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Trace Elements in Clutches of Yellow-Legged Gulls, *Larus cachinnans,* from the Medes Islands, Spain

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Seabird eggs are considered excellent subjects to monitor short-term impact of pollutants in marine environments (Furness 1993, and references therein). The Mediterranean has been shown to be an area with high mercury levels, owing not only to manmade sources, but also to geochemical and geographic features (Lambertini and Leonzio 1986). In the Mediterranean region, the Yellow-legged Gull *Larus cachinnans* is the most common seabird (Bourne 1993), probably due both to its opportunistic feeding habits and to its few specific requirements for successful breeding (Goutner 1992).

The Medes Islands (NE Spain) hold one of the largest *L. cachinnans* populations in the Mediterranean, their diet being highly dependent on refuse tips (Bosch et al. 1994). The aim of the present paper was to determine the concentration of trace elements, particularly Hg and Se, in eggs of this species, including intra- and interclutch variability.

MATERIALS AND METHODS

During the 1992 season, 11 nests (each containing a three-egg clutch) of *L. cachinnans* from the Medes Islands breeding colony, were monitored daily to determine egg characteristics and laying order. Once discovered, eggs were collected (under license) and replaced in the nest by surrogates to avoid disturbance in the laying process. Eggs were transported to the laboratory and kept refrigerated until chemical analysis.

Whole eggs were first weighed (±0.01 g), the eggshell opened along the equator, and the weight of the contents (albumen+yolk) was obtained. The egg content was homogenized, and oven-dried at 60°C to constant weight.

To determine Zn, Cu, Mn and Ca, samples from contents were digested first by 5 ml nitric acid (65%); once the nitric acid was evaporated, 1.5 ml perchloric acid (65%) was added. Concentration of these elements was determined by ICP-AES

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(JOBIN YVON 70). Two replicate subsamples were analyzed and a reference tissue (Bovine Liver 0868, NRCC) was included in each batch of the analysis. Mean recoveries were: 101% (Zn), 93% (Cu) and 98% (Mn).

For mercury, samples of contents were mineralized in hermetic teflon digestors (561R2) by 3 ml nitric acid (65%). To determine Se, an aliquot of the previous sample solution was placed to dry in a sand bath (at 26°C), diluted in de-ionized water, and 1 ml nitric acid (1%) was added. Two replicate subsamples and a reference tissue (DOLT-2, NRCC) were included in each batch of the analysis. Mean recoveries were: 93% (Hg), 97% (Se). Mercury was determined by CV-AAS (PHILLIPS PV9200X) and Se was determined by the Graphite Furnace technique (VARIAN SPECTRA 30/40).

All the analyses were done at the Spectroscopy Service of the University of Barcelona.

Since the concentration of different elements, except selenium, fitted a log-normal model, log-transformed data were used for statistical analysis for those variables. Normality of distribution was assessed by means of NPlot and Shapiro-Wilks test. Interclutch comparisons, to detect a possible female effect on heavy metal levels, were performed using a random effect one-way ANOVA analysis. Intraclutch comparisons, to ascertain the effect of laying order, were examined by two simultaneous paired t-tests, with Bonferroni's correction for each element. The Pearson's correlation coefficient was used to determine the relationship between different elements; also, a Bonferroni's correction was applied to guarantee a table-wise significant level of $\alpha=0.05$.

RESULTS AND DISCUSSION

Descriptive statistics for the concentrations of elements analyzed are provided in Table 1.

Zinc, Cu and Mn concentrations in eggs of Yellow-legged Gulls were similar to those found in the eggs of Audouin's Gull *L. audouinii* from a neighbouring area, the Ebro Delta (Morera et al. 1987). However, the levels of Hg were much lower (ca. 10x). As has been pointed out by several authors (Leonzio et al. 1986, Scheuhammer 1987, Furness 1993), mercury enters eggs in a dose-dependent fashion, fish-eating seabirds reaching the highest body burden for this metal. Therefore, the interspecific difference could be explained by differences between the feeding habits of the two species: while Yellow-legged gulls from Medes Is. feed mainly (more than 60% in dry weight biomass) on garbage from refuse tips (Bosch et al. 1994), the Audouin's Gull at the Ebro Delta feed almost exclusively (95% in dry weight biomass) on fish, particularly clupeiforms (Ruiz et al. 1996, Oro et al. 1997). Similar differences have been found for Se, and though the relationship between Se egg concentration and parental dietary levels has not yet been clearly established in wild birds (Goede 1993), the experiments with caged animals fed known selenium doses suggest a close relationship (Heinz et al. 1987, 1989).

