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Mercury in White Stork (*Ciconia ciconia*) Chick Feathers from Northeastern Mediterranean Areas in Relation to Age, Brood Size, and Hatching Order

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Abstract Mercury (Hg) levels in white stork (*Ciconia ciconia*) feathers collected in the mid-1990s from five northeastern Mediterranean (Greece) areas varied, with mean ranges between 301 ng g^{-1} dry weight (dw) (Pinios River) and 1911 ng g^{-1} dw (Sperchios Delta). A significant increase of Hg levels in chick feathers with age (surrogated by bill size) was found in the Evros and Pinios River areas, a nonsignificant increase in the Amvrakikos Gulf and the Epirus Region, and a marginally significant decrease in the Sperchios Delta area. For combined data of 1993 and 1995, Hg concentrations did not differ significantly in relation to hatching order among broods but differed significantly in relation to brood size being higher in 4-chick broods than those in 3-chick broods. All 10 areas formed 4 groups with levels mutually significantly different. Highest levels were detected in the Evros, Axios, and Sperchios riverine areas, whereas the lowest levels occurred at Drama plain, which lacks large water bodies in its vicinity. Levels were lower than those associated with intoxication to other ciconiiform species.

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Mercury (Hg) has long been known as a ubiquitous element. In the Mediterranean region, natural emissions originate from volcanoes, fumaroles, and solfataras as well as contributions from widespread geologic anomalies (Ferrara et al. 2000). Anthropogenic sources are combustion of fossil fuels, roasting and smelting of ores, kiln operations in cement industry, incineration of wastes, and production of certain chemicals (Pacyna et al. 2001; Pirrone et al. 2003). Due to its high toxicity, Hg poses threats to the health of humans and wildlife. Particularly in the Mediterranean, levels in various fish and shellfish are higher than those from Atlantic areas (Bernhard 1983). High levels of methylmercury taken up through fish consumption result in acute toxicity, leading to symptoms known as Minamata disease (Eisler 1987). Exposure to environmental Hg may have negative effects on human health, such as cognitive deficits, heart attack, and premature death (Swain et al. 2007). Wildlife, particularly birds, has also been affected. In adult birds high (15 ppm wet weight) methylmercury levels in tissues may be lethal; nonlethal lower Hg levels affect reproduction by impairing hatchability and embryonic mortality (Scheuhammer et al. 2007) and also affect immune, detoxification, and nervous systems in young birds (Henny et al. 2002).

Hg (as methylmercury) is biomagnified in avian food chains and can reach high levels, especially in fish-eating birds, showing a clear trophic level relation (Burger and Gochfeld 1997a). Birds are able to excrete, during moulting, a considerable part of their Hg body burden in the form of methylmercury (Thompson and Furness 1989; Lewis and Furness 1991; Lewis et al. 1993). Feathers of young water birds have proved an excellent means of assessing Hg exposure and occurrence of environmental levels because Hg in feathers of growing birds is accumulated from the surroundings of the breeding area during a narrow period of chick growth, thus reflecting local pollution (Becker et al. 1993a; Burger 1993; Monteiro and Furness 1995; Stewart et al. 1997). Consequently, feathers have been used as Hg monitors in long-term environmental-monitoring projects (Thompson et al. 1993).

Ciconiiforms (herons, egrets, ibises, and allies) are primarily fish-eating birds, top predators in aquatic food chains, and therefore have been used as Hg monitors in these environments (Burger and Gochfeld 1993, 1997b; Boncompagni et al. 2003). Although some species have been extensively studied with regard to their feather Hg content, levels in others are relatively unknown. The white stork (*Ciconia ciconia*) is a large wading bird whose populations underwent a large decline between 1970 and 1990. Its population size is large and appears to be increasing; thus, the species is categorised as of "least concern" (Birdlife International 2004, www.birdlife.org). In Greece, its population is between 2000 and 2500 pairs (Tsachalidis and Papageorgiou 1996; Goutner and Tsachalidis 2007). Within a study of the breeding biology of the white stork in Greece (1993 to 1995) (Tsachalidis and Goutner 2002; Goutner and Tsachalidis 2007), chick feathers were collected with the following aims: (1) to show spatial trends in Hg levels; (2) to investigate the extent to which Hg accumulation is affected by chick age, brood size, and hatching order; and (3) to evaluate Hg exposure and its toxicologic significance for the white stork.

