

## **Age-Dependent Accumulation of Cadmium and Zinc in the Liver and Kidneys of Norwegian Willow Ptarmigan**

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During several decades the southern parts of Norway have been considerably affected by long-range atmospheric transport of heavy metals from central Europe (Steinnes 1987; Steinnes et al. 1989). Previous studies have shown elevated levels of cadmium (Cd) in different game species from these areas (Frøslie et al. 1986; Fimreite et al. 1990), and the highest levels have been found in tetraonids such as rock ptarmigan (*Lagopus mutus*) and willow ptarmigan (*L. lagopus*).

Although earlier studies have shown that elevated levels of cadmium in captive birds and mammals may produce sublethal effects such as reduced growth, anaemia, kidney lesions and testicular necrosis (Sarkar and Mondal 1973; Richardson et al. 1974; Cain et al. 1983), and modified behaviour (Heinz et al. 1983), no negative effects have so far been reported in free-living Norwegian game species. Eisler (1985) has suggested that Cd-values > 10 ppm wet weight in liver and kidneys in vertebrates indicate possible Cd-contamination. Using this limit, game species such as tetraonids and cervids are contaminated with cadmium at several locations in Norway (Frøslie et al. 1986; Fimreite et al. 1990).

A number of factors have some effect on metal accumulation in wild animals. Cadmium concentrations may vary among different organs and with the age of the animal (Flick et al. 1971; NRCC 1979). Cadmium accumulates with age, and concentrations of Cd having negative effects may eventually be reached at low exposure levels, provided exposure is of sufficient duration.

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Several studies in birds have shown differences in Cd-concentration in liver and kidneys between juvenile and adult birds (Fimreite et al. 1990; Lock et al. 1992). However, few have examined Cd-accumulation in relation to exact age (Walsh 1990). Here we report levels of cadmium and zinc in liver and kidneys in different age-categories of juvenile and adult Norwegian willow ptarmigan.

## MATERIALS AND METHODS

Thirty-three willow ptarmigan were collected in 1988-89 in Dovrefjell National Park, (62°17'N, 09°39'E), in central Norway. The area is considered not to be affected by long-range atmospheric transport of heavy metals (Steinnes pers. com). All birds were shot either in spring (May-June) or in the autumn (August-September). Age was determined using pigmentation of the primaries (Bergerud et al. 1963). Normally the birds can be classified as juveniles (<12 months) or adults (>12 months). However, by using the time the birds were shot, it was possible to group juvenile birds into two age-categories: 2-3 months and 10-11 months (shot in autumn and spring, respectively). The adult birds could likewise be grouped into age-categories 14<sup>+</sup> months or 23<sup>+</sup> months (shot in autumn and spring, respectively). The ages of adult birds are minimum estimates and the age-categories could include older birds.

The birds were kept frozen (-20°C) until analyses were carried out. The liver and kidneys were digested in concentrated HNO<sub>3</sub>, and cadmium and zinc (Zn) determined by graphite furnace atomic absorption spectrometry. Standard reference materials, National Institute of Standards and Technology, Citrus leaves 1572, Pine needles 1575 and Bovine liver 1577a, were used. The detection limit for Cd was 0.002 ppm. All metal-values are given as ppm (mg kg<sup>-1</sup>) wet weight. The analyses were performed at the Department of Chemistry, University of Trondheim, Norway. To compare age-categories we used ANOVA and Scheffe's multiple-comparison method. Differences were considered significant when P values were less than 0.05.

## RESULTS AND DISCUSSION

In earlier studies of heavy-metal loading in tetraonids in Norway, birds were collected during the autumn hunting season (e.g., Fimreite et al. 1990). In accordance with these studies, we found that among willow ptarmigan collected during autumn, adults (14<sup>+</sup> months) had significantly higher Cd-levels than juveniles (2-3 months). Further, the

Table 1. Mean content of cadmium and zinc (ppm wet weight) in liver and kidneys of willow ptarmigan of different age,  $\pm$  = standard deviation. (n) = number of birds in each age category.

		Age (months)/Season			
		Autumn 2-3	Spring 10-11	Autumn 14 <sup>+</sup>	Spring 23 <sup>+</sup>
Cadmium	Liver	0.11 $\pm$ 0.04 (8) <sup>abc</sup>	5.2 $\pm$ 1.9 (4) <sup>ab</sup>	2.1 $\pm$ 1.6 (14) <sup>b</sup>	3.8 $\pm$ 1.0 (7) <sup>c</sup>
	Kidney	0.37 $\pm$ 0.30 (8) <sup>abc</sup>	21.1 $\pm$ 2.7 (4) <sup>a</sup>	21.4 $\pm$ 12.8 (14) <sup>b</sup>	22.7 $\pm$ 3.2 (7) <sup>c</sup>
Zinc	Liver	28.5 $\pm$ 3.3 (8) <sup>a</sup>	43.7 $\pm$ 15.7 (4) <sup>a</sup>	33.4 $\pm$ 6.7 (14) <sup>b</sup>	32.4 $\pm$ 5.0 (7) <sup>c</sup>
	Kidney	22.2 $\pm$ 1.3 (8) <sup>abc</sup>	42.4 $\pm$ 5.2 (4) <sup>a</sup>	35.6 $\pm$ 7.4 (14) <sup>b</sup>	40.8 $\pm$ 2.8 (7) <sup>c</sup>

Within each line values with identical superscripts indicate significant differences. (ANOVA, Scheffe's multiple-comparison method,  $p < 0.05$ ).

