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Mercury in Feathers of Little Egret *Egretta garzetta* and Night Heron *Nycticorax nycticorax* Chicks and in Their Prey in the Axios Delta, Greece

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Abstract. Mercury concentrations were measured in feathers of little egret and night heron chicks and in their prey in the Axios Delta, Greece. Significantly higher concentrations occurred in night heron than in little egret in 1993. In the night heron the mercury content of feathers was negatively correlated to the size of chicks, possibly due to inhibition of growth. Mercury concentrations were higher than reported for heron feathers in seriously polluted sites in North America and Japan, but the toxic hazard is unclear. Diets differed considerably between the two species due to use of different foraging habitats and this seems responsible for different mercury contents of feathers. Mercury concentrations in the pumpkinseed sunfish *Lepomis gibbosus*, goldfish *Carrassius auratus*, and in dragonfly *Odonata* larvae were the highest among the prey categories. Frogs and water beetles *Dytiscidae* had moderate concentrations whereas saltwater fish and terrestrial prey had very low mercury concentrations. The implication is that the deltaic marshes are the habitat most polluted with mercury. Night heron chick feathers, freshwater fish and dragonfly larvae could be used to monitor mercury contamination in this region, but use of bird feathers alone could give misleading results if changes in diet occurred.

The Mediterranean is an increasingly polluted sea due to its enclosed formation and heavy discharges by surrounding countries (Hernandez *et al.* 1990). Although heavy metals and especially mercury are environmentally harmful (George 1990; Thompson 1990) limited research has been carried out in the Mediterranean and the neighbouring Black Sea basins. High natural levels of mercury occur in some parts of the Mediterranean as a result of mercury ores in the underlying rocks, but in most parts of the world inputs of mercury to the environment from pollution sources considerably exceed natural ones, and are both by long-range atmospheric transport and local riverine inputs (Mason *et al.* 1994; Sorensen *et al.* 1994). In the Mediterranean region, mainly seabird and waterfowl eggs have been used as a monitor for heavy metal pollution (Fossi *et al.* 1984; Lambertini and Leonzio 1986; Focardi *et al.* 1988). Bird feathers are also a useful means to monitor mercury in the environment (Furness *et al.* 1986; Burger 1993; Furness 1993), and can show long-term increases in both mercury pollution from atmospheric deposition (Thompson *et al.* 1992) and local riverine contamination (Thompson *et al.* 1993). Birds diminish their body burdens by placing mercury into growing feathers. All mercury stored in feathers is methyl mercury (Thompson and Furness 1989a, 1989b). Species with slow moult cycles tend to convert methyl mercury into an inorganic storage form (Thompson and Furness 1989a). Inorganic mercury is immolized in the liver and cannot circulate in the blood and become incorporated in feathers (Thompson and Furness 1989b). Mercury present in the egg enters the developing embryo and is incorporated into the first chick down so has little influence on levels in the later growing chick feathers. Mercury in chick feathers is accumulated during the short period of only a few weeks between hatching and development of feathers. As mercury in feathers is accumulated from the birds' diet, measurement of mercury in feathers of bird chicks is a useful tool to indicate the level of pollution in the food chain (Hoffmann and Curnow 1979; Burger 1993; Hahn *et al.* 1993), and can be used to identify local pollution in the foraging area around bird colonies.

In northern Greece, earlier research revealed that rivers carried considerable amounts of heavy metals (Fytianos *et al.* 1986). The water of the river Axios contained the highest amounts of mercury. The Axios river, pouring into the Thermaikos Gulf, created an extensive deltaic area which together with the deltaic areas of neighbouring rivers constitute a Ramsar wetland complex and an important bird area in Europe (Grimmet and Jones 1989). This study was conducted to measure the mercury level in feathers of chicks of heron species and their foods, and to investigate whether such material could be used as a biomonitor of mercury in this system. Night herons *Nycticorax nycticorax* and little egrets *Egretta garzetta* seemed especially useful in this regard since mercury concentrations in heron and egret eggs and feathers have already been reported from some severely polluted and some unpolluted sites elsewhere in the world (Hoffman and Curnow 1979; Doi *et al.* 1984; Cosson *et al.* 1988a; Honda *et al.* 1986; Burger *et al.* 1992; Burger and Gochfeld 1993; Spalding *et al.* 1994).

