreted in the faeces and urine was relatively high (Table 5.5). An average of 76% of the mercury administered was excreted in this way. There was no relationship between mercury excretion and age (Pearson product moment correlations, $r_{wglt}=-0.26$, $n=18$, $P>0.1$, $r_{H+B}=0.26$, $n=18$, $P>0.1$; see Fig. 5.6).

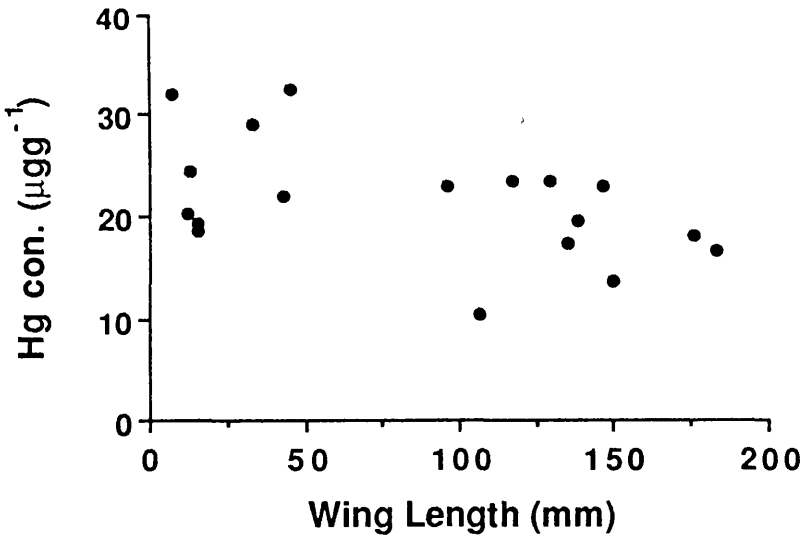
Table 5.5 Mean mercury excretion in Kittiwake chicks of various ages after administration of 1000 µg of methyl-mercury. (all values are corrected for background (control) levels (n=18).

	x	sd
Total Hg excreted (µg)	937.90	33.10
Total Hg excreted as a % of intake	93.90	3.10
Hg excreted in faeces only	764.91	78.42
Hg excreted in faeces as % of intake	76.49	7.84
Total Hg excreted in plumage only	172.96	77.65
Hg excreted in plumage as % of intake	17.96	7.77

Figure 5.5 Concentrations and total amounts of mercury in the plumage of kittiwake chicks fed 1000 µg of methyl mercury, in relation to age as defined by wing length and head+bill measurements.


Hg con. (µgg-1)
40
30
20
10
0
40
50
60
70
80
90
Head & Bill (mm)


Total Hg (µg)
300
200
100
0
40
50
60
70
80
90
Head & Bill (mm)


Hg con. (µgg-1)
40
30
20
10
0
0
50
100
150
200
Wing Length (mm)

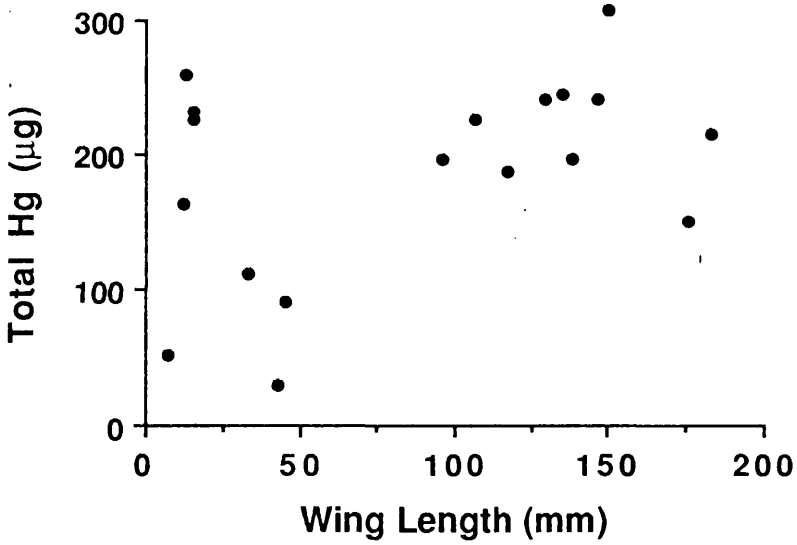
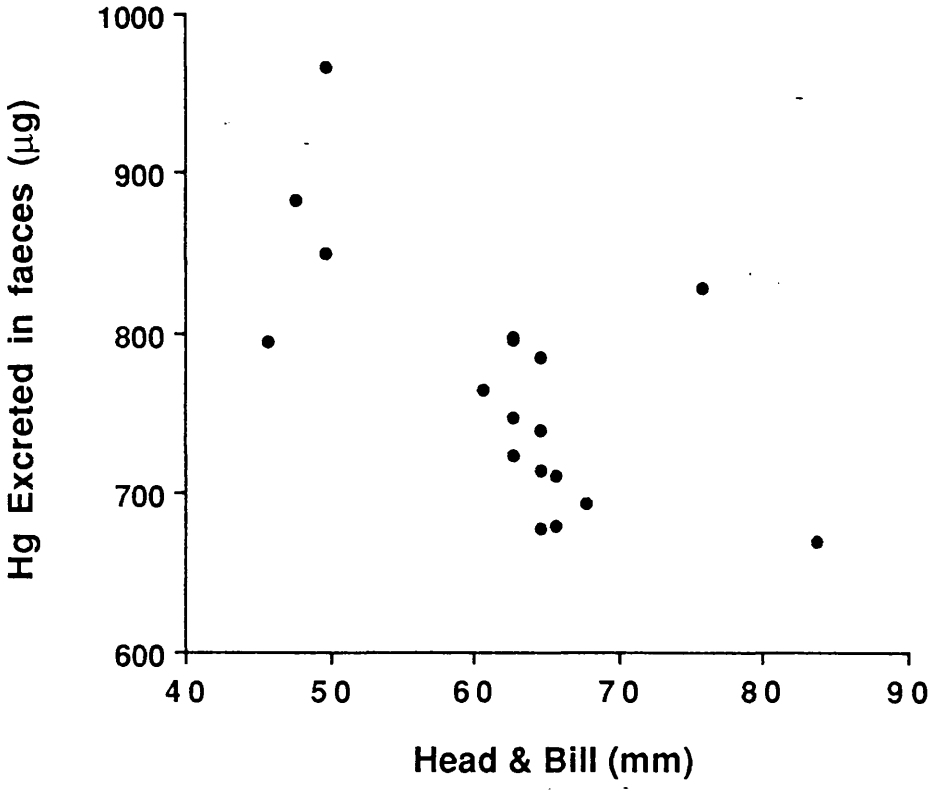
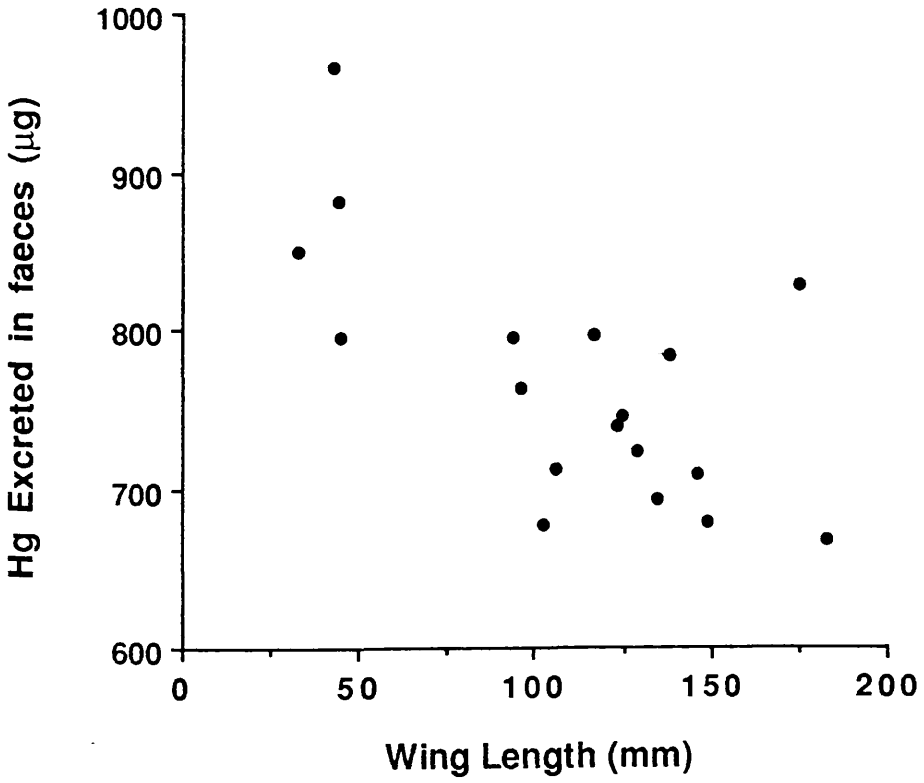

Total Hg (µg)
300
200
100
0
0
50
100
150
200
Wing Length (mm)

Figure 5.6 Amount of mercury excreted by kittiwake chicks fed 1000 µg methyl mercury, in relation to age as defined by wing length and head+bill measurements.


Hg Excreted in faeces (µg)
1000
900
800
700
600
40
50
60
70
80
90
Head & Bill (mm)


Hg Excreted in faeces (µg)
1000
900
800
700
600
0
50
100
150
200
Wing Length (mm)

5.4 DISCUSSION

5.4.1 Effects of age on tissue distribution and excretion

Many studies looking at effects of age on tissue storage and elimination of metals have found higher metal levels in adult birds than pre-fledglings or juveniles (Hoffman & Curnow, 1979; Hulse et al., 1980; Maedgen et al., 1982). This is entirely expected due to the longer exposure time available to the older birds. The period from hatching to fledging is characterised by a high growth rate and a high rate of protein synthesis and this may lead to differences in the handling of metals by growing chicks. A difference in the binding of metals to the plasma proteins or to the body ligands in younger animals may influence the value of metals in the blood as well as the metal distribution and excretion from the body (Jugo, 1977).

Differences in the metabolism of metals between suckling and older rats have been found (Kostial et al., 1978), where younger animals absorb more of the oral dose of mercury, lead, cadmium and manganese than older rats. Whole body retention in sucklings was also higher than retention in the older animals. The percentage of metals in the blood was 2-3 times higher in younger rats and the liver retained 13 times as much of the metal dose of mercury whereas kidney retention was 2-3 times lower in the sucklings compared to older animals. In a review of heavy metals in growing organisms (Jugo, 1977) it was concluded that underdeveloped organisms may be more resistant to the action of heavy metals.

In the present study no difference was found in the retention of mercury by any of the tissues of different aged chicks. Mercury concentration decreased with age but this would

be expected due to the mercury load of the tissue or organ being diluted in older birds due to the increase in mass of the tissue due to growth.

Gochfeld & Burger (1987) found no difference in a variety of metal levels between adult and young common terns Sterna hirundo. This was attributed to either the young being exposed to high doses of metals in early life and therefore rapidly reaching adult levels or to the young being less efficient than the adults in excreting metals. This present study did not find any age-related changes in the excretion of mercury via the faeces in kittiwake chicks.

There was, however, a significant increase in the total amount of mercury excreted into the feathers. Because there was no difference between the mercury retention in the tissues as age increased the mercury entering the feathers may come from one of two sources. Either from supplementary mercury found in the diet (and therefore older birds receiving extra mercury due to the longer exposure time) or from mercury held in the circulating blood. Control chick mercury levels were derived through the maternal contribution to the egg and supplementary levels available in the diet which would have been much the same as that which the experimental birds received and it would be expected that these levels would cancel effects due to outside exposure. It has however been shown that organic mercurials are retained for a long time in the circulating blood (Swensson et al., 1959; Berlin, 1963; Gage, 1964; Yoshino et al., 1966) and Backstrom (1969) showed that after an injection of methyl-mercury, the mercury concentration in the circulating blood of quail Coturnix coturnix decreased very slowly with time. In male birds, blood still contained a tenth of the mercury intake 30

days after administration. Plumage concentrates mercury through the firm bonding to the disulphide bonds of the keratin (Crewther et al., 1965). As the feather grows, mercury circulating in the blood at this time is placed into the growing feathers. Tejning (1967) found that ingested methyl-mercury in the domestic fowl was transported to the blood cells and the internal organs and from there into the growing feathers. In this present study it appears that mercury found in the feathers of older birds may have come from the mercury circulating in the blood at the time of initial feather growth and was not transported from mercury stored in the internal tissues.

The decrease in mercury concentration was likely to have been caused by a dilution effect as the feathers grew. Thompson et al. (in press) found no age-related trend in mercury levels in chick feathers of great skuas Catharacta skua. They suggested this was due to a coupling of an increase in exposure to mercury through the diet, with an increase in size. In this study it appears that the increase in total mercury going into the chicks with increasing age was not sufficient to remove any effect caused by an increase in feather growth.

5.4.2 Tissue distribution and excretion

The distribution pattern of mercury in the internal tissues of the chicks differs from patterns found in many other studies (e.g. Anderlini et al., 1972; Connors et al., 1975; Hulse et al., 1980). In the present study there was no significant difference between the concentrations of mercury in the liver, kidney and pectoral muscle. It has been suggested that the kidney:liver ratio is a useful measure of the acuteness of a birds' exposure to metals (Gochfeld & Burger, 1987; Lewis &

Furness, in press; see also Chapter 7). In wild populations liver levels tend to exceed those of the kidney which in turn are greater than muscle and egg levels (Anderlini et al., 1972; Connors et al., 1975; Blus et al., 1977; Hutton, 1981; Ohlendorf et al., 1985; Honda et al., 1986). Lewis & Furness (in press; see also Chapter 7) found that at experimental exposures higher than natural levels but not sufficient to cause acute toxicity, kidney levels exceeded those of the liver. Here, because no difference between these tissues was found the results may be an indication that levels were approaching toxic thresholds.

Of the body tissues of the kittiwake chicks, skeletal muscle comprises the major storage site for mercury. The remaining carcass after sampling consists to a large extent of muscle and over 17% of the total mercury in the body burden of mercury (minus the feathers) was stored in the carcass. Corresponding figures have reported between 6 and 39% (Braune & Gaskin, 1987; Lewis & Furness, in press). For the size, however, the liver and kidney contained a disproportionally high amount of mercury accumulating over 2% of the mercury burden, once again stressing the capacity of these tissues as a sensitive indicator of metal pollution.

