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Relationships Between Heavy Metal and Metallothionein Concentrations in Lesser Black-Backed Gulls, *Larus fuscus*, and Cory's Shearwater, *Calonectris diomedea*

F. M. Stewart[1]*, R. W. Furness[1], L. R. Monteiro[1,2]

[1] Applied Ornithology Unit, University of Glasgow, Glasgow, United Kingdom G12 8QQ
[2] Department of Oceanography and Fisheries, University of the Azores, 9900 Horta, Portugal

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Abstract. Metallothionein, cadmium, zinc, copper, and mercury concentrations were measured in adult lesser black-backed gulls, *Larus fuscus;* and metallothionein, cadmium, zinc, and copper concentrations were measured in fledgling Cory's shearwaters, *Calonectris diomedea.* In gulls, metallothionein was positively correlated with cadmium (kidney r = 0.83, liver r = 0.46), zinc (kidney r = 0.46, liver r = 0.37), and copper (kidney r = 0.28, liver r = 0.34). Mercury levels in lesser black-backed gulls showed no correlations with metallothionein or with any other metal. In shearwaters metallothionein was positively correlated with cadmium in the kidney (r = 0.41) but not in liver, zinc in kidney (r = 0.43) and liver (r = 0.52), and copper in kidney (r = 0.55) but not in liver. Cadmium levels were the most important factor determining tissue metallothionein concentrations in adult lesser black-backed gulls demonstrating the role of metallothionein in heavy metal detoxification. In fledgling Cory's shearwaters, the most important factor in determining metallothionein concentrations in kidney was copper concentrations, and in liver, zinc concentrations. During the latter phases of chick growth high levels of zinc are required for feather development, and at this time the binding of cadmium may be masked by the presence of a large amount of zinc- and copper-bound metallothionein. These results illustrate disparate roles of metallothionein, the levels of which will be in a state of flux both seasonally and annually.

Cadmium and mercury are heavy metals that are naturally present in the marine environment, but there is evidence that levels are increasing in some areas due to human inputs (Nriagu 1988; Slemr and Langer 1992; Thompson *et al.* 1992). These metals are toxic even at relatively low levels and are known to accumulate in many marine animals, including seabirds, although they have no known biological function (Thompson 1990). From both a biomonitoring and toxicological viewpoint it is important to understand the physiological processes and dynamics of nonessential metals in the organs and tissues of seabirds. There is some evidence that the concentrations found in free-living birds can cause kidney damage. Nicholson *et al.* (1983) found evidence of patchy necrosis in seabird kidney with mean concentrations ranging from 94.5 to 228.0 μg/g cadmium and 5.02 to 13.4 μg/g mercury, but Elliott *et al.* (1992) found no damage apparent with comparable metal concentrations. Cadmium and mercury are bound up within the liver and kidney to a low molecular weight protein called metallothionein (Scheuhammer 1987). Cadmium bound in this way has a long biological half-life and has a tendency to accumulate with age (Scheuhammer 1987). However, recent research has shown seasonal fluctuations in cadmium concentrations in the common guillemot, *Uria aalge,* from northwest Scotland, indicating that in seabirds cadmium turnover may be faster than previously thought (Stewart *et al.* 1994).

Few studies have investigated metallothionein levels in seabirds. A metal-binding protein thought to be metallothionein was first isolated in free-living seabirds by Osborn (1978). Hutton (1981) described a metal-binding protein from the great skua, *Catharacta skua,* kidney cytosol which bound the greater part of the cadmium in the kidney. Metal and metallothionein concentrations were measured in free-living Canadian seabirds by Elliott *et al.* (1992), and in flamingos, *Phaenicopterus ruber,* and little egrets, *Egretta garzetta* overwintering in the Camargue, collected after they had starved and frozen to death (Cosson 1989).

Many laboratory-based studies have induced the synthesis of metallothionein by dosing animals orally or by injection with various heavy metals (Scheuhammer 1987; Sendlebach and Klassen 1988). It is important to understand the responses of both kidney and liver tissue to heavy metal exposure because these are the target organs for cadmium toxicity. Scheuhammer and Templeton (1990) used ring doves, *Streptopelia risoria,* as a bird model to look at metallothionein induction after exposure

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* *Present address:* School of Biological and Medical Sciences, University of St Andrews, Fife, United Kingdom, KY16 9TS

Correspondence to: F. M. Stewart

to elevated dietary cadmium levels. They concluded that changes in zinc and copper concentrations should be considered in addition to cadmium levels when researching metallothionein induction.