Cd-levels were high both in liver and kidneys, indicating Cd-contamination in the area (Table 1).

Although juvenile birds in the autumn had low Cd-levels in kidneys and liver, by spring (age 10-11 months) the levels were significantly higher, and similar to adult birds (Table 1). Compared with mammals like cervids (Wren 1983; Frøslie et al. 1986) and seabirds like the great skua (*Catharacta skua*) (Furness and Hutton 1979), in which a positive correlation between Cd-content and age has been documented, willow ptarmigan show an extremely rapid accumulation through their first winter. However, after reaching a threshold of about 22 ppm in kidneys, no further accumulation seems to occur (Figure 1).

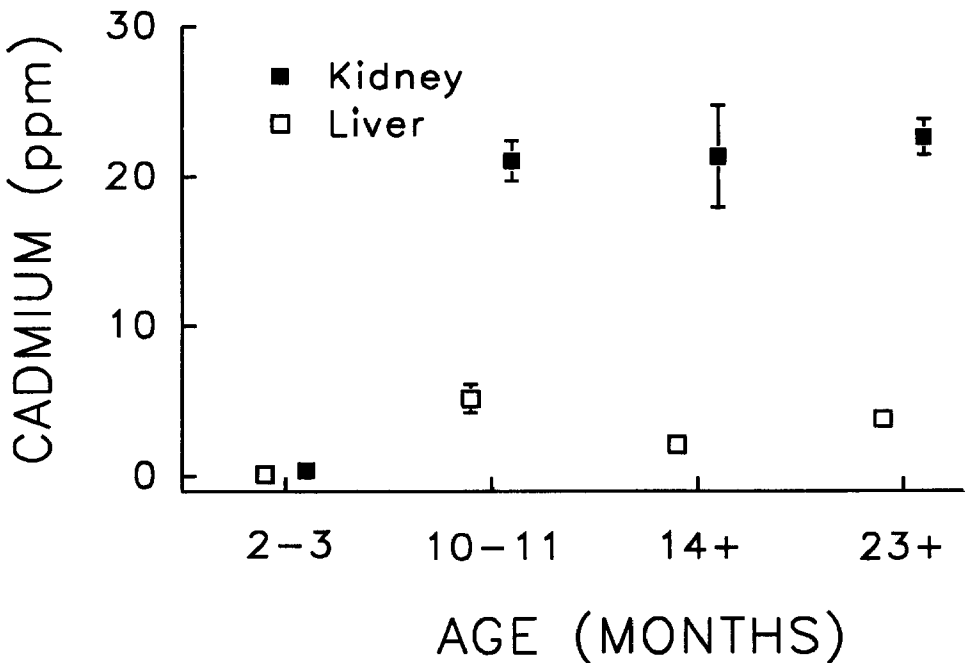


Figure 1. Mean level of cadmium in kidneys and liver (ppm wet weight) in willow ptarmigan of different age. Vertical line =  $\pm 1$  standard error. Number of birds in the different age-categories see Table 1.

There is a similar rapid accumulation of Cd in the liver during the ptarmigans' first winter, and no age-dependent accumulation seems to occur after that (Table 1). Whereas the Cd-level stays stable in the kidneys between seasons after reaching the threshold-value, there is great variation in Cd-levels between seasons in the liver (Figure 1).

The Cd-concentration in liver is significantly higher in spring (10-11 months and 23+ months) than in autumn (14+ months) (Table 1). This indicates an accumulation of Cd in the liver during the winter and an excretion during the summer.

