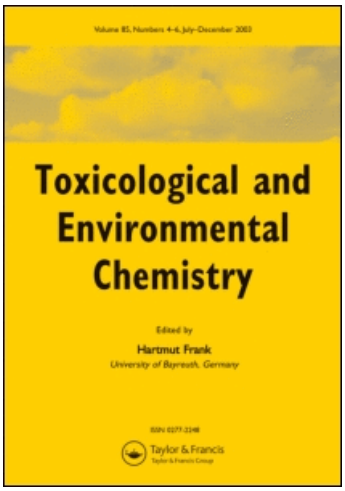

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Vassilis Goutner [a]; Robert W. Furness [b]
[a] Department of Zoology, Aristotelian University of Thessaloniki, Thessaloniki, Macedonia, Greece [b] Applied Ornithology Unit, Graham Kerr Building, University of Glasgow, Glasgow, UK

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FEATHERS OF WHITE STORK *CICONIA CICONIA* CHICKS IN NORTH-EASTERN GREECE, AS INDICATORS OF GEOGRAPHICAL VARIATION IN MERCURY CONTAMINATION

VASSILIS GOUTNER[a,*] and ROBERT W. FURNESS[b]

[a]*Department of Zoology, Aristotelian University of Thessaloniki, GR-54006 Thessaloniki, Macedonia, Greece;* [b]*Applied Ornithology Unit, Graham Kerr Building, University of Glasgow, Glasgow G12, 8QQ, UK*

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Variations in mercury contamination of ecosystems were assessed among areas of northeast Greece by sampling and analysing feathers of White Stork chicks. Mercury concentrations in stork chick feathers were highest in an area known to be affected by riverborne mercury pollution, and were lowest in an inland area away from rivers or lakes. Although regurgitated pellets indicated a predominantly terrestrial diet among White Stork adults, variation in mercury concentrations among localities seemed to relate to pollution of aquatic rather than terrestrial foods of storks. Toxic thresholds of mercury in this species are unknown, but the highest concentrations, observed in chicks from the Axios Delta (up to 4.1 $\mu g \cdot g^{-1}$ in feathers) were well above levels having toxic effects in gamebirds. Mercury concentrations in chick feathers increased slightly with chick age and with brood size, but these trends were small by comparison with variations among localities.

Keywords: Mercury; pollution; bioindicators; stork

INTRODUCTION

Effluent discharge through rivers is a major reason for the pollution of the Mediterranean sea (Hernandez *et al.* 1990). Elevated levels of mercury, a metal particularly harmful for human and wildlife (Ohlendorf *et al.* 1978) occur in water, sediments and biota in countries surrounding the Mediterranean (e.g. Fytianos *et al.* 1986, Focardi *et al.* 1988, Batty *et al.* 1996). Toxic effects of

* Corresponding author.

mercury on birds include embryonic deaths, disturbed cell division resulting in reduced fertility (Ramel 1967), brain lesions, subcutaneous edema and neurological disturbance (Ohlendorf *et al.* 1978, Scheuhammer 1987, Thompson 1996). Reduced egg hatchability and retarded growth may result in reduced fledging success (Heinz 1976, Scheuhammer 1987). Birds and especially waterbirds, accumulate mercury in their bodies through the food chain and are thus being a tool for monitoring pollution in food chains (Thompson *et al.* 1991, 1993, Burger 1993, Hahn *et al.* 1993). Mercury in bird feathers is accumulated during feather development between moult cycles as a means of diminishing their mercury body burdens (Honda *et al.* 1986, Braune and Gaskin 1987) stored in the form of methyl mercury (Thompson and Furness 1989 a, b). In chick feathers, mercury represents that accumulated during the short period from hatching to fledging, plus any input from the egg.

In the Mediterranean although a variety of studies have examined mercury content of waterbird eggs (Lambertini and Leonzio 1986, Focardi *et al.* 1988, Ayas and Kolankaya 1996) bird feathers have scarcely been analysed. Cosson *et al.* (1988 a, b) revealed a high atmospheric pollution in the region of the Camargue, southern France analysing heavy metal deposition on feather surface of Flamingos (*Phoenicopterus ruber*) and Little Egrets (*Egretta garzetta*); and Goutner and Furness (1997) analysed feathers of Little Egret, Night Heron (*Nycticorax nycticorax*) and also their main prey in the Axios Delta, Greece, revealing that, in addition to a considerable mercury pollution of aquatic habitats and some biota the chicks with the highest mercury concentrations had probably been subjected to toxic effects. Use of feathers for monitoring the effects of mercury on breeding success and survival of birds has advantages as feather mercury concentrations correlate with those of internal tissues so no bird killing is necessary (Furness and Hutton 1979, Furness 1993).

