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Mercury and Selenium Levels in Eggs of Common Terns (*Sterna hirundo*) from Two Breeding Colonies in the Ebro Delta, Spain

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Common terns (*Sterna hirundo* Linnaeus, 1758) are seabirds that have been used extensively as indicators and early warning organisms of habitat pollution (Fimreite 1974; Becker 1992; Becker et al. 1993, 1994; Castillo et al. 1994; Burger and Gochfeld 1997). This species have become a valuable indicator of coastal ecosystem health for two main reasons. First, it is a widespread and non-endangered species, so sampling, analysis of contaminants, and intraspecific comparisons among different localities, are feasible. Second, they feed almost exclusively on fish, some of which are also captured for human consumption (i.e., there are implications for human health), and animals situated at the top of marine or terrestrial food webs are at higher risk to suffer the consequences of accumulated environmental pollutants (Scheuhammer 1991; Guitart et al. 1994).

Several chemical elements have been documented to have undesirable effects on the reproduction and survival of certain waterbird populations (Scheuhammer 1991; Furness 1993), and many of them are present and have been reported for the Ebro Delta (NE Spain) (Guitart et al. 1994; Morera et al. 1997; Mañosa et al. 2001).

In this paper, we examine Hg and Se levels in eggs belonging to two breeding colonies of the common tern, which are respectively placed at the northern (Punta del Fangar) and southern (Punta de la Banya) peninsulas of the Ebro Delta. In spite of their proximity, the studied colonies exhibit differences in mean clutch size (Hernández and Ruiz 1999), being significantly larger in the Fangar. The northern colony foraging grounds correspond to a highly productive upwelling area, while those of the la Banya colony are less productive and common terns probably rely, to a higher extent, on fisheries discards (Arcos 2001). Moreover, northern and southern foraging areas are presumably exposed to different pollutant loads, since materials carried by the Ebro River are mainly deposited at the southern hemidelta bay, owing to the Liguro-Provençal-Catalan oceanic current. In order to assess possible differences in diet between these colonies and to evaluate their potential use as a biomarker, the fatty acid composition in egg yolks was also investigated.

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MATERIALS AND METHODS

We analyzed 47 common tern eggs, 34 from the colony of Punta del Fangar and 13 from the Punta de la Banya, collected during the breeding seasons of 1998 and 1999. One of the eggs from the Punta del Fangar was broken during transport, so only the yolk was available. The eggs collected at the Banya correspond to first eggs in the laying sequence, while at the Fangar 26 correspond to first and 8 to second ones. Seven first and second eggs were from the same nests. Eggs were weighed, measured, and kept refrigerated until their analyses.

Chemical determination of Se and Hg was carried by ICP-MS at the Laboratori d'ICP-MS-Thermo Optek of the Autonomous University of Barcelona, using a Thermo Elemental VG PQ ExCell (Winsford, Cheshire, UK) apparatus. Solid samples (0.5 g) of the yolk and albumen of each egg were digested separately in a microwave oven, as most Hg compounds are highly volatile and because this system reduces also risk of external contamination, using 7 ml of concentrated HNO_3. After diluting (with MilliQ water) and calibrating with standards, samples were measured by triplicate: thus, results are the mean of these three analyses. Limits of detection were established at 100 ng/g WW for Se and 0.5 ng/g WW for Hg. For statistical analysis, the non-detectable level (in one sample of Se) was considered as half of the limit of detection. Accuracy of the results was measured by spike recovery and reference material analysis (BCR, CRM 185 - Bovine Liver), and was considered acceptable for both analytes.

Fatty acid composition was examined (derivatizing them to methyl-esters, FAME) in yolk by gas chromatography, using previously described methods (Guitart et al. 1996; Gutiérrez et al. 1997). Analyses were carried out using a Perkin-Elmer model 8500, equipped with a cross linked and fused silica 70% cyanopropyl equivalent modified siloxane (BPX70) column of 25 m, 0.53 mm I.D. and 0.5 μm of film thickness (SGE, Ringwood, Australia), coupled to a flame ionization detector. Initial temperature was 100°C, maintained for 5 min, and run to 240°C at a ramp rate of 2.5°C/min. Composition (in percentages of total FAMEs) was expressed in terms of arithmetic mean and standard deviation.

