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Mercury in feathers of Squacco Heron (*Ardeola ralloides*) chicks in relation to age, hatching order, growth, and sampling dates

V. Goutner [a,*], R.W. Furness [b], G. Papakostas [c]

[a] *Department of Zoology, Aristotelian University of Thessaloniki, GR-540 06 Thessaloniki, Greece*
[b] *Applied Ornithology Unit, IBLS, Graham Kerr Building, Glasgow University, Glasgow G12 8QQ, Scotland, UK*
[c] *Department of Zoology, 203 Natural Science Building, Michigan State University, East Lansing, MI 48824, USA*

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"Capsule": *Feathers from Squacco Heron are appropriate biomonitors of mercury.*

Abstract

We studied the relationships between mercury content of Squacco Heron (*Ardeola ralloides*) chick body-feathers and nestling age, hatching order (seniors–juniors) and growth parameters, and the date of feather sampling in the Axios Delta, northern Greece, in 1993 ($n=75$ chicks) and 1994 ($n=80$). Mercury levels were not significantly correlated with chick age in either year of the study. Most of the variability in mercury (90%) was found among broods, attributable to differential prey selection and/or foraging habitat and patch utilization by parents. Within broods, juniors had significantly higher mercury loads than seniors in 1993, but there was no significant difference between the two in 1994. Correlations of nestling weight and linear measurements corrected for chick age and mercury concentrations were never significant and explained small amounts of variability in chick growth. However, linear measurements corrected for age were significantly higher among seniors in 1993, when those nestlings had lower mercury loads than their siblings. Mercury levels were unaffected by the date of feather collection in 1993, but exhibited a significant increase over time in 1994. This can be attributed to a shift towards more highly contaminated habitats and prey types by foraging parents, resulting from seasonal changes in water level and vegetation cover in important foraging habitats. Feather collection from Squacco Heron nestlings late in the breeding season seems to be an appropriate method for biomonitoring mercury pollution in the Axios Delta. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Mercury; *Ardeola ralloides*; Feathers; Greece

1. Introduction

Mercury is an especially toxic metal for humans and wildlife (Ohlendorf et al., 1978); thus its levels must be appropriately monitored in food chains and in different species (Thompson, 1990). Feathers can be used to evaluate mercury contamination without the need to sacrifice birds, as mercury concentrations in feathers are dose dependent (Burger, 1993; Furness, 1993). Mercury occurring in the environment reaches bird feathers through food chains, making birds valuable indicators of environmental quality (Furness and Camphuysen, 1997; Thompson et al., 1998). Mercury is found in feathers in the form of methyl-mercury (Thompson and Furness, 1989a, b). During the breeding season, the food used by parents to feed their chicks is captured in the vicinity of the nesting areas, meaning that mercury detected in chicks reflects that present in the local environment (Burger et al., 1992; Stewart et al., 1997).

Herons and egrets are especially useful bioindicators of environmental pollution, because of their range in diet and habitats and their longevity (Burger and Gochfeld, 1997). In the Mediterranean region, these birds commonly breed in river deltas, frequently forming dense multispecific colonies surrounded by diverse foraging habitats (Hafner and Fasola, 1992). Mediterranean and Black Sea coastal wetlands have extensively been used by humans for cultivation, grazing, hunting, aquaculture, fisheries and recreation (Pearce and Crivelli, 1994). At the same time, they suffer from pollution by river discharges, as detected in various studies using waterbirds as indicators (e.g. Fossi et al., 1984; Fasola et al., 1987; Ruiz et al., 1992; Albanis et al., 1996; Ayas and Kolankaya, 1996; Morera et al., 1997).

* Corresponding author. Tel.: +3-31-998341; fax: +3-31-998269.
E-mail address: goutner@bio.auth.gr (V. Goutner).

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The Axios Delta in northern Greece was selected for this study because it hosts the largest coastal wetland heronry in the country. This river plays an important role in the lives of the people living along its banks, mainly as a source of water. Its delta supports the greatest rice-cultivated and mussel-cultured areas in Greece. The river, however, originates in the former Yugoslavia and is highly polluted by heavy metals, mainly due to industrial discharges (Fytianos et al., 1986). We studied mercury levels in feathers of Squacco Heron chicks raised in the delta. Mercury dynamics have never been studied in this species before. We were concerned both with the species' value as a bioindicator of mercury pollution in the area, and with the possible effects of mercury on its breeding performance, because the Squacco Heron has low populations (Tucker and Heath, 1994) and enjoys a special conservation status in the European Union (included in the EC Bird Directive 79/409).