The White Stork (*Ciconia ciconia*) is a wading bird which has subjected a considerable decline in many European countries during the course of this century, thereafter needing conservation (Goriup and Schulz 1990). Greece hosts an important part of the European White Stork breeding population (Tucker and Heath 1994). The Greek population declined between 1958 and 1970 by about 73% (Cramp and Simmons 1984), probably due to human induced habitat changes. The potential effect of mercury in the breeding performance of White Storks has never been examined, although previous pollution studies have measured mercury levels in eggs or tissues (Baluja *et al.* 1983, Büthe *et al.* 1989, Hernandez *et al.* 1988). However, chick feathers are particularly useful in providing a measure of local patterns of mercury pollution, since chicks are fed on prey caught by adults from the area close to where they nest.

This study presents data on the mercury levels of White Storks in a variety of areas in northern Greece and evaluates potential effects on the breeding success of these populations.

STUDY AREAS AND METHODS

The areas from which chick feathers were collected were selected based on adequate numbers of White Stork pairs for sampling but also on the accessibility of nests. Additionally, the areas were chosen to represent a different spectrum of habitats to evaluate mercury accumulation by White Storks in a variety of environments. Five areas were sampled, each constituting two to five localities (Figure 1), as described in detail in Goutner and Tsahalidis (1995). We defined sub-sample areas as villages, since most storks placed their nests on human constructions, primarily electric poles and occasionally on churches, houses and trees. Feather sampling took place between 8 and 18 June 1993, a period when chicks at most nests that started normally in the season were well grown and almost fledged. Areas A-E are mainly agricultural. Area A (Figure 1), which included two typical agricultural villages in the plain of Drama town, lacks extensive water bodies nearby. Area B also included two villages situated in the vicinity of the artificial lake Kerkini (7300 ha), a

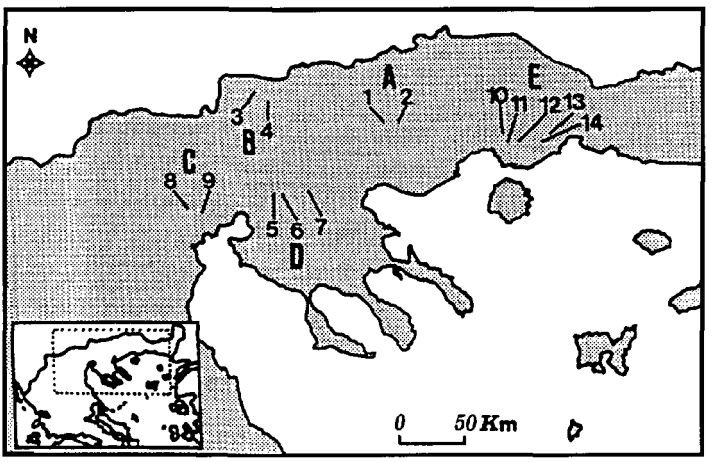

FIGURE 1 The localities sampled (1-14) in the five study areas (A-E) in North-eastern Greece. The insert indicates the area within the context of Greece. Locality names: A villages of Drama plain; 1: Megalokampos, 2: Nikotsara. B, villages of Kerkini lake area; 3: Kerkini, 4: Limnochori, C, villages near Axios river; 8: Anatoliko, 9: Kymina. D, villages of Koronia lake area: 5: Kavalari, 6: Aghios Vassilios, 7: Nymphopetra. E, villages in the Nestos Delta; 10: Pondolivado, 11: Eratino, 12: Ziloti, 13: Dekarho, 14: Maggana.