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Study Area and Methods

The Axios Delta (40° 30′ N, 22° 53′ E) is part of a wetland complex 68.7 km^2 in extent (Athanasiou 1990), situated at the west coast of Thermaikos Gulf, including the estuarine and deltaic areas of the rivers Axios, Aliakmon, Loudias, and Gallikos. The Axios Delta includes a variety of habitats such as saltwater and freshwater marshes, mudflats, lagoons, open sea, vegetated coastal islets, limited sandy shores, ricefields, forested river bank and tamarisk bushland. During the study a mixed colony with about 1,500 pairs of birds occurred in the Delta including species such as the little egret, night heron, squacco heron *Ardeola ralloides*, great cormorant *Phalacrocorax carbo* and spoonbill *Platalea leucorodia.* However, sampling from some of these species was impractical, since nests cannot be visited without risk of chick losses in cormorants in particular. We thus concentrated on two abundant species that could be easily sampled.

We collected, under licence, a small quantity of feathers from 65 little egret and 53 night heron chicks in 1993 and 1994. Chicks were unable to fly and were thus captured with the aid of a pole with a curved wire construction at its end. Feathers were removed from between the shoulders of each bird and were placed in polythene bags. Tarsus and bill were measured on each bird by slide callipers and regression equations, available from previous studies on the species (Kazantzidis, unpubl.) were used to estimate the age of each individual. In the laboratory, feathers were placed in glass tubes and washed with distilled water in an ultrasonic bath for 10 min to remove any surface contaminants. They were then oven-dried at 65°C for about 24 h. Feathers exposed to the atmosphere for ten months have not accumulated measurable quantities of mercury (Lewis 1991, in Furness 1993). The studied material was not exposed to preservatives, thus the washing method was adequate.

The main prey fed to the chicks of the study species was identified in a previous study by examination of chick regurgitations collected in the colony (Kazantzidis unpubl.). Information on the diet of the two heron species was also recorded in the fledging period of 1994 to identify any changes in the birds' diet. On the basis of this information, samples of the most important prey items were collected in 1994 during the fledging period of the chicks from the feeding habitats of each species (Kazantzidis and Goutner 1996). Fish were collected by hand net, professional fishing nets and a long cylindrical net locally called a ''daouli,'' a tool we also used to catch adult frogs. Larvae of aquatic insects, tadpoles and the mosquito fish *Gambusia affinis*, were collected by a circular 1 m^2 throw-trap (Kushlan 1974). Tadpoles and larvae were also collected by a hand net. Mole crickets *Gryllotalpa gryllotalpa* were collected by digging low dykes in the ricefields.

Prey items was killed in the field by placing in a solution of 3-aminobenzoic acid ethyl ester. They were quickly carried fresh to the laboratory, and weighed to the nearest 0.001 g, and were then deep frozen in individual polythene bags in −20°C until analysis. Prior to analysis, prey was thawed and items from all samples were sorted in proportion to their numerical representation in diet samples. These samples were oven dried at 65°C until steady weight was attained (24 h for small items to about a week for larger fish and frogs). The dry weight was recorded on a Mettler balance, the whole prey were homogenized, and 0.1–0.2 g were used for the subsequent analysis. For some prey types of small size, in some of the samples analysed a number of individuals were taken together to reach the weight needed for analysis. Up to 0.15 g of feather samples were also weighed to the nearest 0.001 g.

Weighed samples of both feathers and prey were 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. Hydrogen peroxide was added until any precipitate was fully dissolved and each sample was made up to 40 ml by the addition of distilled water. Samples were separated into two parts of 20 ml each and one of them was analyzed, 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 sucked 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, though we assume that this was all methylmercury as found in previous studies of bird feathers (Burger 1993; Furness 1993). All chemicals used were of Aristar grade, dissolved in double-distilled water. Standard reference materials were included in each batch of samples to check on measurement accuracy.

Data on mercury contents of feathers were analyzed using nonparametric statistical procedures.

Results

Mercury was found in both species' feathers (Table 1). There was a significant difference in mercury content between little egret and night heron in 1993 being greater in the latter ($U = 790.5$, $P < 0.001$, Mann-Whitney U-test) but no difference between species in 1994 ($U = 226$, $P > 0.20$, N.S.). There was no significant difference in mercury content of night heron feathers between the study years ($U = 419$, $P > 0.10$, N.S.), but the difference was highly significant for the little egret, being greater in 1994 ($U = 687.5$, $P < 0.001$).

For the little egret, there was no significant relationship between chick age and mercury content of feathers in 1993 ($r_s = 0.282$, df = 42 N.S., Spearman Rank Correlation Coefficient) or in 1994 ($r_s = -0.166$, df = 18, N.S.). In the night heron this relationship was significantly negative in 1993 ($r_s = -0.422$, df = 31, $P < 0.02$; Figure 1) but insignificantly negative in 1994 ($r_s = -0.022$, df = 18, N.S., Figure 1).

