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Intraspecific Variation of Mercury Contamination in Chicks of Black-Winged Stilt (*Himantopus himantopus*) in Coastal Wetlands from Southwestern Europe

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Birds have been considered useful biomonitors for mercury pollution (Furness 1993). Mercury contamination in birds can easily be assessed through feather sampling. Feather represents the major pathway for elimination of mercury, body feathers can be used for the assessment of whole bird mercury burdens (Furness et al. 1986; Thompson et al. 1990) and mercury in feathers is almost entirely methylmercury (Thompson and Furness 1989). Chicks have certain advantages for monitoring mercury pollution. Chicks are confined to a limited area and mercury uptake during their growth period can be followed. Moreover dynamics of mercury contamination have been well studied in chicks (Lewis and Furness 1991; Becker et al. 1993a; Monteiro and Furness 2001), and chick feather has been considered a good monitoring unit (Becker et al. 1993b). Growing feathers accumulate higher mercury concentrations than those in chick tissues or blood (Lewis and Furness 1991; Burger and Gochfeld 1992), but show a high correlation with mercury levels in tissues and in food (Stewart *et al.* 1997; Monteiro and Furness 2001). For these reasons chick feathers have already been used to study geographic variation between colonies (Goutner and Furness 1998).

The Black-winged Stilt *Himantopus himantopus* is an aquatic species using several breeding areas in coastal wetlands of south-western Europe (Hagemeijer and Blair 1997). Coastal saltpans are an important habitat for aquatic birds in southern Europe (Britton and Johnson 1987; Velasquez 1992) and in particular for Black-winged Stilt (Hagemeijer and Blair 1997). Black-winged Stilt chicks are nidifugous (Cramp and Simmons 1983) and once they leave the nest, stay in a limited area until they can fly (Dubois 1994) which allows chick contamination to be related to where the bird has been reared. The species feeding technique is more specialised than in many other wading species (Espin et al. 1983) and diet is dominated by insects and crustaceans (Serrano et al. 1983; Perez-Hurtado et al. 1997). The main objective of the present work was to study intra-specific variation of mercury contamination in Black-winged Stilt chicks from coastal wetlands of south-western Europe.

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MATERIALS AND METHODS

The study area included 4 wetlands in south-western Europe, Mondego estuary, Tagus estuary, Sado estuary and Formosa coastal lagoon (Figure 1). Sampling sites were represented by coastal saltpans in all wetlands and also by rice-fields in the Tagus estuary. Plumage samples were collected from live chicks (down and body feathers) and from chick corpses (down). Egg sampling occurred only for flooded eggs, and other tissues (liver and carcass) were obtained from corpses of chicks that were found dead. Sampling occurred during breeding periods of 1997, 1998 and 1999. Chick biometry was measured (Svensson 1992). Body weight was also measured using a spring balance. With the exception of plumage, all biological materials were frozen after collection and later defrosted, dehydrated to constant weight in an oven at 45°C and homogenised prior to mercury analysis.

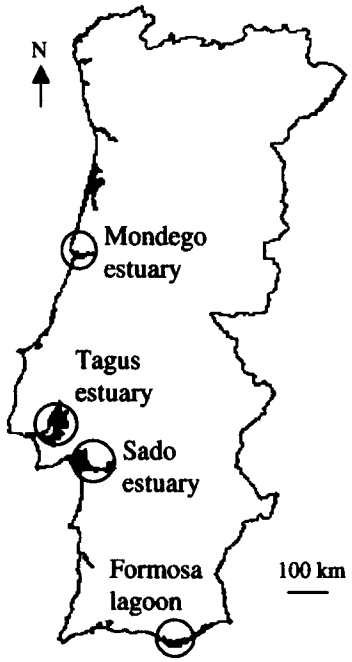

Figure 1. Map of Portugal mainland with the location of the study area.

All samples were analysed for total mercury by Cold Vapour Atomic Absorption Spectroscopy. Samples were digested in a water bath at 70 °C for 6 hours by the addition of concentrated H_2SO_4. $KMnO_4$ 5% was added and the solution kept at 70°C for 2 more hours. The $KMnO_4$ in excess was reduced with $NH_2OH.HCl$ 20%. Reproducibility was checked performing successive measurements with the same sample. Relative standard deviations in the range 3-5% were found. Accuracy of the method was within 10% and was monitored analysing reference materials tuna-muscle-350 (IAEA, Monaco) and RM50 (NBSBM, USA). The

limit of detection, taken as twice the standard deviation of triplicate analysis at blank concentrations (Saltzman et al. 1983) was 0.01 µg Hg / g digested sample. Interference due to matrix and the pre-treatment were assessed by standard additions for the wet mineralization procedure. Recoveries of added inorganic mercury were between 98% and 104% for feathers and average recoveries of 79% were obtained for eggs. Egg concentrations were corrected by a factor of 1.26. Mercury concentration is given on a fresh weight (f.w.) basis for plumage samples and on a dry weight (d.w.) basis for the other samples. Preliminary tests were performed for goodness of fit to normal distribution (Kolmogorov-Smirnov one-sample tests). In cases where the fit was good parametric tests were applied. In the situations where data deviated significantly from normality, non-parametric tests were used (Zar 1984). Statistical tests used the Statistica software package (StatSoft 1995) and were performed with 5% as threshold for significance.

