Arch. Environ. Contam. Toxicol. 33, 71–75 (1997)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Inter- and Intraclutch Variability in Heavy Metals and Selenium Levels in Audouin's Gull Eggs from the Ebro Delta, Spain

M. Morera[1], C. Sanpera[1], S. Crespo[1], L. Jover[2], X. Ruiz[3]

[1] Departament de Biologia Animal, Biologia Vegetal i d'Ecologia, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[2] Departament de Salut Pública i L.S. (Bioestadistica), Facultat de Medicina, Universitat de Barcelona, Avda. Diagonal s/n, 08028 Barcelona, Spain
[3] Departament de Biologia Animal (Vertebrats), Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain

Received: 10 June 1996/Revised: 28 October 1996

Abstract. Heavy metal (Zn, Cu, Mn, Cd, Pb, Hg) and Se concentrations were analyzed in 57 Audouin's gull (*Larus audouinii*) eggs belonging to different clutch sizes. Inter- and intraclutch variability in metal concentrations was investigated as a potential source of bias in the assessment of pollution levels. Moreover, we analyzed the relationship between metal levels in the shell and in the contents, to evaluate the reliability of museum eggshells as indicators of historical changes of these pollutants. An outstanding female effect and/or a laying order effect underlies egg Hg levels; the fact that eggs in a clutch are not independent observations needs to be taken into account both when designing sampling strategies and when performing any analysis or interpretation of the results. The relationship between Hg in shells and contents is not sufficiently accurate to allow the use of egg-shell concentration as a reliable predictor of egg-contents concentration. However, if changes in the ecosystems are large enough it could be used to trace gross historical trends of these pollutants.

The Audouin's Gull (*Larus audouinii*) is one of the rarest gull species in the world, and one of the few SPEC species (Species of European Conservation Concern) belonging to category 1 (species of global conservation concern) in the Mediterranean (Tucker and Heath 1995).

The main potential threat for this species comes from the concentration of 84% of the world population in only two breeding colonies, those of the Ebro Delta and the Chafarinas Islands (Pedrocchi and Ruiz 1995). This situation is particularly sensitive to epidemiological and ecotoxicological risks that may affect such populations during the breeding season (Mace and Collar 1994).

In previous studies both, the incidence of organochlorine compounds at the Ebro Delta colony (Pastor *et al.* 1995a, 1995b) and potential risks derived from blood parasites (Ruiz *et al.* 1995) on these populations during reproduction, have been reported. However, nothing is known about other potentially hazardous elements, such as heavy metals. Mercury pollution is particularly relevant to Audouin's gull, because it is a pelagic predator endemic to the Mediterranean (Ruiz *et al.* 1996), a sea characterized by comparatively high Hg levels (Lambertini and Leonzio 1986; Renzoni *et al.* 1986). Winterquarters of this species are located in the Atlantic coasts of Northern Africa (Oro and Martínez-Vilalta 1994).

Because eggs can adequately reflect the environmental levels of some metals (Hg or Se) they have been used to monitor the exposure of wild bird populations (Furness 1993). However, although eggs have several advantages over tissue samples (Becker 1989; Burger and Gochfeld 1995) they also have some drawbacks, especially when the potential bias associated with inter- and intraclutch variability factors is unknown (Furness 1993). Therefore, the study of these sources of variability is a key factor in the design of sampling procedures to assess the incidence of such pollutants (Becker 1989, 1992).

In this study, inter- and intraclutch variability in concentration of heavy metals (Zn, Cu, Cd, Mn, Pb, Hg) and Se borne by shells and contents of Audouin's gulls eggs at the Ebro Delta was analyzed. Moreover, the relationship between levels of heavy metals in egg contents and in egg shell was examined, in order to assess the reliability of museum eggshells as surrogates of egg-contents, to trace historical changes of those pollutants in ecosystems (Furness 1993).

Material and Methods

Sampling

During the 1992 breeding season, 6,714 breeding pairs nested at the Ebro Delta colonies. Fifty-nine nests (14 one-egg, 27 two-egg, 16 three-egg, and 2 four-egg clutches) of *Larus audouinii* were monitored daily to determine egg characteristics according to laying order. Once discovered, eggs were marked with a felt tip pen on the blunt end. A total of 57 freshly laid eggs were collected (under license): 6 one-egg, 11 two-egg, 7 three-egg, and 2 four-egg clutches, being replaced in the nest by surrogate eggs to avoid interference with the laying process. Eggs were transported refrigerated to the laboratory for further analyses.

