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Cadmium levels in oystercatcher *Haematopus ostralegus* from the German Wadden Sea

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ABSTRACT: Cadmium levels of kidney and liver tissues of 150 oystercatcher *Haematopus ostralegus* frost victims from the German Wadden Sea area were determined by means of Electrothermal Atomization Absorption Spectrometry (ET-AAS). Overall median kidney values amounted to 11.9 μg g^{-1}, those of liver to 4.9 μg g^{-1}. Because of tissue wastage, levels may be somewhat elevated compared to those in healthy birds. Females accumulated less cadmium than males. Accumulation of hepatic and renal cadmium was age-dependent, with subadult birds having significantly higher amounts than juveniles. There was no difference in concentration between subadult and adult birds. Overall, cadmium concentrations in kidney and liver tissues were strongly, positively, linearly correlated. No correlation between feather and tissue cadmium concentrations was found. A possible cadmium regulation mechanism for oystercatchers is discussed.

INTRODUCTION

The Wadden Sea is characterized by a high primary (Cadée & Hegeman 1974a, b) and secondary production (Beukema 1976, Wolff 1983). A considerable proportion of the zoobenthos is consumed by waders (Smit 1983, Baird et al. 1985). Data on the average daily consumption per bird species have shown that about 75% of the total zoobenthic consumption is taken by only 5 carnivorous species, with oystercatcher *Haematopus ostralegus* ranking second (Smit 1983).

Levels of certain heavy metals are often elevated in the sediments as well as in the invertebrate fauna in coastal environments (Förstner 1980, Bryan 1984, Salomons & Förstner 1984). Cadmium is a well-known toxic and nonessential element showing a nearly 20-fold increase in the Wadden Sea within sediments the last 200 yr (Förstner & Reineck 1974). Between sediment, sea water, and biota remarkable interactions exist. Heavy metals bound to the sediment or to suspended particulate matter in the seawater can be accumulated in all marine organisms (Cooke et al. 1979, Loring & Prosi 1986). The action of filter feeding is thought to give rise to this effect, particularly in molluscs, and therefore it is not surprising to find heavy metals often concentrated in the gills (Cooke et al. 1979).

In the Wadden Sea oystercatchers eat mainly molluscs, and to a lesser extent worms and crustaceans. Of the molluscs cockles *Cerastoderma edule*, mussels *Mytilus edulis* and Baltic tellins *Macoma balthica* are the main prey species. The Baltic tellin is taken when cockles are not available. Of the annelid worms, lugworm *Arenicola marina* and ragworm *Nereis diversicolor* are the chief species taken. Other prey species play a minor role (Hulscher 1985). Of all these, mussels are known to be the most sensitive indicators to elevated concentrations of heavy metals, particularly cadmium. Within the food-chain mussels, and to a lesser extent cockles, accumulate high levels or certain heavy metals (von Westernhagen et al. 1978, Buchwald et al. 1985). These and other diets of waders and shorebirds are contaminated with heavy metals (Packer et al. 1970, Evans & Moon 1981, Zunk 1988).

In general, little is known about trace metal pollution in waders. Hutton (1981) investigated the accumulation of heavy metals and selenium in the oystercatcher. Further results are available from other wading species, e.g. dunlin *Calidris alpina*, knot *Calidris canutus*, curlew *Numenius arquata*, curlew sandpiper *Calidris ferruginea*, redshank *Tringa totanus* and bar-tailed godwit *Limosa lapponica* (Ward 1978, Evans & Moon 1981, Goede & de Bruin 1984, 1985, Goede 1985, 1988, Goede & de Voogt 1985, Blomqvist et al. 1987). Because of this lack of information we used an excep-

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tional opportunity to measure cadmium levels in liver and kidney tissues as well as in tail-feathers of oystercatchers.

MATERIALS AND METHODS

Birds. A prolonged frost period in winter 1986/87 caused large parts of the tidal flats in the Wadden Sea to freeze over. Thousands of waders, particularly oystercatchers, died in the German part of the area. Feeding opportunities were dratistically reduced and the body condition of the birds quickly decreased. All frost victims collected had an extremely low mean body weight. Nearly 3000 oystercatchers were found dead within only a few days. Of these, more than 900 were found on high tide roosts on the Isle of Wangerooge. A total of 354 birds were collected immediately after a first die off in January 1987 and studied in detail. All birds were measured, aged, sexed, weighed, and the form of the bill and bill-tip was noted; these results will be given elsewere (Stock unpubl.).

