Ornis Fennica 78:39–43. 2001

# Sexual difference in heavy metal contamination in the liver of tits *Parus* in winter

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*Received 25 November 1999, accepted 18 January 2000*

Although tit *Parus* species are considered suitable as biomonitors for local contamination, sexual differences in their accumulation of heavy metals have not been studied. Here I present data on the concentration (mg $kg^{-1}$ dry weight) of cadmium, copper and zinc in the liver of adult Marsh Tits *P. palustris*, Great Tits *P. major* and Blue Tits *P. caeruleus* caught within a restricted area in central Norway during the winters of 1993–99. The mean concentration of cadmium was slightly higher in adult males than adult females each winter, and males of all the species had significantly higher mean concentrations of cadmium, copper and zinc over the years. The sexual differences may be associated with different metabolic rates in the two sexes.

## 1. Introduction

Birds are considered as useful bioindicators of environmental contaminants such as DDT, pesticides and heavy metals (Burger 1993), and there are abundant data on heavy metal contamination in several species of seabirds (Kim et al. 1996) and birds of prey (Dennemann & Douben 1993, Jager et al. 1996), and also in a few passerine species (see Eens et al. 1999). However, when examining for heavy metal contamination, it is important to be aware of possible differences in the contamination between the sexes. When studying the impact of heavy metal accumulation in Dippers *Cinclus cinclus* during the breeding season, Nybø et al. (1996) found that females had significantly higher concentrations of zinc and copper in their liver than males, while, although not significantly so, the converse was found for cadmium. Relatively high concentrations of zinc and copper in the liver of Pied Flycatcher *Ficedula hypoleuca* and Great Tit *Parus major* nestlings from central Norway (Kålås & Framstad 1993) and of mercury in Pied Flycatcher eggs and nestlings (Rosten et al. 1998) and in eggs of Dippers (Nybø 1996) and Great Tits and Blue Tits *Parus caeruleus* (Dauwe et al. 1999) indicate that females transfer metals to their eggs (but see Leonzio & Massi 1989). Thus, sexual differences in metal accumulation may be due to physiological differences that are particularly pronounced during breeding.

During the winter, however, when small passerines require especially high energy amounts for maintenance, their main challenge is to survive from one day to the next. Hence, it should be expected that the sexes had an approximately equal uptake and accumulation of heavy metals. This study presents data on sexual differences in accumulation of the toxic metal cadmium (Cd) and the essential elements copper (Cu) and zinc (Zn) in livers from male and female Marsh Tits *Parus palustris* and Great and Blue Tits caught during the winter in Trondheim, central Norway.

## 2. Materials and methods

The majority of the birds (62 of 70) were caught at the same site during October–March 1993–99 in a mixed forest area 12 km south of Trondheim city. Since Marsh Tits are territorial during the whole year (Nilsson & Smith 1988) and the Great and Blue Tits visited the study site in pairs, it is suggested that the birds were territorial pairs from the local area. The forest consists mainly of Norway Spruce *Picea abies*, Scots Pine *Pinus sylvestris*, Birch *Betula odorata* and Grey Alder *Alnus incana*. An incinerator plant is situated about 2 km west of the collection site and may be a source of heavy metals. In 1994–95, also four juvenile Marsh Tits and four adult Great Tits were caught in a mixed forest of Birch and Grey Alder about 15 km west of Trondheim city. Both places were situated in the vicinity of urban areas, and the birds may have fed at bird tables. The birds were caught under licence in feeder traps baited with sunflower seeds and classified as juveniles (6–9 months) or adults (older than 16 months). During October–March 1993–99, adult birds of Marsh Tits (12 males, 11 females), Great Tits (12 males, 6 females) and Blue Tits (4 males, 2 females) were caught and analysed. Since the concentration levels of cadmium and copper in livers of juvenile tits change during winter (contrary to no change in adults; Hogstad 1996a), only juveniles caught in December–March (10 males and 13 females of Marsh Tits) have been analysed.