Differences were found between mercury levels in the different feather types. This agrees with other, similar studies (Furness et al., 1986; Braune & Gaskin, 1987; Lewis & Furness, in press) where consistent differences were found, with the head feathers, which usually grow last having less mercury that body and primary feathers due to a gradual depletion of the body mercury pool (Furness et al., 1986). The anomaly, in this study, of primary feathers having marginally lower mercury concentrations than the other feather types may be an indication

of a different pattern of plumage growth. Of the body burden of mercury, 80% was found in the plumage of the chicks which had not yet completed feather growth. This can be compared to figures ranging from 65-93% of the body burden of mercury that has been found in birds after a completed moult (Honda et al., 1986; Braune & Gaskin, 1987; Lewis & Furness, in press). Nearly 18% of the mercury administered was excreted into the plumage of the kittiwake chicks compared to 49% in the plumage of black-headed gull Larus ridibundus chicks (Chapter 6) . This may be due to the fact that the kittiwake chicks have not yet completed their moult or it may be due to a species difference in the handling of metals (Gardiner, 1972).

Excretion rates of mercury have been widely documented (Swensson et al., 1959; Miller, 1960; Swensson & Ulfvarson, 1968) and excretion rates of methyl mercury vary from between 50-95% of the ingested mercury. The kittiwake chicks in this experiment excreted up to 93% of the mercury intake over a period of 10 days which does appear to be remarkably high.

Under natural environmental exposure to toxic metals chicks are likely to have a body burden of metals as a result of metal transfer during egg production and subsequent accumulation due to dietary levels. Earlier findings (e.g Kostial et al., 1978) indicated that in early post natal periods there may be a higher accumulation of metals as a result of higher intestinal absorption and a higher body and organ retention. A high body burden of toxic metals is undesirable at a period of such intense growth and functional development. These findings also indicated that mortality may occur in the young at metal levels previously thought to be harmless in the adult.

5.5 REFERENCES

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CHAPTER 6

Mercury accumulation and excretion in laboratory reared black-headed gull *Larus ridibundus* chicks

This chapter is based on a paper in press in *Arch. Environ. Contam. Toxicol.*

6.1 INTRODUCTION

In recent years concern regarding the long-term effects of environmental contaminants has increased. Mercury is considered one of the most potentially serious pollutants since it has no known biological function, is highly toxic and, unlike other heavy metals, tends to be accumulated through the food chain (Bryan, 1979). Furthermore, inputs of mercury to the environment include a large anthropogenic component (Lantzy and Mackenzie, 1979).

Birds accumulate mercury from their food and eliminate it via faeces, feathers and, in females, eggs (Miller et al. 1960; Barrett et al. 1985; Braune & Gaskin, 1987a). Mercury levels in birds result from a dynamic balance between rates of intake and elimination (Evans & Moon, 1981). Much of the mercury which birds take up from their food goes into their plumage during feather growth (Furness et al, 1986). Once fully grown the feathers become physiologically isolated from the rest of the bird (Voitkevich, 1966) preventing further influx of mercury from the body. Mercury bonded into feather keratin reflects the amount of mercury in the blood at the time when the individual feather was formed, and mercury will begin to accumulate in the body once moult is complete (Braune & Gaskin, 1987a).

All mercury excreted into the plumage is methyl mercury (Thompson & Furness 1989a) and most of the mercury assimilated by seabirds is in this form, although Thompson & Furness (1989b) have shown that species with slow moult cycles, such as large albatrosses, have high proportions of inorganic mercury in the liver. Many papers have examined the role of plumage in the elimination of mercury (Furness et al., 1986; Honda et al,1986; Braune & Gaskin 1987a,b) and in relation to the storage of

mercury in other tissues (Nicholson 1981; Nicholson & Osborn, 1984; Muirhead & Furness, 1988). Braune & Gaskin (1987b) showed that in Bonaparte's gull Larus philadelphia, 93% of the body burden of total mercury in adult birds was redistributed into the feathers. Honda et al. (1986) investigated seasonal changes in mercury accumulation by the black-eared kite Milvus migrans lineatus and showed that 70% of the body's mercury burden was in the feathers, establishing the plumage as a major eliminatory pathway for mercury during moult.

The levels of mercury in bird feathers have been used by numerous authors in the past three decades to indicate the degree of mercury contamination in marine, terrestrial and freshwater ecosystems (Borg 1966; Furness & Hutton 1979, 1980; Appelquist et al., 1985). Such studies have been limited by a lack of knowledge concerning the amounts of mercury being ingested by birds, preventing accurate assessment of mercury pollution levels.

Braune & Gaskin (1987a) constructed a bio-energetics based mercury budget to compare predicted with measured mercury gain or loss in seabirds. This method relied on analysis of prey types for a determination of mercury ingested, which gave only an approximation of the total mercury intake. There is a lack of information regarding mercury deposition in relation to absolute mercury intake in wild birds, especially in relation to moult.

The objective of this study was to examine methyl-mercury deposition in internal tissues, the carcass and in different parts of the plumage of black-headed gull chicks, in order to establish a relationship between mercury intake and the amount deposited in the feathers. This should provide a means of

estimating mercury contamination in the environment more quantitatively than has previously been possible. The study also examined whether or not a relationship exists between methyl-mercury intake and the proportion of mercury deposited in external and internal tissues.

6.2 MATERIALS AND METHODS

6.2.1 Collection of birds and experimental procedure

In May 1989, black- headed gull Larus ridibundus eggs were collected under licence from a colony at the Carron Valley Reservoir, Central Region, Scotland and hatched in a laboratory incubator. Fifteen chicks were randomly assigned to one of three experimental groups, while four chicks acted as a control group. Controls were used to measure background metal levels obtained either from the wild (from the eggs) or from food given in the laboratory. All mercury levels calculated for the experimental groups were corrected by subtracting the mean level found in the control group. The birds were fed on a commercial meat product obtained from Biosure Research Animal Health which on analysis contained less than 0.08µg/g (dry weight) of mercury. The birds had free access to food and fresh water at all times.

Mercury was administered in the form of analytical grade methyl-mercuric chloride solution obtained from B.H.D. Chemicals Limited. The mercury was given in gelatin capsules, each bird received a gelatin capsule containing either 4 µg, 20 µg or 40 µg of methyl mercury every two days for ten days from the age of seven days after hatching. The total dose administered to the respective experimental groups was either 20 µg, 100 µg or 200 µg of mercury and the entire dose was administered before any feather growth was visible. These doses were chosen to be within

the range of exposures naturally experienced by wild gull chicks as estimated from levels measured in studies of gulls (Hutton, 1981).

Gulls were weighed every two days and once head and tail feathers had completely grown (an average age of 30 days) the gulls were sacrificed and stored intact at $-20^{o}C$ prior to further treatments.

6.2.2 Sample Preparation and Mercury Analysis

On thawing, the birds were plucked; the primaries, secondaries, wing coverts, body feathers, head feathers and tail feathers were removed, weighed and stored separately. The kidney, liver and a sub-sample of muscle (pectoralis and supracoracoideus) were dissected out and along with the rest of the body were dried to constant weight in an oven at $50^{o}C$.

Mercury analysis was carried out as described in Chapter 2.

Body burden is referred to as the amount of mercury in the entire body (this includes all internal tissues as well as feathers). All tissues with the exception of feathers are referred to as internal tissues. The carcass is defined as the remains of the bird once the liver, kidney and plumage have been removed.

6.2.3 Statistical Analysis

Data were normally distributed, as determined by Kolmogorov-Smirnov one-sample tests, and satisfied requirements of equality of variance, as determined by Bartlett Box F-tests ($P>0.05$). Differences in mercury concentration according to tissue type and dose were tested by a two-way analysis of variance in conjunction with Tukey's range tests.

6.3 RESULTS

6.3.1 Mercury accumulation in tissues

Table 6.1 shows mercury concentrations in tissues of birds from the three dose groups and the control group. Mercury accumulated differentially in the internal tissues, concentrations in the kidney tending to be greater than liver concentrations which in turn exceeded muscle concentrations. This pattern was consisitent for all dose groups (Two-way analysis of variance, $F_{2,36}$ (TISSUE) = 139.03, P< 0.001 $F_{2,36}$ (DOSE) = 69.13, P<0.001). (No interaction P>0.05)

All feather types contained much higher mercury concentrations than internal tissues (Table 6.1). There were consistent differences between mercury concentrations in different feather types with head feathers which grow last having consistently low mercury concentrations and body feathers and primaries having consistently and significantly higher concentrations (Table 6.1; Two-way analysis of variance, $F_{5,72}$ (TISSUE) = 6.40, P<0.001). ($F_{2,72}$ (DOSE) = 292.0, P<0.001) Body feathers and covert feathers were the major elimination route for mercury in the plumage accumulating up to 20 and 19% of the mercury intake, respectively (Table 6.2). However, the carcass was the major storage site for mercury in the soft tissues, accumulating up to 42% of the mercury intake compared to 0.46% and 2.2% of the mercury intake found in the kidney and liver, respectively.

Fig. 6.1 shows mean mercury concentrations in the primary feathers for the three dose groups. For each dose group there was a progressive and pronounced reduction in the concentration of mercury in the primary feathers as growth sequence progressed. This trend was highly significant for each dose group (Spearman Rank Correlation Coefficient: 20 µg dose: -0.927, 100 µg dose: -0.988, 200 µg dose: -0.988, n=10)

Table 6.1 Mercury concentrations (µg/g) dry weight) in various tissues, in relation to the dose administered.

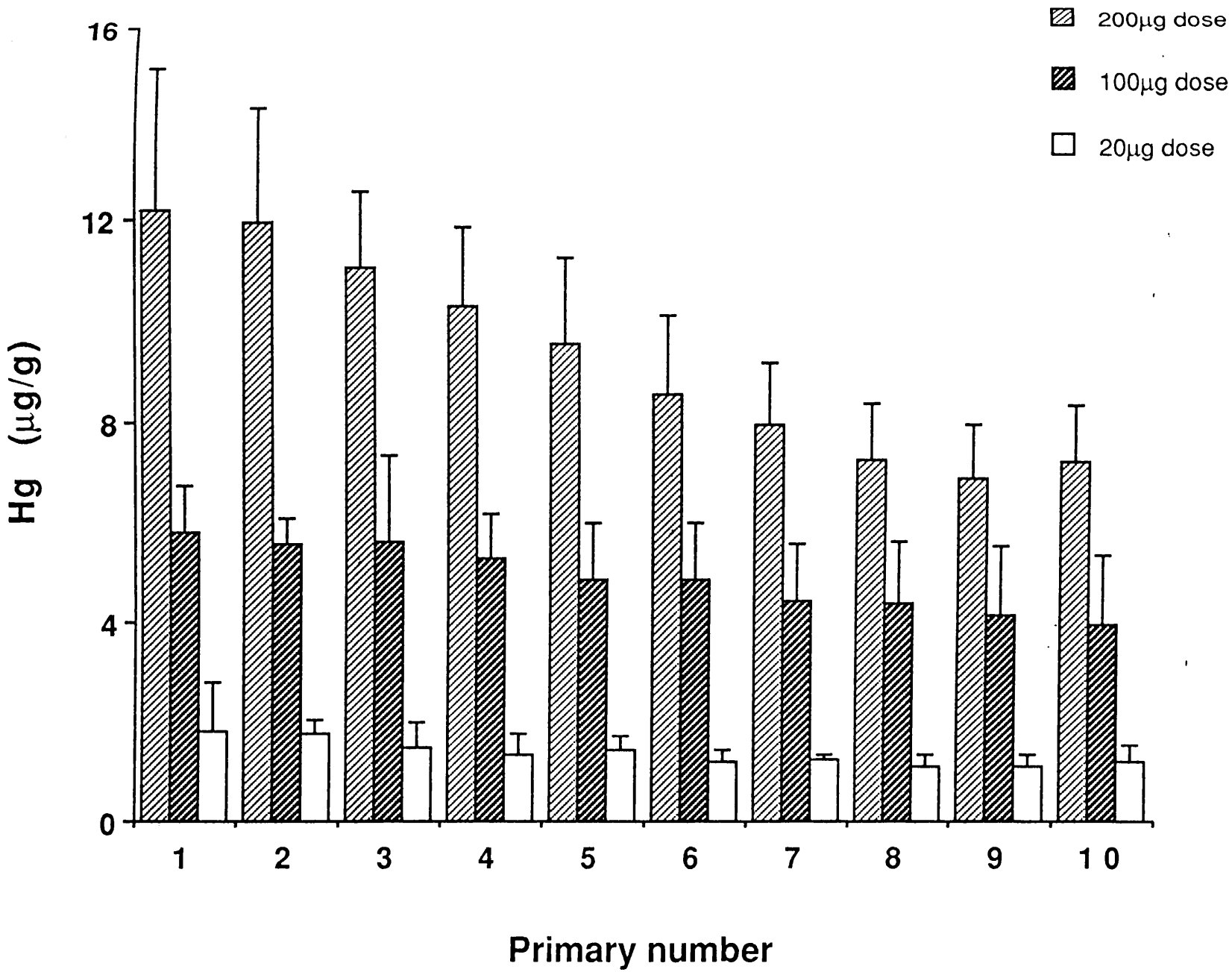
	Dose							
	200 µg		100 µg		20 µg		control	
Tissue	x	sd	x	sd	x	sd	x	sd
Kidney	1.87	0.37	1.04	0.11	0.42	0.09	0.35	0.04
Liver	1.56	0.36	1.05	0.22	0.36	0.09	0.25	0.05
Pectoral muscle	0.59	0.22	0.43	0.22	0.13	0.13	0.11	0.06
Carcass	0.62	0.06	0.45	0.30	0.18	0.73	0.15	0.15
Primaries	10.01	2.04	4.67	0.76	1.33	0.35	0.73	0.13
Secondaries	9.54	1.68	5.18	1.48	1.26	0.04	0.63	0.11
Covert feathers	9.93	1.27	4.82	1.31	1.42	0.26	0.56	0.05
Body feathers	7.76	1.35	3.76	0.66	1.39	0.35	0.64	0.09
Tail feathers	6.83	1.39	3.97	1.83	0.98	0.19	0.41	0.23
Head feathers	6.35	1.30	3.24	0.62	0.99	0.11	0.75	0.12

Table 6.2 Amount of mercury in various tissues expressed as a percentage of the mercury burden in the whole body and as a percentage of the mercury intake

Dose	Tissue								
	Kidney*	Liver	Carcass*	Primary*	Secondary	Tail feathers	Head	Body	coverts
Dry mass of tissues	00.53	02.69	67.72	02.71	01.31	00.83	00.39	4.84	4.30
a.% Body Burden									
20µg	00.58	02.90	39.19	08.47	05.80	03.14	00.80	23.37	15.65
100µg	00.62	03.20	28.84	12.11	08.00	03.00	00.85	19.73	23.65
200µg	00.68	02.51	27.92	11.89	07.07	03.01	00.85	20.80	25.25
b. % Intake									
20µg	00.31	02.20	42.40	06.65	05.36	03.31	00.62	20.62	15.73
100µg	00.31	01.93	27.17	07.79	05.32	01.89	02.05	11.58	15.72
200µg	00.46	01.82	20.19	08.90	05.30	02.24	00.60	14.93	19.15

Differences between dose groups in relation to the proportions of mercury deposited as a percentage of the mercury intake and as a percentage of the body burden were tested by a One-way ANOVA; those tissues marked with * are significantly different ($P<0.01$, $n= 5$ in each dose group)

Figure 6.1 Mean mercury concentrations in primary feathers of black-headed gulls fed 20 µg, 100 µg and 200 µg of methyl mercury.