Organ and feather sampling. From randomly chosen individuals liver (n = 149) and kidney samples (n = 60) were taken. Sampling was done using stainless steel scissors and forceps to avoid contamination. Tissues were stored in polyethylene vessels until subsequent treatment. Additionally the innermost tail-feather from a smaller randomly chosen subsample (n = 30) was collected and placed into labeled plastic bags.

Sample preparation. Immediately after removal, the organ tissues were freeze-dried for 48 h and then homogenized with an agate mill. The dried samples were stored in clean polyethylene vessels at 4 °C. All the laboratory material involved in destruction and storage was cleaned by immersion in 1:20 diluted HNO_3 overnight and subsequently rinsing with deionised water. To avoid further contamination, pulverised samples were handled with teflon-coated instruments. Two aliquots of 5 to 10 mg of dried organ tissues were digested with 100 µl of a mixture of concentrated HNO_3:H_2SO_4 (4:1, Merck Suprapur) in polyethylene cups overnight (Sperling et al. 1977, Sperling & Bahr 1979). Afterwards the cooled samples were diluted with 900 µl deionised water and stored in the same cups at 4 °C. During this process a blank – acid without tissue sample – was also induced every day. These 1:10 diluted samples can be stored for some weeks until further dilution and analysis.

To remove superficial contamination, feathers were sonicated in a 0.01 % Triton-X-100 solution for 2 min. Subsequently the cleaned feathers were dried at 80 °C overnight. The vane was clipped from the shaft with stainless steel scissors. Only the vane was analysed.

Analysis. Electrothermal Atomization Absorption Spectrometry (ET-AAS) with deuterium background correction was applied. Operating conditions for the equipment as well as the temperature program of the atomizer are given in Table 1. Instrument settings were chosen according to manual instructions and optimizing procedures. Due to aging of the graphite tubes, alterations occur and may sometimes lead to signal drift effects and signal reduction. Therefore the tubes were changed after ca 150 firings. To obtain reproducible results numerous control determinations of reference material were required. Consequently every tenth sample cup contained a control sample with a known cadmium concentration. NBS bovine liver 1577a was taken as reference material and analysed daily together with the samples. A stock solution of 1000 mg kg^{-1} Cd was prepared by dissolving metallic cadmium shots (Alfa Products, USA) in 1 % HNO_3 Suprapur (Merck, FRG). The concentration was controlled by titration. Aqueous acidified standards in the range 0 to 3 µg kg^{-1} were prepared daily Since the standard-addition method for large series of determinations with the same matrix is unnecessarily intricate, aqueous acidified standards were chosen to evaluate cadmium concentration. This method is possible when the cadmium concentration of the original sample is high enough to allow a more than 20-fold dilution. In this case the standard curves from acidified solution fit exactly to those obtained from spiked material (Sperling et al. 1977). The detection limit of the method was

Table 1. Instrument settings for the analysis of cadmium in oystercatcher tissues

Instrument	:	VARIAN AA 1275, GTA 95, Graphite-Tube-Atomizer with PSV Auto Sampler and D_2 Background Correction
Wavelength / slit	:	228.8 nm / 0.2–0.3 nm
Lamp current	:	4 mA
Injection	:	10 µl at ambient temperature
Replicate firings	:	2
Determination conditions	:	Absorbance / gasstop / peak height mode
Drying	:	95 °C, ramp 10 s; 120 °C, ramp 5, hold 35 s
Ashing	:	250 °C, ramp 15, hold 16 s
Atomization		1600 °C, ramp 0.7, hold 2 s
Tube cleaning		2500 °C, ramp 3 s

0.02 μg g^{-1}, the repeatability of the determinations 95.4 %. The recovery value amounted to 99.7 %. All tissue and feather concentrations are given as μg g^{-1} dry matter.

Data handling. Since the cadmium concentrations were non-randomly distributed, we evaluated arithmetic means with standard deviations, geometric means, harmonic means and medians of the data set. To randomize the data, decadic logarithmic transformation was carried out. The results were checked by a chi-square adaptation test. Parametric correlation and regression analysis were carried out with these transformed data. Non-parametric tests were done with original data. Calculations were performed with standard statistical procedures using the STATPAK Statistic Package (Northwest Analytical 1983).

