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Mercury in Feathers of Audouin's Gull (*Larus audouinii*) Chicks from Northeastern Mediterranean Colonies

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Abstract. Feathers of Audouin's gull chicks from three Aegean island areas (north Dodecanese, Cyclades, Kythera) Greece, were sampled in 1997 and 1998 and analyzed for mercury. Mean concentrations varied from 0.94 μg/g (Lipsos, Dodecanese, 1998) to 2.14 μg/g (Paros, Cyclades, 1998). Significant differences between years occurred in some regions (Lipsos, Fourni) but not in others (Paros). Within each year, especially in 1998, mean mercury concentrations differed among colonies. Results did not support the prediction that mercury levels would be higher in the north Dodecanese area due to the proximity of the polluted Menderes delta. There was no relationship between estimated chick age and feather mercury contents ($r = -0.04$, NS). Detected mercury levels do not seem to pose any toxic hazard to the Aegean Audouin's gull populations. However, the ease of sampling from gulls indicates that they may be a useful biomonitor of mercury contamination in this region.

Mercury, as a highly toxic metal, has attracted considerable scientific and public concern (Furness 1993). In birds, mercury is associated with a number of adverse effects, such as eggshell thinning, reduced egg production, lighter eggs, smaller clutches, and reduced hatching success and fledging rates (Burger 1993). Mercury binds with feather keratin in the form of methylmercury (Thompson and Furness 1989a, 1989b). Mercury accumulates in growing chick feathers (Thompson *et al*. 1991; Hahn *et al*. 1993). During the chick growth period the food is collected from near the breeding sites (Furness 1993), therefore mercury in chick feathers is derived from local sources. Removing a small quantity of body feathers from chicks has been recognized as a nondestructive technique for the detection of mercury and other heavy metals using birds as biomonitors of the environment and has extensively been used (Burger 1993; Furness and Camphuysen 1997).

Pollution studies in the Mediterranean Sea analyzing seabird eggs or feathers revealed considerable mercury pollution in some parts (Lambertini 1982; Lambertini and Leonzio 1986; Renchoni *et al*. 1986; Leonzio *et al*. 1989; Morera *et al*. 1997). Among the seabirds sampled is the Audouin's gull (*Larus audouinii*), an endangered species breeding only within the Mediterranean (Oro 1998). Most (70%) of its world population (about 18,600 pairs) is concentrated at the Ebro delta colony, Spain, and a generally low number of colonies are scattered mainly on Mediterranean islands (Oro 1998). Recent surveys carried out by the Hellenic Ornithological Society (HOS) discovered that in 1997, the Hellenic seas hosted at least 530 pairs in 20 colonies (HOS, unpublished data).

Mean mercury concentrations of 14.96 μg/g (Lambertini 1982), among the highest reported in bird feathers (cf. Burger 1993), and high levels in eggs (Leonzio *et al*. 1989; Morera *et al*. 1997) coupled with elevated concentrations of organochlorines in its eggs (Pastor *et al*. 1995a, 1995b) posed the necessity of risk assessment of pollutants on the populations of this rare gull (Morera *et al*. 1997). For these reasons we measured mercury levels in Audouin's gull feathers in Aegean colonies to investigate geographical mercury contamination and evaluate its potential hazards for the gulls. We also tested the prediction that elevated concentrations would be expected at the Dodecanese colonies (see below) because of their proximity (ranging 30–90 km) to the delta of Menderes River (Turkey). The Büyük (Big) Menderes River, occupies a drainage area of 24,976 km^2, including tributaries of some other rivers and streams, and many populated towns within its limits, of which most lack wastewater treatment plants. The Menderes River is also being polluted by an increasing number of geothermal energy production plants (Environmental Foundation of Turkey 1995). Organomercurial fungicides used in agriculture in Turkey have been detected in south Turkish wetlands (Ayas and Kolankaya 1996) and would probably be expected in this area.

Study Areas

This study was conducted during the breeding seasons of 1997 and 1998 including Audouin's gull colonies from north Dodecanese, Cyclades, and Kythera island areas, Greece. Audouin's

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gulls nested on small rocky islands. For reasons of species protection the colonies will be given regional names.

In north Dodecanese, three colony areas were studied. In both years, Lipsos colony at the southern part of this area was situated on a peninsula of an island (22.5 ha) with rough terrain and considerable altitudinal differences. Agathonisi colony, at the northeast, was situated on an almost level island (12.7 ha) in 1997, whereas in 1998 on a smaller island (9 ha) with rocky cliffs 8 km distant. During the study years, Fourni colony, at north Dodecanese area, was also situated on different islands with rocky cliffs and rough terrain (extending over 30 ha in 1997 and 35 ha in 1998) 4 km distant from each other.