RESULTS AND DISCUSSION

Mean mercury concentrations in down from chick corpses varied between 0.53 and 2.20 µg Hg / g f.w. Mean mercury concentration in internal tissues from chick corpses varied between 0.02 and 1.36 µg Hg / g d.w. for liver, and between 0.15 and 0.60 µg Hg / g d.w. for carcass. Significant positive correlation was observed between mercury levels in down and liver (Spearman r, r^2= 0.79, P<0.005), liver and carcass (Spearman r, r^2= 0.88, P<0.0001), down and carcass (Spearman r, r^2= 0.95, P<0.0001) from chick corpses. Significant differences were observed between mean mercury levels in down, liver and carcass from chick corpses (Kruskal-Wallis, P<0.05) with the highest mean mercury values in down (Table 1). Mean concentrations of mercury in eggs varied between 0.04 and 1.13 µg Hg / g d.w. No geographic or inter-year variations were found for mercury concentration in eggs (one-way ANOVA, P>0.05). No significant correlation was observed across sites between mercury levels in flooded eggs and feathers from live chicks (Pearson r, P>0.05). Mercury concentrations differed between eggs and chick feathers (t test, P<0.05) and eggs showed the lowest mean mercury levels for each sampling site and period (Table 1).

Despite there being no significant correlation between feather mercury levels and biometry of live chicks (Pearson r, P>0.05), mercury concentration differed significantly between two plumage groups, down from hatchings and feathers from fledglings, in Vau saltpans (t test, P<0.05) and in Giganta rice-fields (t test, P<0.05). The highest mean value in Vau saltpans belonged to hatchling down (2.20 ± 1.26 µg Hg / g f.w.) and the highest mean mercury value in Giganta rice-fields belonged to fledgling feathers (3.41 ± 1.50 µg Hg / g f.w.). Because significant differences were observed between hatchling down and fledgling feather levels in Vau saltpans and Giganta rice-fields, fledgling feather has been chosen as the main monitoring unit in the present study, and significant geographic variation was observed. Mean mercury concentrations in fledgling feather varied between 0.1 and 7.9 µg Hg / g f.w, and it varied significantly between wetlands but not between years (two-way ANOVA, P<0.005). Tagus

estuary showed the highest mean mercury level and differed significantly from other wetlands in 1997 (Tukey, $P<0.05$). Large amounts of mercury were found in Tagus estuary in the 1980s (Figueres et al. 1985; Fowler 1990). The lowest mean mercury level was observed at Mondego estuary and it differed significantly from the other wetlands in 1999 (Tukey, $P<0.05$) (Table 2). Significant differences for mercury concentrations in feathers from live fledglings were also found between sites within the Tagus estuary in 1997 (one-way ANOVA, $P<0.0001$) and 1999 (one-way ANOVA, $P<0.05$) and between sites within Formosa lagoon in 1997 (one-way ANOVA, $P<0.005$). Differences in mercury levels between sites indicate that there is a strong pattern of localized contamination, and fledgling feather samples provided a consistent measure of geographical patterns.

Table 1. Total Hg concentration in egg (μg/g, d.w.), chick feather (μg/g, f.w.) during 1997, 1998 and 1999, in down (μg/g, f.w.), and liver and carcass (μg/g, d.w.) from chick corpses during 1998 at different sites.

year	tissue	Giganta	Vasa-Sacos	Vau-P	Vau-N	Vaia
1997	egg	0.5 ± 0.2 n=3 (0.32–0.69)	0.8 ± 0.4 n=4 (0.43–1.23)	-	-	-
	chick feather	3.4 ± 1.5 n=40 (0.10–7.97)	1.4 ± 0.5 n=11 (0.96–2.12)	-	-	-
1998	down	-	3.0 ± 1.2 n=17 (1.24–4.92)	1.4 ± 0.3 n= 4 (0.96–1.72)	1.2 ± 0.6 n= 7 (0.53–2.10)	-
	liver	-	1.4 ± 0.6 n=16 (0.50–2.38)	0.8 ± 0.4 n= 4 (0.36–1.36)	0.5 ± 0.3 n= 7 (0.23–1.04)	-
	carcass	-	0.9 ± 0.3 n=17 (0.49–1.57)	0.3 ± 0.1 n= 4 (0.18–1.42)	0.3 ± 0.2 n= 7 (0.15–0.60)	-
	egg	0.4 ± 0.1 n=6 (0.29–0.65)	0.5 ± 0.3 n=9 (0.23–1.23)	0.5 ± 0.3 n=24 (0.22–1.25)	0.4 ± 0.3 n=26 (0.05–1.43)	-
	chick feather	2.4 ± 1.2 n=14 (1.11–4.49)	2.9 ± 1.1 n=6 (0.90–4.08)	1.7 ± 0.8 n=21 (0.53–5.57)	2.2 ± 1.6 n=20 (0.36–6.22)	-
1999	egg	-	1.3 ± 0.5 n=16 (0.51–2.54)	-	-	0.6 ± 0.3 n=5 (0.30–1.06)
	chick feather	-	2.1 ± 0.6 n=14 (0.81–3.29)	-	-	2.3 ± 0.7 n=10 (1.09–3.25)