The main prey of the two heron species differed considerably (Table 2). Salt and brackish fish constituted a large proportion of the little egret diet but were not taken by night herons. Little egrets took a greater proportion of amphibians and insects (aquatic and other), apart from mole crickets which were found in a higher proportion in the night heron diet. Phyllopod *Triops cancriformis*, an aquatic crustacean, although recorded in a considerable proportion in the diet especially of the night heron in 1988 and 1990 (Table 2), was not found in regurgitations during 1994.

Analysis of prey items of the heron species studied, revealed that the highest amounts of mercury were found in the freshwater pumpkinseed sunfish *Lepomis gibossus* followed by larvae of dragonflies *Odonata* and the freshwater goldfish *Carassius auratus* (Table 2). Moderate levels were found in tree frog *Hyla* tadpoles and larvae of aquatic water beetles *Dytiscidae*. Marsh frog *Rana ridibunda* adults contained higher amounts than tadpoles probably due to bioaccumulation but both contained much lower amounts than tree frog tadpoles. The lowest amount of mercury of all freshwater prey was found in the mosquito fish. Sand smelt *Atherina boyeri* and Mediterranean toothcarp *Aphanius fasciatus,* fish living in salty water environments, contained low amounts of mercury. Very low concentrations of mercury were found in the mole cricket, a terrestrial insect species.

Table 1. Mercury (µg/g dry mass) in feathers of heron chicks and in their prey in the Axios Delta

	1993			1994		
	Median	Range	N	Median	Range	N
Egretta garzetta	1.69	0.53–8.28	45	3.32	1.40–9.11	20
Nycticorax nycticorax	2.11	0.53–9.54	33	3.01	1.01–9.98	20
Freshwater prey						
Lepomis gibossus				2.31	1.55–2.36	5
Odonata larvae				1.50	0.14–1.92	20
Carassius auratus				0.96	0.85–1.77	11
Dytiscidae larvae				1.12	0.08–1.56	12
Hyla tadpoles				0.84	0.56–1.49	19
Rana ridibunda adults				0.17	0.13–0.44	18
Rana ridibunda tadpoles				0.05	0.00–0.56	20
Gambusia affinis				0.09	0.03–0.23	20
Salt water prey						
Atherina boyeri				0.15	0.06–0.43	19
Aphanius fasciatus				0.00	0.00–0.03	20
Terrestrial prey						
Gryllotalpa gryllotalpa				0.05	0.00–0.18	20

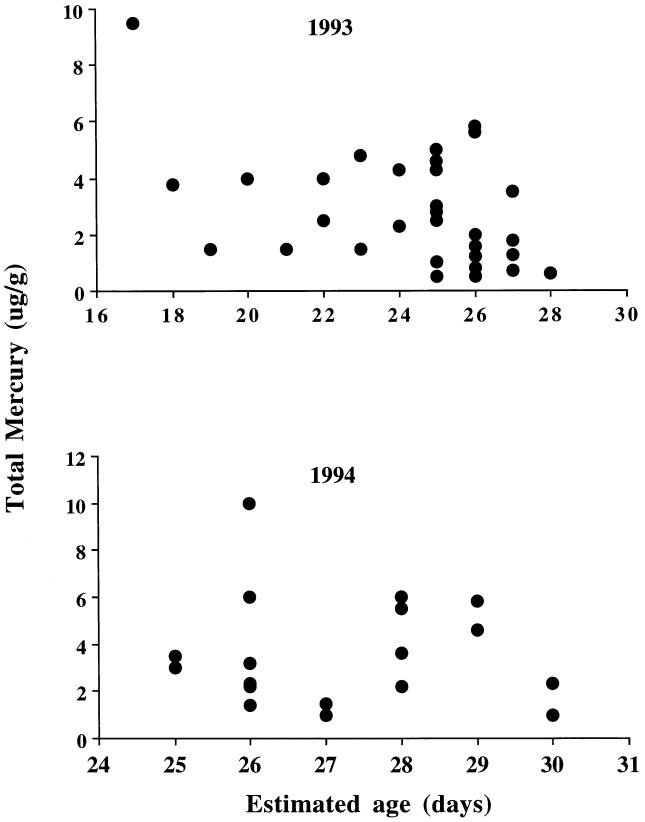

Fig. 1. Concentrations of mercury (µg/g) in body feathers of night heron chicks in relation to chick age (days from hatching) estimated from body measurements, for chicks sampled in 1993 and in 1994 from the Axios Delta wetlands, Greece