In the case of Punta del Fangar, where the first and the second eggs were collected from some nests, the effects of clutch order on Hg and Se concentrations in yolk and albumen were studied with paired t-tests. Differences in Hg and Se concentrations between breeding colonies were studied with t-tests, only considering the first laid eggs. Pearson's correlations between Hg and Se in yolk and albumen were calculated. A compositional analysis was employed grouping fatty acids (FA) as saturates (SAFA), monounsaturates (MUFA), and polyunsaturated (PUFA) n-3 and PUFA n-6, to evaluate differences in FA composition related to Hg and Se concentrations in the egg yolk, and between breeding colonies. Percentages (x_1, x_2,..., x_D) were transformed to log-ratios $y_i = \ln (x_i/x_j)$ ($i = 1, 2,..., D$), and the percentage of saturates was taken as x_j. Log-ratios of FA groups were analyzed with MANOVA with the breeding colony as a factor and Hg and Se concentrations as covariants. Differences in the ratios of the

different groups of FA between breeding colonies were studied with t-test. The effect of clutch order on FA composition was evaluated within those eggs from Punta del Fangar comparing the FA groups ratios between first and second laid eggs with paired t-tests. Calculations were carried out with the SPSS 10.1.4 statistical package.

RESULTS AND DISCUSSION

Mercury and Se concentrations in albumen and in yolk from the two sampling locations are shown in Table 1. Albumen Hg levels in first laid eggs from Punta de la Banya (807 ng/g WW) were higher than those found at Punta del Fangar (485 ng/g WW; $t_{36} = 2.3$, $p = 0.027$). Although levels of Hg were slightly higher in first than in second laid eggs, no statistical differences were found in our sample (paired t-tests, $p > 0.5$). Selenium levels were neither different ($p > 0.2$). Mercury and Se concentrations pooled from the two sites were positively correlated in albumen ($n = 46$; $r = 0.628$; $p < 0.001$) and also in yolk ($n = 47$; $r = 0.465$; $p = 0.001$).

Mercury in eggs of birds is considered to describe recent influences of diet (from food consumed 1-2 weeks prior to egg laying) better than Hg in feathers (Furness 1993; Becker et al. 1994; Burger and Gochfeld 1997; Thyen et al. 2000). As expected, higher levels of Hg have been found in the albumen fraction than in the lipid-rich yolk (Scheuhammer 1991; Furness 1993; Sanpera et al. 2000). Furthermore, levels of Hg in albumen are significantly different for both colonies; since albumen is directly derived from diet (Sanpera et al. 2000), such differences are attributable initially to dietary exposure to this pollutant. Recent studies in birds indicate that the no observed effects level of Hg is about 500 ng/g WW, while critical level that may cause reproductive impairment is above 1,000 ng/g WW (Thompson 1996). There are data on Hg levels in eggs from common terns from a variety of sites, with values ranging from 100 to near 5,000 ng/g WW (Fimreite 1974; Becker et al. 1993; Burger and Gochfeld 1997), although most values are around or below 500 ng/g. The Hg concentrations reported here for the Ebro Delta are thus slightly above the mean values, confirming previous studies of Hg contamination in this zone (Morera et al. 1997; Mañosa et al. 2001).

Selenium is an essential micronutrient, but with a fairly narrow safety range for most domestic and wild animals. High concentrations of Se in irrigation drainwater in some areas of California resulted in elevated Se concentrations in birds (Ohlendorf et al. 1986). Selenium concentrations in eggs reflect dietary exposure, and levels above 1-3 μg/g WW in whole eggs has been suggested as a guideline threshold for reproductive impairment based on studies on a variety of avian species (Ohlendorf et al. 1986; Heinz 1996). In our study, no differences were found between Se concentrations in eggs from the two colonies of the Ebro Delta. However, it should be noted that 8 out of 46 albumen samples (17.4%), and 11 out of 47 yolk samples (23.4%), were >1 μg/g, although none of them show values >3 μg/g. Previous studies carried out in the Ebro Delta on Se concentration

Table 1. Total Hg and Se levels (geometric mean and range) in albumen and yolk of common tern eggs from two colonies of the Ebro Delta.

Sample	Zone	Clutch order	n	Hg (ng/g WW)	Se (ng/g WW)
Albumen	Punta del Fangar	1st	25	485[a] (112-1717)	422 (50-1864)
		2nd	8	442 (254-1408)	447 (164-1215)
		1st+2nd	33	474 (112-1717)	428 (50-1864)
	Punta de la Banya	1st	13	807[a] (164-1907)	578 (109-2431)
	TOTAL	1st+2nd	46	551 (112-1907)	466 (50-2431)
Yolk	Punta del Fangar	1st	26	140 (44-933)	679 (211-1645)
		2nd	8	125 (40-280)	808 (383-1508)
		1st+2nd	34	136 (40-933)	708 (211-1645)
	Punta de la Banya	1st	13	141 (30-1441)	543 (133-2187)
	TOTAL	1st+2nd	47	137 (30-1441)	658 (133-2187)

[a]t-test, $p = 0.027$.

in birds suggest that this element probably is not a contaminant of importance in this wetland (Guitart et al. 1994; Morera et al. 1997), in spite of the fact that interactions with other chemicals can not be discarded (Furness 1993; Burger and Gochfeld 1997).