200µg dose
100µg dose
20µg dose
Hg (µg/g)
16
12
8
4
0
1
2
3
4
5
6
7
8
9
10
Primary number

6.3.2 Effect of dosage on relative accumulation

Table 6.2 shows mercury deposition in all tissues analysed as a percentage of the body burden of mercury and as a percentage of the mercury dose. There was a slight but significant increase in the proportion of mercury deposited in the kidney as a percentage of the total body burden as the dose increased (One-way analysis of variance, $F_{2,14}$= 9.001, $P<0.05$). The amount of mercury in the kidney as a percentage of mercury intake also increased between the two highest dose groups (One-way analysis of variance, $F_{2,14}$= 31.44, $P<0.01$).

The proportional accumulation of mercury in the carcass was also dose dependent. As the dose increased the amount of mercury found in the carcass as a percentage of the body burden decreased (One-way analysis of variance, $F_{2,14}$= 14.878, $P<0.01$), but when the dose was increased from 20 µg to 100 µg the mercury deposited in the primaries as a percentage of the body burden increased significantly (One-way analysis of variance, $F_{2,14}$= 6.797, $P<0.01$, Tukey's range test $P<0.05$), though no further increase was detected at the highest dose level.

6.3.3 Excretion and distribution of mercury in the plumage

Table 6.3 shows absolute and percentages of mercury excreted in faeces and plumage at the three different dose levels. The concentration of mercury administered had no significant effect on the total excretion rate (One-way analysis of variance, $F_{2,14}$= 2.705, $P>0.1$, ns).

An average of 71% of the dose administered was excreted over the fledging period. Of this, 22% was excreted in the faeces while the remainder was lost into the growing feathers. The total plumage, therefore, contained 49% of the mercury administered and 65% of the total body burden of mercury after

the completed moult.

Table 6.3 Mercury excretion in black-headed gull chicks.

Dose	200 µg		100 µg		20 µg	
	x	sd	x	sd	x	sd
Total Hg excreted (µg)	155.1	5.1	80.3	6.8	11.2	6.1
Total Hg excretion as % of intake	77.5	2.1	80.3	6.8	56.3	37.5
Hg excreted via faeces (µg)	52.8	18.3	37.7	9.5	0.7	8.5
excretion in faeces as % of intake	26.4	9.2	37.7	9.5	11.0	37.4
Hg excreted into plumage (µg)	102.3	16.0	44.2	6.5	10.5	5.2
Excretion in plumage as % of intake	51.2	8.0	44.2	6.9	52.6	19.1

6.4 DISCUSSION

In a variety of birds sampled from the wild, liver mercury levels are consistently greater than kidney levels which in turn exceed those in muscle (Hutton, 1981; Noble & Elliot, 1986). This contrasts markedly with experimental studies (Swensson & Ulfvarson, 1968; March et al., 1983; Nicholson & Osborn, 1984) which found higher levels of methyl mercury in the kidney than in the liver, as was the case in the present study. Here the levels of mercury in the kidney were most elevated in relation to the level in the liver among the birds given the highest dose of

mercury. The ratio of levels in the kidney and liver may therefore be an indicator of mercury poisoning. Higher kidney to liver ratios may indicate elevated mercury levels.

Skeletal muscle comprises the major storage site for mercury in the body. Although concentrations of mercury are not as high as in the liver, kidney or feathers, the greater mass of muscle makes for greater total accumulation. One possible explanation for this may be that concentrations in muscle tissue are lower than elsewhere because after the withdrawal of dietary mercury, they are diluted both by muscle growth and by loss from the tissue as existing protein undergoes degradation due to turnover. In muscle tissue, mercury ingested in amounts insufficiently large for maximum binding onto proteins will bind mainly to proteins with a faster turnover (March et al., 1983) and this may explain the higher variation in mercury concentrations in the lowest dose group.

The proportion of mercury deposited in kidney and primaries increased with dose whereas the opposite was found in the carcass. Mercury which would be deposited in the carcass at low levels appears to be placed in these other two tissues at high mercury burdens.

Braune & Gaskin (1987a) showed that 5.8% of the mercury in the body was found in the carcass of juvenile Bonaparte's gulls. The corresponding figure from this study was as high as 39% although the figures for the liver and kidney in this study did not differ from those of the previous experiment (Table 6.2). The discrepancy may be explained by differences in exposure to mercury. In the former study, birds were exposed to naturally occurring mercury levels, which were much lower than the doses given in the present study. It appears that at higher

exposure levels more mercury is stored in other parts of the body, particularly the skeletal muscle, which may once again provide a means of assessing increasing pollution levels.

Excretion of various mercurials has been widely documented for poultry and other captive-bred birds (Miller et al., 1960; Swensson & Ulfvarson, 1968; Backstrom, 1969; Hienz, 1974) and excretion rates of methyl mercury vary from 50-95% of the mercury ingested for up to a period of 8 weeks. In this study 71% of the mercury administered was excreted over approximately the same period. March et al. (1983) found that methyl mercury excretion decreased in growing chickens as the dietary concentration of mercury increased. The present study did not find any significant effect of dose on the rate of mercury excretion but this may have been due to the much lower levels of mercury used.

The pattern of feather growth is such that once mercury has been bound into feather protein it has been effectively excreted and is no longer part of the body load. The reduction in mercury concentration down the primary growth sequence was therefore expected. In young birds growing their first set of primaries the innermost primary is the first grown (as in adult moult) and a higher level of mercury would be present in the blood during its growth. This would gradually be depleted as the body pool of mercury was reduced and so the amount entering the growing feathers during the moult would also decrease (Furness, 1986). Head feathers, which are the last to be grown, have lower mercury concentrations than found in other parts of the plumage. However, body and covert feathers comprised the major storage sites for mercury in the plumage because they had both high concentrations and greater mass than

other types of feather.

Sixty five percent of the body burden of mercury was in the newly grown feathers of the chicks after the completed moult. This compares with the figure obtained by Braune & Gaskin (1987a) of 93% and that obtained by Honda et al. (1986) of 70% for adult birds.

Of the administered mercury, 49% was accumulated in the plumage independent of the dose administered. This figure allows a more quantitative approach to measuring mercury pollution using birds as monitors, since the mercury burden in fledging plumage can be converted to total mercury ingested by using this conversion figure, although the exact figure is likely to vary between species.

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CHAPTER 7

Prolonged retention of methyl mercury by quail *Coturnix coturnix* and the role of eggs in mercury excretion

7.1 INTRODUCTION

The toxicology of organomercurials, particularly the most stable form, methyl mercury, has been much more extensively studied than that of the inorganic forms of the metal (Miller et al., 1960, 1961; Fimreite, 1971; Koeman et al., 1971; Heinz, 1974, 1976; March et al., 1983). Methyl mercury is more slowly metabolised than other organomercurials and has a slower excretion rate. Thus its biological half life is relatively long (up to 2-3 months in a variety of bird species) and it is therefore more likely to become a serious toxic hazard (Swensson & Ulfvarson, 1968; Odsjo & Edelstam, 1975)

The impact of mercury pollution on birds will depend on many factors such as geographical location, time of exposure, interactions with other toxins, form or species of the metal, sex, age, intraspecific variations in diet or species differences in physiology. Migration patterns must also be considered because these mean that some birds stop in areas contaminated more than others due to industrial effluent. Any assessment of the impact of those locally contaminated sites on birds will depend in part upon the accumulation of mercury during short exposure and the retention time of those residues.

Metals which are assimilated by the body are either eliminated through excretion or accumulate in internal tissues. Published studies are conflicting in the data presented on the absorption and excretion of methyl mercury. Berglund & Berlin (1969) showed that 100% of ingested methyl mercury is absorbed in some mammals but Miller et al., (1960, 1961) showed the immediate excretion of a variety of mercurials, including methyl mercury to range from 50-95%.

Mercury, unlike some other metals such as cadmium, can be

transferred to eggs (Sell 1974; White & Finley, 1978). It was found to accumulate particularly in the albumen portion of the egg after dietary exposure to methyl mercury (Backstrom, 1969). In eggs, mercury has also been shown to accumulate in a dose-dependent fashion in response to increasing dietary levels of methyl mercury (Tejning, 1967; Heinz, 1976; March et al., 1983). Thus the mercury content of eggs presents yet another possible indirect measure of dietary mercury levels (Fimreite et al., 1974, 1980; Barrett et al., 1985; Becker et al., 1985; Ohlendorf & Harrison, 1986; Newton et al., 1989).

If birds put mercury into the eggs as implied, it would be expected that differences between male and female mercury burdens would have been observed for birds with high mercury levels (see Chapter 4). The use of feathers as a monitoring tool may be impaired if females excrete high levels of mercury to their eggs, as the feather levels may then give a deflated indication of the mercury consumed between the times of the moult.

This study used quail Coturnix coturnix to examine excretion rates of methyl mercury and to establish if there is a difference between mercury retention by males and females. Mercury levels in quail eggs and the relationship between egg levels and those found in the internal tissues of the female were studied and the use of eggs as a reliable monitor for mercury pollution was assessed.

7.2 MATERIALS AND METHODS

7.2.1 Experimental procedure

Fifty quail were used for the experiment. Female birds reach maturity at 5-6 weeks of age. All birds used here were at

least 8 weeks old. The birds were randomly assigned to one of four groups. Group A consisted of 4 males and 10 females, group B, 6 males and 9 females, group C, 6 males and 4 females and group D, 7 males and 4 females.

120 µg of mercury was administered to each bird in the form of 0.2ml of analytical grade methyl mercuric chloride solution. This was placed in a gelatin capsule and administered orally. One, four, eight and twelve weeks after administration group A, B, C and D were sacrificed, respectively, and stored intact at -20°C prior to further treatment. Each week eggs from all females were collected, marked and stored as above until treated.

Six controls were used to measure background mercury levels obtained either before the birds reached the laboratory or from the food given during the experiment. The birds were fed on a commercial poultry product, which on analysis contained less than 0.01 µg/g of mercury (dry weight). The birds were caged separately and had free access to food and water at all times.

7.2.2 Sample preparation

On thawing, the birds were plucked and the feathers stored separately. The kidney, liver and the left pectoral muscle (pectoralis and supracoracoideus) were dissected out and along with the remainder of the body (known as the "carcass") were dried to a constant weight in an oven at 50°C. These were then ground using a pestle and mortar and a sub-sample of each tissue removed for analysis. Eggs collected from each female were pooled at the end of each week of the experiment. These were opened, the contents removed, homogenised and dried at 50°C. A sub-sample was removed for analysis.

Mercury analysis was carried out as described in Chapter 2.

7.2.3 Calculations and statistical analysis

Data were examined both as concentrations and in absolute terms because, in the case of growing tissue or tissue with a fast turnover, concentration may be declining as a result of loss of mercury from the tissue or as a result of dilution as new tissue is added. All mercury levels calculated for the experimental group were corrected by subtracting the mean level found in the control group. All subsequent calculations use this 'net' mercury level.

Total and faecal excretion values were gained indirectly. Total excretion values were calculated by adding the total amounts of mercury in all the tissues analysed (including the carcass) for each individual bird. This was subtracted from the amount of mercury administered. Data were pooled and the mean obtained. Faecal excretion in regard to males was calculated by subtracting the excretion value via the plumage from the total excretion value. Female excretion was calculated by the following equation:

Faecal excretion = Total Hg excreted - (Amount Hg excreted via the plumage + amount excreted via eggs)

Differences between male and female mercury levels for each experimental group were calculated by Mann-Whitney U-tests, as were any differences in male and female excretion rates. A One-way analysis of variance was used on log transformed data to produce homoscedasticity in conjunction with a Tukeys' range test to test differences between tissue levels.

7.3 RESULTS

7.3.1 Accumulation of mercury in the tissues

Mercury levels in control birds were generally less than 0.02 µg/g for all tissues analysed. Table 7.1 shows net mercury concentrations in the tissues of experimental birds. Mercury accumulated differentially in the internal tissues, concentrations in the kidney being greater than liver concentrations which in turn exceeded muscle and feather concentrations (One-Way analysis of variance, $F_{3,199}$= 2.70, P<0.05). Mann-Whitney U-tests showing differences in tissue mercury concentrations are presented in Table 7.2. One week after administration there was a slight but significant difference in internal tissue concentrations between male and female birds (in all cases Mann-Whitney U-test, P< 0.05). In groups B and C (Four and eight weeks after mercury administration) all tissue concentrations differed highly significantly between the sexes (Mann-whitney U-test, P<0.001). Males were found to have up to ten times the concentration found in females. In group D (12 weeks after mercury administration) no significant differences were found in mercury concentrations in the liver and carcass between the sexes (Mann-Whitney U-test, P> 0.2). However, the concentration in the pectoral muscle and kidney did differ with the female birds having significantly lower concentrations of mercury in their muscle than found in males (Mann-Whitney U-test, P< 0.05).

Table 7.1 Mercury concentration (µg/g) (after corrections for control levels) in tissues of quail 12 weeks after administration of 120 µg methyl mercury chloride.

Time after dosage	n	kidney		liver		pectoral muscle		carcass		plumage	
		x	sd	x	sd	x	sd	x	sd	x	sd
week 1 males	4	5.85	2.11	4.96	1.08	3.09	1.84	2.16	0.51	1.64	1.04
females	10	2.87	2.23	2.37	2.42	1.57	1.50	1.34	1.07	0.93	1.54
Week 4 males	6	3.49	1.24	3.03	0.78	2.26	0.95	1.62	0.96	0.57	0.67
females	9	0.41	0.41	0.11	0.23	0.13	0.23	0.09	0.07	0.32	0.22
Week 8 males	6	1.42	0.63	1.29	0.39	0.75	0.13	0.28	0.18	1.50	1.32
females	4	0.14	0.14	0.16	0.15	0.18	0.18	0.13	0.17	0.67	0.56
Week 12 males	7	1.28	0.79	0.90	0.68	0.76	0.07	0.33	0.15	0.26	0.18
females	4	0.08	0.05	0.12	0.05	0.08	0.07	0.12	0.04	0.14	0.02

Table 7.2 Results of Mann-Whitney U-tests of differences between male and female internal tissue mercury concentrations.