Methods

Study Area and Sample Collection

Back feathers from white stork chicks were collected at five areas in 1995. Each area includes several villages because most birds placed their nests on human constructions, primarily electric poles and occasionally on churches, houses, and trees. These areas are as follows: the Evros River ($n = 1$ village), the Pinios River ($n = 2$), the Amvrakikos Gulf ($n = 2$), the Sperchios Delta ($n = 3$), and the Epirus Region ($n = 4$).

Goutner and Furness (1998) reported Hg levels in feathers of white stork chicks from five different areas in 1993: Drama Plain ($n = 2$ villages), Nestos Delta ($n = 5$), Lake Koronia ($n = 3$), Lake Kerkini ($n = 2$), and Axios River ($n = 2$). Data from these areas were also used in some analyses to increase sample size (see Statistical Analysis subsection for details).

All villages sampled were surrounded by agricultural fields and were associated with wetlands, except for those at Drama plain and the Epirus Region, both of which lacked nearby large water bodies. For details on village names and geographic coordinates, refer to Goutner and Furness (1998) and Tsachalidis and Goutner (2002). All sampled areas are indicated in Fig. 1. Information on the rivers involved in this study is given in Skoulikidis (2009).

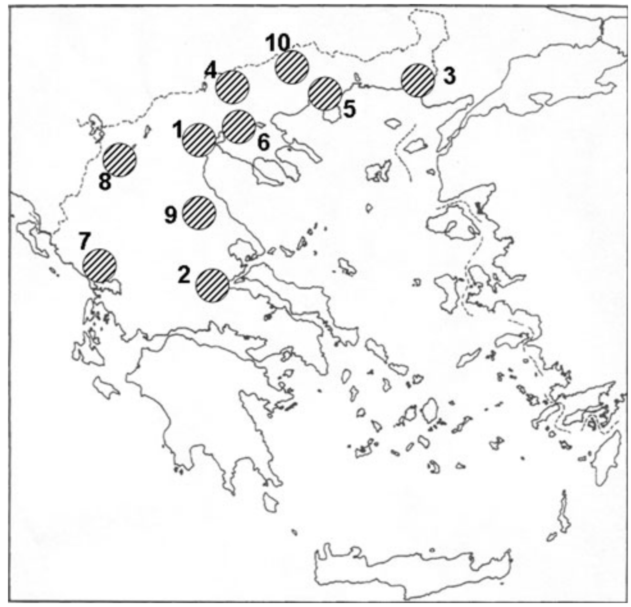

Fig. 1 Map of the areas where white stork chick feathers were sampled. *1* Axios River, *2* Sperchios Delta, *3* Evros River, *4* Lake Kerkini, *5* Nestos Delta, *6* Lake Koronia, *7* Amvrakikos Gulf, *8* Epirus Region, *9* Pinios River, *10* Drama Plain. The areas' numbers refer to their respective row in Fig. 4

Nests were accessed from a hydraulic lift operated by technicians of the Public Electric Corporation. During visits at nests for ringing, biometry, and pellet collection, a small quantity of back feathers was collected from each chick. Feathers were removed from between the chicks' shoulders and were placed in polythene bags. Feather sampling took place when chicks at most nests were well grown. A collection of feathers in more recent years for temporal comparisons was not possible due to the high cost charged by the Public Electric Corporation for the use of equipment and personnel.

Sample Analysis

Samples of 1995 were analysed by the Institute of Avian Research in the ICBM-Terramare in Wilhelmshaven, Germany. Hg measurement was accomplished with the DMA80 Direct Mercury Analyzer (Mikrowellen Labor Systeme, Leutrich, Germany). A single feather was weighed, wrapped in aluminum foil, and introduced into the sample boat. All samples were analysed in duplicate. The analytic performance of this method was evaluated by analysis of certified reference materials and has been proposed as a ready-to-use analytic method by which to analyse a large number of samples in a short time frame (Roy and Bose 2008; Maggi et al. 2009). Furthermore, the laboratory ICBM-Terramare takes part in international intercalibrations (i.e., Quasimeme) with satisfactory results. The detection limit is 0.003 ng total Hg. The concentrations are expressed as ng g^{-1} dry mass.

Samples collected in 1993 were analysed according to Goutner and Furness (1998) using a DA 1500.DP6 Mercury Vapour Detector (Data Acquisition Ltd, Stockport, Cheshire, UK) at the University of Glasgow (Goutner and Furness 1998). To certify results, parallel reference materials were measured.