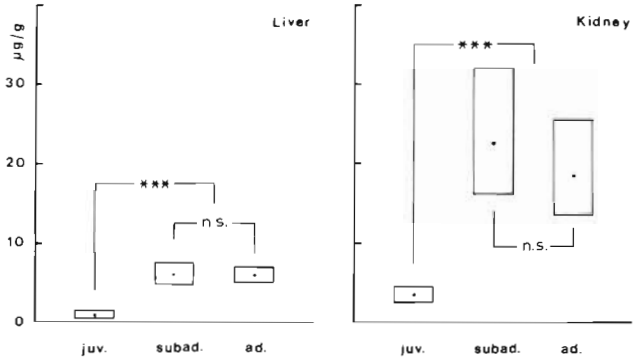

Fig. 1. *Haematopus ostralegus*. Age-dependent cadmium concentration (median and confidence interval; μg g^{-1}) in oystercatcher liver (n = 149) and kidney tissues (n = 59) from the German Wadden Sea. (Mann-Whitney-test; *** $p < 0.001$; ns: not significant)

RESULTS

Cadmium body burden and sex

Tissue and feather cadmium concentrations of oystercatchers from the German Wadden Sea are shown in Table 2. Highest values derived as arithmetic, harmonic or geometric means or medians were found in the kidney, with appreciable amounts in liver, but low levels in feathers. Overall, females accumulated less cadmium than males (Table 2). Differences in liver concentrations between the sexes are significant (Mann-Whitney-test, $p < 0.07$). This is also true when looking at juveniles only (Mann-Whitney-test, $p < 0.04$), but not for subadults and adults.

Age-dependent cadmium accumulation

Fig. 1 shows that accumulation of hepatic and renal cadmium is age-dependent, with subadult birds having significantly higher amounts than juveniles (Mann-Whitney-test, $p < 0.001$). The differences were greatest for the kidney tissues, with an average 6.3-fold difference (Mann-Whitney-test, $p < 0.001$) between juvenile and subadult oystercatchers. The corresponding factor for liver tissues was a 4.2-fold difference ($p < 0.001$). There was no difference in concentrations between subadult and adult birds. Hepatic values in subadult and adult birds were similar whereas renal cadmium concentrations in adult birds were slightly lower than in subadults; however, the differences are not significant.

Table 2. *Haematopus ostralegus*. Cadmium concentration (μg g^{-1} dry matter) in organ tissues and feathers from a total of 149 oystercatchers. HM: harmonic mean; GM: geometric mean; AM: arithmetic mean; ± SD: ± 1 standard deviation; M: median; CM: confidence interval of median. Range is also given

	n	HM	GM	AM	± SD	M	CM	Range
Cadmium – liver (μg g^{-1})								
Juvenile	24	1.15	1.34	1.56	0.92	1.43	1.11–1.79	0.46– 3.83
Subadult	53	3.76	5.62	8.36	8.27	5.98	4.70–7.57	0.76–41.57
Adult	72	4.28	5.64	7.42	6.18	5.89	4.96–6.96	0.97–35.94
Male	85	3.78	5.27	7.58	7.64	5.57	4.65–6.64	1.00–41.57
Female	62	2.21	3.64	5.83	5.83	4.07	3.17–5.17	0.46–32.40
Cadmium – kidney (μg g^{-1})								
Juvenile	20	3.11	3.50	3.94	2.04	3.59	2.82– 4.49	1.24– 9.17
Subadult	21	16.39	22.42	29.05	19.79	22.76	15.94–32.34	5.18–70.87
Adult	19	14.84	18.37	22.39	13.36	18.59	13.45–25.61	5.23–42.03
Male	27	7.28	12.47	20.67	19.57	13.08	8.53–19.80	2.11–70.87
Female	31	6.03	10.19	16.80	16.04	10.77	7.29–15.71	1.24–66.06
Cadmium – feather (μg g^{-1})								
Overall	30	0.13	0.18	0.21	0.11	0.21	0.17– 0.25	0.02– 0.49

Cadmium in tail-feathers

Cadmium concentrations in feather vanes are relatively low. They amounted to 0.21 ± 0.11 µg g^{-1}. Since we found a normal cadmium distribution in the feathers the arithmetic mean gives the best representation of the data (Table 2). We found no significant correlation between cadmium concentrations in the tissues and the feathers (correlation coefficient between feather and liver: $r = -0.24$, $n = 30$; between feather and kidney: $r = -0.27$, $n = 30$).

Inter-organ cadmium correlations

We calculated inter-organ correlations by means of linear correlation analysis with log-transformed data. There was a positive correlation (Fig. 2) between kidney and liver cadmium concentrations ($p < 0.001$). If we subdivided the sample according to sex similar correlations with the same slope of the regression line occurred. The correlation coefficient for males was 0.78 ($n = 27$), and for females 0.88 ($n = 31$). Both correlations were also significant ($p < 0.001$).