2. Study area and methods

The Axios Delta (40°30′ N, 22°53′ E) (Fig. 1) is part of a wetland complex 68.7 km^2 in extent, situated at the west coast of Thermaikos Gulf and including the estuarine and deltaic areas of the rivers Axios, Aliakmon, Loudias and Gallikos. The Axios Delta contains a variety of habitats, such as salt- and fresh water marshes, mudflats, lagoons, open sea, vegetated coastal islets, sandy shores, ricefields, forested river banks and tamarisk bushland. During the study, a mixed colony with about 1500 pairs of birds occurred in the Delta, including Little Egrets (*Egretta garzetta*), Night Herons (*Nycticorax nycticorax*), Squacco Herons (*Ardeola ralloides*), Great Cormorants (*Phalacrocorax carbo*) and Spoonbills (*Platalea leucorodia*) (Goutner and Furness, 1997; Kazantzidis et al., 1997).

The breeding season of the Squacco Heron in the Axios Delta starts in early May and chicks hatch in June. Individually marked nestlings of known hatching date and age were caught with the aid of a long pole bearing a curved wire at its end. Small quantities of body feathers (about 0.3 g, from the mantle) were collected under licence from 75 chicks between 20 June and 11 July in 1993 and 80 chicks between 29 June and 12 July in 1994. Some nestlings were sampled twice in 1994, but only one measurement was used in most analyses (see later). The spatial distribution of nests in the colony site allowed the capture of whole broods with minimal disturbance to their neighbours. Feather samples were preserved in polythene bags. During their collection, we measured nestling weight with Avinet spring scales, and forearm and bill + head lengths using Vernier callipers. Forearm length was measured from the radial side of wrist joint to the ulnar side of the elbow joint and bill + head from the tip of the mandible to the occipital condyle of the cranium. Prey fed to chicks was identified by examining regurgitations produced while handling them. In order to examine whether mercury loads

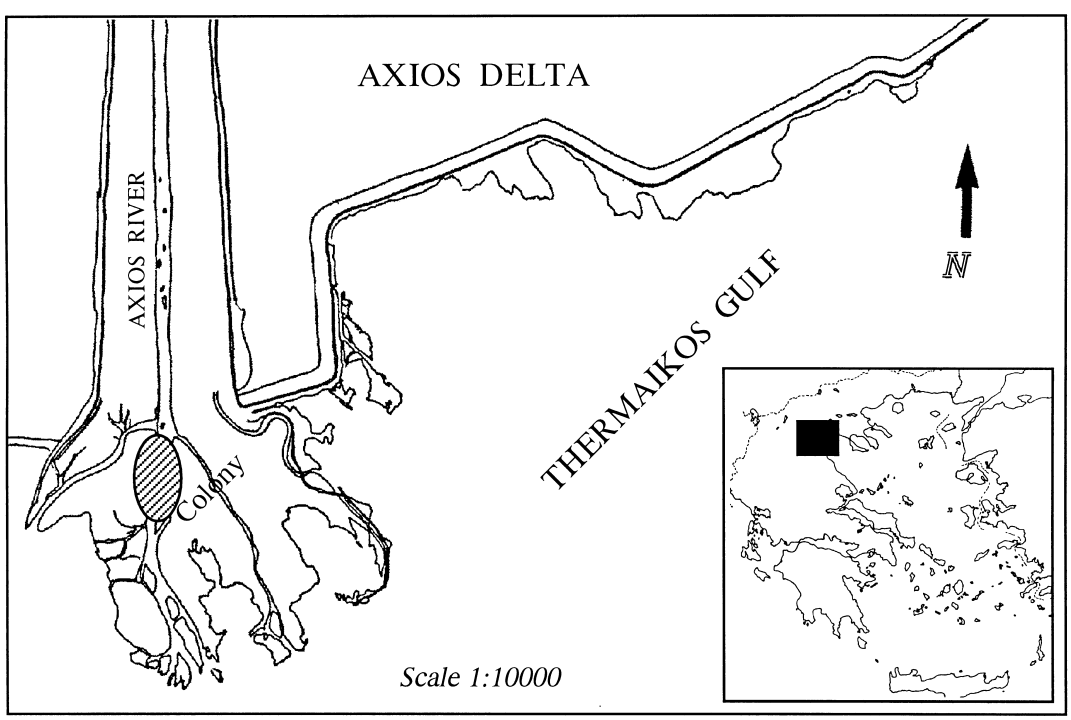

Fig. 1. Map of the study area (Axios Delta) indicating the colony site. The insert places the area within the context of Greece (black square).

affected chick survival, we followed sampled broods until close to fledging (lasting about 45 days), and we also sampled feathers from five other nestlings who were found dead.