Correspondence to: C. Sanpera

Analysis: Eggs were weighed (± 0.01 g) and photographed, the eggshell carefully opened along the equator, and the contents (albumen + yolk) consecutively voided into a flask placed on a balance to obtain the weight of albumen and yolk separately. The yolk plus the albumen (thereafter the egg content) was homogenized, weighed, and oven-dried at 60°C to constant weight. Eggshells (including shell membranes) were gently washed in distilled water, left drying at room temperature for 24 h, weighed, and oven dried at 60°C to constant weight. All concentrations are reported on dry weight basis.

Lead, Zn, Cu, Cd, and Mn were analyzed in the eggshells and contents of all of the eggs, while total Hg and Se were only determined for two- and three-egg clutches.

Determination of Pb, Zn, Cu, Cd, and Mn: Eggshell samples were mineralized by 3 ml nitric acid 65% in pyrex volumetric flasks equipped with an air-cooled condenser. Egg contents were digested in two steps: first, by 5-ml nitric acid (65%); when the nitric acid had evaporated, 1.5 ml perchloric acid was added (65%). The concentration of these elements was determined by ICP-AES (JOBIN YVON 70). Two replicate subsamples were analyzed and a reference tissue (Bovine Liver 0868, NRCC) was included in each batch of the analysis.

Determination of Hg and Se: To determine Hg in eggshell samples, an aliquot was digested by 3 ml nitric acid (65%) in pyrex volumetric flasks equipped with an air-cooled condenser. Egg contents were acidified in hermetic teflon digestors (561R2) by 3-ml nitric acid (65%). To determine Se in contents, 5 ml of the previous sample solution were dried in a sand bath ($T = 26$°C), and later, diluted in deionized 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. Mercury was determined by means of Cold Vapor AAS (PHILLIPS PV9200X) and Se was determined by the Graphite Furnace technique (VARIAN SPECTRA 30/40).

All of the analyses were carried out at the Spectroscopy Service of the University of Barcelona.

Statistics

Interclutch comparisons were performed using a random effect one-way ANOVA analysis on clutches of two and three eggs, to detect a possible female effect for heavy metal levels. To compare the parameters studied among clutch sizes, we performed a one-way ANOVA analysis on mean clutch values, therefore assuming that eggs in a clutch are not independent observations (Becker 1992). Intraclutch comparisons to ascertain a possible laying order effect were performed using a paired *t*-test for two-egg cluthes, and two simultaneous paired *t*-tests, with Bonferroni's correction for three-egg clutches.

Since levels of heavy metals, except for Zn in the eggshell, fitted a log-normal model, log-transformed data were used for statistical analysis by parametric tests.

The relationship between concentrations of trace metals within and between compartments (eggshell and contents) was assessed by Pearson's correlation coefficient with Bonferroni's correction to guarantee a tablewise significance level of $\alpha = 0.05$. The predictive capacity of Hg concentration in shells for Hg concentration in contents was assessed using regression analysis on a subsample formed by one egg per clutch ($n = 17$) selected at random.

Results

Descriptive statistics of trace element concentrations are given in Table 1. Cadmium and Pb concentrations in both shell and contents were below the limit of detection (Pb: 50 ng/g; Cd: 5 ng/g). In some eggshells, Mn concentrations were not detectable (<5 ng/g). For those elements analyzed in both compartments, most of the load of the element was found in the contents (Zn: 97%; Cu: 80%; Mn: 95%; Hg: 99%).

A significant female effect was detected for Hg concentrations in shell and in contents of two-egg clutches, in Cu concentrations in shells of two- and three-egg clutches, and in Se concentrations in contents of three-egg clutches (Table 2).

When examining the effect of clutch size (one-, two-, and three-egg clutches), only Se in the egg content and Mn in shell showed significant differences. Se was clearly higher in three- than in two-egg clutches, while Mn concentrations were higher in two-egg clutches (Table 3).