The description of the detailed composition of FA in yolk of eggs is out of the scope of this paper, and will be published elsewhere. However, in summary, 41 different FAMEs were detected, with oleic, palmitic, stearic and docosahexaenoic acids as the four major components. First laid eggs had lower percentage of PUFA than second laid eggs (Table 2), and this were reflected in the PUFA/SAFA ($t_6 = 3.98$, $p = 0.007$) and PUFA/MUFA ratios ($t_6 = 2.65$, $p = 0.038$). In consequence, comparisons between colonies were only performed for first laid eggs. Differences in FA composition were detected between the two colonies (Table 2), the proportions of PUFA n-3 and PUFA n-6 being higher in yolks from Punta de la Banya than from Punta del Fangar (Table 2; MANOVA, Wilks' $\lambda = 0.368$, $F_{3, 33} = 18.9$, $p < 0.001$). The proportion of PUFA in yolks from Punta de la Banya was also higher, as evidenced by the PUFA/SAFA and PUFA/MUFA ratios, while the PUFA n-3/n-6 ratio was higher in Punta del Fangar (Table 2). FA composition of the egg yolk was not affected by Hg or Se concentrations.

Table 2. Fatty acid composition (in %, classified by chemical groups, mean ± SD) of yolk in eggs of common terns from two breeding colonies at the Ebro Delta.

FA group	Punta de la Banya	Punta del Fangar	
	1st-laid (n = 13)	1st-laid (n = 26)	2nd-laid (n = 8)
SAFA	34.01±4.99[a]	40.16±3.17[a]	35.59±5.15
MUFA	48.26±5.57[a]	53.25±3.35[a]	50.14±5.88
PUFA	17.58±3.15[a]	6.70±3.92[a]	14.34±5.31
PUFA n-3	11.81±2.59[a]	4.78±2.76[a]	10.91±3.50
PUFA n-6	5.77±1.61[a]	1.92±1.54[a]	3.43±2.28
MUFA/SAFA	1.47±0.40	1.34±0.15	1.46±0.45
PUFA/SAFA	0.53±0.12[b]	0.17±0.11[bd]	0.42±0.18[d]
PUFA/MUFA	0.37±0.10[b]	0.13±0.08[be]	0.30±0.14[e]
PUFA n-3/n-6	2.16±0.67[c]	3.48±2.47[c]	4.36±2.76

[a]Compositional analysis, MANOVA, $p < 0.001$; [b]t-test, $p \leq 0.001$; [c]t-test, $p = 0.016$; [d]paired t-test, $n = 7$, $p = 0.007$; [e]paired t-test, $n = 7$, $p = 0.038$.

Different exposure to Hg between the two Ebro Delta common tern colonies can be a consequence of qualitative differences in the exploited food resources or because of distinct levels of pollution at northern and southern feeding areas, or both. Diet can also influence yolk FA composition, thus providing a valuable system to detect variations of feeding habits between groups. In our study, differences in egg yolk FAs between the two colonies are large enough to suggest the existence of dietary differences. At the Ebro Delta, common terns feed mainly on small clupeids, although studies carried out onboard trawlers suggest that fisheries discards are also an important food resource during the breeding season, at least for adult birds (Arcos 2001). PUFA n-3, for which fish are a good source, dominated in both colonies over the n-6 series, indicating that fish is the main resource exploited. As indicated by their lower clutch size, the southern colony exploits less productive feeding grounds; being closer to the main fishing fleet (Arcos 2001), the Punta de la Banya individuals might rely, to a higher extent, on fisheries discards. These results are in agreement with recent studies that have demonstrated that seabirds from the same area which consume fisheries discards are exposed to higher Hg concentration in their diet. This may explain both the observed interspecific differences in Hg loads in the same area (Arcos et al. 2002) and the intraspecific differences between localities (Sanpera et al. 2000).

Fatty acid profile in total lipids has been proposed as a biomarker of response to toxicants in wildlife, after analysis and results obtained in striped dolphins (*Stenella coeruleoalba*) and flamingos (*Phoenicopterus ruber*) exposed to organochlorines and lead, respectively (Guitart et al. 1996; Gutiérrez et al. 1997). In the common tern samples analyzed in this paper, no relationships were found among FA on Hg and Se levels. However, it should be noted that organochlorine and lead concentrations in dolphins and flamingos were much broader in range

than Hg or Se levels detected in common terns, which can explain the lack of connection observed here. However, the analysis of FA and pollutants in the same sample can still be defended because, if not useful to demonstrate that a pollutant (or a group of them) can influence the FA composition (i.e., as a biomarker), it is at least useful to suggest that differences in diet can account for dissimilar degrees of pollutant exposures.

In conclusion, the results of this study indicate that there exist remarkable differences in Hg loads between the two colonies of common terns in the Ebro Delta. Based on the FA profile, these differences may be attributed initially to variations in the diet.

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