Values refer to average, standard deviation, sample size, minimum and maximum.

Table 2. Total Hg concentration in chick feather (μg/g, f.w.) in wetlands during 1997, 1998 and 1999.

wetlands	1997	1998	1999
Tagus estuary	2.8 ± 1.8 n=52 (0.10-7.97)	1.8 ± 1.4 n=60 (0.36–6.22)	2.4 ± 1.0 n=21 (1.10–5.20)
Sado estuary	1.1 ± 0.5 n=23 (0.20–2.54)	1.7 ± 0.7 n=11 (0.54–2.62)	2.1 ± 0.6 n=4 (1.23–2.43)
Formosa lagoon	1.2 ± 0.9 n=21 (0.19–2.54)	-	2.5 ± 1.5 n=11 (0.96–4.45
Mondego estuary	0.7 ± 0.2 n=9 (0.49–0.97)	0.9 ± 0.8 n=34 (0.05–2.19)	0.8 ± 0.6 n=26 (0.07–3.18)

Values refer to average, standard deviation, sample size, minimum and maximum.

According to results in the present study, mercury concentration in down was positively correlated with mercury in liver and carcass, and mercury concentration in down was significantly higher than mercury concentration in other tissues. During its growth period chick plumage retains an important part of the mercury burden in the chick body. In the embryonic development down can have an important effect in decontaminating the body. The burden of mercury can even increase in chicks without the mercury concentration in soft tissues increasing, because mercury is transferred from soft tissues to growing down or feather (Becker et al. 1993a). Although in hatchlings the mercury level is determined mainly by mercury concentration in eggs, in fledglings the mercury uptake by food ingestion is more important. In a well-grown chick the mercury dose present in the egg is a negligible proportion of the total mercury burden in the chick body and the main factor influencing feather mercury level is the mercury entering the body by food ingestion (Becker et al. 1993a).

Feather and egg mercury levels were not significantly correlated across sites, and although feather mercury levels showed geographic variation for Black-winged Stilt chicks, egg levels did not. Other studies have shown that sampling eggs can show local geographical variation in mercury contamination (e.g. Gochfeld 1997; Becker et al. 1998; Braune et al. 2002) but this was not the case in the Black-winged Stilts in this study, perhaps either because levels in eggs were uniformly low, or because stilts may have accumulated resources for egg production while ranging across the region rather than from a local area where they nested. Egg contamination represents about 20% of the female contamination during a limited period before egg laying, and egg mass represents only 2-8% of the mass of a completely grown chick, which explains why egg contamination provides only a trivial part of fledging contamination (Monteiro and Furness 1995). Moreover, mercury acquired from the egg tends to be eliminated in the first down of chicks,

whereas levels in chick feathers reflect chick contamination during the growth period (Becker et al. 1993b). In the case of Black-winged Stilt, this period represents between 3 and 4 weeks (Dubois 1994). During that period the Black-winged Stilt chick has not acquired flight abilities and is limited to the breeding ground. Because they are nidifugous and precocial they feed on the available prey inside that limited area. During the first weeks chicks show a high growth rate and the rapidly increasing body mass may be the cause of the dilution effect, reducing the mercury concentration in chick tissues (Becker et al. 1994).

Although mercury levels in chick plumage did not correlate with chick biometry, which can be used as a proxy for chick age, significant differences in mercury levels were found between down from hatchlings and feather from fledglings, and different patterns were observed between Giganta rice-fields and Vau saltpans. In Vau saltpans feathers from fledglings showed lower mercury levels than down from hatchlings. This pattern can be considered as due to the dilution effect in growing tissues, and is typical for bird species in environments that are not highly contaminated (Becker et al. 1994; Wenzel et al. 1996). In Giganta rice-fields feathers from fledglings showed higher mercury levels than down from hatchlings. In growing chicks, mercury concentration in chick feather reflects the balance between the mercury input from dietary uptake and the dilution effect as a result of increasing body mass (Becker et al. 1994; Stewart et al. 1997). The dilution effect is apparently not seen at highly contaminated sites as Giganta rice-fields and the enhanced input of mercury into the chick body through contaminated food results in a higher level of accumulation of mercury in chicks as they grow.

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