Statistical Analysis

Kania (1988) and Gangloff et al. (1989) found that bill growth of white stork chicks does not reach an asymptote even after 70 days of life, the age at which nestlings are able to fly (Hancock et al. 1992). Tsachalidis et al. (2005) found that, in Greece, the growth of white stork chicks' bill is linear during the first 51 days of life (bill sizes 20 to 140 mm). In our data set, bill length ranged 81 to 155 mm, with most sizes falling within ranges reported in Tsachalidis et al. (2005). Because bill growth is a linear function of age, it can be used as an age surrogate for comparisons. Hatching order was then assigned to each chick within each brood according to bill size. The chick with the largest bill was considered as the first-hatched and so on. Within-area trends in Hg concentration were tested with linear regression (Zar 1999).

Data sets from 1993 and 1995 were combined for subsequent analyses to increase sample size and examine differences between the 10 studied areas. Because samples were taken from nestlings of different age among areas and between and within broods, general linear models (GLMs) were used to control for the effect of age difference on Hg concentrations (Crawley 1993). Only complete broods (all chicks were measured and sampled) were used to test between- and within-brood differences, whereas all data were used for among-areas comparisons. Hg was the response variable, area (fixed effects), hatching order (fixed effects), and brood size (random effects) were the categoric factors, and bill length was the covariate. First, we tested if the covariate had different effects at different levels of the factors (Homogeneity of Slopes model, Statistica GLM procedure). If slopes were homogenous, analysis of covariance (Statistica GLM procedure) was used to evaluate differences in Hg concentrations; if not, the separate slopes model routine was run (Statistica GLM procedure).

Assumptions of normality and equal variances were tested, and Hg data were log-transformed for all analyses. Type III sum of squares was used. Arithmetic and geometric means are given to facilitate comparison with other studies in the literature. All results are reported as means ± SEs unless otherwise stated. Hg concentrations are reported in parts per billion on a dry-weight basis (ng g^{-1} dw). All statistical analyses were performed using Statistica, version 6.0 (StatSoft, Tulsa, OK, USA).

Results

In 1995, Hg levels in back feathers from white stork nestlings (estimated age 26 to 56 days, based on bill length; Tsachalidis et al. 2005) ranged from 59 ng g^{-1} dw (Epirus Region) to 2439 ng g^{-1} dw (Sperchios Delta), with arithmetic mean concentrations for the five study areas ranging from 301 ng g^{-1} dw (Pinios River) to 1911 ng g^{-1} dw (Sperchios Delta; Table 1). Linear regressions of log-transformed Hg concentrations with bill size (i.e., the age surrogate) indicated a significant increase with chick age in the Evros ($R^2 = 0.296$, $a = 2.739$, $b = 0.0039$, df = 1,45, $F = 18.940$, $p = 0.00008$) and Pinios areas ($R^2 = 0.195$, $a = 1.799$, $b = 0.0052$, df = 1,24, $F = 5.834$, $p = 0.0024$). The trend also increases, but not significantly so, at the Amvrakikos Gulf ($R^2 = 0.063$, $a = 1.424$, $b = 0.0079$, df = 1,49, $F = 3.286$, $p = 0.076$) and Epirus Region ($R^2 = 0.044$, $a = 1.423$, $b = 0.0072$, df = 1,40, $F = 1.836$, $p = 0.183$). Hg content decreased with bill length at the Sperchios Delta, although on a marginal significance level ($R^2 = 0.117$, $a = 3.506$, $b = -0.0019$, df = 1,31, $F = 4.105$, $p = 0.051$) (Fig. 2).

Using both 1993 and 1995 data sets, age (bill length surrogated) had no effects on the different categories of the variable area (GLM, Homogeneity of Slopes model: df = 9,356, $F = 1.684$, $p = 0.091$). Significant differences,

Table 1 Mean concentrations (±SE, sample size, range) of Hg (ng g^{-1}) in back feathers of white stork nestlings taken from five areas in Greece in 1995

Mean concentrations	Evros River	Pinios River	Amvrakikos Gulf	Sperchios Delta	Epirus Region
Arithmetic mean	1487 ± 31	301 ± 31	357 ± 48	1911 ± 58	343 ± 53
Geometric mean	1472	270	242	1881	227
n	47	26	51	33	42
Range	1035–1957	146–733	70–1411	1263–2439	59–1218