Weighed samples of feathers were digested in glass flasks in 4 ml of sulphuric acid and 1 ml of nitric acid in a bath of 60°C. Digestion was completed by adding 10 ml of 5% potassium permanganate and standing overnight in a fridge. Then hydrogen peroxide was added until any precipitate was fully dissolved and each sample was raised to 40 ml by addition of distilled water. Samples were separated into two parts of 20 ml each and one of them was analysed. The other was kept in case the analysis should be repeated. To each 20-ml sample were added 20 ml of a reducing stannous chloride solution (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 were taken on total mercury. All chemicals were of Aristar grade, dissolved in double-distilled water. Standard reference materials were included in each batch of samples to check the accuracy of measurements. Mercury concentrations are given as $\mu g\ g^{-1}$ dry weight.

2.1. Statistical procedures

The variability in feather mercury content among siblings with respect to hatching order (hereafter referred to as chick "rank"), and between the 2 years of the study, was analysed with a repeated measures analysis of variance (RM ANOVA), in which "year" would be a random among-broods factor and "rank" a fixed within-broods factor (mixed model RM ANOVA). This model was deemed appropriate because a preliminary examination of the data revealed greater differences in mercury values among broods than among siblings, thus indicating that broodmates' loads depended on the overall mercury levels in the food brought to them by parents. Sampled broods varied in size (two to four chicks), and the hatching order of some siblings was uncertain, due to simultaneous hatching. For these reasons, we grouped siblings in two ranks (seniors and juniors), according to their relative ages, and averaged measurements whenever each rank comprised more than one chick. Broods where all sampled nestlings belonged to the same rank were excluded from analyses. In cases of duplicate measurements (see previous section), we used the values measured at the age closer to those at which the chick's siblings were sampled. Such pairs of values, however, were used to examine possible changes in mercury levels with increasing nestling age with a Wilcoxon rank sum test, due to small sample size.

We examined the relationships between feather mercury content and chick age and feather collection (or sampling) date with regression analyses. A significant association between mercury and either of the other two variables would require the inclusion of the latter as a covariate in the analysis described above. For this reason, we ran a separate regression in each of the four different samples (year×rank combinations) in order to be able to test the homogeneity of slopes across all samples, as required by the analysis of covariance (ANCOVA). To perform the latter test, we specified the following general linear model:

$$\text{Mercury} = \text{Constant} + \text{Sample} + \text{Covariate} + \text{Sample} \times \text{Covariate} + \text{Error},$$

where "Covariate" stands for either chick age or sampling date. A significant Sample×Covariate interaction would indicate slope heterogeneity among the four samples and would preclude their simultaneous comparison with an ANCOVA. This proved to be the case with sampling date, so we were obliged to test the difference in mercury between ranks with paired *t*-tests in each year separately, rather than with the RM ANOVA (details are given in the Results section). Given this, we could evaluate the significance of the year effect on mercury with a simple *t*-test and dispense with the RM ANOVA table. However, we decided to retain it because it allows a comparison between the amounts of variability (% of total sum-of-squares) in mercury that reside among and within broods.

The relationship between feather mercury content and nestling growth was examined with correlation analyses, after removing the effect of chick age. Nestling weight and a linear index of body size (average of the forearm and bill + head measurements) were regressed on chick age (which cost one degree of freedom), and the residuals were correlated with the mercury values, which were independent of age (see Section 3). The slopes and residuals of the regressions of the growth variables on chick age were also compared between nestling ranks in each year (with *F*- and paired *t*-tests, respectively) in order to examine whether differences in mercury levels between ranks might be associated with differences in growth. The paucity of deaths among sampled nestlings precluded any statistical analyses of chick survival with respect to mercury contamination. All statistical procedures followed Zar (1996) and were performed on a SYSTAT (1992) statistical package.

3. Results

Statistics on sampling date, nestling age and feather mercury content (by year and chick rank) are shown in

Table 1. Sampling date does not follow the normal distribution, so we present its median values. Means and standard deviations are shown for the other variables, which are normally distributed.