A significant laying order effect was found for some metals within clutch sizes. In clutches of two eggs, Mn in the content was significantly higher in the a-egg ($t = 3.57$; d.f. = 10; $p = 0.005$). In three-egg clutches, significant differences were found in Hg concentrations in both the shell and contents only between b- and c-eggs (Hg_{shell}: $t = 4.06$, d.f. = 5, $p = 0.005$; Hg_{cont}: $t = 4.47$, d.f. = 6; $p = 0.002$); Hg concentrations decreased with laying order.

Significant positive correlations were found in the egg content between concentrations of Zn, Cu and Mn. No correlation was found between elements in the eggshell. Of the four metals analyzed in both compartments, only Hg showed a significant positive relationship between levels in content and shell (Table 4). The predictive equation of eggshell Hg concentration (ESC) for Hg contents concentration (ECC) is: ESC = 3.03 + 9.41 ECC ($r^2 = 0.33$, $p < 0.016$, $n = 17$) (Figure 1).

Discussion

The requirements and metabolism of essential elements in the egg have been mainly studied in domestic poultry (Richards and Steel 1987; Burley and Vadehra 1989). Except for Hg and Se, few data have been published on other metals in eggs of wild bird populations.

The concentration of Zn in the egg content of *Larus audouinii* (mean: 58.3 µg/g DW) is in the range of values found in Cory's shearwater *Calonectris diomedea* (37.3–64.2 µg/g DW) from various parts of the Mediterranean (Renzoni *et al.* 1986). Copper concentrations in the eggs of seabirds are usually low, between 0.15 and 1.8 µg/g FW (Blus *et al.* 1977; Walsh 1990; Stronkhorst *et al.* 1993), in agreement with Cu values found in our study (mean: 0.61 µg/g).

No data are available on Zn and Cu concentrations in the eggshells of seabirds. Nevertheless, it must be pointed out that the amount in this compartment is negligible when compared to that in the egg contents.

Mn levels in eggs of *L. audouinii* are lower than values reported for *L. argentatus* and *Sterna dougallii* from the east coast of the United States (Burger 1994; Burger and Gochfeld 1995). These authors found that Mn concentrations in the shell and in the content were similar, whereas in Audouin's gull, eggshell concentrations were much lower and, in some cases, the element was not detected.

Several authors have suggested that very little cadmium and lead is transferred from female body to eggs (Furness 1993 and references therein), although Burger (1994) pointed out that egg laying constitutes an excretion method for metals such as Cd and Pb in *L. argentatus.* In reviewing this topic, Scheuhammer (1987) suggests that transfer to the eggs only takes place if

Table 1. Concentration (μg · g^{-1} dry wt) of the different metals analyzed in Audouin's gull eggs from the Ebro Delta

