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Heavy Metal Concentrations in Cory's Shearwater, *Calonectris diomedea,* Fledglings from the Azores, Portugal

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Seabirds have been used extensively as monitors of heavy metals, demonstrating both significant temporal (Thompson et al. 1992) and geographical trends (Walsh 1990). Concentrations of heavy metals are often reported for adult birds but less often for chicks or fledglings (Walsh 1990). However, chicks have been proposed as particularly useful indicators for both baseline pollution studies and monitoring programs, as they concentrate heavy metals during a specific period of time (i.e. hatching to fledging) and from a local and definable foraging area (Walsh 1990). This can be much more valuable than measurements of adult tissue concentrations where it is rarely possible to evaluate the accumulation period or define the feeding area from which metals are accumulated.

The Cory's shearwater, *Calonectris diomedea,* is a long-lived pelagic seabird found in warm marine waters from temperate to sub-tropical zones of the North Atlantic and the Mediterranean (Cramp and Simmons 1977). High concentrations of heavy metals have been reported in tissues of adult Cory's shearwater from the Mediterranean and Salvage islands which were attributed to accumulation from prey items (Renzoni et al. 1986). It is thought that squid form a significant proportion (around 26%) of their prey (Furness 1994). There is also some evidence to suggest that squid accumulate unusually high concentrations of metal, particularly concentrating cadmium in the digestive gland (Martin and Flegal 1975). Squid beaks can remain undigested in predators' stomachs and gizzards for some time after being eaten, being considerably more resistant to digestion than fish otoliths and crustacean carapace. Squid beaks have previously been used successfully to look at the diets of whales, seals, fish and birds (Clarke 1986).

The aims of this study were to analyze the amount of cadmium accumulated by Cory's shearwater chicks in three months from hatching, and measure any interactions with the essential metals copper and zinc. We tested the hypothesis that variations in the cadmium levels accumulated were due to differences in the amount of squid in individual

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chick diets. This assumes that all squid beaks fed to chicks would still be present in the gizzards of chicks at fledging, or at least that the number of beaks in each chick correlated closely with the amount of squid it had been fed.

MATERIALS AND METHODS

In the Azores there are many shearwater colonies situated close to towns. When chicks fledge (always at night) at around twelve-weeks old, some are confused by the bright town lights and crash into street lamps or car headlights, and are killed. A total of 39 fledglings were collected for analysis in October and November 1991 and 1992 on the islands of Faial and Pico.

All specimens were weighed and then stored frozen at -20°C before dissection. Liver, kidney and gizzard were dissected out using stainless steel instruments. Gizzards were stored whole in 70% alcohol for transport to Glasgow. All other tissues were stored and transported frozen. In Glasgow tissues were weighed and samples dried in an oven at 50°C to constant mass, prior to metal analysis. Gizzards were cut open and turned inside out to remove all fragments of squid beaks and other contents. Beaks were sorted and counted, and lower beaks were analyzed using the method of Clarke (1986). Samples of the most intact specimens were sent to Dr. K. Thompson, Ms. B. Santos, Dr. N. Klages and Dr. G. Pierce to confirm identifications of taxa. An attempt was made to measure the most complete beaks of Ommastrephidae. The mass of the squid was estimated by measuring the lower rostral length (LRL) and using the equation $\ln W = 2.714 + 2.93 \ln LRL$ (Clarke 1986). The minimum LRL was measured, as beaks were badly eroded. Consequently sizes of squid are only an estimate.

Cadmium, zinc and copper concentrations in kidney and liver samples were analyzed by Atomic Absorption Spectrophotometry using a Philips PU 2000 A.A.S. Samples of 0.5-1.0g dried tissue were acid digested in 10 ml concentrated nitric acid on a hot plate, by first soaking at 100°C for two hours, then boiling at 120°C for 20 minutes. Samples were diluted to 15 ml, using distilled water. Accuracy and reproducibility of metal determinations were tested by analysing International Atomic Energy Agency horse kidney Reference Material H-8. Recoveries were between 95-105% of the certified value in all cases, and detection limits were 0.014 µg/g for cadmium, 0.01 µg/g for zinc and 0.035 µg/g for copper in the digested sample. All metal concentrations are expressed on a dry weight basis.