The results of the regressions of feather mercury levels on nestling age are shown in Table 2. None of the relationships was significant, slope signs varied inconsistently between years and ranks, and chick age explained only small amounts of variability in mercury, as indicated by the low R^2-values. Since chick age seemed not to affect feather mercury levels within its recorded range, there was no need to include it as a covariate in the RM ANOVA on mercury. Neither would any variability in mean age among samples (Table 1) bias the results of that analysis. The difference between duplicate samples (2nd−1st measurement) taken 4 days apart from nine chicks in 1994 ranged between 0.64 and 6.31 μg g^{-1} and had a median value of 0.25 μg g^{-1}. These differences were not statistically significant (Wilcoxon $Z=1.244$, $n=9$; $P=0.214$).

Feather mercury content was independent of sampling date in 1993, but increased significantly over time in 1994 (regression results are presented in Table 2), when sampling date accounted for about half of the variability in mercury among both seniors ($R^2=0.48$) and juniors ($R^2=0.55$). In 1994, juniors were also sampled later than their senior siblings (Wilcoxon $Z=2.070$, $n=15$; $P=0.038$). Therefore, the comparison of mercury levels between ranks would be biased in 1994, unless mercury values were corrected for sampling date. However, the same correction cannot be applied to all four samples, because the mercury-sampling date regression slope varies significantly among them ($F=6.084$, df = 3,58; $P=0.001$). Table 2 indicates that this is due to an overall difference in slopes between the two years; within each year, the slopes of both ranks are remarkably similar. In accordance with this, the slopes of the two 1994 samples were found statistically homogeneous ($F=0.025$, df = 1,26; $P=0.875$).

Therefore, the comparison of feather mercury content between seniors and juniors was made with paired *t*-tests separately in the two years, using the actual mercury values in 1993, and the residuals of the regression of mercury on sampling date in 1994 (statistics on the latter variable are shown in Table 1). In 1993, juniors had significantly heavier mercury loads than their senior siblings (paired $T=2.245$, df = 17, $P=0.038$), but there was no significant difference between the two in 1994 (paired $T=-0.318$, df = 13; $P>0.755$), when the effect of sampling date on mercury was removed.

There was no significant difference in feather mercury levels between the two study years (see results of RM ANOVA in Table 3). Moreover, in 1994, when mean mercury values were higher than in 1993 in both chick ranks (Table 1), and when there was also a significant seasonal increase in mercury (see above), nestlings were sampled at significantly later dates than in 1993 (Mann–Whitney $U=33.5$, $n=33$; $P<0.001$). Therefore, the difference between the two years' means (as they appear in Table 1) is probably artificially augmented. An ANCOVA would be inappropriate, because the

Table 1
Statistics on sampling date (number of days after 30 April), nestling age (days), feather mercury content (μg g^{-1} Hg, dry wt.) and the residuals of the regression of mercury values on sampling date (for 1994 only) by year and nestling rank[a]

	1993		1994	
	Juniors	Seniors	Juniors	Seniors
A. Sampling date				
n	18	18	15	15
Minimum	51	51	60	60
Maximum	72	70	73	73
Median	59	57	71	69
B. Nestling age				
n	18	18	15	15
Minimum	13.50	13.50	12.50	13.75
Maximum	17.50	18.00	17.00	18.50
Mean	15.36	16.15	14.98	16.08
S.D.	1.04	1.36	1.25	1.55
C. Feather mercury content				
n	18	18	15	15
Minimum	0.71	0.36	1.19	1.12
Maximum	6.54	5.47	6.20	6.09
Mean	3.30	2.79	3.56	3.40
S.D.	1.55	1.36	1.55	1.34
D. Mercury content — sampling date regression residuals				
n	15	15		
Minimum	−2.08	−1.90		
Maximum	1.89	2.33		
Mean	−0.03	0.03		
S.D.	1.05	0.96		

[a] All variables are normally distributed except sampling date.