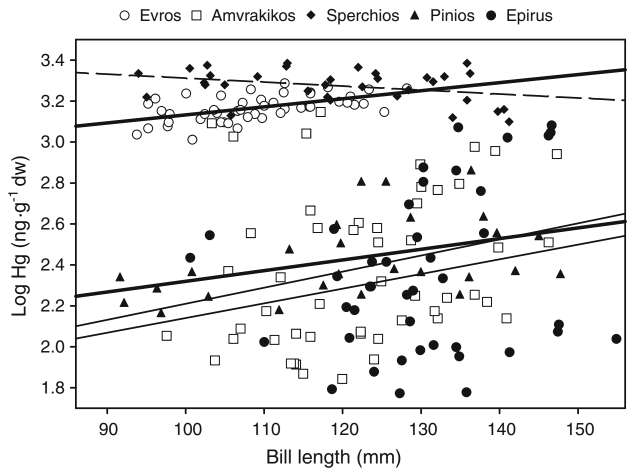

Fig. 2 Hg concentrations of white stork chick feathers, taken from five areas in Greece in 1995, in relation to bill length. *Bold lines* indicate significant increase (Evros, Pinios), and the *dashed line* indicates marginally significant decrease (Sperchios)

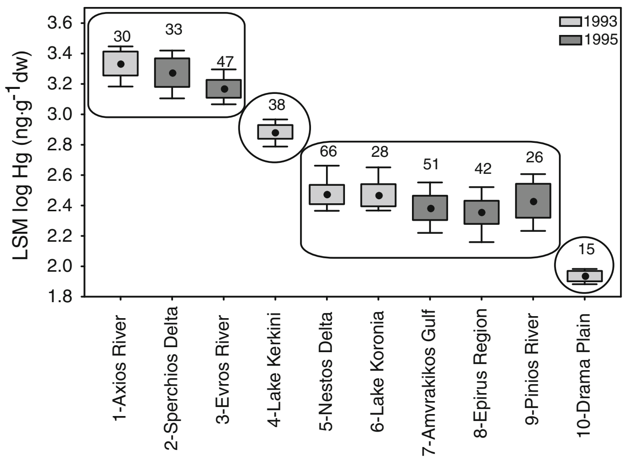

Fig. 4 Hg levels of white stork chick feathers from 10 areas in Greece. Area numbers correspond to those in Fig. 1. Levels are presented in a descent rank to display the 4 significantly differing groups (*circled*). Indicated are the least squares means (LSM) computed for chick with 112.3 mm bill (approximate age 39 days, covariate mean) (*dots*), two SDs (*boxes*), range (*whiskers*), and sample sizes (*above whiskers*)

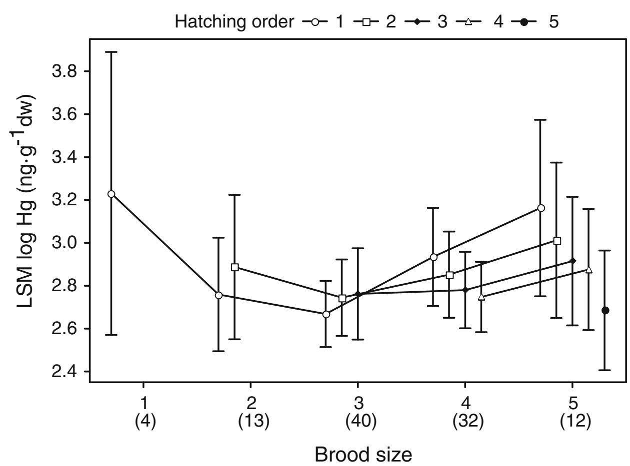

Fig. 3 Interbrood and intrabrood variation of least squares means (LSM) of Hg concentrations of white stork chick feathers, taken from 10 areas in Greece (five in each 1993 and 1995), computed for chicks with 112.3 mm bill (approximate age 39 days, covariate mean). *Vertical bars* denote 0.95 confidence intervals. Sample sizes are given in parentheses

however, were detected between age and different brood size (df = 4,328, $F = 12.138$, $p < 0.00001$) and hatching order (df = 4,328, $F = 3.133$, $p = 0.015$).

Thus, in a further separate slopes model (GLM), Hg concentrations differed significantly in relation to brood size (GLM, separate slopes model: df = 4,308, $F = 32.906$, $p < 0.00001$) but not with hatching order (GLM, separate slopes model: df = 4,308, $F = 0.294$, $p = 0.878$) or their interaction (GLM, separate slopes model: df = 6,308, $F = 0.194$, $p > 0.900$) (Fig. 3). Post hoc comparisons with Tukey's Honestly Significant Differences (HSD) test for unequal sample sizes showed that Hg levels in four-chick broods were significantly higher than those in three-chick broods ($p = 0.01$). Among all other brood sizes, Hg levels did not differ significantly (Tukey's HSD test: $p > 0.100$ for all other pairwise comparisons).