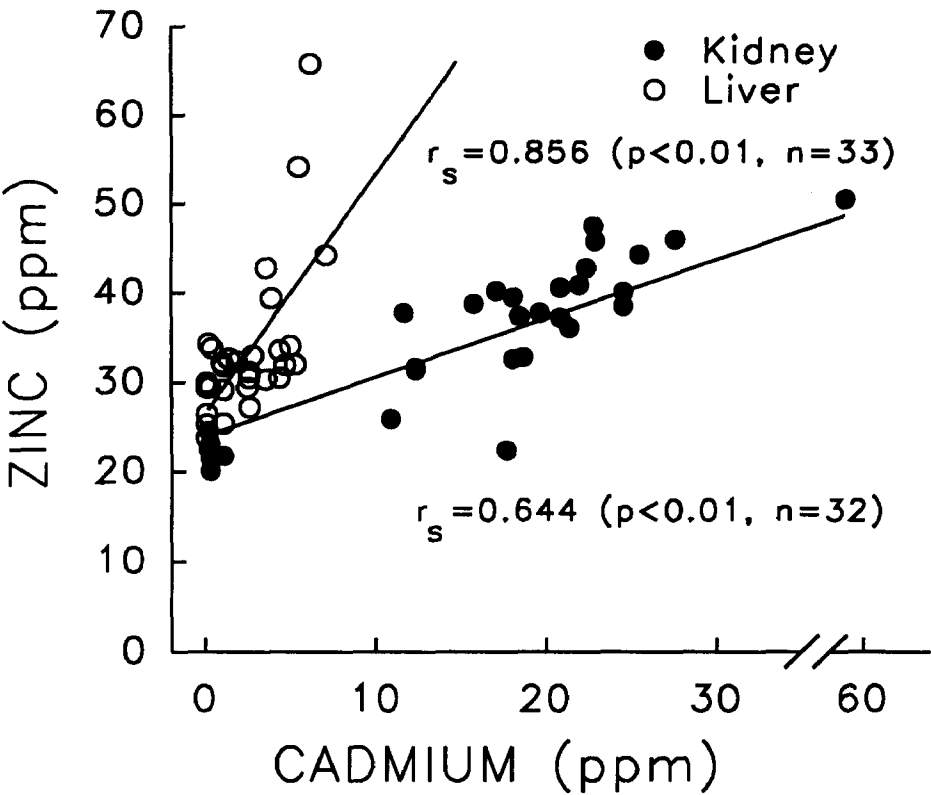


Figure 2. Relationship between the level of zinc and cadmium (ppm wet weight) in liver and kidney tissue in willow ptarmigan.  $r_s$  = Spearman rank correlation coefficient.  $n$  = number of birds.

In several bird species there is a close correlation between tissue concentration of zinc and cadmium, and zinc is believed to be important in detoxification of Cd (Walsh 1990). Similar to cadmium, tissue levels of Zn were significantly lower in age-category 2-3 months compared with the other age-categories (Table 1). Further, zinc also showed a rapid accumulation during the first winter. Contrary to Cd a seasonal difference seems to exist in Zn-level in the kidneys, but not in the liver (Table 1). However, this difference in seasonal development between Cd and Zn in kidneys and liver did not abolish the positive correlation between the two metals in either tissue (Figure 2).

As no net accumulation of Cd seems to occur after the ptarmigans' first winter, this implies that; after a threshold is reached in liver and kidneys, willow ptarmigan excrete the same amount of Cd as is taken in through the food each year. This indicates that age-dependent accumulation of Cd in willow ptarmigan shows a different pattern than that observed in several seabirds (Walsh 1990).

In a study of herring gulls (*Larus argentatus*), Nicholson (1981) found no age-dependent accumulation of cadmium, mercury, or zinc. In studies where no age-dependent accumulation of Cd has been found, this is suggested to reflect a low dietary exposure. This is possibly not the case in willow ptarmigan. The rapid increase in Cd level in kidneys and liver during the ptarmigans' first winter indicates rather heavy Cd exposure. Also, the tissue burden of Cd is much higher in willow ptarmigan than in herring gulls (liver:  $\bar{x} < 0.60$  ppm; kidneys:  $\bar{x} = 3.1$  ppm, calculated from Nicholson (1981)), and comparable to levels found in great skuas (liver:  $\bar{x} = 2.4$  ppm; kidneys:  $\bar{x} = 18.7$  ppm, calculated from Furness and Hutton (1979)). The original data for herring gulls and great skuas are presented as ppm dry weight, but results here are presented as ppm wet weight using the conversion factors 0.32 and 0.23 for liver and kidneys, respectively (Scanlon 1982).

The difference in accumulation pattern found between different bird species, and even between individuals within species, could be caused by differences in geological, dietary, and physiological parameters (Walsh 1990). To avoid tissue damage caused by excessive levels of heavy metals, different mechanisms for detoxification and excretion of heavy metals have evolved. For Cd the detoxification involving zinc and metal-binding proteins, metallothioneins, are considered most important (Walsh 1990).

One of the most significant excretion mechanisms of mercury (Hg) in many bird species is the shedding of feathers during moulting (e.g., Braune and Gaskin 1987). The mechanism of accumulation of Cd in feathers and the significance of moulting for excretion of Cd is not well known. Willow ptarmigan moult 3-4 times/year and replace 100-120 g feathers, averaging 20-25% of their body weight. Some preliminary results from willow ptarmigan from Dovrefjell indicate that these birds excrete substantial quantities of Cd through moulting (Nygård unpubl.).

Willow ptarmigan start moulting into their winter plumage in September and stay white until May the following spring. During the next four months they moult 2-3 times. This spring and summer moult coincides with the reduction in liver Cd-burden, whereas the buildup of high levels of Cd in liver and kidneys in juvenile birds, as well as in liver of adult birds, is seen during winter when the ptarmigan has the same plumage for 8 months. Hence, in willow ptarmigan shedding of feathers during moult seems to be an important way to excrete cadmium. After a threshold of Cd is reached in liver and kidneys, moult may counteract further age-dependent accumulation.

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