The present study was undertaken to investigate metal and metallothionein concentrations, and inter- and intra-tissue relationships between metallothionein, the essential metals zinc and copper, and the nonessential metals cadmium and mercury. Free-living birds of two species were analyzed: breeding adult lesser black-backed gulls, *Larus fuscus,* obtained from culls in northwest England and southeast Scotland, and Cory's shearwaters, *Calonectris diomedea,* from the Azores, which had died at fledging. This presented an opportunity to investigate aspects of metal dynamics in healthy free-living seabirds without the need to kill birds for study.

Materials and Methods

Lesser black-backed gulls were obtained from two separate culls, one at Abbeystead, Lancashire, England, and the other at Inchmichery, Firth of Forth, Scotland, both in June 1992. Breeding birds had been killed by baiting with alphachloralose at the nest, and a total of 58 gulls were obtained. Freshly dead Cory's shearwaters were collected in October/November 1992 on the Azores islands of Faial and Pico. In the Azores many shearwater colonies are situated close to towns. When chicks fledge (always at night) at around 12 weeks old, considerable numbers are confused by the bright town lights; they crash into street lamps or car headlights and are killed. An advertisement was placed in the local newspaper by L. M., and a total of 35 freshly dead birds were handed in or collected by searching the streets early in the morning. Confused fledglings handed in alive were released somewhere dark that evening.

Gulls were dissected fresh, using stainless steel instruments, and whole kidney and liver tissues were removed and weighed. Subsamples were taken and frozen for subsequent metallothionein analysis. Subsamples also were taken and dried in an oven at 50°C to constant mass prior to metal analyses. Shearwaters were weighed and stored frozen at −20°C before dissection. After thawing, the whole liver and kidney were removed and weighed, subsamples were taken for metal and metallothionein analysis, and samples were transported frozen to Glasgow for analysis.

Cadmium, Zinc, and Copper Analyses

Samples of 0.5–1.0g dried tissue (kidney or liver) were acid-digested in 10 ml concentrated nitric acid on a hot plate, by first soaking at 100°C for two h, then boiling at 120°C for 20 min. Samples were diluted to 15 ml using distilled water. Metal concentrations were analyzed by atomic absorption spectrophotometry using a Philips PU 2000 A.A.S. Accuracy and reproducibility of metal determinations were tested by analyzing International Atomic Energy Agency horse kidney Reference Material H-8. Detection limits in the digested sample were 0.014 μg/g for cadmium, 0.01 μg/g for zinc, and 0.035 μg/g for copper. All metal concentrations were expressed on a dry weight basis.

Mercury Analysis

Only adult gulls were analyzed for mercury, and a total of 28 birds were sampled. Total mercury in acid-digested samples was determined by a cold vapor technique using a Data Acquisition Ltd. DA 1500-DP6 Mercury Vapour Detector (Furness *et al.* 1986). Accuracy and reproducibility of mercury determination were tested by analyzing International Atomic Energy Agency horse kidney Reference Material H-8.

Table 1. Metal and metallothionein concentrations in lesser black-backed gulls. Concentrations are given as μg/g dry weight for metals and μg/g wet weight for metallothionein; n = 58 for all samples except mercury (n = 21 for kidney, n = 28 for liver)

Organ	Metallothionein	Cadmium	Zinc	Copper	Mercury
Kidney					
Mean	137.60	28.86	118.98	13.51	1.84
S.D.	67.76	18.57	33.41	3.95	1.19
Range	39–360	9–106	75–208	8–28	1–4
Liver					
Mean	27.17	2.83	61.21	12.56	1.55
S.D.	19.51	1.71	19.65	3.43	1.09
Range	7–110	1–9	22–136	7–23	0–5

Metallothionein Analysis

Metallothionein concentration was determined by the silver saturation method of Scheuhammer and Cherian (1986, 1991) as modified by Scheuhammer (1988). The only further modification was the use of heparinized sheep rather than human blood. The reproducibility and accuracy of this method was tested by analyzing purified horse kidney metallothionein obtained from Sigma Chemicals.

Statistical Analysis

Preliminary tests of the goodness of fit of data to normal distributions were performed using Kolmogorov–Smirnov one-sample tests. All data appeared normal with the exception of kidney and liver cadmium concentrations in Cory's shearwaters. These data were logarithmically transformed and analyses were made using parametric statistics. Data were expressed in μmol/g dry weight for direct comparison between metal and metallothionein concentrations.

No differences in metal concentrations were detected between the Inchmichery and the Abbeystead collections of lesser black-backed gulls, and there were no sex differences; so the data were pooled for analyses.

All statistical tests were performed using the SPSS/PC+ package.

Results

Metal and Metallothionein Concentrations

Metal and metallothionein data are shown in Table 1 for adult lesser black-backed gulls and Table 2 for fledgling Cory's shearwaters. In gulls, liver and kidney concentrations of cadmium, zinc, and metallothionein were quite variable, although the levels were consistently higher in kidney than in liver. Copper and mercury concentrations were similar in the two organs. Concentrations of cadmium (kidney mean 28.86 μg/g, liver mean 2.83 μg/g) were considerably higher than concentrations of mercury (kidney mean 1.84 μg/g, liver mean 1.55 μg/g).