	N	Zn Shell mean (S.D.)	Zn Contents mean (S.D.)	Cu Shell mean (S.D.)	Cu Contents mean (S.D.)	Mn Shell mean (S.D.)	Mn Contents mean (S.D.)	Hg Shell mean (S.D.)	Hg Contents mean (S.D.)	Se Contents mean (S.D.)
All eggs	56	6.58 (2.12)	58.32 (7.17)	2.14 (0.70)	2.58 (0.29)	0.29 (0.44)	1.69 (0.35)	0.22 (0.11)	5.06 (1.50)	4.12 (1.45)
C.Size 1	6	6.28 (2.24)	58.26 (6.11)	2.02 (0.72)	2.73 (0.27)	0.23 (0.30)	1.51 (0.29)			
C.Size 2	21	6.79 (1.86)	57.55 (5.42)	2.22 (0.89)	2.50 (0.29)	0.45 (0.62)	1.61 (0.29)	0.22 (0.13)	5.01 (1.54)	3.59 (1.54)
a-egg	10	6.90 (1.91)	59.53 (5.17)	2.29 (0.81)	2.61 (0.31)	0.41 (0.28)	1.74 (0.26)	0.22 (0.13)	5.23 (1.30)	4.06 (1.72)
b-egg	11	6.69 (1.90)	55.56 (5.15)	2.15 (0.58)	2.38 (0.23)	0.49 (0.85)	1.49 (0.27)	0.22 (0.13)	4.77 (1.77)	3.12 (1.25)
C.Size 3	21	6.36 (2.52)	57.84 (6.87)	2.24 (0.73)	2.60 (0.28)	0.15 (0.21)	1.77 (0.37)	0.21 (0.10)	5.10 (1.54)	4.66 (1.26)
a-egg	7	5.99 (2.36)	59.90 (9.29)	2.21 (0.53)	2.48 (0.13)	0.14 (0.22)	1.69 (0.34)	0.27 (0.13)	6.01 (1.90)	4.73 (1.42)
b-egg	7	5.65 (1.52)	56.16 (4.37)	2.06 (0.54)	2.58 (0.37)	0.20 (0.26)	1.78 (0.41)	0.21 (0.05)	5.20 (1.19)	4.38 (1.34)
c-egg	7	7.46 (3.33)	57.46 (6.57)	2.46 (1.03)	2.73 (0.27)	0.12 (0.13)	1.84 (0.39)	0.14 (0.03)	4.08 (0.84)	4.90 (1.13)
C.Size 4	8	6.81 (1.80)	61.72 (12.08)	1.74 (0.63)	2.61 (0.30)	0.30 (0.31)	1.83 (0.43)			
a-egg	2	7.92 (0.46)	71.17 (21.28)	1.76 (0.55)	2.45 (0.04)	0.29 (0.41)	1.58 (0.27)			
b-egg	2	7.90 (2.68)	56.16 (5.68)	2.32 (0.76)	2.48 (0.22)	0.33 (0.47)	1.66 (0.68)			
c-egg	2	4.80 (1.42)	52.59 (0.78)	1.07 (0.18)	2.58 (0.41)	0.35 (0.50)	1.90 (0.28)			
d-egg	2	6.62 (0.54)	66.96 (8.57)	1.81 (0.52)	2.95 (0.28)	0.24 (0.16)	2.18 (0.46)			

Table 2. Results of analysis of variance for female effect in two- and three-egg clutches

	Two-egg (n = 10)		Three-egg (n = 7)	
	F-value	Prob.	F-value	Prob.
Zn shell	1.26	0.36	0.90	0.51
Zn contents	1.22	0.37	2.36	0.08
Cu shell	8.33	0.0008	5.76	0.0041
Cu contents	0.79	0.63	2.49	0.074
Mn shell	0.49	0.83	2.14	0.17
Mn contents	1.82	0.17	2.16	0.11
Hg shell	7.73	0.0017	0.17	0.97
Hg contents	5.79	0.0038	0.95	0.49
Se contents	0.35	0.94	5.76	0.004

Table 3. Results of the analysis of variance for clutch size effect

			95% CI of the Estimated Mean		
	F-value	Prob.	One-egg n = 6	Two-egg n = 11	Three-egg n = 7
Zn shell	0.512	0.60	3.92–8.64	5.94–7.64	5.21–7.51
Zn content	0.030	0.97	51.84–64.68	55.14–59.95	54.72–60.97
Cu shell	0.309	0.74	1.27–2.78	1.91–2.53	2.00–2.41
Cu content	1.803	0.18	2.45–3.01	2.37–2.63	2.47–2.73
Mn shell	3.803	0.03	0–0.55	0.17–0.73	0.06–0.25
Mn content	1.801	0.18	1.21–1.81	1.48–1.74	1.60–1.94
Hg shell	0.281	0.60		0.16–0.28	0.16–0.26
Hg content	0.057	0.81		4.32–5.68	4.40–5.80
Se content	7.099	0.01		2.91–4.28	4.07–5.25

concentration in the tissues of the female are high. None of these metals was detected in eggs of Audouin's gulls, suggesting that these metals do not constitute a hazard for this species.

Mercury concentrations in eggs of *L. audouinii* from the Ebro Delta are amongst the highest values reported for seabirds, together with those provided by Leonzio *et al.* (1989) for the same species in other parts of the Mediterranean; Se levels are similar to those reported by these authors. Renzoni *et al.* (1986) found that Hg concentrations in eggs of Cory's shearwater from the Mediterranean were higher than in the Atlantic colonies of the same species and suggested that the origin of this Hg is natural resulting from the greater geochemical activity in this sea and from the lower turnover of water masses. Nevertheless, even if Hg concentrations are high, its relationship with hatching failures has not yet been clearly established (Heinz 1979; Fimreite *et al.* 1980; Barrett *et al.* 1985; Thompson *et al.* 1991; Becker *et al.* 1993). In our study, no case of reproductive impairment in the colony that could be associated with high Hg levels was observed.