Group/tissue	n_1	n_2	U_1	P
Group A				
Kidney	6	4	0.0	0.01
Liver	"	"	1.0	0.02
Pectoral M.	"	"	2.0	0.03
Carcass	"	"	1.0	0.02
Plumage	"	"	7.5	1.00
Group B				
Kidney	9	6	0.0	0.02
Liver	"	"	0.0	0.00
Pectoral M.	"	"	0.0	0.00
Carcass	"	"	0.0	0.00
Plumage	"	"	12.0	0.71
Group C				
Kidney	6	4	0.0	0.04
Liver	"	"	0.0	0.04
Pectoral M.	"	"	2.0	0.01
Carcass	"	"	0.0	0.00
Plumage	"	"	8.0	0.40
Group D				
Kidney	7	4	2.0	0.03
Liver	"	"	6.0	0.20
Pectoral M.	"	"	1.0	0.04
Carcass	"	"	5.5	0.55
Plumage	"	"	7.0	0.88

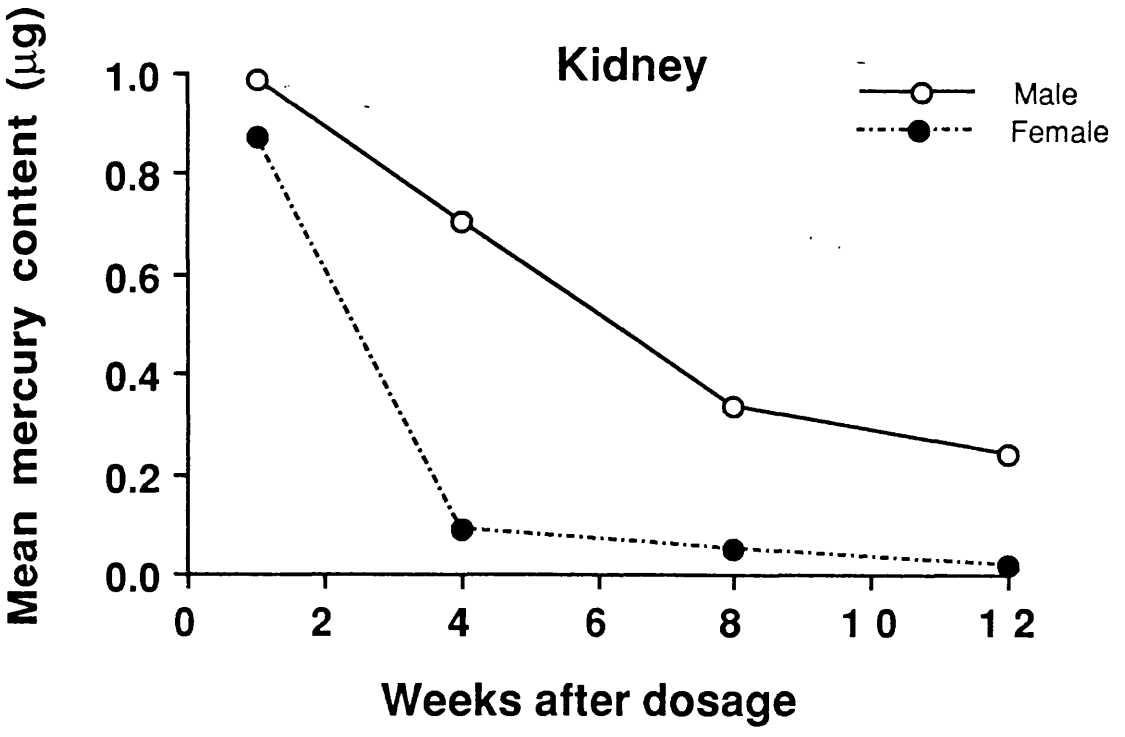
Table 7.3 shows the percentage of the mercury intake present in the tissues analysed. The carcass was the major storage site for mercury in the soft tissues, accumulating nearly 60% of the mercury dose in the male birds and 25% in the females during the first week compared with only 0.8% and 3.0% of the mercury administered found in the kidney and liver, respectively, of the male birds and 0.7% and 3.4% in female birds.

Table 7.3 Mean percentage of the mercury intake found in various tissues of quail fed 120 µg of mercury in the form of methyl mercuric chloride.

Weeks after dosage		n	Tissue: Kidney	liver	Pectoral muscle	Carcass	plumage
Week 1	male	4	0.83	3.05	6.06	59.45	3.35
	female	10	0.72	3.35	3.63	25.08	1.05
Week 4	male	6	0.59	1.08	4.81	49.89	2.51
	female	9	0.08	0.20	0.42	05.09	2.01
Week 8	male	6	0.28	1.12	1.65	14.48	2.53
	female	4	0.07	0.25	0.41	04.28	1.97
Week 12	male	7	0.02	0.67	1.76	10.64	1.10
	female	4	0.05	0.48	0.29	02.53	1.08

Figures 7.1 & 7.2 show micrograms of mercury in internal tissues for 12 weeks after dosage. The birds accumulate mercury rapidly in their internal tissues and lose it relatively slowly. There was a significant difference between total amounts of mercury in all tissues of the male and female birds (Mann-Whitney U-test, in all cases $P<0.01$) except in the kidney where no significant difference between the sexes was found in the first week after mercury administration (Mann-Whitney U-test, $n_1=4$, $n_2=10$ $U_1=7.0$, , $P>0.2$). After this time the same pattern was seen in the actual amounts of mercury in the internal tissues as was reported for mercury concentrations in

Figure 7.1 Micrograms of mercury in the kidney and liver of quail for up to twelve weeks after dosage.


Kidney
Male
Female
Mean mercury content (μg)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Weeks after dosage

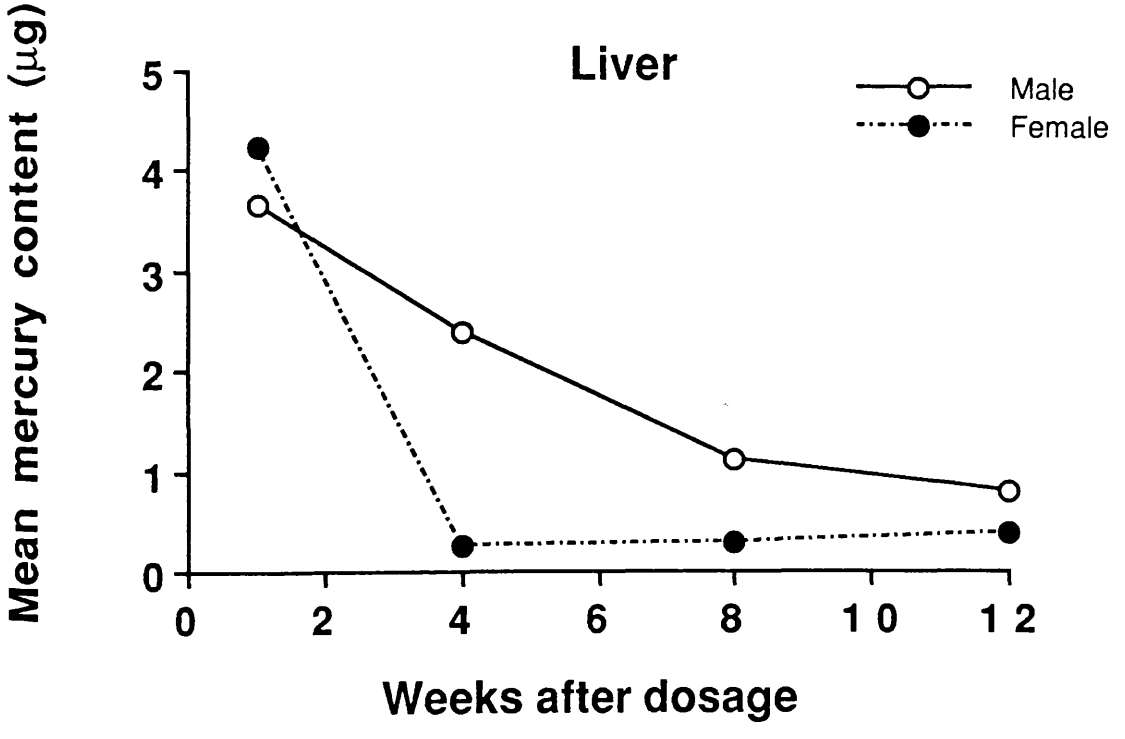
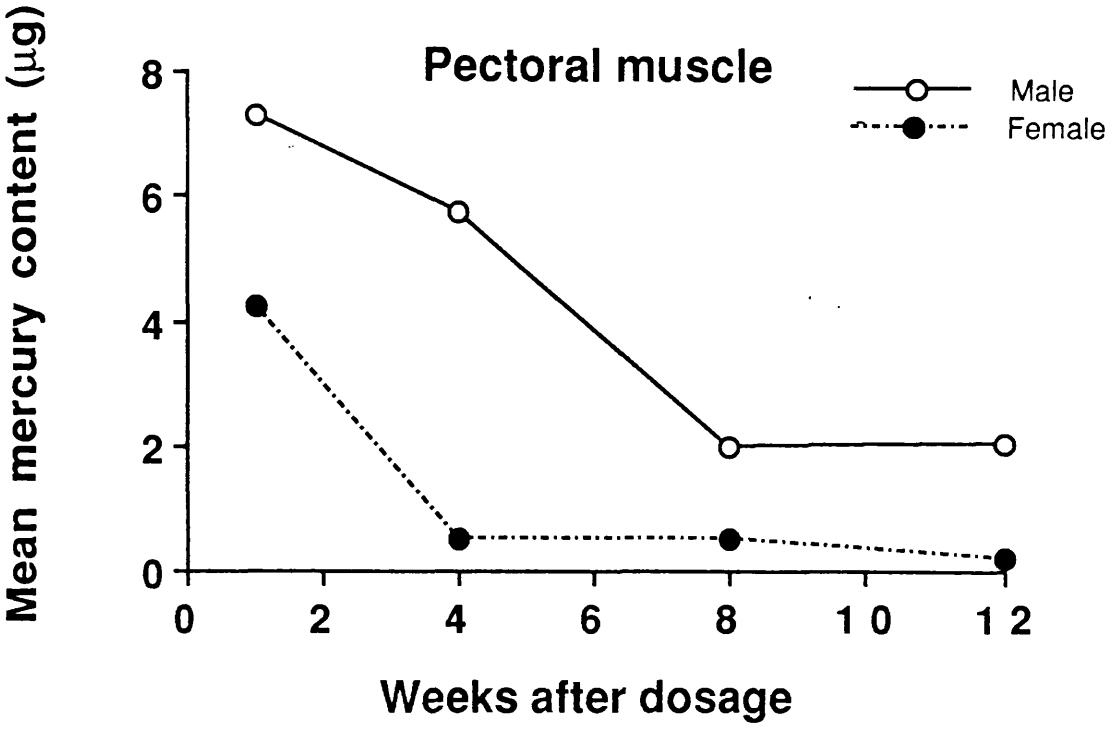
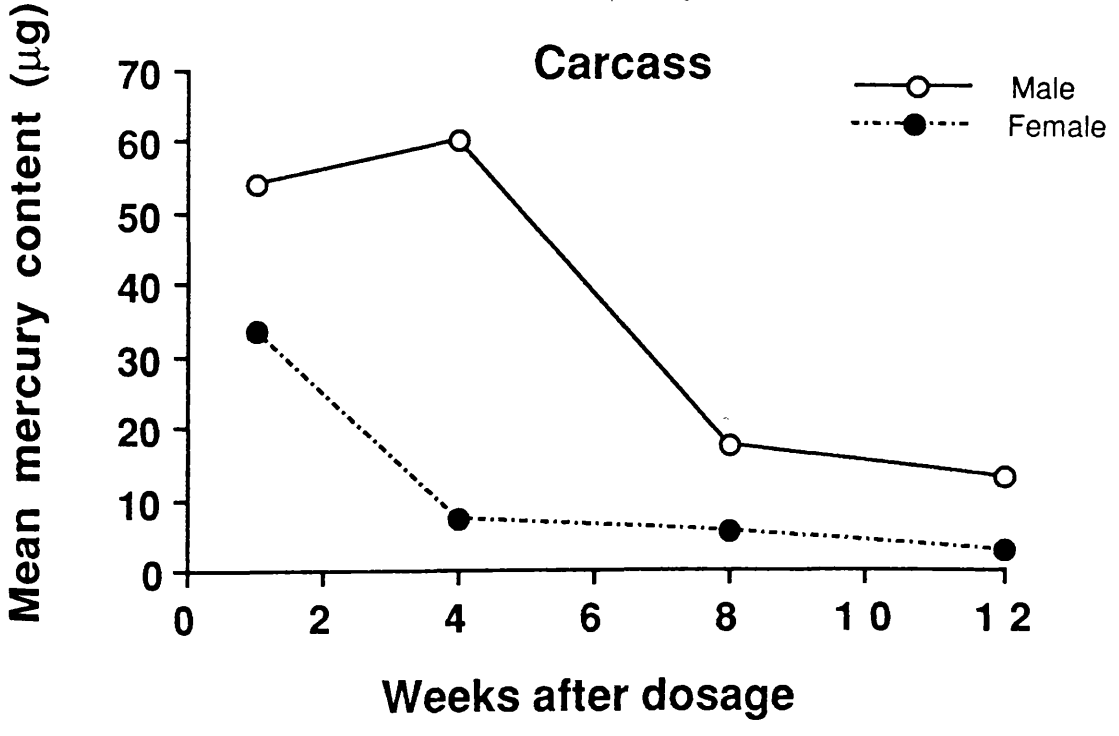

Liver
Male
Female
Mean mercury content (μg)
5
4
3
2
1
0
0
2
4
6
8
10
12
Weeks after dosage

Figure 7.2 Micrograms of mercury in the pectoral muscle and carcass of quail for up to twelve weeks after dosage.


Pectoral muscle
Male
Female
Mean mercury content (μg)
8
6
4
2
0
0
2
4
6
8
10
12
Weeks after dosage


Carcass
Male
Female
Mean mercury content (μg)
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
Weeks after dosage

these tissues with the exception of the carcass where total amounts of mercury present did differ between the sexes 12 weeks after administration (Mann-Whitney U test, $n_1=4$, $n_2=7$, $U_1=0.0$, , $P< 0.05$).

7.3.2 Mercury excretion

Table 7.4 shows mercury excretion in the birds up to 12 weeks after administration. There was no difference between male and female excretion via the plumage (see Table 7.2, Mann-Whitney U-test, $P> 0.1$). Approximately 2% of the mercury intake was excreted in this way.