The Cory's shearwaters aged approximately 12 weeks had already accumulated considerable amounts of cadmium in both kidney and liver tissue. As in the adult gulls, the shearwater

Table 2. Metal and metallothionein concentrations in fledgling Cory's shearwater. Concentrations are given as μg/g dry weight for metals and μg/g wet weight for metallothionein; n = 34 for liver, n = 35 for cadmium, zinc, and copper in kidney, n = 33 for metallothionein in kidney

Organ	Metallothionein	Cadmium	Zinc	Copper
Kidney				
Mean	24.76	9.31	114.94	12.59
S.D.	16.58	10.07	24.39	3.54
Range	3–66	2–41	62–173	7–23
Liver				
Mean	106.05	2.03	176.43	13.29
S.D.	40.59	2.78	48.61	7.38
Range	16–196	0.4–13	29–92	6–44

fledglings had higher cadmium concentrations in the kidney (mean 9.31 μg/g) than in the liver (mean 2.03 μg/g), and again there was a broad range of concentrations, with two individuals accumulating 40.64 and 40.47 μg/g cadmium in kidney, and 11.59 and 13.29 μg/g in liver, respectively. The concentrations in these two individuals created the skewed cadmium distribution in the sample. Zinc concentrations in the liver were much higher than those in the kidney, and copper concentrations were similar in the two organs.

Inter-Organ Relationships

In lesser black-backed gulls mercury, cadmium, and copper, and metallothionein showed strong positive relationships between levels in the liver and kidney: mercury r = 0.75, n = 21, $P < 0.001$; cadmium r = 0.68, n = 58, $P < 0.001$; copper r = 0.29, n = 58, $P < 0.05$; and metallothionein r = 0.34, n = 58, $P < 0.05$. There was no relationship between zinc concentration in the liver and kidney, r = 0.15, n = 58, N.S.

In the Cory's shearwater, cadmium, copper, and zinc showed a positive relationship between levels in the liver and kidney: cadmium r = 0.73, n = 34, $P < 0.001$; copper r = 0.43, n = 34, $P < 0.05$; zinc r = 0.41, n = 34, $P < 0.05$. However, there was no significant relationship between metallothionein levels in the two organs, r = −0.06, n = 33, N.S.

Metal and Metallothionein Relationships

Using stepwise multiple regression analysis for the lesser black-backed gull sample, cadmium concentrations alone explain 70% of the variation in kidney metallothionein concentrations and 21% of the variation in liver metallothionein, with none of the other metals significantly improving the model (Table 3). Figure 1 illustrates the relationship between cadmium and metallothionein in the kidney. The relationships were quite different in fledgling Cory's shearwaters. In the kidney, copper was the single most important factor explaining metallothionein levels, accounting for 30% of the variation. In contrast, in the liver zinc was the most important influence, explaining 27% of the variance. In neither case was any further variation explained by concentrations of the other metals. Figure 2 illustrates the relationship between zinc and metallothionein in the liver.

Table 3. Multiple regression equations to describe the relationship between metals and metallothionein in the tissues (k = kidney tissue, l = liver tissue); * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Multiple regression	
Equation	r value
Lesser black-backed gulls	
MT(k) = 3.04 Cd(k) + 55.89	0.83***
MT(l) = 5.31 Cd(l) + 12.12	0.46***
Cory's shearwater	
MT(k) = 0.46 Cu(k) − 0.02	0.55***
MT(l) = 0.08 Zn(l) + 0.009	0.52**

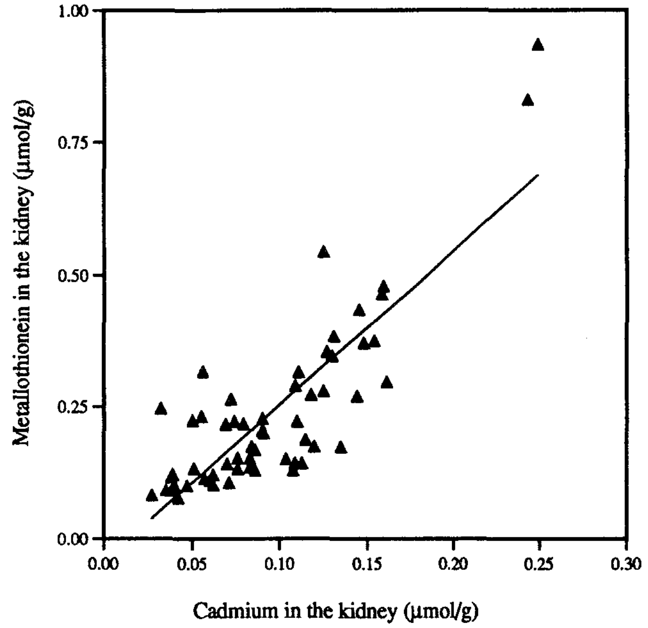

Fig. 1. The relationship between cadmium and metallothionein in the kidney of lesser-black backed gulls