Table 2
Results of regression of feather mercury contents on nestling age and sampling date by year and nestling rank (sample sizes as in Table 1)

	1993		1994	
	Juniors	Seniors	Juniors	Seniors
A. Nestling age				
Slope	−0.163	0.127	0.339	−0.391
R^2	0.012	0.016	0.075	0.205
F	0.196	0.267	1.052	3.351
df	1,16	1,16	1,13	1,13
P	0.664	0.613	0.324	0.090
B. Sampling date				
Slope	−0.059	−0.052	0.237	0.223
R^2	0.049	0.042	0.545	0.481
F	0.828	0.703	15.589	12.042
df	1,16	1,16	1,13	1,13
P	0.376	0.414	0.002	0.004

Table 3
Repeated measures ANOVA on feather mercury content by year (among-broods factor) and nestling rank (within-broods factor)[a]

Source	Sum of squares	% of total variability	df	Mean square	*F*	*P*
Total	136.24	100.0	65			
Among broods						
Year	3.14	2.3	1	3.143	0.815	0.373
Error	119.48	87.7	31			
Within broods						
Rank	1.83	1.3	1			
Year×rank	0.50	0.4	1			
Error	11.29	8.3	31			

[a] Rank effect and year×rank interaction not tested, because results would be biased (explanation in text).

relationship between mercury levels and sampling date is heterogeneous between years (see earlier). However, since the inflated difference in mercury between years was found non-significant, the actual (smaller) one must also be the same.

The RM ANOVA on mercury by year and chick rank (Table 3) also revealed that only 10% of the total variability in mercury is found within broods (between senior and junior siblings), whereas 90% of it resides among broods. It must be admitted that the difference in sampling dates between years and between ranks in 1994, in association with the seasonal increase in mercury levels in 1994, would tend to inflate the size of the year and rank effects in the RM ANOVA table. Since, however, both have relatively low sum-of-squares values and, moreover, one of the them is an among- and the other a within-broods term, any bias in the comparison made above must be minor.

Table 4 presents the slopes of the regressions of nestling weight and linear index on age, which indicate growth rates within the sampled age intervals, and the results of their comparisons between chick ranks in each year. Regression slopes did not vary significantly between senior and junior siblings for either growth variable in either year of the study. Table 5 shows statistics on the above regressions' residuals, which depend on the amount of growth achieved by nestlings up to their sampling time, and the results of their comparisons between chick ranks. The only significant trend was that seniors had higher linear index values than their junior siblings in 1993, when the latter had significantly heavier mercury loads.

The results of the correlations between the residuals of the above regressions and mercury values are shown in Table 6. Correlation coefficients were generally low, statistically insignificant and varied in sign. Therefore, within samples, chick growth and mercury levels seem to be independent of each other. The effect of mercury on nestling survival could not be statistically evaluated, since only one of the sampled chicks died, falling victim to a predator. Its mercury value was high (6.07 μg g^{-1}), but not the highest in its brood. The mercury loads of five dead chicks (outside the monitored broods) were rather low (1.31–2.73 μg g^{-1}, median 2.31 μg g^{-1}), and their death causes were accidents ($n=3$), brood reduction ($n=1$) and uncertain ($n=1$). Nestling deaths were attributed to accidents when their bodies were found hanging from branches or tangled in the vegetation without bearing wounds (that might indicate predation).

Table 4
Results of regression of nestling weight and linear index on chick age and comparison of slopes between sibling ranks (sample sizes as in Table 1)

	1993		1994	
	Juniors	Seniors	Juniors	Seniors
A. Weight				
Slope	5.83	7.55	6.90	5.26
S.E.	4.60	2.45	3.53	2.27
F	0.119		0.160	
df	1,32		1,26	
P	0.732		0.693	
B. Linear				
Slope	2.64	3.19	2.77	1.90
S.E.	0.60	0.37	0.45	0.37
F	0.655		2.242	
df	1,32		1,26	
P	0.424		0.146	

Table 5
Statistics (mean and standard deviation) by year and rank on the residuals of the regressions of nestling weight and linear index on chick age and results of paired *t*-tests on residuals between chick ranks (sample sizes as in Table 1)

	1993		1994	
	Juniors	Seniors	Juniors	Seniors
A. Residuals of regression of nestling weight on chick age				
Mean	−0.50	0.50	−0.24	0.24
S.D.	19.24	13.37	15.97	12.74
T	0.192		0.100	
df	16		13	
P	>0.850		>0.900	
B. Residuals of regression of linear index on chick age				
Mean	−0.58	0.58	2.77	1.90
S.D.	0.60	0.37	0.45	0.37
F	0.655		2.242	
df	1,32		1,26	
P	0.424		0.146	

4. Discussion

Feather mercury concentrations were independent of nestling age in our samples, as evidenced both by