The birds were frozen at –20 °C within a few hours of being killed, and kept frozen until analysed for cadmium, copper and zinc. The liver was digested in concentrated $HNO_3$ and then evaporated. The concentration of metals was then determined by atomic absorption spectrometry. Standard reference materials were analysed concurrently with the samples. All values were within certified limits. All metal values are given as p.p.m. (mg $kg^{-1}$) dry weight. Statistical tests are two-tailed.

## 3. Results

### 3.1. Sexual differences

The adult males of the three species showed slightly higher mean concentrations of cadmium in their liver compared to females in each winter (Table 1) and the sexes differed significantly over the years (Wilcoxon signed-rank test; Z = –2.20 – –4.20, P < 0.001). Although the sexual differences in the concentrations of copper and zinc were less consistent, males of all species had significantly higher values over the years (Z = –2.20 – –4.20, P < 0.05 – P < 0.001).

No difference in the mean metal concentration was found between the three species over the six winters (one-way ANOVA, males: cadmium: $F_{2,27} = 3.01$; copper: $F_{2,23} = 0.88$; zinc: $F_{2,27} = 0.72$; females: cadmium: $F_{2,18} = 1.03$; copper: $F_{2,16} = 0.56$; zinc $F_{2,18} = 0.95$, all values n.s.).

In juvenile Marsh Tits, the concentration of cadmium was highest in males in both winters studied, significantly so for 1993–94 (Mann-Whitney U-test; Z = –2.55, P = 0.01) and for the pooled sample across the two winters (Z = –2.31, P < 0.05). The situation was more complex for copper and zinc.

### 3.2. Variations between winters

No variation in the mean metal concentration between years was found for adult male Marsh Tits for cadmium (one-way ANOVA; $F_{4,11} = 1.87$, n.s.) and zinc ($F_{4,11} = 0.55$, n.s.), but the copper concentration varied ($F_{4,11} = 9.05$, P < 0.001). Female Marsh Tits showed an annual variation for cadmium: $F_{4,10} = 5.99$, P < 0.05), but not for copper ($F_{4,10} = 2.23$, n.s.) or zinc ($F_{4,10} = 0.42$, n.s.). Thus, the concentration of the heavy metals in male and female Marsh Tit livers seems relatively stable over the years.

## 4. Discussion

Blue Tits have been found to accumulate lead (Pb), zinc and copper in feathers to a greater extent than Great Tits, possibly because of a higher metabolic rate of the smaller-sized Blue Tits (Eens et al. 1999). No such between-species difference was found in my study (Hogstad 1996a, this study). However, as Eens et al. (1999) did not look for differences between sexes, and the sample size from my study site is small, more data is needed

to clarify this disagreement.

Sexual differences in heavy metal concentration in birds during the breeding season may be related to different foraging habits and to physiological differences (Finley & Dieter 1978, Kendall & Scanlon 1981, Nybø et al. 1996). Furthermore, if the dietary metals accumulate in the liver of birds and are partly transferred to eggs (Nybø 1996, Rosten et al. 1998), it may be suggested that the effect of egg laying should be reflected also in the winter levels of metals in females. However, the higher concentration of cadmium in juvenile males than in juvenile female Marsh Tits found in this study, weaken this suggestion.

Hunger and energetic stress influence the

Table 1. Mean concentrations (mg $kg^{-1}$ dry weight) of cadmium (Cd), copper (Cu) and zinc (Zn) in the liver of male (m) and female (f) tits *Parus* caught in Trondheim during the winters of 1993–99. Bold numbers denote the highest values. Sample sizes in parentheses. Asterisks denote significance levels in a Wilcoxon signed-rank test; *=P<0.05, **=P<0.01, ***=P<0.001