Although cadmium concentration was the single most important influence on metallothionein concentration in lesser black-backed gull kidney, there were strong correlations both between metallothionein and the other metals, and between the different metals (Table 4). Zinc concentration was highly correlated with metallothionein concentration, as was the concentration of copper, although less significantly. A similar pattern was seen in the liver, with many significant metal–metal and metal–metallothionein correlations (Table 4). However, mercury concentrations showed no significant relationship with cadmium, zinc, copper, or metallothionein levels.

The results for the Cory's shearwaters were rather different. The single most important influence on metallothionein concentration in the kidney was copper (Table 3), but again there were significant correlations between zinc and metallothionein, and between cadmium and metallothionein levels (Table 4). However, in the liver zinc was the sole influence on metallothionein (Table 3), and neither cadmium nor copper correlated with metallothionein levels. Again, several metal–metal relationships were significant (Table 4).

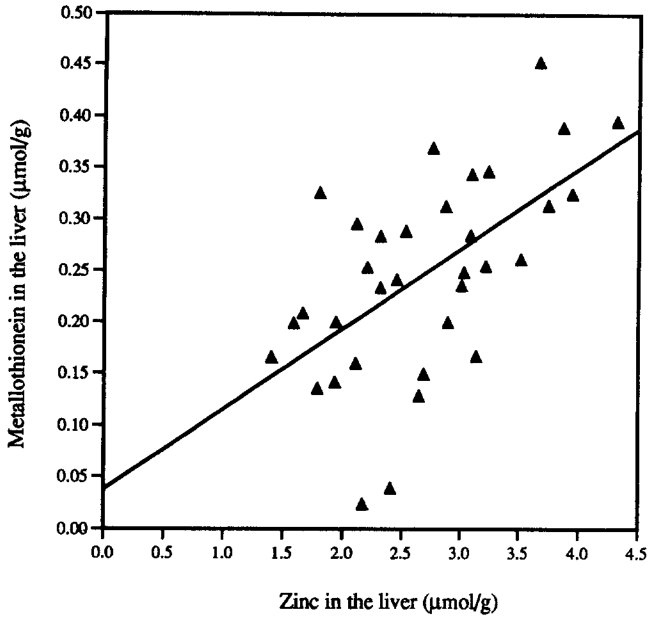

Fig. 2. The relationship between zinc and metallothionein in the liver of Cory's Shearwater

Table 4. Pearson correlation coefficients to describe the relationships between metals and metallothionein (performed on molar data)

Correlation Parameter	Correlation Coefficient	
	Gulls	Shearwaters
Kidney		
MT–Cd	0.83***	0.41*
MT–Zn	0.46***	0.43*
MT–Cu	0.28*	0.55***
MT–Hg	N.S.	N.A.
Cd–Zn	0.46***	0.40*
Cd–Cu	0.40***	0.56***
Cd–Hg	N.S.	N.A.
Zn–Cu	0.54***	0.57***
Zn–Hg	N.S.	N.A.
Cu–Hg	N.S.	N.A.
Liver		
MT–Cd	0.46***	N.S.
MT–Zn	0.37***	0.52**
MT–Cu	0.34**	N.S.
MT–Hg	N.S.	N.A.
Cd–Zn	0.49***	0.35*
Cd–Cu	0.37**	0.69***
Cd–Hg	N.S.	N.A.
Zn–Cu	0.45**	N.S.
Zn–Hg	N.S.	N.A.
Cu–Hg	N.S.	N.A.

*P < 0.05, **P < 0.01, ***P < 0.001; N.A. = not analyzed, N.S. = not significant.