Table 6
Results of correlations between feather mercury content and the residuals of the regressions of nestling weight and linear index on chick age (sample sizes as in Table 1)

	Sample	*r*	df	*P*
A. Weight				
	Junior 93	−0.167	16	0.507
	Senior 93	−0.293	16	0.238
	Junior 94	0.465	13	0.081
	Senior 94	−0.193	13	0.491
B. Linear index				
	Junior 93	0.153	16	0.545
	Senior 93	0.217	16	0.387
	Junior 94	0.204	13	0.465
	Senior 94	−0.296	13	0.285

regression analyses (Table 2) and by comparisons between duplicate measurements taken 4 days apart from nine young herons. It must be admitted that this apparent independence may be due merely to the small range of chick ages in our samples (less than 5 days; Table 1). Goutner and Furness (1997), for instance, found a significant drop in feather mercury with increasing age in Night Heron nestlings from the same colony in a sample where chick ages spanned 10 days, but no relationship between these variables in a sample where the range of recorded ages was only 5 days.

Nevertheless, even studies including nestlings of highly variable ages have failed to demonstrate any association between feather mercury levels and chick age (e.g. Thompson et al., 1991, Great Skuas; Stewart et al., 1997, Arctic Skuas and Arctic Terns). Such a constancy of mercury concentrations during the nestling period has been attributed to proportional increases in both total mercury loads and the mass of growing tissues (Thompson et al., 1991). Gradual accumulation of mercury and an increase in its concentration during early life has been found in Great Egrets in Korea (Honda et al., 1986), in three ardeids in the USA (Hoffman and Gurnow, 1979) and, recently, in Great Egrets (*Ardea albus*) in Florida (Sepulveda et al., 1999). Conversely, negative trends in feather mercury concentrations with chick age have been found in three seabirds in the Azores (Monteiro et al., 1995) and in Kittiwakes and Great Skuas in the Shetlands (Stewart et al., 1997). The authors have attributed these to a 'dilution effect', occurring when the rate of mass increase of growing tissues exceeds the rate of mercury deposition in them.

The latter hypothesis suggests that, among nestlings of similar ages, the faster-growing ones should have lower mercury concentrations. However, after controlling for age, we were unable to find significant associations between mercury values and either growth variable in any of our samples (Table 6). Still, the variability in mercury among samples could be related to differences in growth between chick ranks. In 1993, senior nestlings had significantly lower mercury levels than juniors, but growth rates during the sampled age intervals did not vary significantly between the two (Table 4). However, senior linear index values corrected for age, which depend on individuals' growth rates prior to sampling time, were significantly higher than juniors' (Table 5), in agreement with the prediction made above. No such trend was found in chick weight (Table 5), possibly because its measurements tend to fluctuate according to the amount of food present in the nestling's alimentary canal (Rising and Somers, 1989). Faster senior growth rates are a common phenomenon among ardeids, including the Little Blue Heron (Werschkul, 1979), Purple Heron (Moser, 1986), Great Egret (Mock, 1985; Custer and Peterson, 1991), Snowy Egret and Night Heron (Custer and Peterson, 1991; Erwin et al., 1996). In 1994, when we found no significant difference between nestling ranks in any growth measure (Tables 4 and 5), mercury levels were also similar between ranks. These results are consistent with the hypothesis that increased growth rates 'dilute' mercury loads in nestling tissues, and indicate that the higher mercury values of juniors in 1993 may be attributed to their slower growth rates.

Differences in mercury levels between nestling ranks could possibly arise also from the unequal distribution among siblings of prey types carrying variable mercury loads. The analysis of 295 prey items from Squacco Heron nestling regurgitations collected in 1993 (Papakostas and Goutner, unpublished), revealed a diverse diet, consisting of *Rana* frogs and tadpoles (relative frequency 35%), larval and adult aquatic insects (mostly Dytiscidae and Hydrophyllidae, 30%), mole crickets (*Gryllotalpa gryllotalpa,* 20%), small freshwater fish (8.5%) and various aquatic invertebrates (6.5%). These prey types do vary in mercury content, as analyses of samples collected in 1994 showed (Goutner and Furness, 1997), and can be ranked with respect to median mercury values as follows: dytiscid larvae (1.12 μg g^{-1}) > *Rana* adults (0.17) > *Gambusia affinis* (0.09) > *Rana* tadpoles = mole crickets (0.05). Several studies of ardeids (e.g. Fujioka, 1985; Mock, 1985; Ploger and Mock, 1986) have shown that senior nestlings have primary access to parental feedings, something which also seems to occur in the Squacco Heron (Papakostas, pers. obs.). However, there is no information in the literature indicating that certain prey types are preferred by chicks of a certain rank. Therefore, it seems more likely that prey items are distributed among Squacco Heron siblings randomly with respect to their mercury loads.