Table 1. Element concentration ($\mu g.g^{-1}$ d.w.) in the egg contents of *L.cachinnans*.

	N	Zn median mean (S.D)	Cu median mean (S.D)	Mn median mean (S.D)	Hg median mean (S.D)	Se median mean (S.D)	Ca median mean (S.D)
Total	33	54.4 55.1 (10.0)	2.34 2.28 (0.26)	1.67 1.71 (0.32)	0.46 0.52 (0.29)	1.48 1.44 (0.38)	2,310 2,354 (525)
a-egg	11	49.1 51.6 (7.1)	2.39 2.35 (0.28)	2.03 1.94 (0.28)	0.61 0.65 (0.32)	1.45 1.41 (0.27)	2,305 2,505 (812)
b-egg	11	52.5 55.3 (12.0)	2.37 2.31 (0.22)	1.67 1.65 (0.35)	0.53 0.50 (0.26)	1.26 1.33 (0.35)	2,331 2,304 (275)
c-egg	11	57.8 58.5 (10.0)	2.11 2.19 (0.26)	1.49 1.55 (0.21)	0.38 0.41 (0.25)	1.61 1.60 (0.49)	2,246 2,253 (329)

Our results for Hg are in the lower range of values reported for Yellow-legged Gulls in the Mediterranean, while Se levels are similar (see Lambertini and Leonzio 1986, and Focardi et al. 1988). Nevertheless, most of these samples were taken without considering the relevance of inter- and intraclutch variability sources of heavy metal levels in eggs (see e.g., Becker and Sperveslage 1989, Becker 1992, for *L. argentatus*, and Morera et al. 1997, for *L. audouinii).*

We found a significant effect of the laying female on the element concentrations in the eggs (Table 2), thus indicating that metal status of the female is reflected, to some extent, by levels found in their clutch. To our knowledge, the female effect for the other elements analyzed (Zn, Cu, Mn, Se and Ca) has not been previously reported in other wild birds. However, further studies are needed to establish adequately the transfer of these elements from the female to their eggs.

Copper, Mn and Hg levels decrease with the laying sequence (Table 3). In all cases the a-egg concentrations were higher when compared to the c-egg, Hg showing the highest difference. Our results agree with those of Becker (1992) in *L. argentatus* and Morera et al. (1997) in *L. audouinii.* Calcium showed also the same trend, though differences were not significant.

Table 2. Results of analysis of variance for female effect.

	F value	Prob.
Zn	2.57	0.03
Cu	2.46	0.04
Mn	2.67	0.03
Hg	11.99	<0.001
Se	3.14	0.01
Ca	2.77	0.02

Only two significant correlations were found for a table-wise α=0.05. Both, Zn and Cu concentrations showed a positive significant correlation with Ca (Zn-Ca: r= 0.48; Cu-Ca: r= 0.64), probably linked to vitellogenins activity during yolk formation (Burley and Vadehra 1989).

Table 3. Mean difference between eggs and results of the t-test for the laying order effect.

Difference	**Cu**	**Mn**	**Hg**
a-b	1.7% (ns.)	14.94% (*)	23.07% (*)
b-c	5.19% (n.s.)	6.06% (n.s.)	18% (ns.)
a-c	6.80% (*)	20.10% (*)	36.92% (*)

* Significant for α=0.05, Bonferroni corrected.

No significant correlation between Hg and Se was found. The relationship between both elements in seabirds remains unclear. In both, liver and eggs, such relationship has been found to be highly variable (Ohlendorf, 1993). In any case several authors postulate that even if the relationship is lacking, a potential protective effect of Se against Hg could not be discarded (Crivelli et al., 1989; Nielsen and Dietz, 1989; Walsh, 1990). Other authors, however, found that the presence of methylmercury in the diet greatly enhanced the storage of selenium in tissues of mallards and, despite counteracting the toxic effects of mercury in adults, can worsen the deleterious effects of mercury on reproduction (Heinz and Hoffman, 1996).

Magat and Sell (1979) found that most of the Hg in the egg is associated to ovoalbumin in the egg-white, while Se is mainly bound to the egg-yolk fraction. However, in mallards *(Anas platyrhynchos),* Heinz et al. (1987, 1989) reported that Se accumulation in the different fractions of the egg, depends greatly on Se chemical form.

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