Table 2. The diet of heron chicks in the Axios Delta. Data are frequencies of occurrence (% of a particular item in the total number of items from regurgitations)

	E. garzetta (1988–1990)	*N. nycticorax* (1988, 1990)
Fresh water fish	6.2	6.6
Salt and brackish water fish	32.0	—
Amphibians (frogs and tadpoles)	19.2	6.3
Orthoptera (*G. gryllotalpa*)	2.7	21.6
Other insects adults and larvae, mostly aquatic[a]	34.2	13.9
Various	5.7	51.6[b]
Totals	2595	974

[a] Mainly Dytiscidae and Hydrophilidae.
[b] Mainly *Triops cancriformis.*

Discussion

The results show that while chick feathers may be used to indicate the amount of mercury pollution in the Axios Delta wetlands, dietary changes do hinder interpretations. Feather analysis showed that there were differences in mercury content between species in one of the two study years, and a change between years in one species. 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), so these differences can be attributed to the fact that the two species have different diets, use different foraging habitats, and will switch diet according to prey availability. In the Axios Delta, the little egret forages in habitats such as saltmarshes, freshwater and ricefields (Kazantzidis and Goutner 1996) whereas the night heron never uses salty habitats but instead uses mainly ricefields and the river bed (Kazantzidis, unpubl.). A higher mercury content in night herons than in little egrets may be attributed to differences in the type of the prey taken by the two species: mercury content of pumpkinseed sunfish and goldfish, constituting important freshwater fish prey of night herons were considerably higher than that of mosquito fish that is mainly taken by the little egret. Fish from salty habitats, also important in little egret diet, contained very low amounts of mercury (Table 1; and Goutner, Kazantzidis, and Papakostas, unpubl.). Invertebrates could well play their role in the mercury content of feathers: Odonata larvae for example, contained considerable amounts of mercury which means that extensive feeding upon them would result in increasing levels of mercury in feathers. Swanson *et al.* (1972) found increased levels of mercury in livers of ducks that fed on aquatic invertebrates.

In the little egret chicks, mercury levels were independent of age. In the night heron mercury content of feathers reduced as they grew in one of the two study years but not in the other. Although it is expected that mercury content in chick feathers may vary a little with age (Furness 1993), absence of this relationship in 1994 might be attributable to the type of sample containing ages of limited spectrum in comparison to the sample of 1993. By inspecting Figure 1, it seems that the significant relationship found in 1993 appears to be due to a single point of a sample from the youngest chick. Nevertheless if this value is removed the relationship still remains significant

($rs = -0.365$, $df = 30$, $P < 0.05$) suggesting a true negative relationship between the age and mercury content in the night heron chicks. Honda *et al.* (1986) found that in the eastern great white egret (*Egretta alba modesta*) mercury content in feathers increased with age. They suggested that the age of exposure is a dominant factor in the mercury content of feathers. If so, it seems that the youngest night heron chicks in 1993 were exposed to higher mercury concentrations than the oldest but this did not happen to little egrets probably due to differences in foraging habits of adults. Younger night herons may have received higher amounts of mercury than older due to a change in the type of food provided by their parents. Dietary changes during the breeding season have been documented for the night heron (Fasola *et al.* 1981) and for other herons (Lowe 1983; Ruiz 1985). The difference found between years in mercury content of little egret feathers may also be due to changes in the diet between years. Considerable yearly changes in particular types of prey have been recorded in other herons, such as the grey heron *Ardea cinerea* (Draulans *et al.* 1987).

The mercury levels found in the feathers of the night heron chicks in the Axios Delta were a little higher than those found in chicks of the same species at Lake Erie in Canada (Hoffman and Curnow 1979). They were similar to levels found in feathers of adult night herons from the very polluted area of Shiranui Sea in Japan (Doi *et al.* 1984), though in almost all birds studied, mercury concentrations in feathers of chicks are less than in feathers of adults at the same site. Burger and Gochfeld (1993) reported mercury concentrations in night heron chicks from Szechuan and little egret chicks from Hong Kong that were almost identical to our results for these same species, but they found rather lower concentrations of mercury in night heron chicks from Hong Kong. Cosson *et al.* (1988a, 1988b) present data on mercury concentrations in little egret and flamingo *Phoenicopterus ruber* from the Camargue region of the Mediterranean, but it is difficult to compare our data with theirs. Their feather samples came from fully grown birds killed by severe winter weather. Mercury concentrations in flamingo feathers were around 1–2 µg/g while those in little egret feathers were reported as around 5 µg/kg but this is presumably an error in units and should be read as 5 µg/g. These were from birds of unreported age and so, assuming that most were adults and that levels in adults exceed those in chicks, represent a similar level of contamination to that found in our little egrets from a different Mediterranean wetland. Burger *et al.* (1993) reported rather lower levels of mercury in feathers of wood stork *Mycteria americana* chicks from Florida and Costa Rica.