| Species | | 1993–94 | | 1994–95 | | 1995–96 | | 1996–97 | | 1997–98 | | 1998–99 | | P | 1993–99 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | m | f | m | f | m | f | m | f | m | f | m | f | | m | f |
| **Adults** | | | | | | | | | | | | | | | | |
| Marsh Tit | Cd: | **0.56** | 0.55 | - | - | **0.36** | 0.35 | **0.52** | 0.41 | **0.52** | 0.34 | **0.39** | 0.23 | *** | **0.44** | 0.38 |
| | | (2) | (2) | | | (4) | (2) | (2) | (3) | (1) | (2) | (3) | (2) | | (12) | (11) |
| | Cu: | 10.4 | **13.3** | - | - | **17.4** | 15.2 | **20.2** | 17.0 | 15.3 | **18.6** | **24.3** | 24.2 | *** | **18.2** | 17.6 |
| | | (2) | (2) | | | (4) | (2) | (2) | (3) | (1) | (2) | (3) | (2) | | (12) | (11) |
| | Zn: | **74.3** | 72.1 | - | - | **86.0** | 80.5 | **78.3** | 72.8 | **66.2** | 62.8 | **83.1** | 71.3 | *** | **80.4** | 72.0 |
| | | (2) | (2) | | | (4) | (2) | (2) | (3) | (1) | (2) | (3) | (2) | | (12) | (11) |
| Great Tit | Cd: | - | - | **0.62** | 0.46 | **0.54** | 0.43 | - | - | - | - | **0.40** | 0.30 | *** | **0.52** | 0.42 |
| | | | | (4) | (2) | (4) | (3) | | | | | (4) | (1) | | (12) | (6) |
| | Cu: | - | - | - | - | 17.0 | 17.0 | - | - | - | - | **22.6** | 11.2 | ** | **19.8** | 15.6 |
| | | | | | | (4) | (3) | | | | | (4) | (1) | | (8) | (4) |
| | Zn: | - | - | **73.5** | 72.5 | 83.0 | **89.3** | - | - | - | - | **68.6** | 74.9 | *** | 75.0 | **81.3** |
| | | | | (4) | (2) | (4) | (3) | | | | | (4) | (1) | | (12) | (6) |
| Blue Tit | Cd: | - | - | - | - | **0.38** | 0.32 | - | - | - | - | **0.27** | 0.24 | * | **0.35** | 0.28 |
| | | | | | | (3) | (1) | | | | | (1) | (1) | | (4) | (2) |
| | Cu: | - | - | - | - | **15.1** | 13.9 | - | - | - | - | **17.9** | 15.2 | * | **15.8** | 14.6 |
| | | | | | | (3) | (1) | | | | | (1) | (1) | | (4) | (2) |
| | Zn: | - | - | - | - | 77.7 | **84.0** | - | - | - | - | **91.3** | 61.9 | * | **81.1** | 73.0 |
| | | | | | | (3) | (1) | | | | | (1) | (1) | | (4) | (2) |
| Tits | Cd: | **0.56** | 0.55 | **0.62** | 0.46 | **0.43** | 0.39 | **0.52** | 0.41 | **0.52** | 0.34 | **0.38** | 0.25 | *** | **0.46** | 0.38 |
| | | (2) | (2) | (4) | (2) | (11) | (6) | (2) | (3) | (1) | (2) | (8) | (4) | | (28) | (19) |
| | Cu: | 10.4 | **13.3** | - | - | **16.3** | 15.9 | **20.2** | 17.0 | 15.3 | **18.6** | **22.7** | 18.7 | *** | **18.4** | 16.7 |
| | | (2) | (2) | | | (11) | (6) | (2) | (3) | (1) | (2) | (8) | (4) | | (24) | (17) |
| | Zn: | **74.3** | 72.1 | **73.5** | 72.5 | 82.6 | **85.5** | **78.3** | 72.8 | **66.2** | 62.8 | **76.8** | 69.8 | *** | **78.2** | 75.0 |
| | | (2) | (2) | (4) | (2) | (11) | (6) | (2) | (3) | (1) | (2) | (8) | (4) | | (28) | (19) |
| **Juveniles** | | | | | | | | | | | | | | | | |
| Marsh Tit | Cd: | **0.82** | 0.43 | **0.49** | 0.47 | | | | | | | | | | **0.62** | 0.44 |
| | | (4) | (8) | (6) | (5) | | | | | | | | | | (10) | (13) |
| | Cu: | 11.4 | **12.0** | **15.4** | 12.1 | | | | | | | | | | **12.2** | 12.0 |
| | | (4) | (8) | (1) | (4) | | | | | | | | | | (5) | (12) |
| | Zn: | **66.4** | 66.3 | 68.5 | **72.9** | | | | | | | | | | 67.6 | **68.8** |
| | | (4) | (8) | (6) | (5) | | | | | | | | | | (10) | (13) |