Eggs from different clutches may present differences in metal concentrations, probably related with the age of the laying female or with other physiological parameters (Barrett *et al.* 1985; Becker *et al.* 1989). In seabirds, Becker (1992) found interclutch differences in Hg concentrations, both in *L. argentatus* and in *S. hirundo*, as well as decreasing Hg levels according to the laying sequence (a-, b-, c-eggs). In *L. audouinii*, a

Table 4. Correlation coefficient values among metals within eggshell (SH) and egg contents (CT), and between concentrations in eggshell and egg contents for each metal. Tablewise significance levels: *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, n.s. = not significant

	Zn (SH)	Cu (SH)	Mn (SH)		Zn (CT)	Cu (CT)	Mn (CT)	Hg (CT)
Cu (SH)	0.201 n.s.			Cu (CT)	0.369 ***			
Mn (SH)	0.115 n.s.	−0.063 n.s.		Mn (CT)	0.380 ***	0.285 **		
Hg (SH)	−0.071 n.s.	0.080 n.s.	0.237 n.s.	Hg (CT)	−0.066 n.s.	−0.022 n.s.	0.226 n.s.	
				Se (CT)	0.130 n.s.	0.332 **	0.105 n.s.	0.165 n.s.

Zn (CT) / Zn (SH)	0.081 n.s.	Cu (CT) / Cu (SH)	0.029 n.s.
Mn (CT) / Mn (SH)	0.299 *	Hg (CT) / Hg (SH)	0.400 **

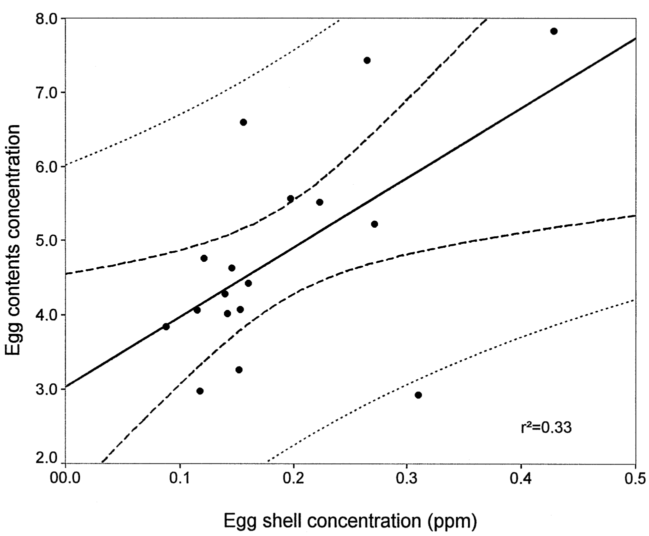

Fig. 1. Regression line of egg contents on eggshell Hg concentrations (solid line). 95% confidence bands for the regression line (dashed line). The prediction of Hg concentration in the egg content based on its Hg shell concentration is not enough accurate to be used for practical purposes, as can be seen from plotted prediction limits (95%) (dotted line)

significant female effect on Hg concentrations was detected in two-egg clutches, but not in clutches of three eggs. This results from the smaller variability between a- and b-eggs in clutches of two (a/b: 9%), whereas the decreasing Hg concentrations in three-egg clutches was much greater (a/b: 15%; b/c: 27%; a/c: 47%), thus indicating that larger sample sizes would be needed to detect female effect in that case.

If, as pointed out by various authors, the levels of Hg in the egg are determined by Hg levels in the female diet during the pre-laying season (Walsh 1990; Furness 1993), the decrease in Hg concentrations between a- and c-eggs would be related to the depletion of food intake during the laying process, a hypothesis in agreement with the decrease of courtship feeding rate after the laying of the first egg, because of male mateguarding behavior (Salzer and Larkin 1990). Moreover, this effect was probably enhanced by the food shortage induced by a trawler moratorium coinciding with the egg-laying period in 1992 (Oro *et al.* 1996), since Audouin's gull diet depends largely on fisheries discards (mainly clupeoids, perciforms, and pleuronectiforms) at this colony (Ruiz *et al.* 1996).