The interactions of area with both brood size and hatching order were not significant (GLM, separate slopes model: brood size df = 2,150, $F = 1.336$, $p = 0.301$; hatching order df = 21,150, $F = 7.199$, $p > 0.900$).

Data from all areas were then pooled together to examine geographic differences in Hg levels (Fig. 4). Hg levels were found to be significantly different among the 10 study areas (ANCOVA, df = 9,365, $F = 93.391$, $p < 0.0001$). Post hoc Tukey unequal N HSD test indicated 4 groups with increasing Hg levels: Drama < Amvrakikos, Pinios, Epirus, Nestos, and Koronia < Kerkini < Evros, Axios, and Sperchios. The differences between areas in different group areas were all $p < 0.0001$. Within-groups differences were not significant. Highest levels were found at some river areas and the lowest (Drama) at dry habitats (i.e., lacking water bodies in their vicinity). Lakes (Koronia, Kerkini) were in the intermediate Hg-level group.

Discussion

Concentrations

Hg levels detected in white stork chick body feathers from Greece can be considered low to moderate. Compared with some common ciconiiform species' levels (mostly from the 1990s), lowest mean white stork levels are within the lower part range of the primarily insectivore cattle egret (*Bubulcus ibis*), whereas maxima are in the mid-range of

Table 2 Levels of Hg detected in feathers of ciconiiforms from various geographic areas[a]

Species	Mean or range of means	Ranges	Area	Collection year	Reference
Herons					
Black-crowned night heron (*N. nycticorax*)	300–1700	200–3800	Honk Kong	2000	Connell et al. (2002)
	810–1250[b]	177–5180	Delaware and Chesapeake Bays	1998	Golden et al. (2003)
	810–1690[b]	597–1950	United States	1998–1999	Custer et al. (2008)
	820–1800[c]		Szechuan, China and Honk Kong	1992	Burger and Gochfeld (1993)
	1982[b]	1244–2640	Italy	1994	Fasola et al. (1998)
	2140	2100–2200	Iran	2005	Zolfaghari et al. (2009)
	2110–3010[c]	530–9980	Axios Delta, Greece	1993–1994	Goutner and Furness (1997)
	35,250[b]	21,930–67,070	Carson River, Nevada, United States	1998	Henny et al. (2002)
Pond heron (*Ardeola bacchus*)	970[c]		Szechuan, China	1992	Burger and Gochfeld (1993)
Purple heron (*Ardea purpurea*)	1600–8160		Ebro Delta, Spain	2006	Barata et al. (2010)
Squacco heron (*Ardeola ralloides*)	2790–3560	360–6540	Axios Delta, Greece	1993–1994	Goutner et al. (2001)
	880	860–910	Iran	2005	Zolfaghari et al. (2009)
Little bittern (*Ixobrychus minutus*)	770	460–940	Iran	2005	Zolfaghari et al. (2009)
Egrets					
Little egret (*Egretta garzetta*)	310–360[b]	40–580	Pakistan	1999–2000	Boncompagni et al. (2003)
	563		Bali	1990s?	Burger and Gochfeld (1997b)
	1500[c]		Honk Kong	1992	Burger and Gochfeld (1993)
	1600–1700		Ebro Delta, Spain	2006	Barata et al. (2010)
	1690–3320[c]	530–9110	Axios Delta, Greece	1993–1994	Goutner and Furness (1997)
	2587[b]	1296–9076	Italy	1994	Fasola et al. (1998)
	300–4100	0–7100	Honk Kong	2000	Connell et al. (2002)
Cattle egret (*Bubulcus ibis*)	60		Sulawesi	1990s?	Burger and Gochfeld (1997b)
	100[b]	30–300	Pakistan	1999–2000	Boncompagni et al. (2003)
	383		Bali	1990s?	Burger and Gochfeld (1997b)
	770	750–800	Iran	2005	Zolfaghari et al. (2009)
	1000[c]		Honk Kong	1992	Burger and Gochfeld (1993)
Intermediate egret (*Egretta intermedia*)	340[b]	140–1190	Pakistan	1999–2000	Boncompagni et al. (2003)
	1127		Bali	1990s?	Burger and Gochfeld (1997b)
Great egret (*Ardea alba*)	260–740[c]		Honk Kong	1992	Burger and Gochfeld 1993
	1600–6250		Florida Everglades, United States	2006–2007	Herring et al. (2009)
	3200–4400		Florida Everglades, United States	1999–2000	Rumbold et al. (2001)