The differences in feather mercury content between nestling ranks, though significant in 1993, made up only 10% of the total variability in mercury, while the remaining 90% resided among broods (Table 3). This

implies a high level of variability in the mercury content of the food brought to different broods by their parents. Given the diversity of Squacco Heron diet at the Axios Delta, and the range of mercury concentrations across prey taxa (see earlier), the above phenomena could result from parental preferences for certain prey types or foraging habitats, where some prey taxa may be more abundant or readily available than others. Variability in overall mercury levels among habitat types or patches could produce similar effects, if parents show preferences for certain foraging habitats or sites. We lack data on individual parents' feeding habits and foraging habitat or site fidelity, but the following information lends some support to these arguments. The type of prey captured by herons may vary according to the hunting method used, as evidenced by Great Blue Herons in British Columbia (Forbes, 1987). Squacco Herons employ a variety of hunting methods, as standing in wait for prey and slow walking (Voisin, 1991), but also actively pursuing prey (Goutner, unpublished). However, we do not know if individuals tend to specialize in these methods. Squacco Herons in the Axios Delta feed mainly in ricefields and vegetated irrigation and drainage canals, and, to a smaller extent, along the river banks (Papakostas, pers. obs.). All these habitats receive river water, which carries mercury originating mainly from industrial discharges in the former Yougoslavian region (Fytianos et al., 1986). The canals, however, also receive effluents from a number of local point sources. Although the concentration and dynamics of mercury in these environments are at present unknown, they are likely to vary according to each habitat's physical properties and biotic communities. Mercury uptake by benthic macroinvertebrates and fish depends on sediment concentrations (Baluja et al., 1983; Becker and Bigham, 1995), whereas aquatic insects accumulate metals in proportion to the latter's concentration in the water (Nehring, 1976). Precipitation of suspended particles is generally inversely related to the rate of water flow. In our case, water flows faster in the river than in the canals and is stagnant in the ricefields. Mercury concentrations in the water column will depend on losses to the sediment and on evaporation rate, which should be faster in shallow areas like ricefields. Differences in biotic communities and food chains among these environments are also likely and they could influence the mercury levels of Squacco Heron prey taxa. In the Axios Delta, habitat-dependent pollution by organochlorine compounds has been detected in heron and tern eggs (Albanis et al., 1996; Goutner et al., 1997).

The overall difference in feather mercury content between 1993 and 1992 was not found to be significant (Table 3), but different seasonal patterns in mercury were observed in the two study years. No change in mercury levels was observed during the 3 weeks of feather sampling in 1993, but a significant increase in mercury loads over time was found in the shorter, 2-week sampling period of 1994 (Table 2). Therefore, there must have been a shift towards more highly contaminated prey types or habitats/patches by foraging parents in the latter period, or a rapid increase in environmental mercury pollution at some time prior to it (enough to allow the augmented mercury loads to reach young Squacco Herons through the food chain). The fact that this temporal variability was observed only in one of the study years, though both sampling periods overlapped, indicates that its causes do not recur regularly. As mentioned earlier, the main source of mercury pollution in the Axios Delta are industries in the former Yougoslavian region, which discharge effluents in an uncontrolled and unpredictable way (Fytianos et al., 1986). Thus, rapid changes in overall mercury levels at the Delta may occur at any time. Different habitats probably vary in their overall mercury levels (see earlier) and the numbers of Squacco Herons who forage in them vary seasonally (Papakostas, pers. obs.). Ricefields attract many feeding herons after being flooded in late April or early May, but are visited by fewer birds after the end of June, when the growth of riceplants begins to impede the herons' foraging activities. This results in higher heron concentrations in the irrigation and drainage canals and, secondarily, the river banks. During the herons' breeding season, the canals' water levels fluctuate (but mostly drop), depending on water influx from the river and diversion towards fields, and on evaporation. Water level changes affect heron prey density and availability, which in turn may influence feeding herons' concentrations and behaviour, as has been shown for the Little Egret (Hafner and Britton, 1983; Erwin et al., 1985; Kersten et al., 1991).