RESULTS AND DISCUSSION

Fledgling Cory's shearwaters (12-weeks old) had accumulated measurable amounts of cadmium, zinc and copper in both kidney and liver tissue (Table 1). Although the cadmium levels measured in the fledglings conformed to a normal distribution, they showed a wide range of concentrations with kidney values from 1 to 40 µg/g (Fig. 1) and liver

concentrations from 1 to 9 µg/g (Fig. 2), and a correspondingly high coefficient of variation (CV) (Table 1).

Table 1. Concentrations of cadmium, zinc and copper in Cory's shearwater fledglings. Concentrations are given as µg/g dry weight, n = 39.

	Cadmium	Zinc	Copper
Kidney			
mean	11.28	110.68	21.16
S.D.	8.74	32.35	7.99
C.V.	77.5	29.2	37.8
range	1 - 40	40 - 194	3 - 45
Liver			
mean	3.03	198.86	18.86
S.D.	1.72	80.44	5.29
C.V.	57.0	40.4	28.0
range	1 - 9	39 - 389	12 - 30

Cory's shearwater fledglings accumulated a mean cadmium concentration of 11.28 µg/g in kidney tissues and 3.03 µg/g in liver tissues in the three months from hatching to fledging. Virtually all of the cadmium would have been accumulated after hatching as eggs contain almost no cadmium. Less than the 0.03 µg/g detection limit were reported for Cory's shearwater egg contents by Renzoni et al. (1986). Cadmium concentrations in the fledglings were lower than values measured in adults sampled on Atlantic and Mediterranean islands (Renzoni et al. 1986). Mean adult concentrations in kidney were 214.17 µg/g in the Salvages, 42.82 µg/g in Majorca, 106.21 µg/g in Linosa, and 187.32 µg/g in Crete. Mean adult liver concentrations were 26.48 µg/g in the Salvages, 7.52 µg/g in Majorca, 13.91 µg/g in Linosa, and 55.92 µg/g in Crete. Copper concentrations were not analyzed in adult shearwaters from the Atlantic and Mediterranean but the levels here are comparable to others reported in the literature for seabirds (Thompson 1990: Lock et al. 1992). Zinc concentrations in the kidney were similar to adult values recorded in Majorca and Linosa, but lower than in the Salvages and Crete. In comparison, zinc concentrations in the liver of Cory's shearwater fledglings were higher than zinc concentrations in adult Cory's shearwater from Salvages, Majorca and Linosa, but similar to five adults sampled in Crete. Adult birds from Crete had high zinc concentrations (mean value = 181.33 µg/g), probably in association with high concentrations of cadmium (mean value = 55.92 µg/g), although correlation coefficients were not calculated. This is quite different in the immature shearwaters where concentrations of zinc in the liver were high (mean value = 198.86 µg/g), but cadmium concentrations were low (mean value = 3.02 µg/g) with no significant correlations between the metals (Table 2).