In the Cyclades islands area, situated south east of the Dodecanese study area, Mykonos colony, situated at north Cyclades area, was made on an big island (1,500 ha). Amorgos colony, at southeastern part of this area, was made on an island about 25 ha. These two colonies were only studied in 1998. Paros colony, at the central part of the area, was made on an island extending over 22 ha and was studied in both years.

In the Kythera region, south of Peloponnesus, we studied Kythera colony (1997), located on a small (~3 ha), rocky island situated near the island of Kythera.

Detailed description of 1997 Dodecanese and Kythera colony sites is given in Goutner *et al*. (2000).

Materials and Methods

A small quantity of body feathers (from the mantle; Furness *et al*. 1986) were collected under license from a sample of chicks in each colony in June 1997 and 1998. Feather samples from each chick were preserved in a separate polythene bag. Chick weight was taken during feather sampling using portable electronic balances. Chick ages were estimated using the equation (Oro *et al*. 1996):

$$\text{Weight} = 18.51 \times \text{Age} - 43.39,\ r = 0.651,\ n = 80,\ p < 0.05$$

Prey fed to chicks was identified by examination of different types, such as food remains, regurgitates, dry boli, and pellets (Gonzales-Solis *et al*. 1997), collected during the chick growth period and later identified in the laboratory. In June 1998, fish species of those constituting prey of Audouin's gull were collected from trawler discards in the vicinity of Dodecanese colonies. Fish were placed in polythene bags and were subsequently deep frozen. In the laboratory they were thawed, weighed, and then freeze-dried to constant dry mass.

Between 50 and 100 mg of weighed feather or fish sample was placed in a 50-ml PTFE beaker with 10 ml of concentrated nitric acid. The beaker was covered with a watch glass, and the digest was gently boiled for 2 h. Samples were then left to cool for half an hour, after which 0.3 ml of hydrogen peroxide was added, and the beaker was returned to the hotplate for another hour. The digest was made up to 50 ml with distilled water in a volumetric flask and reduced using 2% tin (II) chloride in 10% hydrochloric acid. Total mercury concentrations were measured by atomic fluorescence spectrophotometry using a PSA 10.004 Vapour Generator coupled to a PSA 10.023 Merlin Fluorescence Detector (PS Analytical Orpington, Kent). Detection limits on a typical run (10 ng/g calibration) were less than 0.1 ng/g, which is an order of magnitude lower than lowest concentrations measured in this study. Precision and accuracy of the method were tested using standard reference materials (TORT-2 lobster hepatopancreas ±95% CI = 0.26 ± 0.03, n = 17, certified value = 0.27 ± 0.06) and replicate samples. Mercury levels are expressed in μg/g on a dry weight basis.

Feather mercury data were normally distributed, so we used parametric procedures throughout. Colony year data and intercolony data were compared using Student *t* tests and ANOVAs, respectively, using the Scheffe test to assess differences. Mercury contents were regressed on estimated chick ages.

Results

Dodecanese Colonies

Mean mercury contents of feathers from Fourni colonies differed significantly between 1997 and 1998 ($t = 5.408$, $df = 34$, $p < 0.0001$) with higher mean levels found in 1997 (1.68 μg/g) (Table 1). Significant differences between years were also detected in mean mercury contents at the colony of Lipsos ($t = 4.693$, $df = 28$, $p < 0.0001$) with the higher mean found in 1997 (1.71 μg/g). Nonetheless this difference was insignificant between years at the colonies of Agathonisi area ($t = 1.706$, $df = 21$, NS).

Cyclades Colonies

Mean mercury contents of feathers at the colonies of Paros were not significantly different between years ($t = -1.917$, $df = 14$, NS) (Table 1). The highest mean levels of all colonies studied were found within this area (Paros: 2.02 μg/g, Mykonos: 1.76 μg/g).

Kythera Colony

At Kythera colony the mercury contents of chick feathers were of the lowest found during the study (Table 1).

In 1997 the overall variation among all colonies in mercury concentrations of feathers was significant ($F = 3.580$, $df = 5, 77$, $p = 0.006$) due to the difference between Agathonisi (mean of 1.69 μg/g) and Kythera colony (1.20 μg/g) (Scheffe test). In 1998, this difference was highly significant ($F = 12.387$, $df = 4, 49$, $p < 0.0001$) due to significant differences between Mykonos colony (1.76 μg/g) and Fourni colony (1.02 μg/g); Mykonos and Lipsos colonies (0.94 μg/g); between Paros (2.02 μg/g) and Fourni colonies; and also between Paros and Lipsos colonies (Scheffe test). The mercury content of feathers between Dodecanese and other colonies did not differ significantly ($t = -1.51$, $df = 139$, NS).

We did not find any relationship between the feather mercury content and the age of the chicks ($r = -0.04$, NS).