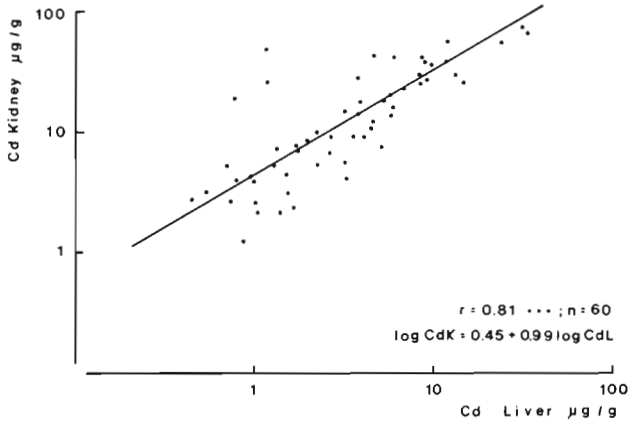

Fig. 2. *Haematopus ostralegus*. Relation between cadmium concentration in kidney versus liver tissues of all oystercatchers examined ($n = 60$). Logarithmically transformed data. (••• $p < 0.001$; CdK: cadmium in kidney; CdL: cadmium in liver)

A different picture emerges when comparing the 3 age classes (Fig. 3). Correlation coefficients and significance levels between kidney and liver tissue cadmium concentrations increased with the age of the birds: juveniles, $r = 0.49$, $p < 0.05$, $n = 20$; subadults, $r = 0.59$, $p < 0.01$, $n = 21$; adults, $r = 0.90$, $p < 0.001$, $n = 19$. The slopes of the regression lines behave differently. For juvenile and subadult birds the ratio of liver to kidney concentration was similar: only a weak renal cadmium increase occurred with rising hepatic concentrations. The regression coefficient amounted to 0.61 in juveniles, and 0.51 in subadults. In adult birds the slope is in accordance with an assumed reference line (equal cadmium level in both organs). The regression coefficient amounted to 1.15.

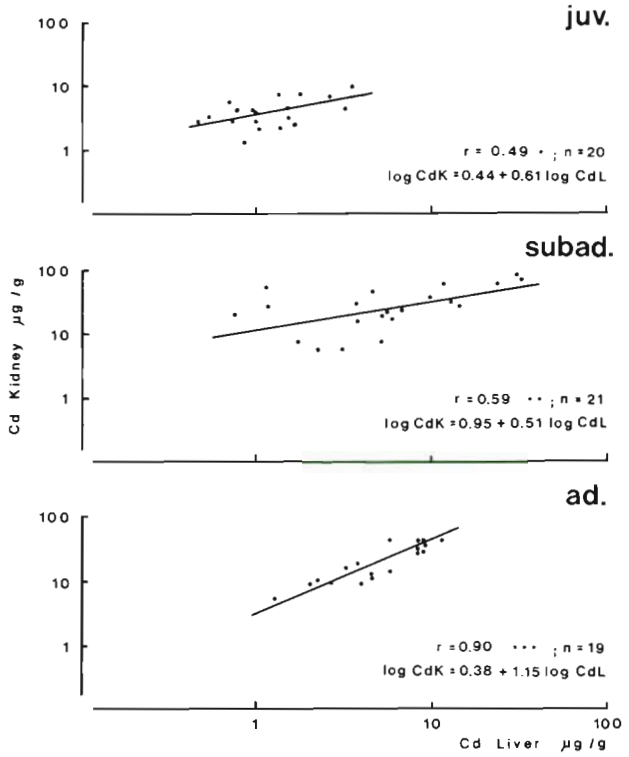

Fig. 3. *Haematopus ostralegus*. Relation between cadmium concentration in kidney versus liver tissues in juvenile, subadult, and adult oystercatchers. Logarithmically transformed data. (• $p < 0.05$, •• $p < 0.01$, ••• $p < 0.001$)

DISCUSSION

Once absorbed, cadmium is retained in the body and usually only a small proportion is excreted. Nordberg (1974) quoted a mean retention rate of 0.5 to 3% in humans which depends on the protein, calcium, and vitamin D content of the food. Evans et al. (1987) investigated curlews in Great Britain and calculated a retention rate of less than 1%.