Table 7.4. Mercury excretion in quail after administration of 120 µg of mercury in the form of methyl mercuric chloride

Weeks after dosage		Total Hg excreted µg		Hg excreted via plumage µg		Hg excreted via faeces* µg
	n	x	sd	x	sd	x
week 1 male	4	36.77	09.49	2.11	1.26	34.6
female	10	77.71	11.76	2.89	2.52	37.3
Week 4 male	6	51.94	34.73	3.80	4.53	48.1
female	9	113.00	05.66	2.34	1.55	52.9
Week 8 male	6	100.24	08.14	4.43	4.52	95.8
female	4	113.65	03.21	3.73	3.01	52.6
Week 12 male	7	103.04	05.47	3.28	2.66	99.7
female	4	113.88	02.95	0.99	0.31	57.8

(*See text for calculation of faecal excretion rate)

Total amount of mercury lost through excretion was

significantly different between the sexes for the first eight weeks after the dose was administered (Mann-Whitney U-test, $P<0.02$, in all cases). Female birds lost over 80% of the mercury intake in the first 4 weeks after dosage. Males lost only 40%. After eight weeks, female excretion slowed dramatically and little mercury was lost after this time.

Of the mercury intake, 30% was lost in faeces and urine one week after administration. After four, weeks approximately 40% of the intake was lost in this way. However, eight weeks after dosage, male and female excretion began to differ significantly. Male birds lost nearly 80% of their mercury intake in the faeces compared to only 44% lost by female birds in faeces. Faecal excretion rate slowed down by the 12th week after dosage with only another 4% lost by males (to 84%) and 3% by females (to 48%).

Table 7.5. Amounts of mercury in eggs of quail administered 120 µg of methyl mercuric chloride

No. weeks after dosage	Total mercury µg		Mercury conc. µg/g		% of Mercury uptake
	x	sd	x	sd	x
0	37.52	6.95	3.65	0.77	31.27
1	11.15	4.92	1.67	1.29	09.29
2	09.67	7.75	0.59	0.24	08.06
3	04.07	3.17	0.25	0.16	03.39
4	00.24	0.25	0.09	0.06	00.20

NB. After week 4, mercury in the eggs was undetectable.
(Detection level < 0.05 µg/g)

Table 7.5 shows mercury deposition in the eggs of the

quail. Initial mercury concentrations in the eggs were over 3.5 µg/g. Approximately 30% of the females' mercury intake was lost into the eggs just one week after administration. Five weeks later over 40% of the females' intake was lost in this way. After this period no more mercury was detected in the eggs of laying females.

7.4 DISCUSSION

Quail were used in this experiment because they were convenient laboratory animals, being relatively inexpensive and easy to obtain and keep. However, they provide only a poor model of a typical bird as used in mercury monitoring. They are terrestrial, domesticated and unlike most birds, females produce on average one egg per day for a prolonged period. These facts must be taken into consideration when examining the results in relation to natural mercury contamination in birds.

Quail accumulate mercury rapidly in their internal tissues and lose it relatively slowly. Loss of mercury from the liver and kidneys did not follow a biphasic pattern that has been reported in some studies (Swensson & Ulfvarson, 1968; Gardiner, 1972) but showed the same pattern reported by Miller *et al.*,(1961).

In studies administering mercury to birds, kidney concentrations always exceed liver concentrations which exceed muscle concentrations (Swensson & Ulfvarson, 1968; March *et al.*, 1983; Nicholson & Osborn, 1983). However in birds sampled from the wild, liver levels usually exceed those found in the kidney (Blus *et al.*, 1977; Connors *et al.*, 1975; Honda *et al.*, 1986; Muirhead & Furness, 1988). It was proposed in an earlier study looking at mercury accumulation in black-headed gull *Larus*

ridibundus chicks (See Chapter 6) that high kidney to liver ratios reflect elevated environmental mercury levels. Although the birds in this study showed no signs of mercury poisoning (reduced food intake, weight loss, progressive weakness in the wings and legs and inability to coordinate muscle movement (Borg et al., 1966; Tejning, 1967) the level of mercury given to them was higher than natural, terrestrial, environmental levels (e.g Hutton, 1981). Therefore this study offers further evidence that higher kidney to liver ratios may be an early sign of mercury poisoning evident at levels of mercury below those at which neurological symptoms arise.

Skeletal muscle comprises the major storage site for mercury in the body. Although concentrations of mercury in muscle are not as high as in the liver and kidney, the greater mass of muscle makes for greater accumulation.

The excretory mechanisms available to birds are transportation of mercury to the feathers at times of new feather growth, excretion via the urine and faeces or, as in female birds, mercury may be lost into the eggs. The values for feather concentrations of mercury showed varying degrees of contamination according to how much feather loss and regrowth occurred during the experiment. Loss of mercury through excretion into the plumage was minimal (approx. 2% of the intake) because normal seasonal moult was not taking place. However, quail tend to lose odd feathers when in cages and so some mercury was lost into growing feathers in all experimental groups. Excretion appeared to be negatively exponential, the mercury being excreted in an amount per unit time which appears to be proportional to the mercury concentration currently in the soft tissues of the body. This implies a logarithmic excretion

model and is consistent with what has been previously found in rats and poultry (Swensson & Ulfvarson, 1968).

Twelve weeks after administration of mercury, the birds in this study had eliminated up to 95% of the mercury intake. Male birds excreted over 85% of the mercury intake in their faeces alone. Female birds excreted less this way due to the large amount lost in the eggs. The results presented here differ from those of Tejning (1967) who stated that elimination of mercury with excrement in domestic fowl is of little significance after consumption of methyl mercury has ceased. In this study about 12% of the mercury intake was lost in this way. Eskerland & Nafstad (1978), however, reported a daily excretion rate of 11-21% of the mercury intake in poultry. Ulfvarson (1965) found that non-moulting pheasants could eliminate a high proportion of the mercury ingested within 1 month after mercury administration and after 5 months no mercury at all could be detected in their tissues. It appears that with levels of mercury low enough not to cause poisoning, a single dose of mercury can be excreted from the body and therefore may not be a major environmental hazard at low levels.

This poses the question of how mercury is lost in the faeces a relatively long time after administration. The absorption of ingested methyl mercury is reported to be very rapid (Backstrom, 1969). Backstrom (1969) found that one day after administration very little mercury could be detected in the digestive canal. However a marked uptake and retention of mercury was seen in the epithelium of the oesophagus. Small quantities of mercury could be excreted from the epithelium along with the loss of the cells during the course of the digestive process. However from previous studies it appears that

relatively high quantities of absorbed mercury are excreted via the glands of the alimentary canal (Tejning, 1967).

Egg-producing female birds can excrete high amounts of mercury in a shorter time span than male birds and this may have implications for mercury monitoring using bird feathers. A moult usually follows breeding and if feathers are sampled after this time the levels in the feathers may not be reflecting the previous year's contamination as would be expected. Braune & Gaskin (1987) found that in sampling female Bonapartes' gulls Larus philadelphia after egg laying the female feather mercury levels were significantly lower than that of the males. This must therefore be a consideration when using birds' feathers to monitor mercury pollution.

Excretion of mercury into eggs appeared to be substantial in female quail, over 40% of the mercury intake was lost in this way. Unfortunately this present study did not analyse the two components (albumen and yolk) of the egg separately. Thus it was not determined if mercury concentration was restricted to the albumen portion of the egg as was previously suggested (Backstom, 1969). These birds are extraordinary in that they can lay up to one egg per day for a prolonged period and their capacity to excrete mercury is therefore greater than for other birds.

Initial levels in the eggs may be derived totally from the diet (i.e from the orally administered mercury). However, comparatively large amounts of mercury going into the eggs appears to be derived from store tissues which accumulated the metal after the initial dose and not from the diet. Which store tissues are involved in such processes is not known.

Backstrom (1969) showed that mercury particularly

accumulated in the albumen portion of the egg. The albumen is manufactured in the oviduct of the female where no long term storage of egg white protein is seen, rather, the bulk of the protein appears to be transported to the oviduct only once the yolk is ovulated (Gilbert, 1971). In red-billed queleas Quelea quelea it was found that protein used in production of the yolk is derived from the protein store in flight muscle and proteins of the albumen may come from other muscles or protein stores (Jones & Ward, 1976). It was only the mercury levels in the pectoral muscle and carcass that differed at the end of the experiment and therefore it seems probable that the high mercury levels found in the eggs came from both the flight muscles (which may be responsible for small quantities of mercury in the yolk) and the muscle stores found on the rest of the carcass of the bird.

Evidence from this experiment may go some way to supporting the use of birds' eggs to sample for environmental mercury contamination as it appears that eggs will reflect the contamination of the female very well.

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CHAPTER 8

Mercury retention in males and females of two species of seabird and the use of eggs in monitoring mercury pollution

8.1 INTRODUCTION

In recent decades mercury pollution monitoring has increased due to mercury being regarded as one of the more toxic of marine pollutants. Seabird feathers have often been used as indictors of toxic chemicals in the environment (Robinson et al., 1967; Anderlini et al., 1972; Connors et al., 1975; Furness & Hutton, 1980; Appelquist et al., 1985; Furness et al., 1990). However, the levels in feathers are the product of the build up of the mercury intake during the period between moults and as such they have limitations as indicators of localised contamination.

Bird eggs have become a popular method of monitoring environmental pollution (Fimreite, 1971; Barrett et al., 1985; Becker et al., 1985; Newton & Haas, 1988; Newton et al., 1989). Eggs when used as a monitor for environmental contamination have several advantages over previous methods (see Chapter 1) however, they do have some limitations. They are representative of only part of the population and for only part of the year and very little is known about the relationship between the mercury levels in internal tissues of the female and that in the egg. If mercury was being deposited into the egg in proportion to the female's body burden it may be expected that a high inter-clutch variation would be found as the levels in the first egg may be higher than that in successive eggs.

Becker (1989) suggested that egg contamination stems to a great extent from the feeding grounds near the breeding site where the pollutants are ingested during the period immediately prior to egg laying. He also suggested that migrating species may also be valid indicators of the contamination levels in their breeding grounds during the pre-laying period as

considerable quantities of food are required to produce eggs (characterised by courtship feeding in many species) and as a result the egg residues reflect the local contamination of the food web rather than stored mercury accumulated during the non breeding season.

If female birds could excrete some of the mercury contamination they have consumed locally during the period prior to egg laying, it might be expected that in the subsequent moult, compared to males, female birds would put less mercury into their feathers. Braune & Gaskin (1987) found that adult female Bonaparte's gulls Larus philadelphia had significantly lower mercury concentrations in their primary feathers than male birds of equal age. They suggested that adult females may have reduced their body burden of mercury through egg-laying to approach the level found in immature birds. Most authors that have examined sex effects for mercury contamination have found no differences between males and females (see Chapter 4).

There have been several studies emphasising comparisons of one or more metals within one species (Connors et al., 1975; Nicholson, 1981; Custer & Mulhern, 1983; King et al., 1983) or among several species (Hulse et al., 1980; Cheney et al., 1981; Hutton, 1981; Gochfeld & Burger 1987). Studies have shown that near shore predatory birds such as cormorants Phalacrocorax auritus and terns Sterna hirundo and S. paradisaea have higher mercury levels in their feathers than do pelagic predatory birds such as kittiwakes Rissa tridactyla (Braune, 1987), and top fish-eating birds have higher metal levels than those birds eating lower down the food chain (Fimreite, 1974). Any differences in metal bioaccumulation between species have been accounted for by relating the heavy metal load of the bird to

their diet. Species differences due to other physiological causes have generally been overlooked due to the lack of data on the actual metal intake of the birds.

Body burdens and tissue distribution of a given heavy metal depend on the intensity and time course of exposure, the form or species of that metal, interactions with other toxins and a variety of other factors intrinsic to the host. Therefore it may be hypothesised that different bird species given the same mercury load would show characteristic differences in metal concentrations.

The aims of this study are to investigate the capacity of bird eggs to reflect environmental mercury levels using two species of seabird. Sex differences in regard to mercury concentrations are also investigated along with differences in the metal levels of the two species.

8.2 MATERIALS AND METHODS

8.2.1 Experimental procedure

Experiment 1.

In May 1990 (under licence) shags Phalacrocorax aristotelis were captured at their nest site, at a colony on Foula, Shetland. The birds were ringed, weighed and measurements of wing length and 'head plus bill' length taken. Ten males and ten females were administered 2000 µg of methyl-mercury in the form of analytical grade methyl mercuric chloride solution. Each dose was given in two gelatin capsules. Six other shags were also captured and ringed as controls. Mercury was administered to the birds caught at nests where clutches had not yet been completed. The nests were marked along with any eggs found there. Each egg was given a number and a letter to denote the sequence of laying

and whether the egg was laid pre or post mercury administration. Once adults had been dosed the nests were visited every 48 hours and any new eggs found there marked. Birds in the experimental group laid up to three eggs after mercury administration. When no new egg had been laid over a 96 hour period the adult birds were captured as soon as possible and sacrificed by cervical dislocation. The mean interval between dosage and death was 25 days. The eggs were removed and along with the adult bird, stored, intact at -20°C prior to further treatment. Due to difficulty in recapturing the birds only seven of the ten females and four of the males were sacrificed along with four of the control birds. Nevertheless the eggs from all experimental and control clutches were taken.

Experiment 2.

Also under licence on Foula in May 1990, kittiwake adults were captured at the nest using a noose on a 9 metre fishing pole. The nests were marked and the birds ringed and weighed and 'head plus bill' and wing measurements taken. Ten males and ten females were administered with the same amount of mercury in the same form as given to the shags in Experiment 1. Mercury was administered at least one week before egg laying commenced. Ten birds to be used as a control were also captured and ringed. Birds were released and watched for approximately 30 minutes to ensure they did not regurgitate the capsules. Due to the difficulty in removing eggs from the nests of this cliff-dwelling species, chicks were taken instead. Two eggs were usually laid from which only one chick typically survived until the date they were sacrificed. As soon after hatching as possible chicks were noosed out of the nest and killed by cervical dislocation (chicks ranged from a few days to 10 days

old). Adults were also taken, this was approximately 47 days after dosage. These were then stored intact at -20°C until further treatment.

8.2.2 Sample preparation and mercury analysis

On thawing both species of birds were dissected and the entire kidney, liver and left pectoral muscle (pectoralis and supracoracoideus) were removed. These were dried to a constant weight in an oven at 50°C. Eggs were opened and the yolk and white removed, homogenised and dried to a constant weight. One whole shag was plucked and the carcass (whole body minus plumage, kidney, liver and left pectoral muscle) dried to a constant weight and then homogenised. All tissues were then analysed for mercury.

Kittiwake young were opened and dried intact. Once a constant weight was achieved the carcasses (the entire bird including plumage) were completely homogenised and a sub-sample removed for analysis. Loss of water from the eggs during incubation requires that a dry weight concentration should be used only (Potts, 1968). Each egg was analysed separately. The total amount of mercury contained in the whole clutch was derived by summing all the mercury present in each egg of that clutch.