It is difficult to assess the toxic hazard presented by the mercury concentrations reported in this study. Terrestrial and freshwater birds appear to be much more sensitive to mercury toxicity than are seabirds, and experimental studies resulting in tissue concentrations of mercury similar to those reported here led to reduced chick growth, impaired brain development and lesions in nerve tissues (Heinz 1979; Scheuhammer 1987; Thompson 1996). Spalding *et al.* (1994) suggested that herons in southern Florida with mercury concentrations exceeding 6 µg/g wet mass in the liver died as a consequence of mercury-induced illness. This liver concentration equates to about the highest levels in our heron chick feather samples, suggesting that the most contaminated individuals may be exposed to toxic effects of mercury. The higher mercury concentration in feathers of apparently younger heron chicks (as estimated from measurements) could have arisen as a result of growth inhibition by mercury, a known toxic effect, though we have no way of testing this possibility with our present data.

The amounts of mercury found in adult marsh frogs were within the range found in *Rana* frogs at Donana wetland, Spain (0.08–0.39 µg/g) (Baluja *et al.* 1983; Rico *et al.* 1987). Mercury levels in mosquito fish from Lahontan Reservoir, Nevada were much higher than in this study (0.53 µg/g) (Cooper 1983). Mercury concentrations found in pumpkinseed sunfish in our area are much higher than those found by Wren and MacCrimmon (1983) in samples of muscle tissue from precambrian shield lakes in Canada (between 0.02 and 0.54 µg/g). Mercury concentrations in the sand smelt in the Donana National Park, Spain, were considerably higher (0.23–0.41 µg/g Baluja *et al.* 1983) than in sand smelts from our study area.

The amounts of mercury in the two main fish prey species of the night heron namely goldfish and pumpkinseed sunfish were high. The mercury takeup is dependent on the position of fish, as of other biota, in the food web and is influenced by their diet (Ratkowsky *et al.* 1975; Baluja *et al.* 1983; Rico *et al.* 1987) and by a variety biotic and abiotic factors (summarized in Wren and MacCrimmon 1983) of which in freshwater fish, water properties, especially hardness and acidity, play an important role (Wren and MacCrimmon 1983; Allard and Stokes 1989; Wren *et al.* 1991). The mercury concentration in the pumpinseed sunfish muscles is greater at lower pH (Wren and MacCrimmon 1983). Goldfish retain mercury in particular organs (Weisbart 1973), which could be related to metabolic rate or factors controlling the clearance rate of methylmercury (Sharpe *et al.* 1977). Apart from physiological and other mechanisms controlling concentration of mercury in their bodies, the main diet of the goldfish and pumpkinseed sunfish is aquatic invertebrates (Scott and Crossmann 1973). Total mercury concentrations in certain freshwater benthic macroinvertebrates have been found to be closely related to that in sediments (Becker and Bigham 1995), which means that mercury in these two fish species—and further in night heron feathers—could well reflect the pollution of the Axios river bed. Batty *et al.* (1996) found particularly high concentrations of mercury in eels *Anguilla anguilla* from the Camargue, France, indicating that Mediterranean wetland also to be highly contaminated with this metal.

Some aquatic insects accumulate heavy metals in relative proportion to the metal concentration in the water (Nehring 1976), but generally the content of trace elements in aquatic insects and other invertebrates differs among taxa and is governed by biological and environmental factors (Jackson 1988; Cain *et al.* 1992).

The levels of mercury found in the two study species feathers and in some of their prey suggest that, as detected in water and sediments ten years ago (Fytianos *et al.* 1989), the aquatic habitats of the Axios Delta remain polluted by mercury, clearly detected in the food chain of herons and in heron feathers. This study suggests that night heron feathers from samples of chicks, preferably of a wide range of ages, can be used to monitor mercury pollution in the freshwater habitats (the Axios river and associated waters) in this region. Water beetle larvae, the pumpkinseed sunfish, goldfish and tree frog tadpoles also show

high concentrations of mercury and are easily sampled, and so could also be used as monitors. A combination of monitoring heron chick feather mercury and mercury in these prey would provide a better indication of mercury pollution than monitoring bird feathers alone.

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