reservoir collecting the water of the river Strymon for agricultural purposes and also being a wetland protected by the Ramsar Convention (Zalidis and Mantzavelas 1994). Area C, included two agricultural villages situated in the vicinity of the river Axios, a very polluted river (Fytianos *et al.* 1986) discharging into the Thermaikos Gulf creating together with another three rivers an extensive deltaic region protected by the Ramsar Convention. Area D, included three villages situated around lake Koronia, a shallow eutrophic lake which extends over 4420 ha, which in combination with the neighbouring lake Volvi, is protected by the Ramsar Convention (Zalidis and Mantzavelas 1994). Area E, included five agricultural villages situated in a very extensive deltaic area of the river Nestos (12000 ha) a Greek Ramsar wetland made up by a variety of terrestrial and aquatic habitats (Jerrentrup 1986).

A total of 181 chicks were sampled from all localities. Access to nests was possible through a "cherry picker" operated by technicians of the Public Power Corporation, after power was interrupted for various periods of time. We collected, under license, a small quantity of back feathers from each chick. Feathers were removed from between the shoulders of each bird and were placed in polythene bags. Bill was measured on each bird by slide calipers, and a regression equation, available from a previous study on the species (Goutner and Tsahalidis 1995), was used to estimate the age of each bird. A number of chicks were already fledged and capable of flying but were sampled as they did not leave the nest when approached. In the laboratory, feathers were placed in glass tubes and washed with distilled water in an ultrasonic bath for 10 min to remove surface contaminants. They were then oven-dried at 65 °C for about 24 h. No preservatives were used on the study material.

Washed feather samples 0.1–0.2 g were weighed to the nearest 0.001 g. Weighed samples were then digested in glass flasks in 4 ml sulphuric acid and 1 ml nitric acid at 60 °C. Digestion was completed by adding 10 ml of 5% potassium permanganate and standing overnight in a fridge. Then drops of hydrogen peroxide were added until any precipitate was fully dissolved and each sample was made up to 40 ml by addition of distilled water. Samples were separated into two parts of 20 ml each and one of them was analysed, whereas the other was kept in case the analysis should be repeated. To each 20 ml sample was added 20 ml of reducing agent (stannous chloride solution made up as 136 g of stannous chloride dissolved in 400 ml hydrochloric acid and 400 ml water) in a Dreschel flask. Mercury vapour was pumped through magnesium perchlorate (drying agent) into a Data Acquisition Ltd DA 1500.DP6 Mercury Vapour Detector, calibrated by using mercury nitrate standards (Furness *et al.* 1986). Measurements made were of total mercury assuming that it was all methylmercury as found in previous studies

(Thompson and Furness 1989a, Burger 1993, Furness 1993). All chemicals used were of Analytical grade, dissolved in double-distilled water. Standard reference materials were included in each batch of samples to check on measurement accuracy, following the protocol of Furness *et al.* (1986), Thompson and Furness (1989a, b).

Since in waterbirds the feeding habits have a strong influence on mercury content of feathers (Hoffman and Curnow 1979, Doi *et al.* 1984, Braune 1987, Furness 1993) we collected pellets regurgitated by chicks to check their dietary spectrum. Fifteen to 20 pellets were collected in each area including pellets from all nests sampled. Pellets were analysed in the laboratory using reference books and reference material.

Mercury concentrations detected were transformed to common logarithms for comparisons among sampling areas and different brood sizes using Analysis of Variance (ANOVA), locating differences using the Scheffe-test. Regression of transformed mercury feather content on chick age and brood size was used to test relationship between these parameters.

RESULTS

Mercury levels detected in White Stork feathers averaged between 0.09 and 2.16 $\mu g \cdot g^{-1}$ (Table I). Considerable differences were found among areas, the differences being highly significant (ANOVA, $F = 161.87$, $df = 180$, $P < 0.001$). In area C (Axios river) mean mercury content was considerably higher than in all other areas. This was followed by areas B and D (lakes Kerkini and Koronia), and finally by areas E and D (Nestos Delta and dry agricultural land). Differences between these three groups were significant (Scheffe-test).

The diet of White Storks, as indicated by pellet analysis, was constituted mainly by insect prey in which coleopterans and orthopterans dominated (Table II). Aquatic prey formed 16% of prey items in area E, but was limited in the other areas.