Mercury analysis was carried out as described in Chapter 2. All mercury levels calculated for the experimental groups were corrected by subtracting the mean levels found in the control group.

8.2.3 Statistical analysis

Data were tested for normality by Kolmogorov-Smirnov one-sample tests. Because data from eggs of the same clutch were

not independent, differences between A, B and C eggs were examined by subtracting mercury levels of eggs in the same clutch from each other and testing for a distribution around a mean of zero using a one sample t-test. Differences in mercury levels according to tissue type were tested by a One-way analysis of variance using log. transformed data to produce homoscedasticity in conjunction with Tukey's range tests. Differences in mercury concentration between sex, species, experimental and control eggs were assessed using a Two-sample t-test.

8.3 RESULTS

8.3.1 Mercury accumulation in tissues

Mercury concentrations and total amounts of mercury in the tissues of shags are given in Table 8.1a and of kittiwakes in Table 8.1b. Mean mercury concentrations and total amounts of mercury are given in Table 8.1c. Mercury accumulated differentially in the internal tissues. In both species there was no significant difference between the concentrations of mercury in the kidney or the liver though both these concentrations exceeded those found in the muscle (One-way analysis of variance, kittiwakes: $F_{2,57}=31.13$, shags: $F_{2,32}=22.75$, Tukey's range test $P<0.05$).

From a visual inspection of the data there appeared to be a difference between the concentrations of mercury in the liver and kidney. This was tested using a Wilcoxans matched pair test, a significant difference between these two tissues was found with concentrations in the kidney being greater than concentrations in the liver ($Z= -2.93$, $P<0.01$).

Table 8.1a Concentration (µg/g) and total mercury (µg) in the tissues and eggs of experimental and control male (M) and female (F) shags after administration of 2000 µg of methyl-mercury.

Sex	Days between dose & death	Liver		Kidney		Pectoral muscle		A egg		B egg		C egg	
		µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg
Experimental.													
F	22	14.95	258.19	19.01	90.67	4.59	113.24	1.96	11.89	1.66	10.51	1.63	8.49
F	-	9.92	121.72	11.62	59.96	2.12	56.16	1.89	8.28	1.38	6.51	0.78	4.31
F	21	12.43	251.46	16.03	83.19	6.15	149.32	1.04	6.47	2.42	16.34	1.24	7.31
F	13	13.27	277.21	15.23	99.60	5.20	134.99	1.76	11.49	0.95	6.58	1.30	8.07
F	13	10.63	198.25	11.88	64.63	5.00	140.80	1.59	10.16	2.01	13.33	2.73	16.90
F	19	12.30	262.11	13.44	81.31	5.17	140.62	3.60	18.61	2.37	14.22	3.16	17.19
F	20	13.44	262.89	18.37	118.67	4.95	128.65	1.79	9.42	3.38	22.85	3.16	21.61
F	--	---	---	---	---	--	---	2.68	15.01	1.15	6.82	1.08	7.24
F	--	---	---	---	---	--	---	1.71	5.44	1.29	4.17	--	--
F	--	---	---	---	---	--	---	1.09	6.55	1.65	9.98	2.24	13.01
F	--	---	---	---	---	--	---	1.57	6.55	1.72	9.98	1.18	5.93
M	37	11.53	210.07	14.15	82.49	4.85	151.71						
M	33	11.70	224.99	19.28	108.39	4.64	116.56						
M	36	6.29	125.14	20.11	109.60	5.18	149.81						
M	25	11.94	247.39	18.52	118.53	6.16	204.02						
Controls													
F	--	3.41	30.01	4.81	26.74	1.14	23.32	1.75	9.29	1.44	7.27	1.48	7.97
F	--	2.81	46.79	5.36	30.39	1.53	38.98	1.27	7.20	1.23	7.08	1.18	6.61
F	--	---	---	---	---	--	---	1.49	9.74	1.14	7.23	1.63	11.18
M	--	3.59	66.31	5.11	27.13	1.15	26.34	1.30	7.16	1.46	9.64	2.16	14.13
M	--	3.02	27.90	4.31	16.59	1.39	23.96	1.49	9.29	0.99	7.41	1.25	8.21

There was a significant difference between liver and kidney concentrations, Wilcoxons matched pairs $Z = -2.93$ $P < 0.01$

Table 8.1b Concentration (µg/g) and total mercury (µg) in the tissues and eggs of experimental and control kittiwakes after administration of 2000 µg of methyl mercuric chloride.

Sex	Days between dose & death	Liver		Kidney		Pectoral muscle		Chick	
		µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg
Experimental.									
F	47	24.50	73.99	17.76	12.79	6.68	46.36	0.79	164.93
F	"	14.04	66.69	16.27	17.08	5.55	52.67	--	---
F	"	17.60	57.02	13.03	14.46	5.50	48.07	15.37	81.92
F	"	20.11	73.00	17.28	15.90	7.75	51.07	9.98	85.73
F	"	14.31	65.68	15.38	14.61	6.09	40.86	1.41	45.88
F	"	17.89	55.99	17.44	17.09	7.17	45.89	--	---
F	"	19.22	64.96	13.84	10.10	6.52	47.87	19.10	118.99
F	"	26.41	82.39	12.66	10.64	5.77	38.77	--	---
F	"	21.45	82.79	43.79	34.59	14.03	81.65	0.94	77.72
F	"	---	---	---	---	---	---	1.38	56.99
F	"	---	---	---	---	---	---	4.18	95.93
M	"	15.04	51.88	16.89	15.37	6.64	52.65		
M	"	19.00	67.26	23.78	28.29	7.91	65.89		
M	"	16.15	53.13	15.75	12.12	7.35	44.25		
M	"	24.23	73.90	28.67	28.38	11.09	85.17		
M	"	3.47	13.64	4.75	4.61	3.23	28.97		
M	"	15.73	60.71	14.18	14.04	6.22	49.51		
M	"	12.12	43.99	12.71	12.46	4.38	32.37		
M	"	26.34	95.88	20.09	18.48	8.37	66.21		
M	"	16.66	66.64	18.84	18.08	7.29	43.59		
M	"	21.13	96.78	18.57	19.13	9.72	93.80		
Control.									
F	"	3.35	13.64	2.72	1.87	1.90	14.44	0.22	2.40
F	"	5.65	22.54	2.34	1.80	1.15	6.37	0.97	14.48
F	"	--	---	--	--	--	---	1.41	14.27
F	"	--	---	--	--	--	---	1.08	10.03
F	"	1.25	5.84	2.10	1.49	2.18	22.80	1.07	7.02
M	"	3.59	14.58	5.36	5.09	1.53	10.91	0.27	8.06
M	"	3.89	13.93	4.32	3.41	1.53	14.93	2.74	15.43
M	"	3.41	13.30	5.11	5.77	1.39	10.12		
M	"	2.93	10.31	3.47	2.12	1.41	9.29		

Table 8.1c. Concentrations and total mercury in tissues in male and female shags and kittiwakes administered 2000 µg methyl mercuric chloride.

Bird/sex		Tissue: Liver x	Liver sd	Kidney x	Kidney sd	Pectoral muscle x	Pectoral muscle sd
Shags/							
Female (7)	µg/g	11.99	02.59	15.08	02.95	04.03	02.09
	µg	233.12	55.17	85.43	20.15	270.16	70.90
Male (4)	µg/g	10.52	02.41	18.02	02.66	05.21	00.67
	µg	201.90	53.42	104.50	15.44	311.05	72.27
Kittiwakes/							
Female (9)	µg/g	19.50	04.18	18.61	09.64	07.23	02.66
	µg	69.16	09.71	16.36	07.29	100.71	25.07
Male (10)	µg/g	16.99	06.40	17.42	06.44	07.22	02.32
	µg	62.38	24.48	17.10	07.26	112.48	42.77

There was no significant difference in metal levels in the internal tissues of male and female birds in either of the study species (t-test using separate or pooled variance estimate as appropriate, $P>0.2$ in all cases).

There was a significant difference between the levels of mercury in the liver and pectoral muscle of shags and kittiwakes (t-test using pooled variance estimate, $P<0.05$) but kidney mercury levels did not differ significantly (t-test, $P>0.05$). There was a significant difference in the total amounts of mercury in all the tissues analysed between the two species (t-test, $P<0.01$), with the shag tissues having up to four times

the amount of mercury found in the same tissues of Kittiwakes

The percentage of the mercury intake found in the tissues of the experimental birds is presented in Table 8.2.

Table 8.2 Amount of mercury deposited in the tissues of shags and Kittiwakes administered 2000 µg of methyl mercuric chloride expressed as a percentage of the mercury intake (corrected for 'background' levels).

Species		Liver		Kidney		Pectoral muscle	
	n	x	sd	x	sd	x	sd
Shag	11	8.95	2.71	3.09	1.27	10.69	3.55
Kittiwake	20	2.75	0.72	0.72	0.33	04.21	1.69

Of the tissues analysed the pectoral muscle contained the most mercury, accumulating over 10% of the mercury intake in shags and 4% in the kittiwakes. The ratio of kidney:liver:pectoral muscle mercury quantities as percentages of the mercury intake approached a 1:3:4 ratio for both species of seabird.

8.3.2 Mercury assimilation

An estimate of mercury assimilation was carried out using two methods of calculation. An indirect method of calculation was used on the kittiwake data due to the kittiwake carcasses being unavailable for whole carcass analysis. These calculations are only an estimate to enable some idea of total mercury excretion to be assessed.

Calculations

Concentration of mercury in carcass of 1 shag= 2.9 µg/g (dry weight)

Dry weight of shag= 450 g

Therefore total mercury in carcass= 1305 µg Hg

Total amount of mercury in shag= (total mercury in carcass + mercury present in internal organs)= 1908µg

Mean percentage of dose excreted over 25 day period= 5%

Based on other work with quail Corturnix corturnix and black-headed gulls Larus ridibundus (Chapters 6 and 7) there is an approximate ratio of 73:6:5:1 for the amount of mercury contained in the carcass:pectoral muscle:liver:kidney.

Therefore approximate mercury present in kittiwake carcass

= 1051 µg

Total amount of mercury in kittiwake =(total mercury in carcass plus mercury present in other organs)

= 1239 µg

Mean percentage of dose excreted over 47 day period= 38%

8.3.3 Mercury accumulation in eggs and chicks

Concentrations and total amounts of mercury in chicks and eggs of the experimental birds are shown in Table 8.3. The mercury levels in eggs laid by experimental shags after dosing showed no significant difference from those levels found in the control eggs (t-test, $P > 0.1$). However, the levels of mercury found in kittiwake chicks of the experimental group did differ significantly from those levels in the control group (t-test, $P<0.01$), the concentrations of mercury of the experimental chicks being approximately six times that found in the controls. The mercury accumulation of the chicks was equal to 2% of the

administered dose.

Table 8.3 Concentrations and total mercury in experimental and control eggs (mean of clutch) or chicks of shags and kittiwakes

Species		n	Concentration µg/g		Total mercury µg	
			x	sd	x	sd
Shag	Experimental	11	1.71	0.49	32.15	13.14
	Control	5	1.38	0.20	25.88	03.81
Kittiwake	Experimental	8	6.64	7.27	91.01	37.37
	Control	7	1.11	0.84	10.30	05.65

Table 8.4 shows mercury levels in eggs of dosed shags in relation to sequence of laying. There was no significant difference in the mercury levels between the eggs of the same clutch (One-Sample t-test P>0.5 see 8.2.3).

Table 8.4 Concentrations and total mercury in the A, B and C egg from shags fed 2000 µg methyl mercuric chloride.

	A Egg(n=11)		B Egg(n=11)		C Egg(n=10)	
	x	sd	x	sd	x	sd
Concentration µg/g	1.88	0.72	1.82	0.70	1.85	0.90
Total mercury µg	9.99	4.06	11.03	5.39	11.01	5.80

8.4 DISCUSSION

The interspecific variation in mercury concentrations in the two species analysed here may be mainly due to the differences in the interval between mercury administration and death of the bird. However, the variation is in keeping with similar findings of other investigations (Fimreite et al, 1974; Holt et al., 1979; Hutton, 1981; Delbeke et al., 1984; Muirhead & Furness, 1988). Here, authors recognised the difference in mercury concentrations between species as a result of variation with diet.

Muirhead & Furness (1988) when looking at a variety of different species in relation to zinc, cadmium and mercury levels came to the conclusion that dietary and feeding differences between species were important in determining levels of cadmium, those species with high levels tending to feed on squid. For mercury levels, Gough Island seabirds showed no pattern between species that could be linked even tentatively with diet. Nor did variations occur in parallel with variations in cadmium levels. It does appear therefore that constraints on elimination of mercury from the body may be as important as dietary intakes.

In the present study, variation in mercury levels between the two species may be a factor of the time of exposure to methyl-mercury. However, other physiological, interspecific factors may also influence the extent to which mercury accumulates in the body. Interspecific differences may also arise as a result of differential metabolic capacities (Knight et al., 1981; Knight & Walker, 1982a,b). This in turn would effect the amount of mercury going into the plumage at times of new feather growth. It might be suggested that kittiwake

physiology deals with mercury slightly differently to that of the shag.

There was no difference between male and female mercury retention in either of the species studied. Braune & Gaskin (1987) found significant differences in levels of mercury between male and female Bonaparte's gulls, the female birds being presumed to have deposited some of the mercury load into the eggs. Gochfeld & Burger (1987) found significant differences in levels of several metals between male and female ducks, although mercury levels were the same in both sexes. Hutton (1981) found differences in the levels of zinc and cadmium between male and female oystercatchers Haematopus ostralegus and Hoffman & Curnow (1979) found higher levels of mercury in female herons. However, Furness et al. (1990) found no differences in mercury levels in the body feathers of male and female red-billed gulls Larus novaehollandiae scopulinus and in most studies the levels of metals have not differed between the sexes, as found for the shags and kittiwakes, although mercury was being deposited in the eggs.

The levels of mercury in eggs and chicks of the control birds reported here are rather higher than those found in comparable studies (Fimreite et al., 1974, 1980; Ryder, 1974; Becker, 1989). However, these levels are unlikely to be affecting reproductive success of the birds as mercury levels up to 2 µg/g were found in 24 herring gull Larus argentatus eggs where hatchability appeared normal (Vermeer, 1971).

Many authors have used bird eggs to monitor the environment for mercury pollution (Fimreite, 1971; Barrett et al., 1985; Newton & Haas, 1988; Becker, 1989) assuming that the eggs reflect the mercury in the area at the time of egg production.

For example, Becker (1989) showed using bird eggs that there were spatial trends along the Wadden Sea coasts showing serious contamination of the birds. These trends correlated with sites of high industrial output. However, there is little information on whether levels in eggs actually reflect local contamination or show contamination of the bird some time prior to the breeding season.