Discussion

Metal and Metallothionein Levels

Cory's shearwater fledglings accumulated a mean cadmium concentration of 9.31 μg/g in kidney tissues and 2.03 μg/g in liver tissues in the three months from hatching to fledging. It should be noted that none of this was a consequence of transferred maternal cadmium burden as eggs contain very little cadmium [less than the 0.03 μg/g detection limit were reported for Cory's shearwater by Renzoni *et al.* (1986)]. Fledgling cadmium concentrations in this study were lower than values recorded for adults sampled at the Salvage Islands in the Atlantic, and Majorca, Linosa, and Crete in the Mediterranean (Renzoni *et al.* (1986). This is in accordance with most other studies comparing adult and chick metal levels, in which chicks appear to accumulate metals with age (Maegden *et al.* 1982; Reid and Hacker 1982; Honda *et al.* 1986; Blomquist *et al.* 1987; Walsh 1990; Burger *et al.* 1994). Perhaps surprisingly, the two fledglings with the highest metal loads had levels comparable to mean concentrations of adults from Majorca (42.8 μg/g, in kidney and 7.5 μg/g, in liver) (Renzoni *et al.* 1986). Indeed, Cory's shearwater fledglings accumulated a higher concentration of cadmium than some adult seabirds, including pelicans, gannets, cormorants, ducks, gulls, terns, and auks (Walsh 1990). Shearwaters, in common with other petrels, albatrosses, penguins, and skuas, tend to have high natural concentrations of cadmium in their tissues (Walsh 1990; Elliott *et al.* 1992). This usually is attributed to a diet consisting of prey items with high cadmium concentrations, particularly squid (or in the case of skuas, bird meat). Values of cadmium in the digestive gland of the squid, *Nototodarus gouldi,* ranged from 19 to 110 μg/g (Smith *et al.* 1984), and Martin and Flegal (1975) measured concentrations of cadmium of 71–694 μg/g in *Ommastrephes bartrami,* 42–1,106 μg/g in *Symplectoteuthis oulananiensis,* and 33.0–233.1 μg/g in *Loligo opalescens.* These rather limited data indicate that squid can concentrate large amounts of cadmium, possibly accounting for the levels measured in their avian predators.

Although lesser black-backed gulls' cadmium concentrations have not been reported previously, kidney and liver levels in this study were comparable to those of other larids (Hutton 1981; Nicholson 1981; Walsh 1990). Concentrations may be slightly elevated in species that forage on human refuse sites but they would almost certainly not be high enough to cause any toxic effects (Scheuhammer 1987). Mercury levels were low, similar to those reported previously (Thompson *et al.* 1992). Copper concentrations in adult gulls and fledgling shearwaters (Tables 1 and 2) were very similar, in agreement with values reported in the literature (Thompson 1990). Zinc concentrations can show quite large intra- and interspecific variation (Hutton 1981; Nicholson 1981; this study). Zinc concentrations in the kidney of fledgling shearwaters were similar to values recorded for adult Cory's shearwater in Majorca and Linosa, but lower than those from the Salvages and Crete (Renzoni *et al.* 1986). In comparison, liver zinc concentrations in Cory's shearwater fledglings were higher than those in adult Cory's shearwater from the Salvages, Majorca, and Linosa, but similar to levels in five adults sampled in Crete. These had particularly high zinc concentrations (mean values of 181.33 μg/g), probably in association with high concentrations of cadmium (mean value 55.92 μg/g) (Renzoni *et al.* 1986). This was different in the immature shearwaters in which concentrations of zinc in the liver were high (mean value 176.43 μg/g), but cadmium concentrations were low (mean value 2.03 μg/g).

Quantitative studies of metallothionein in seabird tissues are rare. Lesser black-backed gull concentrations are comparable to data on herring gulls, *Larus argentatus* [kidney cadmium =

11–69 μg/g (dry weight), metallothionein = 33.9–377 μg/g (wet weight), liver cadmium = 1.0–6.3 μg/g (dry weight); liver metallothionein levels were not analyzed] (Elliott *et al.* 1992). Concentrations in the fledgling shearwaters were comparable, although, in contrast, concentrations of metallothionein in the liver were much higher than those in the kidney. Cosson (1989) reports far higher concentrations of both metals and metallothionein-like proteins in flamingo and little egrets (metallothionein-like protein levels in liver of flamingo were from 2,090 to 4,840 μg/g, kidney from 530 to 1,070 μg/g, and little egret liver concentrations from 720 to 1,560 μg/g wet weight). Zinc concentrations in these birds were also extremely high, up to 600 μg/g (wet weight) in flamingo liver (Cosson *et al.* 1988). These birds had starved and frozen to death, and were collected subsequently, which may account for the extraordinary levels.