plasma levels of "stress" hormones like corticosterone and testosterone (e.g. Silver et al. 1979, Silverin et al. 1984), which show higher levels in dominant individuals (Silverin et al. 1984). It may therefore be speculated whether the sexual difference in the metal concentration is linked to different hormonal levels in the tits due to their social status.

The tits were caught within a restricted geographical area, and sexual differences in metal concentrations due to the sexes having different diets, seem unlikely. It is more reasonable to suggest a relationship between the sexual difference in cadmium concentration in the birds and sexual dissimilarity in their metabolic rates and food consumption.

Marsh, Great and Blue Tits, as well as Willow Tits *P. montanus*, live in small winter flocks where males dominate females within an age group (Saitou 1979, Hegner 1985, Hogstad 1987a, Nilsson & Smith 1988). As found for Willow Tits (Hogstad 1987b), it is highly probable that males of the other tit species studied have significantly higher oxygen-consumption rates than females during daytime in winter, in adults as well as in juveniles. If the food items of the tits contain heavy metals (cf. Hogstad 1996b), a higher food consumption in males than in females due to different foraging activity, may therefore result in a slightly higher accumulated concentration of heavy metals in male livers than in female livers. The average growth rate of induced tail feathers in winter was higher in Willow Tit males than in females, indicating that males have the highest nutritional status (Hogstad 1992). This supports the suggestion that males have a higher food intake.

No signs were observed whether the analyzed birds suffered from toxicities of heavy metals, and any physiological effects of the sexual difference in the metal concentrations in the tits are unknown. However, because tit *Parus* species has been suggested as biomonitors for local contamination (Eens et al. 1999), the sexual differences in accumulation of heavy metals should be taken into account.

*Acknowledgements:* I thank R. Langvatn, S. Nybø, H.C. Pedersen, B. Silverin for comments on the manuscript, and R. Binns for improving the English. Financial support was given by The Royal Norwegian Society of Sciences and Letters, Trondheim Energy Board and The Museum NTNU. ALLFORSK Department of Ecotoxicology carried out the chemical analyses.

## Sammanfattning: Skillnader mellan könen hos mesar i ackumulering av tungmetaller i lever

Mesar anses vara välägnade som biomonitorer för påvisning av lokal förorensning av tungmetaller i naturen. Det tas emellertid sällan hänsyn till eventuella skillnader mellan könen i ackumuleringen av metallerna. I denna studie visas att koncentrationen av kadmium (Cd), koppar (Cu) og zink (Zn) i lever hos adulta entitor (*Parus palustris*), talgoxar (*P. major*) och blåmesar (*P. caeruleus*), fångade inom ett begränsat område i mellersta Norge vintrarna 1993–1999, var högre hos hannar än hos honor. Hos juvenila entitor hade hannarna högre kadmiumnivåer än honorna. Orsaken antas bero på metaboliska skillnader mellan könen. Vid jämförande studier av kontaminering av tungmetaller, baserad på mesar, måste det därför tas hänsyn till fåglarnas ålder och kön.

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