Cadmium absorption takes place mostly in 2 ways: from the air by inhalation or by oral uptake. It is transported via the blood to other parts of the body where 50 to 75% is stored in only 2 organs, the kidney and the liver. Here the cadmium is bound to metallothionein, a cadmium-specific binding protein (Nordberg 1974, Stoeppler 1984, Herbert et al. 1988). The kidney cortex is of critical importance and functions as a long-term store. The kidney therefore accumulates cadmium for the whole life span (Nordberg 1974).

In birds little is known about the effect of cadmium. Lofts & Murton (1967) and White et al. (1978) found that

high doses of cadmium caused lesions and atrophy of the testes in pigeons *Columba livia* and mallards *Anas platyrhynchos*. White et al. were able to show that mallards fed with high cadmium doses lost their spermatogenetic activities. Nicholson & Osborn (1983) showed patchy nephrotoxic lesions due to natural cadmium exposure in pelagic seabirds. In view of histological nephrotoxicities Nicholson et al. (1983) compared kidney tissues of seabirds from an uncontaminated colony with those of cadmium-dosed starlings and mice. They found that the cadmium concentrations at which damage began were below those presently considered as relatively safe for human beings. When assessing the potential impacts of environmental pollutants such as heavy metals on animals Di Giulio & Scanlon (1985) concluded from their findings in mallards that interactions exist between contaminants and naturally occurring environmental stressors.

When measuring heavy metals in tissues of birds it is usual to choose healthy individuals. This is done because other individuals, e.g. birds starved to death, as in our case, could show tissue wastage. To check whether age-related different tissue losses occurred we measured the total liver weight from a smaller subsample of 10 adult and 10 juvenile oystercatchers. Liver weight averaged 7.8 ± 0.54 g in adults and 7.5 ± 0.9 g in juveniles. Furthermore the fat-free dry liver weight in both age classes amounted to 1.5 ± 0.3 % of fat-free dry body weight (Nehls unpubl.). Thus we can assume that our results remain comparable between age classes. However it must be taken in account that our measurements of renal and hepatic cadmium concentrations may be raised by a certain degree of tissue wastage. This must be considered when direct comparisons are made with levels of heavy metals in healthy birds.

In contrast to other waders, oystercatchers have elevated renal and hepatic cadmium concentrations. The internal distribution patterns found in this study agree with those found in other studies (Hutton & Goodman 1980, Hutton 1981, Evans et al. 1987). Oystercatchers in this study showed a 2-fold elevated cadmium concentration in liver tissues and a nearly 3-fold elevation in kidney tissues in comparison with other wading species taken as healthy birds from different localities in the Wadden Sea (Ward 1978, Evans & Moon 1981, Goede & de Bruin 1984, 1985, Goede 1985, Goede & de Voogt 1985, Blomqvist et al. 1987, Evans et al. 1987, Goede et al. in press). To what degree this elevation is due to a tissue wastage remains unclear. According to results obtained by Hutton (1981), the same species from tidal flats in the UK had somewhat higher cadmium levels than birds from the German Wadden Sea. Heidmann et al. (1987) investigated residues of chlorinated hydrocarbons and heavy metals in a small number of oystercatchers ($n = 15$) from the same locality as our specimens. They found a high PCB and mercury concentration in liver tissues but a negligible cadmium content in the same organ. Since no data about the age of the birds were given, and also because of the small number of birds examined, it is difficult to comment on the low concentration of 1.4 $\mu g\ g^{-1}$ dry matter (recalculated) in that study

In any case, the highest renal cadmium concentration found in this study (70.9 $\mu g\ g^{-1}$) is still lower than the concentrations found to cause histological kidney lesions in other seabirds (Nicholson & Osborn 1983, Nicholson et al. 1983). In considering these results it must be borne in mind that extrapolations from one species to another will be influenced by various parameters. Age, sex, feeding habits, locality of collection, time of year and the species itself may all have an effect. Data on the effect of feeding habits on cadmium tissue content will be published elsewhere (Stock unpubl.). Ward (1978) and Osborne (1979) found seasonal variations in the tissue metal content of birds, and Evans et al. (1987) found site-dependent differences in the heavy metal concentration of organ tissues in waders on wintering grounds in the UK.