Experimental shag egg levels did not differ from those of the controls. Both experimental and control birds showed mercury contamination with the experimental birds being slightly higher but not significantly different from the control birds. This may suggest that the mercury going into the eggs is derived from a store tissue prior to the date of mercury administration. In some species the contamination of the females may originate from the wintering areas and from migration routes. During the pre-laying period considerable quantities of food are required to produce eggs (Krapu, 1981) and as a result the egg residue may reflect the contamination of the food web some time prior to egg laying. Therefore the difference in mercury levels in eggs of shags and kittiwakes might be explained by differences in their methods of egg production.

The daily maximum cost of egg production has been estimated to be between 50-70% of the daily energy intake at constant body weight in the Anatidae (King, 1973). Nutrients for reproduction must either be drawn from the body reserves or obtained from the food resources at the time of breeding or both. Among arctic-nesting geese that breed in environments where food is scarce at the time of nesting it has been shown that stored nutrient reserves contribute substantially to reproduction (Barry, 1962; Hanson, 1962; Ankney & MacInnes, 1978; Raveling, 1979) and lipid

reserves of mallards Anas platyrhynchos contribute substantially to the needs of mallard pairs during reproduction (Krapu, 1981), also protein required by female mallards is obtained principally from the diet.

Female pintails Anas acuta and wood ducks Aix sponsa similarly obtain protein needs for egg formation primarily through dietary intake rather than endogenous reserves (Krapu, 1974; Drobney, 1977). If this situation was similar for shags it may account for the lower than expected levels of mercury in the eggs due to the proteins used in egg production being drawn from the diet immediately prior to egg laying. If this were indeed the case it would mean that eggs would be an excellent indicator for localised contamination monitoring. The mercury administered in this experiment may have been given at too early a date to be incorporated into the eggs or it may have accumulated too rapidly in the internal tissues to be utilised in egg formation.

Kittiwakes' egg formation may be similar to that found by Jones & Ward (1976) and Kendall et al.(1973) in queleas Quelea quelea where proteins used in the production of the yolk came from protein reserves stored in the flight muscle but proteins used in albumen formation (where most of the mercury is concentrated (Backstrom, 1969)) came from other muscles or protein stores in the body. The ecology of the birds does not predict such a dichotomy in egg formation but there is still a lack of information about the egg production process of different species.

Kittiwake chicks of the experimental birds showed a significant difference in their mercury levels from those of the control group. This does suggest that the mercury from the female is being transported to the chicks. However, no

differences were found between mercury levels in male and female kittiwakes. This suggests that the mercury may have been depleted from a store tissue not analysed here. Other experiments (see Chapter 7) showed that female quail that had been given high doses of methyl mercury lost high amounts of mercury from the "carcass" (remainder of bird once feathers, liver, kidney and a sample of the pectoral muscle were removed, it was also the major store tissue for the metal) during egg production. It is proposed that the mercury found in the chicks of kittiwakes came from a protein store situated somewhere on the carcass and not from the internal tissues.

The value of 2% of the mercury intake going into the chicks may give an indication of the contamination of the breeding females. However no relationship between internal tissue mercury levels of the female and the chick mercury levels could be drawn because all the experimental females received the same dose.

This study found a low within-clutch variation in concentrations of mercury. Coulson et al. (1968) also found that within clutch variation in concentrations of organochlorines in shags was significantly less than the between-clutch variation. This suggests that any egg in the clutch can be taken to be representative of that clutch.

Unfortunately at present there are virtually no data to show relationships between heavy metal levels in eggs and the levels in the plumage of birds. It does seem probable that the two may provide rather different results since feather levels appear to relate to a large extent to intake of mercury since the last moult (Furness et al., 1986; Honda et al., 1986; Braune & Gaskin, 1987) whereas levels in the eggs appear to reflect dietary mercury levels during the period of egg development.

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CHAPTER 9

Mercury levels in eggs, internal tissues and feathers of herring gulls *Larus argentatus* from the German Wadden Sea coast.

9.1 INTRODUCTION

To study the impact of pollution there is a great need for materials indicating or monitoring the occurrence of toxic substances. In order to survey the situation, a reliable monitor has to be available for regular sampling and concentration must be related to the contamination level.

Birds are among one of the most closely examined taxa in which heavy metal exposure and toxicity have been studied (Bagley & Lockey, 1967; Anderlini et al., 1972; Martin & Coughtrey, 1975; Holt et al., 1979; Simpson et al., 1979; Buhler & Norheim, 1981; Furness & Hutton, 1980). Seabirds are often considered to be good indicators of marine pollution for a variety of reasons (see Chapter 1).

Both tissues and feathers have been widely used in the past two decades to assess heavy metal contamination in birds (Berg et al., 1966; Borg et al., 1970; Fimreite et al., 1971; Furness et al., 1986; Honda et al., 1986a,b). However it is only comparatively recently that bird eggs have been sampled to show geographical variation in metal levels (Fimreite et al., 1974, 1980; Parslow & Jefferies, 1975; Blus et al., 1977; Holt et al., 1979; Ohlendorf et al., 1982; Becker et al., 1985; Ohlendorf & Harrison, 1986; Becker, 1989).

Bird eggs have several advantages as a monitoring tool (see Chapter 7 and 8). However it is not clear whether the metal levels found in eggs come from the wintering area and migration routes of the female or from the actual breeding ground. There is also conflicting evidence as to how important they are in the process of metal excretion. The transferred mercury content to the eggs has been reported to be small compared to the female's body burden of mercury and thus the

removal of mercury through egg-laying was reported to be negligible in some bird species (Helander et al., 1982; Honda et al, 1986a; see Chapter 8). However, other authors have reported that mercury loss through egg laying is indeed another way the female can utilise removal of a considerable part of her body burden of toxic material (Becker et al., 1985; Braune & Gaskin, 1987; see Chapter 7).

Many studies have demonstrated positive correlations between mercury concentrations in feathers and those of the internal tissues (e.g. Furness & Hutton, 1980; Hutton, 1981; Olhendorf et al., 1986). Several authors have claimed that there is a ratio of 7:3:1 for mercury concentration (fresh weight) in feather, liver and muscle tissue, respectively (Johnels & Westermark, 1969; Jensen et al., 1972; Westermark et al., 1975) and several other authors have used this to facilitate a conversion of mercury concentration measured in one tissue to estimate levels in another (Berg et al., 1966; Borg et al., 1970; Buhler & Norheim, 1981; Appelquist et al., 1985).

The ability to predict internal tissue mercury contamination on the basis of easily collectible samples such as feathers and eggs, would greatly enhance their value as a means of assessing mercury burdens of birds. At present there are virtually no data to show relationships between heavy metal levels in eggs and plumage. It does seem probable that the two would provide rather different results since mercury levels in the feathers appear to relate to a great extent on the mercury intake since the last moult (Furness et al., 1986; Honda et al., 1986b; Braune & Gaskin, 1987), whereas levels in eggs have been reported to reflect dietary mercury levels during the period of egg development (Becker et al., 1985).

Reproductive processes have le d to differences in mercury dynamics between males and females in higher vertebrates. Methyl mercury can cross the placental barrier as demonstrated in both experimental studies (Nordberg & Skerfving, 1972) and field investigations (Reijnders, 1980; Ronald et al., 1984). Methyl mercury has also been reported to be excreted in the milk of lactating female marine mammals (Kim et al., 1974; Born et al., 1981). Since losses of mercury via these routes are unavailable to males and as eggs may also be classed in this category one might predict that there would be differences between the sexes with respect to mercury level fluctuations over the breeding season.

Since most bird species undergo a complete post-breeding (autumn) moult, there may be pronounced changes in the mercury content of internal tissues of such birds during the course of the breeding season and subsequent moult. There is little evidence of differences in mercury levels between the sexes (see Chapters 4 and 8) although differences between male and female birds have been reported for other metals (Gochfeld & Burger, 1987; Hutton, 1981). Only one study has shown lower levels of mercury in the plumage of female birds after egg laying than those levels in male birds from the same colony (Braune & Gaskin, 1987).

The North Sea is internationally important both for fish stocks and because it supports important populations of sea and shorebirds (Tasker & Pienkowski, 1987). It has been shown that the Wadden Sea coast shows clear signs of contamination of breeding birds and the rivers Elbe and Weser are major sources of this pollution (Becker, 1989). North Sea pollution has been increasing and it has become important to establish exactly the

degree of present contamination. Seabirds can be used as a monitor and one of the most abundant species in this area is the herring gull Larus argentatus. It is a predator at the top of the food web which, besides feeding in coastal waters, also feeds at rubbish tips. The immature birds disperse from the breeding area, but return on maturation (Spaans, 1971). Adults are resident all year round, therefore analysis of contamination in the tissues and eggs may provide a useful indication of local environmental pollution.

The aim of this study is to examine relationships between tissue concentration (both internal tissues and feathers) and egg levels as well as differences between male and female herring gulls and to assess the value of eggs as a monitor of local mercury contamination. Finally these data were used to estimate mercury intake and excretion over the year and thus give a more quantitative estimate of contamination of herring gulls in the German North Sea area.

9.2 MATERIALS AND METHODS

9.2.1 Sample collection

At a growing colony situated at Minsener Oldeoog (53°46′N/8°00′E), on the German Wadden Sea coast, 37 adult herring gulls were trapped between the 31st May and 3rd June, 1990, during incubation using drop traps set over the nest. At most sites the female was taken but for 10 clutches both members of the pair were trapped. The individuals were killed and stored in cool conditions prior to further treatment. The largest egg laid was taken from 25 of the available clutches determined by measuring the egg. The largest egg is usually the first laid (Mollering, 1972) and in most cases the first laid egg should

have been collected, which have been reported to be the most heavily contaminated (Becker et al., 1989).

Three to six days after death the birds were dissected and a sample of the liver, pectoral muscle (pectoralis only) and ovary removed. A sample of 4-10 body feathers along with primary 1 (from only some birds) was also taken. All of these tissues were placed in glass vials and frozen at ca. -18°C. All eggs were marked, wrapped in aluminium foil and also frozen.

9.2.2 Sample preparation

All internal tissues (liver, ovary and pectoral muscle) were thawed and removed from vials, weighed and dried in an oven at 50°C to a constant weight. They were then ground using a pestle and mortar and a sub-sample removed for analysis.

Eggs were thawed, opened and internal contents removed and weighed. These were dried as above until a constant weight was achieved and a sub-sample taken for mercury analysis.

Feathers were washed using the procedure outlined in Chapter 2. Body feathers were analysed whole. Primary feather 1 (the primary nearest to the body, the first moulted) was also analysed. This was generally too large for analysis and so was split down the middle of the shaft using a scalpel blade. This procedure removed any differences caused by mercury concentrations varying between the top portion of the shaft and the lower section. The left portion of the split feather was then analysed (see Chapter 3).

Mercury analysis was carried out as described in Chapter 2.

9.2.3 Calculations and statistical analysis

All calculations are given on a dry weight basis to avoid variation caused when using a wet weight (Adrian & Stevens,

1979). Eggs of wild birds collected for measuring pollutants vary from nearly fresh weight to essentially dry weight, so that objective comparisons cannot be made on the basis of weight of contents at time of collection (Stickel et al., 1973). Eggs are especially susceptible to variation as up to 20% of water is lost during incubation and more is lost due to processes such as freezing.

All data were tested for normality using Kolmogorov-Smirnov one-sample tests. Subsequent statistical analyses were performed using non-parametric procedures. Comparisons between levels in male and female tissue samples were made using Mann-Whitney U-tests, since the distribution of some of the mercury levels was skewed. Relationships between various tissues were tested using Spearman rank correlation.

9.3 RESULTS

9.3.1 Mercury levels in tissues

Mean mercury concentrations within the samples analysed are given in Table 9.1. Mercury distribution patterns in the liver were found to deviate from a Gaussian distribution. The wide range of mercury concentrations within the sample from one breeding population are also a feature of note.

Mercury levels in the internal tissues analysed showed no statistical difference between male and female birds (Mann-Whitney U-test, Pectoral muscle, n_1= 10, n_2= 27, U_1= 115, U_2=237, P>0.1, liver, n_1= 10, n_2= 27, U_1= 106, U_2= 172, P>0.1). Body feather concentrations were also not statistically significantly different between the sexes (Mann-Whitney U-test, n_1= 9, n_2= 23, U_1= 84.5, U_2= 190.5, P>0.1). There was however, a significant difference between males and females in the levels

of mercury in the primary feather samples (Mann-Whitney U test $n_1=6$, $n_2=12$, $U_1=13$, $U_2=80$, $P<0.05$).

Table 9.1 Mercury concentrations(µg/g) dry weight in tissues collected at a colony from the German Wadden Sea coast in relation to sex.

Tissue	n	mean	sd	median	range
Females					
Body feather	23	4.87	2.04	4.79	2.15-09.40
Primary 1	12	5.84	2.34	5.66[a]	3.28-10.03
Pectoral muscle	27	2.00	0.75	1.89	1.04-04.17
Liver	27	4.37	1.76	4.36	1.44-09.29
Ovary	26	1.87	0.67	1.86	0.96-03.37
Egg	26	1.43	0.64	1.30	0.58-03.01
Males					
Body feather	9	6.41	2.39	6.25	3.65-10.94
Primary 1	6	9.59	3.51	9.70[a]	4.19-13.35
Pectoral muscle	10	2.56	1.17	2.26	1.41-05.32
Liver	10	4.72	2.90	4.01	1.31-12.08

a=Intersex difference Mann-Whitney U-test, $n_1=6$, $n_2=12$, $P<0.05$

Table 9.2 shows the coefficients and associated probabilities for Spearman rank correlations between mercury concentrations in the tissues analysed for adult female herring gulls. Egg mercury levels were not related to pectoral muscle, ovary or feather levels although they were significantly and positively correlated with mercury concentrations found in the liver ($n=25$, $r_s=0.42$, $P<0.05$). Liver levels were highly

Table 9.2 Spearman rank order correlation coefficient matrix for adult female herring gulls.

		Liver	Pectoral muscle	Ovary	primary 1	Body feather
Egg	(rs)	0.42	0.35	0.25	0.09	0.02
	(P)	0.03	0.09	0.23	0.80	0.92
	(n)	(25)	(25)	(24)	(12)	(21)
Liver			0.35	0.63	0.37	0.38
			0.06	0.00	0.24	0.08
			(27)	(26)	(12)	(23)
Pectoral muscle				0.16	0.42	0.28
				0.46	0.18	0.19
				(26)	(12)	(22)
Ovary					0.15	0.27
					0.66	0.24
					(12)	(22)
Primary 1						0.76
						0.00
						(12)

correlated with those levels found in the ovary (n=26, r_s=0.63, P<0.001), although no other tissues showed a significant relationship.