TABLE I Mercury levels ($\mu g \cdot g^{-1}$) in feathers of White Stork chicks in the five study areas (A-E. See Figure 1 for names of the areas)

Area	*Habitat*	*Mercury* ($\mu g \cdot g^{-1}$) *(geometric means)*	*Range*	*N*
A	dry agricultural	0.09	0.02-0.21	15
B	freshwater lake	0.76	0.00-1.19	41
C	river	2.16	0.83-4.09	30
D	freshwater lake	0.48	0.00-1.17	39
E	river delta	0.24	0.03-0.80	56

TABLE II Diet of White Storks (% of prey items) in the study areas (A-E) as recorded by analysis of pellets

	Areas				
	A	*B*	*C*	*D*	*E*
Coleoptera	36.5	30.5	77.7	36.7	57.7
Orthoptera	61.3	61.9	21.4	53.9	25.5
Hemiptera (aquatic)	0.7	5.6	—	—	15.6
Insect larvae partly aquatic	1.4	1.0	—	7.4	0.3
Bivalvia	—	—	—	0.8	0.1
Other	—	1.0	0.9	1.1	0.8
Total number of prey items	137	197	224	621	771

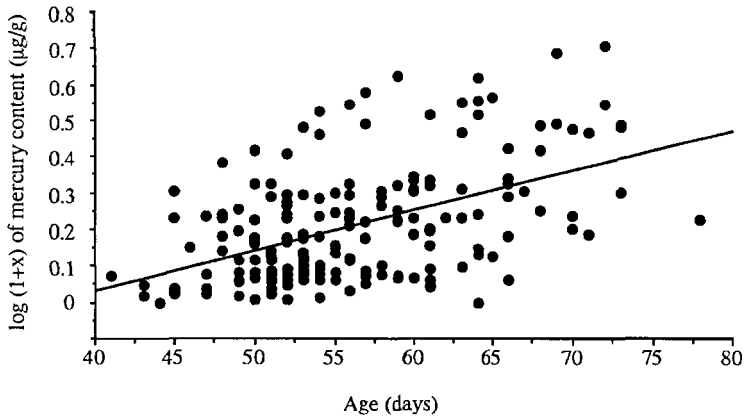

FIGURE 2 Mercury content of White Stork chick feathers in relation to age ($y = 0.011x - 0.404$, $R^2 = 0.241$).

TABLE III Mercury levels ($\mu g \cdot g^{-1}$) in feathers of White Stork chicks in relation to brood size

Brood size	*Mercury* ($\mu g \cdot g^{-1}$) *(geometric means)*	*Range*	*Number of storks*
2	0.80	0.10-3.18	16
3	0.48	0.00-4.09	95
4	0.92	0.00-3.13	37
5	0.79	0.19-1.18	31

There was a highly significant positive relationship between the chick age and mercury content of their feathers ($F = 56.91$, $df = 180$, $P < 0.001$, Figure 2).

Mercury content was also related to brood size (ANOVA, $F = 6.11$, $df = 178$, $P = 0.0006$, Table III): chicks in broods of three had significantly less

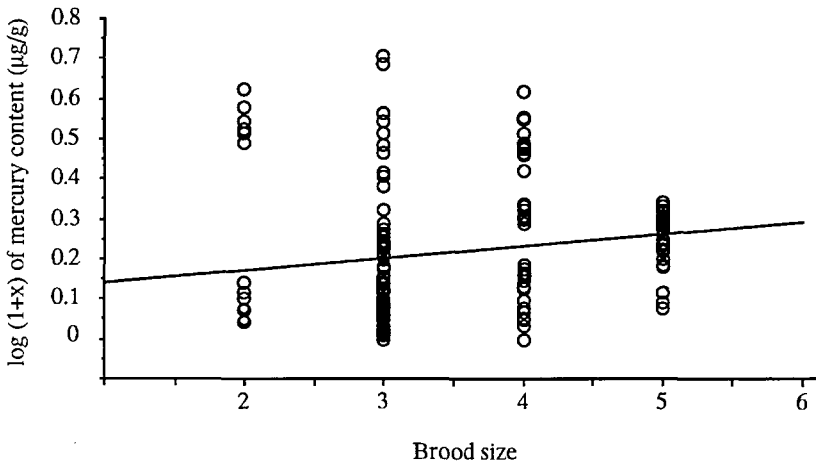

FIGURE 3 Mercury content of White Stork chick feathers in relation to brood size ($y = 0.03x + 0.11$, $R^2 = 0.027$).

mercury content in their feathers than broods of four but differences were not significantly between other groups (Scheffe-test). There was an overall significant increase of mercury content in the chicks of larger broods $F = 4.94$, $df = 178$, $P = 0.027$, Figure 3).