Induction of Metallothionein

Many experimental studies have looked at the accumulation of heavy metals and the induction of metallothionein by injecting or dosing animals with varying amounts of cadmium (Scheuhammer 1987). On subcutaneous injection of cadmium chloride the kidney of rodents is less responsive than the liver and produces less metallothionein (Olafson 1981; Onosaka and Cherian 1981; Sendelbach and Klassen 1988). These studies did not consider changes in zinc and copper concentrations despite their possible role in the induction of metallothionein (Scheuhammer and Templeton 1990). Scheuhammer and Templeton (1990) fed ring doves three levels of dietary cadmium. Feeding birds dietary cadmium at low doses is superior to the injection of cadmium, as it involves the natural absorption and uptake processes. The resulting differences between liver and kidney metallothionein synthesis were accounted for by increases in zinc and copper concentrations. For each μmol of metallothionein in the liver, 7.6 μmol of excess metal was accumulated; and for each μmol of metallothionein in the kidney, 8.8 μmol excess metal was accumulated (cadmium plus zinc plus copper) when compared to the control group. A direct comparison cannot be made with this study, as the lesser black-backed gulls had accumulated metals naturally. However, for every 1 μmol of metallothionein in the kidney, 3 μmols of cadmium were bound; and for every 1 μmol of metallothionein in the liver, 5 μmols of cadmium were bound (Table 3). The remaining metal–metallothionein binding sites probably would be taken up by zinc and copper ions, which would account for their coaccumulation.

Metal and Metallothionein Relationships

Mercury is found in approximately equal quantities in the organic (methyl) form, and in the inorganic form in lesser black-backed gull kidney and liver (Thompson *et al.* 1990). Inorganic mercury can induce metallothionein synthesis and will bind readily with the protein in mammalian kidney (Piotrowski *et al.* 1974; Scheuhammer 1987). In addition, mercury is stated to have the highest affinity for metallothionein, greater than cadmium, zinc, and copper (Cosson *et al.* 1991). The mercury concentrations measured in this study did not show any relationship with metallothionein or any other metals analyzed. This could be due to the rather low levels accumulated, a low proportion of the mercury in the inorganic form, or possibly the small sample size. However, Elliott *et al.* (1992) found significant mercury and metallothionein correlations only in Atlantic puffin, *Fratercula arctica,* kidney, and not in other species studied (Leach's storm petrel, *Oceanodroma leucorhoa,* double-crested cormorant, *Phalocrocrorax auritus,* and herring gull, *Larus argentatus*), although Atlantic puffin had amongst the lowest concentrations among the species studied. Mercury also was not associated with metallothionein-like proteins in fulmar, *Fulmarius glacialis,* or great skua (Osborn 1978, Hutton 1981). A further factor to consider is the coaccumulation of mercury (thought to be inorganic) with selenium in internal tissues (Scheuhammer 1987), a well-established fact in mammal studies but so far not clearly demonstrated in seabirds. The mercury–selenium association in internal organs is thought to be a protective mechanism against the toxic effects of mercury (Cuvin-Aralar and Furness 1991). The complex relationships between organic (methyl) and inorganic mercury and selenium and metallothionein, and the physiological functions and dynamics in seabirds are not clearly understood and certainly deserve further study, perhaps in birds with a higher proportion of inorganic mercury in their tissues.

The strong correlation between cadmium and metallothionein concentrations in liver and kidney of lesser black-backed gulls is in agreement with that found in Leach's storm petrel ($r = 0.692$), Atlantic puffin ($r = 0.845$), and herring gull ($r = 0.866$), but not in double-crested cormorant (Elliott *et al.* 1992). The importance of long-term cadmium accumulation in the induction of metallothionein demonstrates the role of this protein in cadmium detoxification, binding and storing it to eliminate its toxic potential. Although Elliott *et al.* (1992) did not measure liver metallothionein levels, the higher hepatic zinc levels recorded were thought to be associated with metallothionein induction due to chronic cadmium accumulation. However, in fledgling Cory's shearwaters the metal–metallothionein correlations were quite different. High zinc concentrations in the liver were associated with high metallothionein concentrations, rather than with cadmium levels, which showed no significant correlation with metallothionein concentrations. In the kidney, copper concentrations were mainly associated with metallothionein concentrations, and zinc and cadmium had significant but weaker correlations with the protein. Therefore, in Cory's shearwater fledglings, the metallothionein appeared to function primarily as a zinc and copper store. The lack of a significant correlation between levels of metallothionein in kidney and liver demonstrates the independent functioning of the protein between the two organs.

The fledglings were growing plumage, and most birds still had small amounts of chick down on their bodies. Experimental work has shown that high levels of zinc are needed for feather growth, and zinc deficiency results in a frayed feather condition (Supplee *et al.* 1958; Sunde 1972). High concentrations of zinc in the liver prior to moult have been reported in sparrows, *Passer domesticus* (Haarakangas *et al.* 1974) and starlings, *Sturnus vulgaris* (Osborn 1979). Honda *et al.* (1986) found that, in the eastern great white egret, *Egretta alba modesta,* concentrations of metals fluctuated during the different phases of chick growth. Fluctuations in cadmium concentrations mirrored fluctuations in zinc and copper concentrations, which were attributed to the growth of flight feathers in the latter sample of chicks. These results concur with those shown here.