There was a strong correlation between mercury levels found in the body feathers and mercury levels in the primary feathers of the adult female herring gulls (n=12, r_s=0.76, P<0.01).

Coefficients and associated probabilities for Spearman rank order correlations between mercury concentrations in the combined tissue samples analysed for all adult herring gulls are shown in Table 9.3. The significance of the relationship between body feather and primary feather levels increased greatly due to the stronger correlations found for male birds (n=18, r_s=0.70, P<0.001).

Table 9.3. Spearman rank order correlation coefficient for adult herring gulls

	Liver	Pectoral muscle	Body feather
Primary 1 (rs)	0.26	0.48	0.70
(P)	0.31	0.04	0.00
(n)	(18)	(18)	(18)
Liver		0.45	0.45
		0.01	0.01
		(37)	(32)
Pectoral muscle			0.52
			0.00
			(32)

Once the data sets for both the male and female herring gulls were combined the trend for the levels found in the liver and body feathers and levels between body feathers and pectoral muscle noted previously in Table 9. 1 become significant. Significant relationships between levels in the liver and the pectoral muscle are also noted.

Relevant mercury concentration ratios are given in Table 9.4. The ratio of mean feather concentration:mean egg concentration ranged from 3.7 to 5.5 according to which feather was used.

Table 9.4. Ratios of mercury levels in tissues of individual herring gulls sampled soon after egg laying (all levels on a dry weight basis)

Tissue	Mean Ratio	S.D	n
Body feather:Egg	3.7	2.2	21
Primary:Egg	5.5	4.0	12
Liver:Egg	3.3	1.7	25
Pectoral muscle:Egg	1.5	0.7	25
Ovary:Egg	1.5	0.9	25

9.3.2 Mercury excretion in herring gulls

Since herring gulls undergo a post-nuptial moult there will be pronounced changes in the mercury concentrations of the internal tissues. From these data it is possible to estimate the percentage of the body burden excreted in one breeding season via the eggs and the plumage. Honda *et al.* (1986b) showed that 70% of the body burden of mercury was displaced into the newly grown feathers after a complete moult in the black-eared kite *Milvus migrans lineatus*. Braune & Gaskin (1987) showed that in the Bonaparte's gulls *Larus philadelphia*, 93% of the mercury body burden was present in the plumage and similar calculations by Lewis & Furness (in press) showed that approximately 65% of the mercury body burden was distributed into the growing feathers of black-headed gull *Larus ridibundus*, chicks. This

study also demonstrated that 49% of the mercury intake was shed into the growing feathers. Using these values it may be possible to estimate total mercury excretion and ultimately total mercury intake of the adult herring gulls. Furness et al. (1986) suggested that body feathers would provide the most representative sample for estimating whole-bird mercury content and because the levels of these feathers did not differ between the sexes these levels have been used in the calculations.

A mean weight of German herring gulls was determined from the birds collected from Minsener, Oldeoog, 1990 (Female mass= 829 +/- 49g, n=29, Male mass= 1022 +/- 55g, n= 13).

Calculations

Mean weight of German herring gull= 925.5 g

Weight of plumage = 10% of weight of bird

Therefore, weight of plumage= 92.55 g

Mean concentration of mercury in body feathers= 5.64 µg/g

Therefore, approximate amount of mercury in the plumage= 522 µg

Approximately 76% of the body burden goes into the plumage (mean percentage of published results), therefore the body burden of mercury prior to moult= 522/76%

= 687 µg

Therefore, body burden of mercury after moult= 687-522= 165 µg

Mean total amount of mercury in 1 egg of herring gull= 24.45 µg (data from this Chapter)

Therefore mean total amount of mercury in 3 egg clutch=73.35 µg.

If 522 µg of mercury is excreted into the post-nuptial plumage and 73.35 µg of mercury is excreted in an average clutch approximately 760 µg is excreted by female herring gull over one breeding season. (Males excrete only 522 µg).

Now, if 49% of the mercury intake goes into the plumage after a full moult, approximately 1065 µg of mercury was ingested by the adult herring gull in one year previous to the breeding season.

This means that females can excrete approximately 71% of their mercury intake in one breeding season compared to the 49% excreted by males, a difference of 22%.

Second estimate of mercury depollution by the egg.

Mean Hg in female liver = 4.37 µ/g dry weight

Mass of female liver = 19 g dry weight

Therefore total amount of mercury in liver = 83 µg

Now 36.4% of the body's mercury content was estimated to be in the liver (Braune & Gaskin, 1987)

This would result in 230 µg mercury present in the body of the female herring gull

Total mercury in clutch = 73 µg

Therefore, percentage of the body burden of mercury loss by the clutch = 73/(230 + 73) = 24%

Both of these figures correspond, approximately, to the difference in percentage between male and female herring gulls (see Section 9.3.1).

9.4 DISCUSSION

Avian eggs have been widely used for the detection of toxic chemicals including organochlorines and mercury (Blus et al., 1977; Helander et al., 1982; Barrett et al., 1985; Becker et al., 1985). As in the case for the plumage, mercury is reported to accumulate in eggs in a dose-dependent fashion in response to increasing dietary levels of methyl-mercury (Tejning, 1967; Heinz, 1974; March et al., 1983). Thus the mercury content of

eggs presents yet another possible indirect measure of dietary mercury levels. Previously, however, little information existed on the relationship between mercury levels in eggs and those found in other tissues such as the liver and the feathers. There is also a lack of literature available on the source of the mercury found in the eggs.

Becker et al. (1985, 1989) reported that levels of mercury in eggs probably reflect the mercury intake of the female bird immediately prior to egg laying, a period of high food intake in larids. Fimreite (1971) and Becker et al. (1989) found that there was a significant positive relationship between the mercury content of the clutch and that of the female, which supports the idea that egg levels reflected the mercury in the female at times of egg-laying.

From this present study feather mercury levels of the female birds and those found in their eggs are not correlated. This may imply that the source of the mercury contamination of the feathers and the eggs are rather different. Mercury in the feathers reflect the level circulating in the blood at the time of feather growth and this depends both on the amount of methyl mercury in the food and on the amount of methyl mercury stored in the soft tissues between moults. Feather levels therefore mainly reflect mercury intake since the previous moult (Furness et al., 1986; Honda et al., 1986b). If mercury in eggs was also from the same source as that found in the feathers a relationship between egg and feather levels might be expected. The present results suggest that this is not so and therefore egg levels may indicate mainly mercury ingested immediately prior to egg-laying and therefore local contamination at the breeding site, whereas feathers may be a better monitor of

contamination of the previous year's intake or, in the case of other species, their wintering areas. Hence as some bird species (e.g lesser snow geese Chen caerulescens caerulescens) feed very little during egg-laying and incubation (Ankey, 1978) and utilise nutrient reserves during this period, and as the half-life of methyl mercury in birds has been estimated to be about 4-5 weeks (Ulfvarson, 1962) it would seem likely that in such species the mercury content of the eggs would reflect the contamination of their wintering grounds more than that of the breeding site.

Egg and liver concentrations are correlated (see also Becker et al., 1989). This was expected due to a high proportion of yolk synthesis taking place in the liver. Kuwatara (1970) reported that most of the mercury found in eggs was found in the yolk. However, Backstrom (1969) showed that a high proportion of the mercury present in eggs of quail Cotournix cotournix was situated in the albumen. If this were the case here a correlation would have been expected between the pectoral muscle and the egg (flight muscles serve as the major protein store for albumen production. (Kendall et al., 1973; Jones & Ward, 1976). However, because only total egg content was analysed in this study it was not possible to ascertain in which part of the egg the mercury was situated.

Egg and oviduct levels were not expected to correlate due to samples being taken after egg laying. Proteins are only transported to the oviduct a short time before egg production and therefore mercury contamination here would not be expected to be reflected in egg production (Gilbert, 1971).

The feather:egg ratio of mercury levels for the herring gull was 3.7 to 5.5 (according to feather type used) and 3.3 for

liver:egg concentration. This as far as I am aware has not until now been estimated. It is not known if the conversion factors relating mercury concentration in different tissues are prone to variation. Thompson et al. (1990) concluded that conversion factors relating mercury concentration in different tissues are susceptible to interspecific variation in the chemical form of the mercury present and should only be used as a rough means to gain an impression of levels in various tissues. Because mercury in the feathers and eggs is mainly in the methyl form (Kiwimae et al., 1969; Thompson & Furness, 1989) this may indicate that this ratio may be a more robust measure and sampling of such tissues for mercury analysis does avoid complications arising from seasonal variations in levels in soft tissues as well as removing the need to kill birds.

Most studies of mercury contamination have either not examined for differences between the sexes or have found no sex effect on mercury levels. However Braune & Gaskin (1987) did find significantly lower levels of mercury in female Bonaparte's gulls than in the males when they examined for contamination using the primary feathers, which agrees with the results presented here. Primary feathers are usually the first feathers moulted and therefore the first regrown and it may be for this reason that highest levels are recorded for these feathers (Gochfeld, 1980; Buhler & Norheim, 1981; Furness et al., 1986). It appears therefore that adult males approached similar mercury levels as those found in the females only after eliminating large amounts of mercury into the first few developing feathers. There was a tendency of male tissues to have higher mercury levels but this was not significant. Therefore, reduction of the body burden of mercury due to elimination via the eggs was

mainly reflected in the pattern of mercury concentration of the first-grown primary feathers. In mallards Anas platyrhynchos Heinz (1976) found higher mercury levels in the liver of males than in females after egg laying. However, most studies looking at tissues other than primary feathers have found no differences between the sexes and, therefore, it seems likely that the effects of egg-laying could be rather small and may only be likely to be reflected in the first few primaries or in tissues where artificially high mercury levels have been imposed.

Using the data set from the present study it was possible to produce a rough estimate of the amount of mercury ingested and excreted. Female birds were shown to excrete over 20% more of their mercury intake than male birds. It was also estimated that these Herring gulls ingested over 1000 µg of mercury in one year, giving some indication of the pollution problems of the Wadden Sea coast. No evidence is yet available to indicate if this is causing any toxic effects in the population or whether the figure is high compared to other gull populations

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CHAPTER 10

GENERAL DISCUSSION

In attempts to quantify geographical variation in mercury levels in the environment and assess the impact of anthropogenic inputs of metals as well as gaining information regarding the dynamics of mercury, seabirds as biological indicator organisms offer several advantages and mercury concentrations in eggs and tissues of seabirds have been analysed on a large scale since the mid 1960's, particularly in the northern hemisphere.

Most studies have not been specifically designed to quantify environmental variations in metals levels but are primarily concerned with possible adverse effects of elevated metal levels. Also, past multiple tissue studies have made little attempt to investigate inter-tissue correlations between concentrations in particular seabird tissues and total metal burden in the tissues or whole body. This study aimed to draw attention to factors that need to be taken into consideration in designing programmes to monitor geographical or temporal variations in marine levels of mercury using seabirds.

Variations in the age composition of a sample of breeding birds could potentially contribute to apparent differences in tissue concentrations of metals between species, population and years. The availability of a sample of known age red-billed gulls provided a rare opportunity to investigate age-related trends in mercury levels of the plumage. The results obtained would suggest that straightforward accumulation of mercury, as noted for many species of marine fish and mammals, is not the only pattern observed in higher organisms. For many bird species that moult regularly there appears to be no danger of an age accumulation effect. However in choosing species to examine possible differences in mercury concentrations between populations, information regarding moult patterns of the study

species should be taken into account.

Although many studies have aimed to show geographical 'hot-spots' of mercury pollution by presenting data of mercury concentrations found in seabird tissues, little information has been presented to try to quantify environmental mercury levels using biological indicators. It was hoped in Chapter 6 to offer a formula to relate mercury levels found in the plumage of seabirds to the mercury intake of the bird. This figure is however an experimental and approximate estimate and further experiments using a wide variety of bird species are required to confirm such findings before this figure can be used with any confidence.

In past studies examining internal tissue concentrations in populations of seabirds the kidney/liver ratio was close to unity with the liver level usually being slightly higher. However in the dosed birds of the present experiments there was a general trend for kidney concentrations to be greater than those found in the liver. The levels in the kidney were also found to be elevated as the mercury intake was increased (see Chapter 6). This would suggest that the ratio of mercury concentrations in the kidney/liver can be used as an indicator of methyl mercury poisoning. This would provide most useful results when large numbers of dead birds can be easily collected (e.g due to beach wrecks). However, possible influences of starvation must be taken into consideration in metal analysis of birds found dead. Even if mercury does not appear to be freely mobilised in starved birds, changes in the tissue weight may alter the metals' concentration without altering the tissue burden.

Given that mercury concentrations tend to be higher in

feathers renewed early in a moult sequence (Chapters 3, 6 and 9) the average mercury concentration of the plumage can in fact alter. If individual feathers tend to be replaced at the same stage at each moult period, average concentration will be reduced after such high mercury feathers are dropped but before they are regrown. As the moult proceeds this effect may be well reversed. It seems likely, therefore, that feather mercury concentration will be most consistent in samples taken outside the moult period.

Although evidence is conflicting, egg concentrations seem to reflect mercury from recent dietary uptake rather than from accumulated mercury present in tissue stores. Eggs may thus provide a particularly good indicator of mercury exposure in the vicinity of a breeding colony in the immediate pre-laying season. However, it seems likely that there are species specific factors in egg formation processes that should be taken into consideration when assuming the sources of egg contamination and further work to investigate the mercury dynamics associated with egg production in birds is required to produce a more complete picture.

Some studies have suggested that the loss of mercury from the body via egg production is negligible but from the results gained in Chapters 7 and 9 it does appear that eggs play a more important role in mercury excretion with respect to the female bird than previously assumed, potentially increasing their mercury excretion by over 20%

Given that gender differences were found in the mercury levels of some tissue (see Chapters 7 and 9) mercury levels between populations may differ substantially according to time of sampling and the adult sex ratio of populations. For example

if females are sampled after the egg laying period the overall mercury concentration for that colony may be deflated due to loss of mercury into the eggs. Although this effect does seem small in many cases (see Chapters 4 and 8) it would seem to be a wise precaution to sample immediately prior to or outside the breeding season, or at least to pay attention to the dates of sampling when comparing between data sets.

The suggestion that atmospheric deposition accounts for a subtantial part of feather metals levels for metals other than mercury may mean that mercury is the only metal for which analysis of feathers can provide a clear picture of variations in food-chain contamination over temporal or spatial scales.

The facts that different species of seabirds appear to deal with ingested mercury in rather differing manners (see Chapter 8) and that there are a wide variety of factors affecting metal distribution (relating to the metal, the sex or environmental influences) means that there is a great potential for future work investigating such factors more fully. It also begs that the same questions relating to other heavy metals, such as cadmium and lead are also more fully investigated.