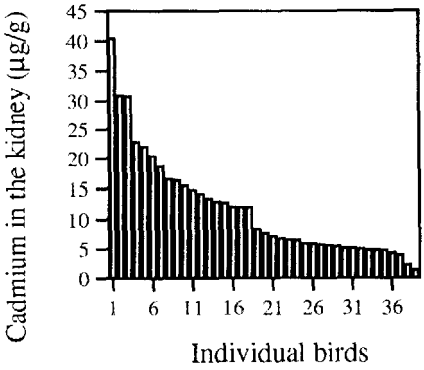

Figure 1. Individual concentrations of cadmium in Cory's shearwater kidney

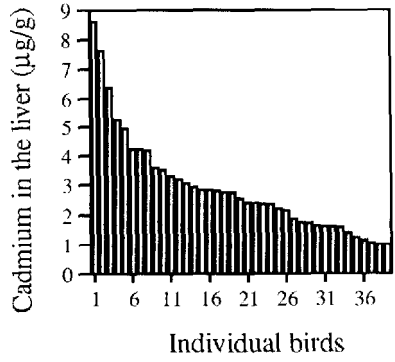

Figure 2. Individual concentrations of cadmium in Cory's shearwater liver

High concentrations of zinc were probably due to metabolic requirements during the fledglings' development. It has been shown experimentally with poultry that high levels of zinc are needed for feather growth, and indeed deficiency in zinc causes a frayed feather condition (Sunde 1972). Zinc has also been found in high concentrations in the liver prior to molting in free-living birds such as sparrows (Haarakangas et al. 1974) and starlings (Osborn 1979).

The concentrations of metals accumulated showed few inter-metal and inter-organ correlations (Table 2). Cadmium concentrations in the liver and kidney were significantly correlated ($r = 0.42$, $n = 39$, $P < 0.05$), and cadmium and zinc concentrations were significantly correlated in the kidney ($r = 0.56$, $n = 39$, $P < 0.001$). Cadmium and copper concentrations were correlated in the liver ($r = 0.40$, $n = 39$, $P<0.05$). Cadmium, zinc and copper are all bound onto metallothionein and are often found to accumulate in parallel (Walsh 1990). However, metal levels will show fluctuations during chick growth and development, which would account for the lack of correlations found here (Stewart 1996).

Cory's shearwater fledglings from the Azores accumulated a higher concentration of cadmium in three months than was found in many studies of either young or adults of other seabirds, including pelicans, gannets, cormorants, eider ducks, gulls, terns and auks (Walsh 1990). Shearwaters, along with other petrels, albatrosses, penguins and skuas, tend to have high concentrations of cadmium in their tissues (Thompson 1990; Lock et al. 1992). These birds are thought to accumulate cadmium from their diet, which consists of prey items which concentrate cadmium. Squid form a major portion of the diet of albatrosses and some petrels, but there is little information on the concentrations of heavy metals in these organisms. Concentrations of cadmium in the digestive gland of the squid *Notodarus gouldi* ranged from 19-110 µg/g (Smith et al. 1984), and Martin and Flegal (1975) measured concentrations of cadmium of 71-694 µg/g in *Ommastrephes bartrami,* 42-1106 µg/g in *Symplectoteuthis oulananiensis* and 33.0-233.1 µg/g in *Loligo opalescens.*

These citations illustrate that squid can certainly concentrate large amounts of cadmium and this may account for the high concentrations measured in their predators. However, no such relationship has ever been quantified.

Table 2. Correlation coefficients between metals and tissues of Cory's shearwater (n = 39). N.S. = not significant.

Relationship	Correlation coefficient
Cd in liver and kidney	0.42, $p < 0.05$
Zn in liver and kidney	0.10N.S.
Cu in liver and kidney	0.15 N.S.
Cd - Zn in kidney	0.56, $p < 0.001$
Cd - Cu in kidney	0.07 N.S.
Zn - Cu in kidney	0.25 N.S.
Cd - Zn in liver	-0.03 N.S.
Cd - Cu in liver	0.40, $p < 0.05$
Zn - Cu in liver	0.05 N.S.

Of the 39 fledglings obtained in the study, all had at least one squid beak in the gizzard. The number of beaks ranged from one to nineteen, with the mean number being six (s.d. = 4.09, n = 39). Squid beaks were badly eroded and each gizzard contained a number of large fragments and many small fragments. Many of the gizzards also contained small plastic pellets but no other food remains. The beaks were tentatively identified as being Family Ommastrephidae, but some were thought to be Cranchiidae, *Taonis sp.*, an Octopoteuthidae, and *Gonatus sp*. Expert opinion was that beaks were too badly eroded for further identification or detailed analysis. From the measurements of the most intact Ommastrephids, most squid had a wet mass of from 40-180 g, with the exception of the largest which was estimated at 370 g. There was no significant relationship between the number of squid individuals had eaten and the concentration of cadmium accumulated, although correlations were positive as predicted (kidney r = 0.15, n = 39, N. S.; liver r = 0.07, n = 39, N. S), Unfortunately the degree of erosion of the beaks made accurate measurement impossible, preventing a test for the relationship between individual cadmium burdens and the total mass of squid ingested.