Table 2 continued

Species	Mean or range of means	Ranges	Area	Collection year	Reference
Snowy egret (*Egretta thula*)	30,640[b]	14,680–59,770	Carson River, Nevada, United States	1998	Henny et al. (2002)
Storks					
Marabou stork (*Leptoptilos crumeniferus*)	810	240–2000	Uganda	2003	Hollamby et al. (2004)
Wood stork (*Mycteria americana*)	1230–5670[d]		Georgia and S. Carolina	1997–1999	Gariboldi et al. (2001)
White stork (*C. ciconia*)	90–2160[b]	20–4090	North Greece	1993	Goutner and Furness (1998)
	242–1881[b]	59–2439	West, central, east Greece	1995	This study

[a] All values have been transformed to ng g^{-1} dw

[b] Geometric means

[c] Medians

[d] LSM

those found in the black-crowned night heron (*Nycticorax nycticorax*) and great and little egrets (*Ardea alba* and *Egretta garzetta*, respectively) in various part of their range (Table 2). Some of these levels might not be comparable with those of white storks because the relevant feather samples were collected in more recent years, when Hg emissions have been decreased (Pacyna et al. 2001, 2006; Swain et al. 2007). Nevertheless, this fact is not reflected in more recent measurements, denoting that more important for Hg accumulation is the position of each particular species in the food chain.

Hg Concentrations Relative to Chick Age

Whereas Hg levels found in water bird down are closely associated with levels in the egg (Becker et al. 1993b), those found in growing feathers are largely due to Hg ingested in food collected and fed by the parents during the chicks' relatively short period of growth (Becker et al. 1994). The white storks data had different patterns of Hg accumulation in relation to age. First, in two areas (Amvrakikos and Epirus, Fig. 2), levels were unrelated to age. Like this study, no age-associated Hg increase in chicks was found in Greece for little egret (Goutner and Furness 1998) and Squacco heron (*Ardeola ralloides*, Goutner et al. 2001). This relation can be found when, during a given time period, the amounts eliminated and ingested are similar. Consequently, there will be no change in levels of feather types subsequently growing (Becker et al. 1994). An apparent independence may also be due to the narrow range of chick ages in the samples (Goutner et al. 2001; Rumbold et al. 2001): Due to high rates of growth, feeding, and depuration, even small differences in age distribution can greatly influence where a chick is located in the uptake curve (Rumbold et al. 2001).

Second, there was a negative relation between Hg levels and chick age in one area (Sperchios, Fig. 2). This is probably the most commonly reported relation during this growth phase. The decrease of Hg concentrations in feathers during the advancing course of plumage development indicates depletion of the body Hg pool (Becker et al. 1994). It has been found in the black-crowned night heron (Goutner and Furness 1997), Cory's shearwaters (*Calonectris diomedea*), roseate tern (*Sterna dougallii*), and common tern (*Sterna hirundo*) chicks (Monteiro et al. 1995) as well as in common guillemot (*Uria aalge*), kittiwake (*Rissa tridactyla*, Stewart et al. 1997), and little tern (*Sterna albifrons*, Tavares et al. 2005). This kind of relation is explained by the "dilution effect," i.e., Hg dilution in tissues occurs during the phase of rapid growth related to a high rate of protein synthesis in chicks and loss from the tissue as existing protein undergoes degradation due to turnover (Lewis and Furness 1991; Stewart et al. 1997; Tavares et al. 2005).

Third, a significant Hg increase with age was found in two areas (Evros and Pinios, Fig. 2), in concordance with the 1993 general tendency (Goutner and Furness 1998). Similar relations were found in the great egret (Rumbold et al. 2001; Herring et al. 2009). Both studies rely on Monteiro and Furness (1995) in explaining that increases in Hg concentration with age of nestlings require that the natural growth dilution be overcome by a certain level of Hg exposure, which is found in heavily contaminated environments. For the related Korean species, *Egretta alba modesta*, Honda et al. (1986) suggested age or exposure time as dominant factors in Hg increase with age. In addition, a positive correlation was found in marabou storks (*Leptoptilos crumeniferus*), which was attributed to a long fledging period and consequent greater dietary exposure and potential accumulation of Hg in the growing feather with age (Hollamby et al. 2004). In addition, increased Hg concentrations with age found in common tern chicks in polluted areas was attributed to enhanced introduction of Hg into the young by contaminated food (Becker et al. 1993b). In these cases, the ingested Hg quantities exceed those excreted, resulting in higher levels in the feathers growing later (Becker et al. 1994).