Intermetal Relationships

Significant positive correlations between zinc and cadmium have been reported in many seabird species, particularly in kidney tissue (Scheuhammer 1987; Walsh 1990; this study). This makes physiological sense, as these metals (and copper) are bound onto metallothionein and have a tendency to accumulate in parallel. Cadmium and copper concentrations in liver and kidney also are correlated in this study but are rare in the seabird literature, zinc–copper and cadmium–copper correlations were found in brown pelicans (Ohlendorf *et al.* 1985), and many other significant correlations are seen in this study (Tables 3 and 4). Recent work has shown seasonal variations in mercury, cadmium, and zinc concentrations in the kidney and liver of common guillemots (Stewart *et al.* 1994). Feather growth during moult, changes in nutrient metal requirements during breeding, and changes in the diet of birds during the winter all may have an effect on metal concentrations (Stewart *et al.* 1994). Metallothionein can be synthesised rapidly or degraded in response to such changes in metal levels (Karin 1985), and would account for the range of intermetal relationships in this study as well as in the literature.

The primary role of metallothionein remains controversial. Karin (1985) and Cosson *et al.* (1991) state that metallothionein serves as the major storage form for the essential metals zinc and copper, and argue that detoxification is not the primary function of metallothioneins but merely a fortuitous interaction. Cosson *et al.* (1991) regards the labeling of metallothionein as a detoxification protein a misuse of language. In addition, Karin (1985) states that nonessential metal ions are not present at high enough levels to exert a selection pressure strong enough for the existence of a special detoxification system. However, the levels to which seabirds are exposed are high enough to cause toxicity in humans and laboratory animals (Scheuhammer 1987), but there is no evidence that seabirds are thus affected. Therefore, it is likely that they have evolved mechanisms to tolerate metals, one such mechanism being metallothionein production. Since seabirds (and indeed many marine animals) have evolved with nonessential metals ubiquitous in their environment, it is possible that metallothioneins have evolved specifically for both homeostatic and detoxification roles.

The results of this study indicate two distinct roles for metallothionein in the physiology of seabirds: as a detoxification mechanism in adult gulls, and as a storage protein for zinc and copper during growth in young shearwaters.

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References

Blomquist S, Frank A, Petersson LR (1987) Metals in liver and kidney tissues of autumn migrating dunlin, *Calidris alpina* and curlew sandpiper *Calidris ferruginea* staging at the Baltic sea. Mar Ecol Prog Ser 35:1–13

Burger J, Pokras M, Chafel R, Gochfeld M (1994) Heavy metal concentration in feathers of common loons (*Gavia immber*) in the northeastern United States and age differences in mercury levels. Environ Monit Assess 30:1–7

Cosson RP (1989) Relationships between heavy metal and metallothionein-like protein levels in the liver and kidney of two birds: the greater flamingo and the little egret. Comp Biochem Physiol 94C:243–248

Cosson RP, Amiard JP, Amiard-Triquet C (1988) Trace elements in little egrets and flamingo of Carmargue, France. Ecotoxicol Environ Safe 15:107–116

Cosson RP, Amiard-Triquet C, Amiard JP (1991) Metallothionein and detoxification. Is the use of detoxification proteins for MTs a language abuse? Water Air Soil Pollut 57:555–567

Cuvin-Aralar MLA, Furness RW (1991) Mercury and selenium interaction a review. Ecotoxicol Environ Safe 21:348–364

Elliott JE, Scheuhammer AM, Leighton FA, Pearce PA (1992) Heavy metals and metallothionein concentrations in Atlantic Canadian seabirds. Arch Environ Contam Toxicol 22:63–73

Furness RW, Muirhead SJ, Woodburn M (1986) Using bird feathers to measure mercury in the environment: relationships between mercury content and moult. Mar Pollut Bull 17:27–30

Haarakangas H, Hyvärinen H, Ojanen M (1974) Seasonal variations and the effects of nesting and moulting on liver mineral content in the house sparrow *Passer domesticus L.* Comp Biochem Physiol 47A:153–163

Honda K, Min BY, Tatsukawa R (1986) Distribution of heavy metals and their age related changes in the eastern great white egret *Egretta alba modesta,* in Korea. Arch Environ Contam Toxicol 15:185–187

Hutton M (1981) Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environ Pollut (A) 26:129–145

Karin M (1985) Metallothioneins: proteins in search of function. Cell 41:9–10

Maegden JL, Hacker CS, Schroder GD, Weir FW (1982) Accumulation of lead and cadmium in the royal tern and sandwich tern. Arch Environ Contam Toxicol 11:99–102

Martin JH, Flegal AR (1975) High copper concentrations in squid livers in association with elevated levels of silver, cadmium and zinc. Mar Biol 30:51–55