Since an outstanding female effect and/or a laying order effect underlie egg Hg levels, the fact that eggs in a clutch are not independent observations needs to be taken into account both when designing sampling strategies and when performing any analysis and interpretation of the results, as already pointed out by Furness (1993) regarding heavy metals and by Pastor *et al.* (1995a) in relation to organochlorines.

The positive relationship between Zn, Cu, and Mn levels found in the egg content can be mediated by vitellogenin, which carries Zn and Cu from the liver to the maturing oocyte. In the egg white, ovoalbumin and conalbumin binds both Zn, Cu, and Mn (Richards and Steel 1987; Eisler 1993). No correlation between Hg and Se in the egg content was found. Conversely to findings in sea mammals (Koeman *et al.* 1975), the relationship between both metals in seabirds is not clear, although this does not constitute evidence that no protective effect of Se against Hg is operating (Crivelli *et al.* 1989; Nielsen and Dietz 1989; Walsh 1990).

On the other hand, it is worth mentioning the positive significant correlation between Hg concentrations in the shell and in the content (Figure 1). This relationship is not sufficiently accurate to allow the use of eggshell concentration as a reliable predictor of egg-content concentrations. However, it could be used to trace historical trends in ecosystems, provided that the changes were large enough.

Acknowledgments. Daniel Oro collected eggs at the Ebro Delta. V. Pedrocchi and M. Bosch helped in the laboratory tasks. The authors are grateful to the P.N. Delta de l'Ebre staff for logistic facilities. English text was improved by Robin Rycroft (Escola Idiomes Moderns, Universitat de Barcelona). Funds were provided by DGICYT grant PB91-0271 of the Spanish Government.

References

Barrett RT, Skaare JU, Vader W, Froslie A (1985) Persistent organochlorines and mercury in eggs of Norwegian seabirds 1983. Environ Pollut 39:79–93

Becker PH (1989) Seabirds as monitor organisms of contaminants along the German North sea coast. Helgol Meeresunter 43:395–403

Becker PH (1992) Egg mercury levels decline with the laying sequence in Charadriiformes. Bull Environ Contam Toxicol 48:762–767

Becker PH, Conrad B, Sperveslage H (1989) Chlororganische Verbindungen und Schwermetalle in weiblichen Silbermöwen (*Larus argentatus*) und ihren Eiern mit bekannter Legefolge. Die Vogelwarte 35:1–10

Becker PH, Schuhman S, Koepff C (1993) Hatching failure in common terns (*Sterna hirundo*) in relation to environmental chemicals. Environ Poll 79:207–213

Blus LJ, Neely BS, Lamont TG, Mulhern B (1977) Residues of organochlorines and heavy metals in tissues and eggs of brown pelicans, 1969–73. Pestic Monit J 11:40–53

Burger J (1994) Heavy metals in avian eggshells: another excretion method. J Toxicol Environ Health 41:207–220

Burger J, Gochfeld M (1995) Heavy metal and selenium concentrations in eggs of herring gulls (*Larus argentatus*): Temporal differences from 1989 to 1994. Arch Environ Contam Toxicol 29:192–197

Burley RW, Vadehra DV (1989) The avian egg: Chemistry and biology. John Wiley, New York

Crivelli AJ, Focardi S, Fossi C, Leonzio C, Massi A, Renzoni A (1989) Trace elements and chlorinated hydrocarbons in eggs of *Pelecanus crispus*, a world endangered bird species nesting at Lake Mikri Prespa, northwestern Greece. Environ Pollut 61:235–247

Eisler R (1993) Zinc hazards to fish, wildlife, and invertebrates: A synoptic review. Contaminant Hazard Reviews, Biol Rep 10, 106 pp

Fimreite N, Kveseth N, Brevik EM (1980) Mercury, DDE, and PCBs in eggs from a Norwegian gannet colony. Bull Environ Contam Toxicol 24:142–144