These findings suggest that in Evros and Pinios areas, the chicks were fed more polluted food as they grew. Pellet analysis from all feather sampling areas indicated that the major components were insects, particularly coleopterans and orthopterans, but in proportions that were different among some of the areas (Tsachalidis and Goutner 2002). The composition of the diet cannot explain the variability in Hg patterns found. In addition, although there were indications that prey, such as fish, was fed to chicks, this prey type was not found in pellets (Goutner and Furness 1998; Tsachalidis and Goutner 2002). Thus, compared with what pellet analyses have shown, it seems that Hg in white stork feathers rather reflects aquatic pollution of their breeding areas. Nevertheless, as recently indicated, terrestrial prey, such as orthopterans, from the habitats adjacent to polluted rivers may transfer considerable amounts of Hg to the terrestrial birds (Cristol et al. 2008). This perspective cannot be excluded as a way of Hg transfer to white stork in the polluted rivers of Evros and Pinios, although further research should clarify the prey types that contribute to the increase of white stork chick Hg body burden with increasing age.

In this study, when controlling for age, Hg levels did not significantly vary with hatching order, both across and within broods, as was also found in herring gull (*Larus argentatus*), black-headed gull (*L. ridibundus*), and common tern (Becker et al. 1994). Figure 3, however, shows a tendency of decreasing Hg levels with hatching order, which was also shown in herring gull and common tern down (Becker et al. 1994). In squacco heron, the differences in feather Hg concentration between nestling ranks made up only 10% of the total variability in Hg, whereas the remaining 90% resided among broods (Goutner et al. 2001), implying a high level of variability in the Hg content of the food brought to different broods by their parents.

In white stork, between-brood Hg variability was significant, with mean concentrations being higher in four-chick than in three-chick broods. White storks with four or five hatchlings could be individuals of high parental quality laying more eggs, thus promoting chick survival, and offering other food than white storks with three hatchlings (Sasvári and Hegyi 2001). Therefore, the differences found can be attributed to different levels of exposure. Temporal and spatial variation in habitat contamination may be significant factors (Custer et al. 2008; Padula et al. 2010). Hg is a spatial highly variable contaminant, and even birds from the same colony could well forage in different areas varying in contamination levels (Rumbold et al. 2001). Regarding white storks in Greece, differences may have been induced by the different proportions of main prey types among areas and major habitats (Tsachalidis and Goutner 2002). Inter- and intra-area differences in Hg contamination, as well as other factors unknown at present, may have contributed to the variation found in this study.

Spatial Trends

Hg is deposited in natural ecosystems through natural processes and human activities. Despite considerable pollution research on the rivers and lakes of Greece (summarised in Konstantinou et al. 2006), Hg pollution studies are rare and were only conducted in northern Greek water bodies in the 1980s (Fytianos et al. 1986). Axios River was reported as the water body most polluted with Hg, whereas Lake Koronia and Nestos River water samples indicated lower mean values. A pattern similar to this was found in white stork feathers in this study.

The significant differences in Hg pollution found among the 10 study areas suggest the influence of other than geologic factors (Karamanis et al. 2008). Multiple local or remote anthropogenic factors may significantly contribute to Hg pollution. Therefore, it is difficult to identify site-specific sources of pollution. Remote pollution is mainly transported through air and water. Especially in the Mediterranean, Hg deposition is greatly affected by air mass transportation of particulate and elemental Hg from northern and northeastern Europe. In addition, wet deposition is the most efficient removal pathway of atmospheric Hg, predicted in highest levels compared with mountainous areas such those in Greece, as expected, due to the higher precipitation usually occurring there (Pirrone et al. 2003). Mean annual precipitation in the river basins studied are ranked as Arachthos [91.2 mm (Amvrakikos system)] > Pinios (72.0) > Nestos (64.8) > Sperchios (64.4) > Axios and Evros (both 62.9) > Strymon [60.8 (Lake Kerkini system)] (Skoulikidis 2009). These values do not seem in accordance with the mean values of Hg in stork feathers (Fig. 4) i.e., precipitation in Amvrakikos and Pinios with lower feather Hg levels is much higher than Sperchios, Axios, and Evros (with highest feather Hg levels). This may suggest that wet atmospheric deposition was not reflected in the stork feather samples.