Cadmium concentrations in feathers are hardly to be found in the literature. Goede & de Bruin (1984) and Goede & de Voogt (1985) found cadmium concentrations in the vane of juvenile and newly formed adult primary flight feathers in the range 0.07 to 0.49 $\mu g\ g^{-1}$. They took feather samples from dunlin, knot and bar-tailed godwit. Our findings are within this range and can be considered as relatively low. No correlation between tissue cadmium and feather content was found. The same was reported by Osborne et al. (1979) in 3 pelagic seabird species and from several wader species from the Wadden Sea area (Goede & de Bruin 1984, Goede & de Voogt 1985). Based on the findings of the latter authors and the present results we conclude that feathers are of no value as indicators in monitoring tissue cadmium concentrations in birds. On the other hand there is evidence that the cadmium content of standardized feathers reflects environmental contamination with cadmium, as recently found in magpies *Pica pica* (Hahn & Ellenberg in press).

Cadmium was found in lower amounts in the livers of female oystercatchers than in males. When lumping all age-classes together a weak significant level appears (Mann-Whitney-test, $p < 0.08$). This is in contrast to the findings of Hutton (1981). He found significantly higher renal cadmium in females but an equal hepatic cadmium level. He assumed that this was due to increased intestinal calcium absorption during eggshell building. An induced calcium-binding protein during this phase has a similar affinity to cadmium, and consequently cadmium uptake should be increased. Cadmium can

be excreted via urine, and possibly through nasal salt glands and preen glands (Goede & de Bruin 1984, Evans et al. 1987). To a lesser extent a seasonal excretion occurs through the feathers during moult.

Oystercatchers show sexual size dimorphism, and from cage experiments and field observations it is known that females may take different foods than males. They feed more often on open tidal flats than males which occur more often on musselbeds (Swennen et al. 1983, Hulscher 1985). These differences in feeding habits, and consequently food types, could also explain the differences in the cadmium content of the sexes.

Cadmium is known to occur at higher concentrations in adults than in juvenile birds. This has previously been found in oystercatcher (Hutton 1981), bar-tailed godwit (Evans & Moon 1981), dunlin and curlew sandpiper (Blomqvist et al. 1987), and in a number of other birds and sea mammals (Frank & Borg 1979, Furness & Hutton 1979, Karlog et al. 1983, Lang 1985). Although in this study subadult oystercatchers had a higher renal and hepatic cadmium content than juveniles, there was no difference between the cadmium levels in subadult and adult birds. This pattern is difficult to explain though the following 4 hypotheses are suggested. (1) The high metabolic rate in subadult birds during continued growth would cause an increased cadmium concentration. Frank (1986) recently could show that a strong cadmium increase in juvenile eider ducks takes place within the first 10 d of their life. Renal cadmium values reached a level equal to the mean kidney level of adult birds from the same study area. (2) Very high cadmium concentrations are known to cause histological lesions of renal tubuli in humans (Maker 1981). This leads to tissue, and thus cadmium, loss (Roels et al. 1981). Consequently the tissue concentration could also be reduced in older birds. (3) Cadmium is a toxic metal and very old birds (adults) may have died as a result of strongly elevated cadmium concentrations and would therefore be missing from the sample. (4) There is the interesting possibility that oystercatchers may have a cadmium regulation mechanism. A similar age-related concentration pattern to that found in this study was demonstrated in herring gulls *Larus argentatus* (Zunk 1984). Birds of known age, subdivided into 6 age classes, showed a steady renal and hepatic cadmium increase up to age 3 yr but afterwards this increase ceased. This is in agreement with our findings and those of Nicholson (1981). Herring gulls aged between 4 and 11 yr did not show any age-related increase in cadmium levels in the liver and kidney tissues. Nicholson assumed that these birds were receiving a level of exposure low enough for them to balance cadmium input with excretion. Although cadmium concentrations in herring gulls in both studies were somewhat lower than those recorded in oystercatchers, it is possible that both oystercatchers and herring gulls are able to cope with the toxin (Stock unpubl.).

Overall, cadmium concentrations in kidney versus the liver tissues were strongly, positively and linearly correlated. This holds for both sexes. When looking at the different age classes we found different slopes of the regression lines. Juvenile and subadult birds were similar but show a reduced renal to hepatic cadmium ratio compared to adults. Similar regressions and correlations to those found in this study were also reported by Kühnast (1984) in eider ducks. Further direct comparisons with other bird species are not possible because logarithmically transformed data were rarely used. Nevertheless, for oystercatchers the results obtained here imply that cadmium metabolism may be better regulated with increasing age. This effect is age-, and not concentration-related, because cadmium levels in subadult and adult birds are equivalent, whereas the regression lines are different. This is also known in humans, where the renal to hepatic ratio of cadmium changes with age (Elinder 1985).

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