Furness RW (1993) Birds as monitors of pollutants. In: Furness RW, Greenwood JJD (eds) Birds as monitors of environmental change. Champan and Hall, London, p 86

Heinz GH (1979) Methyl mercury: Reproductive and behavioral effects on three generations of Mallard ducks. J Wildl Manag 43:394–401

Koeman JH, van de Ven WSM, de Goeij JJM, Tjioe PS, van Haften JL (1975) Mercury and selenium in marine mammals and birds. Sci Total Environ 3:279–287

Lambertini M, Leonzio C (1986) Pollutant levels and their effects on Mediterranean seabirds. NATO ASI Ser 12:359–378

Leonzio C, Lambertini M, Massi A, Focardi S, Fossi C (1989) An assessment of pollutants in eggs of Audouin's gull (*Larus audouinii*), a rare species of the Mediterranean sea. Sci Total Environ 78:13–22

Mace GM, Collar NJ (1994) Extinction risk assessment for birds through quantitative criteria. Ibis 137 (Supplement 1, 1995): 240–246

Nielsen CO, Dietz R (1989) Heavy metals in Greenland seabirds. Meddr Gronland, Biosci 29, 26 pp

Oro D, Martínez-Vilalta A (1994) Migration and dispersal of Audouin's Gull *Larus audouinii* from the Ebro Delta colony. Ostrich 65:225–230

Oro D, Jover L, Ruiz X (1996) The influence of trawling activity on the breeding ecology of a threatened seabird, Audouin's gull *Larus audouinii*. Mar Ecol Prog Ser 139:19–29

Pastor D, Jover L, Ruiz X, Albaigés J (1995a) Monitoring Organochlorine Pollution in Audouin's Gull eggs: The relevance of sampling procedures. Sci Total Environ 162:215–223

Pastor D, Ruiz X, Barceló D, Albaigés J (1995b) Dioxins, furans and AHH-active PCB congeners in eggs of two gulls species from the western Mediterranean. Chemos 31:3397–3411

Pedrocchi V, Ruiz X (1995) On the current status of Audouin's gull *Larus audouinii* in the Mediterranean. In: Tasker ML (ed.), Threats to seabirds: Proceedings of the 5th International Seabird Group Conference. Seabird Group, Sandy, p 40

Renzoni A, Focardi S, Fossi C, Leonzio C, Mayol J (1986) Comparison between concentrations of mercury and other contaminants in eggs and tissues of Cory's shearwater *Calonectris diomedea* collected on Atlantic and Mediterranean islands. Environ Pollut 40:17–35

Richards MP, Steel NC (1987) Trace element metabolism in the developing avian embryo: A review. J Exp Zool Supp 1:39–51

Ruiz X, Oro D, González-Solís J (1995) Incidence of a *Haemoproteus lari* parasitemia in a threatened gull: *Larus audouinii*. Orn Fenn 72:159–164

Ruiz X, Oro D, Martínez-Vilalta A, Jover L (1996) Feeding ecology of Audouin's gulls (*Larus audouinii*) in the Ebro Delta. Colon Waterbirds 19 (Special Publ 1):68–74

Salzer DW, Larkin GJ (1990) Impact of courtship feeding on clutch and third-egg size in Glaucous-winged Gulls. Anim Behav 39:1149–1162

Scheuhammer AM (1987) The chronic toxicity of aluminium, cadmium, mercury and lead in birds: A review. Environ Pollut 46:263–295

Stronkhorst J, Ysebaert TJ, Smedes F, Meininger PL, Dirksen S, Boudewijn TJ (1993) Contaminants in eggs of some waterbird species from the Scheldt Estuary, SW Netherlands. Mar Pollut Bull 26:572–578

Thompson DR, Hamer KC, Furness RW (1991) Mercury accumulation in great skuas *Catharacta skua* of known age and sex, and its effects upon breeding and survival. J Appl Ecol 28:672–684

Tucker GM, Heath MF (1995) Birds in Europe: Their Conservation Status. Birdlife Conservation Series 3, Birdlife International, Cambridge, UK

Walsh PM (1990) The use of seabirds as monitors of heavy metals in the marine environment. In: Furness RW, Rainbow PS (eds) Heavy metals in the marine environment. CRC Press Inc, Boca Raton, FL, p 183