High mercury concentrations in body tissues may cause growth inhibition and developmental abnormalities in chicks (Connors et al., 1975; Gochfeld, 1980; Wiemeyer et al., 1984). Widely ranging levels of feather mercury have been reported from various Ciconiiforms. In the southwest Lake Erie region, Hoffman and Curnow (1979) found that median mercury concentrations in chick feathers ranged from 1.10 $\mu g\ g^{-1}$ in the Great Blue Heron to 2.74 $\mu g\ g^{-1}$ in the Night Heron. In Korea, Honda et al. (1986) found mean levels of 1.19 $\mu g\ g^{-1}$ Hg in the feathers of Great Egret nestlings prior to fledging. Mercury contents in Cattle Egret chick feathers from parts of three distant regions (USA, Puerto Rico, Egypt) varied from 0.331 $\mu g\ g^{-1}$ in Cairo, Egypt, to 3.484 $\mu g\ g^{-1}$ (10.5 times higher) at Aswan, also in Egypt (Burger et al., 1992). Burger and Gochfeld (1993) found that, among five heron and egret species in China (Hong Kong and Szechuan), mean chick-feather mercury values varied from 0.27 (Great Egret) to 2.4 $\mu g\ g^{-1}$ (Pond Heron, *Ardeola bacchus*). These sources, however,

do not specify whether such mercury concentrations resulted in chick developmental impairments. Although mercury levels detected in Squacco Heron nestlings (Table 1) did not seem to affect their growth during the sampling period (Table 6), they are among the highest reported for heron chicks. The mercury levels detected in the feathers of chicks found dead were lower than all sample means, suggesting that none of them could have died as a result of mercury contamination. The highest mercury values measured in individual Squacco Heron chicks were 9.75 μg g^{-1} in 1993 and 9.68 μg g^{-1} in 1994, but such cases were very rare. Some Night Heron chicks in 1994 had even higher feather mercury concentrations (maximum 9.98 μg g^{-1}), which may have resulted in growth inhibition of apparently younger chicks (Goutner and Furness, 1997). Such mercury concentrations in nestlings may have adverse long-term effects, especially since adults tend to accumulate even higher mercury levels than nestlings and juveniles (Hoffman and Curnow, 1979; Honda et al., 1986; Burger et al., 1992). Mostly juvenile Great White Herons (*Ardea herodias occidentalis*) with more than 6 μg g^{-1} mercury in their livers suffered from mortality due to chronic, often multiple, diseases (Spalding et al., 1994). Such birds, however, would have feather mercury levels of about 12.5 μg g^{-1}, because liver mercury concentration is usually only 48% of that in feathers (Burger, 1993).

According to the results of this study, the pollution by mercury of the Axios Delta may be considered high. Therefore, mercury monitoring in this area should be continued for both human protection and bird conservation. When using bird feathers for monitoring mercury levels in the environment, it is essential to choose a suitable indicator species (Burger, 1993; Furness, 1993). One criterion for this selection is the level of mercury bioaccumulation, which seems to be higher in Squacco Heron nestlings than in other young ardeids studied simultaneously in the same colony. Median values of feather mercury content in Little Egret chicks were 1.69 μg g^{-1} in 1993 and 3.32 μg g^{-1} in 1994, while those of Night Heron nestlings were 2.11 and 3.01 μg g^{-1}, in the same years (Goutner and Furness, 1997). Mean values in Squacco Heron samples varied from 2.79 to 3.56 μg g^{-1} in the same period (Table 1). A species used as an indicator should also have a stable diet (Furness, 1993), and the Squacco Heron may be more suitable than sympatric ardeids in the Axios Delta also in this respect. It feeds exclusively in freshwater habitats (Papakostas and Goutner, unpublished), as in other parts of Europe (Fasola, 1994), thus showing lower foraging habitat diversity than the Little Egret, which also uses saline environments (Kazantzidis and Goutner, 1996). Moreover, it forages mostly solitarily and presents a more uniform and stable distribution over its feeding grounds (Papakostas, pers. obs.) than the Little Egret which exhibits flocking behaviour and high variability in foraging distribution (Kazantzidis and Goutner, 1996). Night Herons, on the other hand, may also feed mainly in freshwater habitats, but their partially nocturnal habits (Voisin, 1991) make them difficult to observe, thus creating some uncertainty about their feeding habits and diet. For these reasons, the Squacco Heron appears to be a better bioindicator of mercury pollution in freshwater habitats.

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