The most important fish prey found in the colonies of Audouin's gull in the Dodecanese were *Boops boops*, *Chromis chromis*, *Spicara maena*, mugilids, and *Sardina pilchardus*. Mean mercury concentrations of the fish prey collected in 1998 (namely, *B. boops*, *C. chromis*, and *S. maena*) were similar ($F = 0.20$, $df = 2, 15$, NS, Table 2).

Discussion

Spatial and Temporal Differences in Feather Mercury Contents

Differences were detected in feather mercury content in both years among colonies and between years in some regional

Table 1. Mercury (μg/g) in Audouin's gull feathers from the Aegean colonies studied

Colonies	Year	Mean	SD	n	Minimum	Maximum
Dodecanese colonies						
Fourni	1997	1.68	0.29	15	1.28	2.42
Fourni	1998	1.02	0.40	21	0.36	1.73
Agathonisi	1997	1.69	0.39	16	0.96	2.28
Agathonisi	1998	1.38	0.42	7	0.72	1.93
Lipsos	1997	1.71	0.48	20	0.32	2.55
Lipsos	1998	0.94	0.27	10	0.61	1.46
Cyclades colonies						
Paros	1997	1.43	0.26	6	0.97	1.70
Paros	1998	2.02	0.38	10	1.13	2.45
Amorgos	1997	1.42	0.29	20	0.88	2.04
Mykonos	1998	1.76	0.71	8	0.40	2.58
Kythera colony						
Kythera	1997	1.20	0.33	8	0.76	1.77

Table 2. Mean mercury content (μg/g) of some fish prey of Audouin's gulls in the Dodecenese region

Fish Prey	Mean	SD	n	Minimum	Maximum
Boops boops	0.13	0.08	10	0.04	0.26
Chromis chromis	0.15	0.06	6	0.09	0.25
Spicara maena	0.16	0.01	2	0.15	0.16

colonies (Fourni and Lipsos) but not in others (Agathonisi and Paros). Geographical and/or yearly differences in feather mercury content of chicks are widely known in a variety of bird studies. In ciconiiforms, such differences usually reflected the pollution load of an area due to industrialization or high human population level (Burger *et al*. 1992, 1993, Burger and Gochfeld 1993, 1997). Herring gull chick-feather mercury variations in the Great Lakes, Canada, were explained by mercury differences in the main fish prey (Struger *et al*. 1987). Differences in feather mercury load of herring gull chicks found between 1990–1993 at Long Island, NY, were attributed to a different exposure from prey (Burger 1995). Regional differences in the nestling feathers of ospreys (*Pandion haliaetus*) at Great Lakes reflected local dietary conditions (Hughes *et al*. 1997).

Body mercury levels are dependent on rates of mercury elimination and uptake from food (Becker *et al*. 1994). In a laboratory experiment, the amount of mercury ingested by black-headed gull chicks determined the amount excreted into growing feathers (Lewis and Furness 1991), implying that species concentration in feathers reflect dietary intake of mercury. Thus, the relative mercury uptake would depend on the relative prey amounts chicks were fed. If these amounts were different, they could partly explain the changes observed in mercury content of feathers. Prey composition and availability was probably different between years, and this could explain observed differences. Fish prey seems to be the main contributor of mercury in the diet of Audouin's gull, which also feeds on insects, birds, small mammals, molluscs, and probably plants (olives) (HOS, unpublished). For a better understanding of the dynamics of mercury uptake by this gull, adequate quantitative information is needed on the diet of the Audouin's gull in the study area and probably the contribution of some other prey types. Dietary analyses (HOS, unpublished) suggest that most prey types taken by these gulls originate from natural habitats, and, additionally, no Audouin's gulls have been observed during the breeding season in rubbish dumps on nearby islands. Therefore, mercury in Audouin's gull chick feathers reflects that available in the surrounding seas. Although adults have been sometimes seen to seize prey at the vicinity of colonies, the extent of their feeding grounds and distances covered searching for prey are still unknown for most of the colonies studied. Gulls usually forage relatively near their colonies—herring gull: about 10 km (Morris and Black 1980); Mediterranean gull: 11.4 km (mean); and black-headed-gull: 5.8 km (mean, Fasola and Bogliani 1990). If in our study areas, Audouin's gulls move within these distances, then mercury is derived from local pollution unless pollution is transferred through currents from greater distances. Although some of the highest means were found in the Cyclades colonies, overall our data did not support the prediction about a possible higher pollution at the Dodecanese colonies. The source of mercury pollution in Cyclades is at present unknown. Nevertheless, the highest values found at Paros and Mykonos colonies reflect that within our study area, at least seasonally, these areas are of the mostly populated as hosting hundreds of thousands of tourists in each spring and summer. We also suspect that this pollution occurs along the sea channel between Dodecanese and Cyclades used intensively for national and international navigation traffic.