DISCUSSION

Although, with the exception of some samples, the mercury concentration in White Stork feathers was low compared with concentrations in egrets and herons (Goutner and Furness 1997), the mean concentrations were considerably different among study areas. The highest value found in the vicinity of the Axios Delta is in agreement with a study reporting that even in the early 1980s this river had much higher mercury pollution than did other rivers and lakes in northern Greece (Fytianos *et al.* 1986). Additionally in that study mean mercury concentrations in unfiltered water samples ranked as Axios > Lake Koronia > Nestos river, like mean mercury concentrations in White Stork feathers ranked in this study. On the other hand, as mercury reaches feathers through diet, the dietary data rather reflect a terrestrial than an aquatic diet. Coleopteran prey, mainly Carabidae and Scarabaeidae, and Orthoprera, mainly grasshoppers and Mole Crickets (*Gryllotalpa gryllotalpa*) reflect the use of a wide range of agricultural land. It would be expected that a considerable amount of aquatic prey would be found in pellets in the area C (Axios river).

In contrast, aquatic prey seems to be absolutely lacking in stork pellets from this area. Some types of prey such as insect larvae, amphibians, earthworms and snails may well be underestimated in White Stork pellets, being fully digested (Tibor 1984, Lazaro 1986, Muzinic and Rasajski 1992). Our observations on White Storks in the areas of the Axios river, and lakes Koronia and Kerkini have shown that White Storks do eat fish, at least those discarded by fishermen in local ports and on river edges. In addition, at the villages of lake Kerkini, many of the stork chicks sampled carried fish scales on their bills, although no fish were found in pellets collected from their nests. It is possible that fish bones are entirely digested or are crushed and destroyed easily and thus could not be found and collected. This idea is supported by Muzinic and Rasajski (1992) who found that pellets containing mostly fish remains always involved some mammal remains making them more compact. Table II shows that no mammals were found in pellets so those containing fish remains may have been lost. During the course of the present study we analysed samples of 11 prey types used by breeding herons in Axios (Goutner and Furness 1997). Of these prey, the Pumpkinseed Sunfish (*Lepomis gibbosus*), Goldfish (*Carassius auratus*), and also insect larvae and Tree Frogs (*Hyla arborea*) tadpoles, were the most polluted by mercury. Thus it is probable that highly polluted aquatic prey may has been taken from the Axios area without being identified due to bias in pellet analysis.

In the area of the Nestos Delta, despite a considerable proportion of aquatic prey found in the stork diet, we detected the lowest mean value of mercury in feathers among the four study areas with extensive aquatic bodies nearby (B, C, E, D) suggesting a low aquatic pollution by mercury, as Fytianos *et al.* (1986) found in the 1980s in water. The lowest amount of mercury was detected in the samples from a terrestrial habitat (A), suggesting that there is almost no mercury pollution in the limited water bodies of that area but also that little mercury was taken by White Storks through terrestrial prey. Unlike other countries where mercury compounds in seed-dressing agents have been used (Westermark *et al.* 1975) or are still is use (Ayas and Kolankaya 1996), in Greece such compounds are not used in agriculture. Additionally, even in the polluted Axios area the mercury levels found in Mole Crickets (a main stork prey) were negligible (Goutner and Furness 1997). Thus it is improbable that significant amounts of mercury were accumulated from terrestrial insect prey in White Storks in our study areas.