Dietary data for Cory's shearwater are scarce. Further studies on Cory's shearwater would be necessary to sample crop contents to obtain squid beaks before they are worn down by the action of the gizzard. However, it would seem from the size estimation made here that fledglings are being fed reasonably large squid. Furness (1994) sampled voluntary regurgitations and also used a warm saline off-loading technique to collect 195 food samples from Cory's shearwater at colonies in the Azores in 1989-91; 26% of these contained squid of around 40-140 g. A diet consisting of 25% squid of this size could feasibly account for the high concentration of cadmium in fledglings. If squid fed to shearwater chicks are assumed to be

mainly or wholly Ommastrephids, (this study, Furness 1994), some calculations can be made.

Cory's shearwater chicks reach a mass of around 1100 g. The estimated daily food intake of Cory's shearwater chicks is 100 g / night, and if chicks take 60 days to reach maximum weight this requires a total of 6,000 g of food (Hamer and Hill 1993; Monteiro *unpublished data).* If 26% of this diet were squid, and using a value of 6,000 g for the total food intake, this suggests that chicks consume 1,560 g of squid. This corresponds to at least ten squid of around 156 g. This is not unrealistic given that the number of beaks per gizzard in this study ranged from 1-19, and the estimated size of squid ranged from 40 to 180 g. In squid cadmium is concentrated almost exclusively in the digestive gland, and this is estimated at around 3% of squid body weight giving a value of 46.8 g for the total mass of digestive gland ingested by the chicks, corresponding to a dry weight of 8.6 g (wet:dry ratio = 5.44, Martin and Flegal (1975). Martin and Flegal (1975) measured cadmium concentrations in Ommastrephid squid and found a wide range of concentrations from 71 to 694 µg/g, with a mean value of 287 µg/g. These values can be used for three separate estimates of the total amount of cadmium likely to be accumulated by a Cory's shearwater chick in twelve weeks, shown in Table 3. The mean dry weight of kidney and liver tissues in the Cory's shearwater sample were calculated, and it was assumed that half the cadmium burden would be accumulated by each organ (Scheuhammer 1987).

Table 3. Estimation of cadmium concentrations in fledgling Cory's shearwaters accumulated from squid in their diet

	Minimum	Mean	Maximum
Squid cadmium concentration =	71 µg/g	287 µg/g	694 µg/g
Total metal ingested = (digestive gland x metal concentration)	610.6µg	2468.2µg	5968.4µg
Amount absorbed = 0.5 % (*Stewart, unpublished data,* Scheuhammer 1987)	3.10µg	12.34µg	29.84µg
Concentration in kidney =	1.77µg/g	7.05µg/g	17.05µg/g
liver =	0.69µg/g	2.74µg/g	6.63µg/g
(kidney dry wt = 0.875g, liver dry wt = 2.25g)			

These figures are only estimates. The calculation assumes that the fish making up the remaining 75% or so of the shearwaters' diet would contain very low concentrations of cadmium. Cadmium concentrations in fish muscle are usually low and, although they tend to be higher in liver and kidney, they are much less than reported for squid (Thompson 1990).

Further work on Cory's diet would be required for a more detailed analysis. However, these calculations illustrate that a diet of around 26% squid could result in the accumulation of cadmium burdens close to these measured in the fledgling shearwaters in this study.

Further studies on chick diet including analysis of regurgitations could give more information on the dynamics and accumulation of cadmium in these shearwaters. This, in turn, would allow a greater insight into the baseline levels of metals which can be accumulated in a relatively unpolluted environment such as the Azores.

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