Chick Age and Feather Mercury Contents

Whereas in adult larids feather mercury content is independent of age (red-billed gull, Furness *et al*. 1990; common tern, Burger *et al*. 1994) in chicks the situation sometimes differs. Thus, with increasing age, chicks of herring gull and common

tern had less mercury in their plumage, but in the black-headed gull such differences were not found (Becker *et al*. 1994). A drop of mercury was also found in feathers at fledging time in the great white egret (*Egretta alba modesta*) (Honda *et al*. 1986), whereas in the white stork (*Ciconia ciconia*) mercury increased with the chick age as a result of longer exposure due to a very prolonged fledging period (Goutner and Furness 1998). No age-related trend of mercury in the feathers of the little egret (*Egretta garzetta*) occurred (Goutner and Furness 1997). Lack of such a trend in the feathers of the great skua (*Catharacta skua*) chicks was attributed to both an increase in exposure to mercury as chicks grew (due to an increase in food intake), and a simultaneous increase in chick size (Thompson *et al*. 1991). Probably this was also the case for the Audouin's gull chicks in the Aegean.

Toxicological Impacts

Mercury levels exceeding 2–5 μg/g are considered toxic for wild birds (Ohlendorf and Gochfeld 1997). Beyer *et al*. (1997) suggested that mercury content of 9 μg/g or more in feathers may be associated with toxic effects in wading birds. Goutner and Furness (1998) summarized a variety of studies indicating that toxic effects of mercury may well vary among different bird species. Herring gulls seem to have relatively high threshold toxicity levels (eggs hatch between 0.5 and 2 μg/g) in comparison to game birds where such levels cause hatching failure (Vermeer 1971). In another study, Vermeer *et al*. (1973) suggested that levels of 2–16 μg/g in herring gull eggs did not seem to have been associated with any adverse effects. The yellow-legged gull (*Larus cachinnans michahellis*), a species sympatric to Audouin's gull, shows lower levels of mercury probably in large part due to its dependence on refuse tips for food. In this bird, mercury residues ranged from a mean of 0.52 μg/g (Sanpera *et al*. 1997) to a mean of 3.50 μg/g (Focardi *et al*. 1988) in eggs and a mean of 8.76 μg/g in chick feathers (Lambertini 1982). No toxic effects were detected by the authors. Leonzio *et al*. (1989) reported high concentrations in eggs of Audouin's gull in some parts of the Mediterranean, and Morera *et al*. (1997) found a mean of 5.06 μg/g (dry weight) in eggs from the Ebro delta, being one of the highest reported for larids. Feathers have fivefold higher concentrations of mercury than eggs (dry mass) from the same individual (herring gull, Lewis *et al*. 1993). Thus, concentration of 5.06 μg/g reported in eggs from the Ebro delta Morera *et al*. (1997) probably imply concentrations of above 25 μg/g in body feathers of adult females in this area. Despite these high levels, Morera *et al*. (1997) did not notice any adverse effects on reproduction. Lambertini (1982), though reporting a mean of 14.96 μg/g mercury in Audouin's gull chick feathers, did not mention any hazard on them. This review suggests that the Audouin's gull seems to be very resistant to mercury pollution. Probably this gull being an endemic species of the Mediterranean environment is well adapted to the elevated concentrations of mercury encountered in this region. Therefore, mercury levels detected in chick feathers in the Aegean seem too low to create any hazard to them.

Using Audouin's Gull as a Mercury Biomonitor

The Aegean Sea, being a relatively closed part of the eastern Mediterranean, receives the discharges of some major rivers from Greece and Turkey and considerable fluxes from the Black Sea through Dardanelles (Stergiou *et al*. 1997). Mercury has not yet been monitored in the Hellenic marine environment. Considering Audouin's gull as a bioindicator, it may present the following advantages. As a top predator of small sized surface-living fish, it accumulates mercury from the epipelagic zone, integrating this metal from lower trophic levels, thus, mercury in chick feathers reflects contamination in this marine zone (Monteiro and Furness 1997). Additionally, mercury concentrations show less individual variation in gull feathers than found in their fish prey due to the size/age accumulation in fish that does not occur in birds. Audouin's gulls breed in many widely scattered colonies representing many different parts of a diverse marine environment. Yearly mercury differences detected, may, on one hand, indicate sensitivity of this gull to changing mercury concentrations in its environment, but, on the other, suggest the need for monitoring over a series of years (see also Burger 1995). The remoteness of breeding islands and considerable logistics involved may be a disadvantage, and Audouin's gull is a sensitive species from the conservation point of view, thus disturbance at breeding sites should be avoided. HOS surveys between 1997–1999 discovered new colonies across the Aegean; thus, a mercury monitoring program could involve those representing areas of particular interest where chick feathers could be collected and analyzed on a yearly basis, for example in combination with a chick-ringing program.

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