Marine birds are especially resistant to toxic effects of mercury (Furness *et al.* 1986, Thompson *et al.* 1993, Thompson 1996). The White Stork, a wading bird with a partly aquatic diet, does not feed on marine prey (Cramp and Simmons 1984) and may not be adapted to exposure to elevated amounts

of mercury as marine birds are. Toxic thresholds in the White Stork are unknown. In Lower Saxony (Germany) in none out of nine White Stork chicks were mercury levels in the liver higher than 0.5 $\mu g \cdot g^{-1}$ and (as in eggs) in most were less than 0.2 $\mu g \cdot g^{-1}$ (Büthe *et al.* 1989). In two studies carried out in the Donana Park, Spain, mean mercury levels in White Stork eggs were 0.198 $\mu g \cdot g^{-1}$ (Baluja *et al.* 1983) and 0.21 $\mu g \cdot g^{-1}$ (infertile eggs, Hernandez *et al.* 1988). Ohlendorf *et al.* (1978) state that normal levels in liver or kidney are less than 1 $\mu g \cdot g^{-1}$. Mercury levels of 0.5–1.5 $\mu g \cdot g^{-1}$ decrease hatchability in Pheasant (*Phasianus colchicus*) eggs (Fimreite 1971). In contrast, total mercury levels ranging from 2 to 16 $\mu g \cdot g^{-1}$ had no observable effect in Herring Gulls (Vermeer *et al.* 1973). In the Common Tern (*Sterna hirundo*) a lower hatching success and reduced fledging rate were associated with mercury concentrations in eggs between 1.0 and 3.6 $\mu g \cdot g^{-1}$ (Connors *et al.* 1975). In spite of this, in some albatross feathers mean mercury content exceeds 20 $\mu g \cdot g^{-1}$ but these levels are considered natural (Thompson *et al.* 1993).

Hoffman and Curnow (1979) suggested that hepatic mercury median levels of 0.96 (0.56–2.31) $\mu g \cdot g^{-1}$ in nestling Great Blue Herons did not contribute to mortality. The mean levels of hepatic mercury from dead, injured, or terminally ill ciconiiform birds collected in parts of south Florida (regions of Lake Okeechobee-Water Conservation area and Everglades National Park-western Florida Bay) were in most species lower than mean values recorded in this study in areas B, C and D (Sundlof *et al.* 1994). Nevertheless in other parts of their study area and in some birds, especially in the Great Blue Heron, they found concentrations higher than 30 $\mu g \cdot g^{-1}$ clearly associated with overt neurological impairment. Spalding *et al.* (1994) suggested that herons with mercury concentrations exceeding 6 $\mu g \cdot g^{-1}$ in the liver died by chronic diseases.

It is quite clear that mercury levels that are harmful to one bird species may not be to another (Thompson 1996). In heron chicks mercury concentrations in wing feathers were higher than in liver, in ratios from 1.15 (Great Blue Heron) to 5.17 (Night Heron) (Hoffman and Curnow 1979, Honda *et al.* 1986). In ciconiiform birds and in the White Stork in particular, the mercury levels in feathers indicating potential problem for the species are not known. If the White Stork is sensitive to the effects of mercury at the level of about 1 $\mu g \cdot g^{-1}$ of hepatic mercury, then by using the above mentioned ratio of 1.15, 32 (18%) of White Stork chicks in our study area probably had more than 1 $\mu g \cdot g^{-1}$ mercury in liver; that is they may have been exposed to adverse effects. It is noteworthy that 29 (91%) of these samples were from the area of Axios river with a supposed mean "liver" content of 2.01 (range 1.23–3.56) $\mu g \cdot g^{-1}$. In our study area a number of White Stork chicks may be exposed to toxic

levels of mercury in the area of the Axios river but in other areas this seems to be a negligible hazard.

White Storks, and other ciconiiforms, bioaccumulate mercury so that adults bear higher concentrations in tissues and organs than nestlings or young individuals (Hoffman and Curnow 1979, Honda *et al.* 1985, 1986, Büthe *et al.* 1989, Sudlof 1994). We found mercury content of feathers to increase significantly with the age of White Stork chicks. Similarly, Honda *et al.* (1986) found that in nestling Great White Egret (*Egretta alba modesta*) mercury in feathers (and organs) increased with age suggesting that exposure time is a dominant factor for mercury accumulation in these birds. Nevertheless, a positive correlation with age in ciconiiform chicks may not be found: in Little Egret chicks in the Axios area no statistical relationship was found between these two parameters whereas in Night Heron chicks from the same colony it was negative, and, in the latter case, it may have been due to growth inhibition of youngest chicks due to mercury intoxication (Goutner and Furness 1997).

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