Nicholson JK (1981) The comparative distribution of zinc, cadmium, and mercury in selected tissues of the herring gull *Larus argentatus.* Comp Biochem Physiol 68C:91–94

Nicholson JK, Kendall MD, Osborn D (1983) Cadmium and mercury nephrotoxicity. Nature 304:633–635

Nriagu JO (1988) A silent epidemic of environmental global metal poisoning? Environ Pollut 50:139–161

Ohlendorf HM, Anderson DW, Boellstorff DE, Mulhern BM (1985) Tissue distribution of trace elements and DDE in brown pelicans. Bull Environ Contam Toxicol 35:183–192

Olafson RW (1981) Differential pulse polarographic determination of murine metallothionein induction kinetics. J Biol Chem 256: 1263–1273

Onosaka S, Cherian MC (1981) The induced synthesis of metallothionein in various tissues of rats. 1. Effects of repeated injection of cadmium salts. Toxicol 14:254–253

Osborn D (1978) A naturally occuring cadmium and zinc binding protein from the liver and kidney of *Fulmaris glacialis,* a pelagic North Atlantic seabird. Biochem Pharmacol 27:822–824

——— (1979) Seasonal changes in the fat, protein and metal content of the starling *Sturnis vulgaris.* Environ Pollut 19:145–155

Piotrowski JK, Trojanowska B, Wiewska-Knypl JM, Bolanowska W (1974) Mercury binding in the kidney and liver of rats repeatedly exposed to mercuric chloride: induction of metallothionein by mercury and cadmium. Toxicol Appl Pharmacol 27:11–19

Reid M, Hacker CS (1982) Spacial and temporal variation in lead and cadmium in the laughing gull, *Larus atricilla.* Mar Pollut Bull 13(11):387–389

Renzoni A, Focardi S, Fossi C, Leonzio C, Mayol J (1986) Comparison between concentrations of mercury and other contaminants in eggs and tissues of Cory's shearwater *Calonectris diomedea*, collected on Atlantic and Mediterranean islands. Environ Pollut (A) 40:17–35

Scheuhammer AM (1987) The chronic toxicity of aluminium, cadmium, mercury and lead in birds: a review. Environ Pollut 46:263–295

——— (1988) The dose dependent deposition of cadmium into organs of Japanese quail following oral administration. Toxicol Appl Pharmacol 95:153–161

Scheuhammer AM, Cherian MG (1986). Quantification of metallothionein by a silver-saturation method. Toxicol Appl Pharmacol 82:417–425

Scheuhammer AM, Cherian MG (1991) Quantification of metallothionein by a silver saturation technique. Meth In Enzymology 205:78–83

Scheuhammer AM, Templeton DM (1990) Metallothionein production: similar responsiveness of avian liver and kidney to chronic cadmium administration. Toxicol 60:151–159

Sendelbach LE, Klassen CD (1988) Kidney synthesises less metallothionein than liver in response to cadmium chloride and cadmium-metallothionein. Toxicol Appl Pharmacol 92:95–102

Slemr F, Langer E (1992) Increase in global atmospheric concentrations of mercury inferred from measurements over the Atlantic ocean. Nature 355:434–436

Smith JD, Plues L, Heyraso M, Cherry RD (1984) Concentrations of the elements Ag, Al, Ca, Cd, Cu, Fe, Mg, Mn, Pb and Zn and the radionuclides ^{210}Pb and ^{220}Po in the digestive gland of the squid, *Notodarus gouldi.* Mar Environ Res 13:55–68

Stewart FM, Thompson DR, Furness RW, Harrison N (1994) Seasonal variation in heavy metal levels in tissues of common guillemots, *Uria aalge,* from northwestern Scotland. Arch Environ Contam Toxicol 27:168–175

Sunde ML (1972) Zinc requirements for normal feathering of commercial leghorn-type pullets. Poult Sci 51:1316–1322

Supplee WC, Combs GF, Blamley DL (1958) Zinc and potassium effects on bone formation, feathering and growth of poults. Poult Sci 37:63–67

Thompson DR (1990) Metal levels in marine vertebrates. In: Furness RW, Rainbow PS (eds) Heavy metals in the marine environment. CRC Press, Boca Raton, FL, pp 143–182

Thompson DR, Stewart FM, Furness RW (1990) Using seabirds to monitor mercury in marine environments. Mar Pollut Bull 21:339–342

Thompson DR, Furness RW, Walsh PM (1992) Historical changes in mercury concentrations in the marine ecosystem of the north and northeast Atlantic ocean as indicated by seabird feathers. J Appl Ecol 29:79–84

Walsh P (1990) The use of seabirds as monitors of heavy metals in the marine environment. In: Furness RW, Rainbow PS, (eds) Heavy metals in the marine environment. CRC Press, Boca Raton, FL, pp 183–204