According to Skoulikidis (2009) the highest levels of heavy-metal pollution occur in transboundary rivers entering Greece. Water-pollution transport from neighbouring Serbia, the former Yugoslav Republic of Macedonia, Bulgaria, and Turkey can explain the increased Hg in the Axios and Evros river areas. Ferro-alloys, smelter and fertilizer plants disposing their solid waste near the river beds, untreated industrial and domestic wastewater discharging into the rivers, agricultural runoff and illegal landfills contaminating surface and ground water are the main remote sources of Hg pollution in the Axios and Evros areas (Erkmen and Kolankaya 2006; Milovanovic 2007; Skoulikidis 2009). Transboundary pollution may also affect Nestos River, with industrial effluents from timber industries and uranium mining at Eleshnitza, Bulgaria (Skoulikidis 2009). However, in recent years, Hg concentration in fish of Nestos River were low, not exceeding World Health Organization and United States Environmental Protection Agency health guidelines, and were suitable for human consumption (Christoforidis et al. 2008). In contrast, higher Hg concentrations were expected in Pinios feather samples not only due to higher precipitation but also receiving the effluents of two large towns, namely, Tricala and Larissa, and of some small industries (Bellos and Sawidis 2005). Nevertheless, discharge greatly decreases during the late spring and summer months (Bellos and Sawidis 2005), probably reflecting lower Hg availability in the environment during a period coinciding with stork chick growth. Karamanis et al. (2008) found that heavy-metal pollution in Pinios and Louros (Amvrakikos system) rivers was lower than that found in polluted areas.

The high feather Hg concentrations found in the Sperchios Delta area are difficult to explain. Point sources are unlikely to have significantly contributed to Hg load because there is no industry or large towns associated with the river catchment basin. In addition, the Sperchios River catchment is entirely Greek and does not receive transboundary pollution through water transport. Remote Hg pollution transfer through air and wet deposition cannot be considered higher than other studied areas due to lower mean precipitation values. The high Hg values associated with the Sperchios area may be associated to the high geothermal activity in this area: In Greece, the geothermal areas are frequently located in regions of Quaternary or Miocene volcanism (Mendrinos et al. 2010).

The increased Hg concentrations in the Lake Kerkini area are mainly due to pollution transferred by the Strymon River from nearby Bulgarian industries (Skoulikidis 2009), a fact that also explains the high Hg concentrations compared with other areas, especially Lake Koronia (the other lake in the sample), which is contaminated by local agricultural runoff, animal husbandry effluents, and industry wastewater (Gantidis et al. 2007).

Hg concentrations were the lowest in white storks from agricultural areas, namely the Epirus Region and Drama Plain areas. This can be explained by the lack of nearby water bodies near storks' breeding sites, suggesting a terrestrial diet, and the fact that the use of Hg compounds in agriculture has not been reported in Greece. Hg in white storks depending on terrestrial foraging habitats may be due to long-term atmospheric deposition. Haidouti et al. (1985) also found low Hg contents in soil profiles from 11 Greek agricultural areas (25 to 98 ng g^{-1}), which is within the ranges found in uncontaminated soils elsewhere in the world.

Toxicologic Significance

For some birds, adverse effects have been associated with total Hg concentrations of 5000 ng g^{-1} in feathers (Eisler 1987) but sensitivity to Hg is different among species (Heinz et al. 2009). Studies in ciconiiforms also indicated potential differences: Snowy egrets (*Egretta thula)* with mean Hg levels of 30,000 ng g^{-1} dw in feathers and black-crowned night herons with 35,000 ng g^{-1} presented adverse health effects (Henny et al. 2002; Table 2). The highest values of approximately 10,000 ng g^{-1} median Hg concentrations in feathers of black-crowned night heron chicks from the Axios Delta were reported to result in chick growth inhibition (Goutner and Furness 1998). In the same area, squacco herons had 2790 to 3560 ng g^{-1} median Hg concentrations in feathers (maximum 6540 ng g^{-1}) without visible effects on growth (Goutner et al. 2001). However, Connell et al. (2002) suggested that the threshold for adverse effects is 3000 to 5000 ng g^{-1} Hg in feathers of ardeids.

To our knowledge, there are no other studies reporting Hg levels in white stork feathers but only a few studies of eggs and livers (see Goutner and Furness 1998). According to the above mentioned, it seems that the Hg levels detected in the white stork feathers in Greece were lower than those associated with adverse effects. Nevertheless, the threshold levels of adverse effects to white stork are currently unknown. Heinz et al. (2009) categorised bird species according to their sensitivity to Hg intoxication during experimental injection of methylmercury in laboratory-incubated eggs. It is noteworthy that, among the five species that appeared more sensitive, three were ardeids, namely white ibis (*Eudocimus albus*, $LC_{50} = 0.22$), snowy egret ($LC_{50} = 0.15$), and tricolored heron (*Egretta tricolour*, $LC_{50} = 0.22$). This suggests that at least several ciconiiform species may exhibit increased sensitivity; therefore, Hg levels that have been considered safe for the white stork might in fact be unsafe.

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