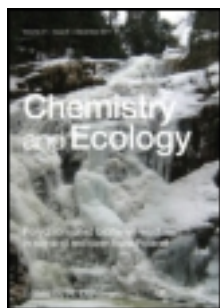


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Chemistry and Ecology

Publication details, including instructions for authors and subscription information:

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Version of record first published: 23 Nov 2012.

To cite this article: Vassilis Goutner, Peter H. Becker & Vasilios Liordos (2012): Low mercury contamination in Mediterranean gull *Larus melanocephalus* chicks in Greece, *Chemistry and Ecology*, DOI:10.1080/02757540.2012.744828

To link to this article: <http://dx.doi.org/10.1080/02757540.2012.744828>



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Low mercury contamination in Mediterranean gull *Larus melanocephalus* chicks in Greece

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(Received 12 June 2012; final version received 18 October 2012)

Mercury (Hg), a toxic heavy metal harmful to animals, commonly occurs in Mediterranean ecosystems. Mediterranean gull *Larus melanocephalus* numbers have declined considerably in Greece since the 1990s. Mediterranean gull chicks' back feathers were therefore collected from three Greek colonies in different years between 1999 and 2009 to assess Hg pollution in this larid. Hg concentrations varied with arithmetic means ranging between 815 ng g⁻¹ dry weight (dw) (Lafri Lagoon in 2000) and 1264 ng g⁻¹ dw (Evros Delta in 2000), and values were independent of chick age, although significant among-year and among-site variations were found. Hg concentrations in Greek Mediterranean gull chick feathers were generally low and in fact lower than those associated with adverse effects in other bird species. Comparisons with other studies revealed that Hg concentrations in Mediterranean gulls from Greece were mostly lower than those found in other gull species elsewhere in the world. This study presents the first data on Hg contamination in the Mediterranean gull. The results might be useful in understanding and evaluating the birds' exposure to this highly toxic pollutant and its impact on both local populations and the wider ecosystem.

Keywords: Mediterranean gull; mercury; wetlands; Greece

1. Introduction

Mercury (Hg) is a highly toxic heavy metal, widely occurring in the Mediterranean environment due to both natural emissions such as volcanoes, fumaroles, solfataras and geological anomalies [1], and anthropogenic sources from industry and waste incineration [2,3]. In fact, Hg concentrations in various seabirds, fish and shellfish have been found to be higher in the Mediterranean than in Atlantic areas [4,5]. In the Mediterranean in particular, Hg deposition is greatly affected by air mass transportation of particulate and elemental Hg from northern and northeastern Europe. Wet deposition is the most efficient removal pathway for atmospheric Hg, and is predicted to occur in the highest levels over mountainous areas such those in northern Greece, as expected, due to the higher precipitation usually occurring there [3].

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In aquatic systems, inorganic Hg is biotransformed into methylmercury with bioaccumulative capacities [6]. Methylmercury is biomagnified in avian food chains and can reach high concentrations, especially in fish-eating birds, showing a clear trophic level relationship [7]. Birds reduce their Hg body burden by excreting considerable amounts of methylmercury in feathers during moulting [8–11]. In addition, Hg concentrations in the first down of chicks are a consequence of Hg levels in the egg, whereas concentrations in the feathers of chicks are largely due to Hg ingested in food [12,13]. Therefore, growing feathers accumulate Hg through the chick feeding at the breeding area during a short period of growth and can be used to assess and monitor local Hg pollution [5,11,14–17]. The negative effects of Hg on human health [18,19] have prompted ever-increasing interest in environmental quality which has led to a large number of studies on the monitoring and evaluating of Hg pollution in habitats, using waterbirds as biomonitors [20–24].

The Mediterranean gull *Larus melanocephalus* at first mainly inhabited the Black Sea and Mediterranean areas, but since the mid-1980s it has shown an impressive expansion of its range, breeding in many European countries [25]. The Greek breeding population reached a maximum at the end of the 1980s, decreasing considerably thereafter. Among the 25 countries in the breeding range, Greece is one of the seven hosting >1000 pairs [26]. The Axios and Evros Deltas were the most important breeding areas in Greece, although occasional breeding takes place in some other coastal wetlands ([27] and unpubl. data).

Despite the plethora of studies on Hg contamination for many waterbird species, including larids, none has yet been conducted on the Mediterranean gull. Greece is situated within the Mediterranean basin, which is contaminated with Hg to a great extent. The first toxicological assessment of Hg in the Mediterranean gull will provide useful information on the Hg burden in the wider environment and will allow for comparisons with other gull species. Our understanding of the biogeochemical cycle of a hazardous heavy metal in the Mediterranean ecosystem, as well as of the ecology of the respective species, will be increased. The aims of this article are therefore to: (1) measure Hg concentrations in Mediterranean gulls from three Greek wetlands in different years, through analysis of chick feathers; (2) estimate trends in Hg pollution among sites and years; and (3) compare Hg concentrations in Greek Mediterranean gulls with those in other larid species reported in the literature.

2. Materials and methods

2.1. Study area and sampling

Mediterranean gulls were studied in the Axios and Evros Deltas, and at Lafri Lagoon (Figure 1). These areas have been designated as Wetlands of International Importance under the Ramsar Convention. The Axios Delta (40°27' to 40°38'N, 22°33' to 22°52'E) belongs to a large wetland complex of 68.7 km², situated near the city of Thessaloniki, formed by the rivers Axios, Loudias and Aliakmon. The Evros Delta at the Greek–Turkish border (40°44' to 40°51'N, 25°53' to 26°8'E) is an extensive area (190 km²) including diverse habitats. Lafri Lagoon (40°59'50"N, 25°02'40"E) is a small lagoon (1.1 km²) within the wetland complex of Lake Vistonis and Porto Lagos lagoons.

Feathers from different parts of seabird plumage show considerable differences in Hg concentrations; however, back feathers have the lowest Hg variability [28]. Intra-individual variability has also been found in the body feathers of adult seabirds [29]. Therefore, for samples to be comparable, only back feathers (between the shoulders) were collected from Mediterranean gull chicks. Sampling bouts were carried out at similar dates in several years between 1999 and 2009: during five years in the Axios Delta (2003, 2006, 2007, 2008, 2009), three years in the Evros Delta (1999, 2000, 2003) and one year at Lafri Lagoon (2000). In the Axios Delta, the gulls also bred in 2004 and 2005, but there was no sampling programme in these years, whereas in

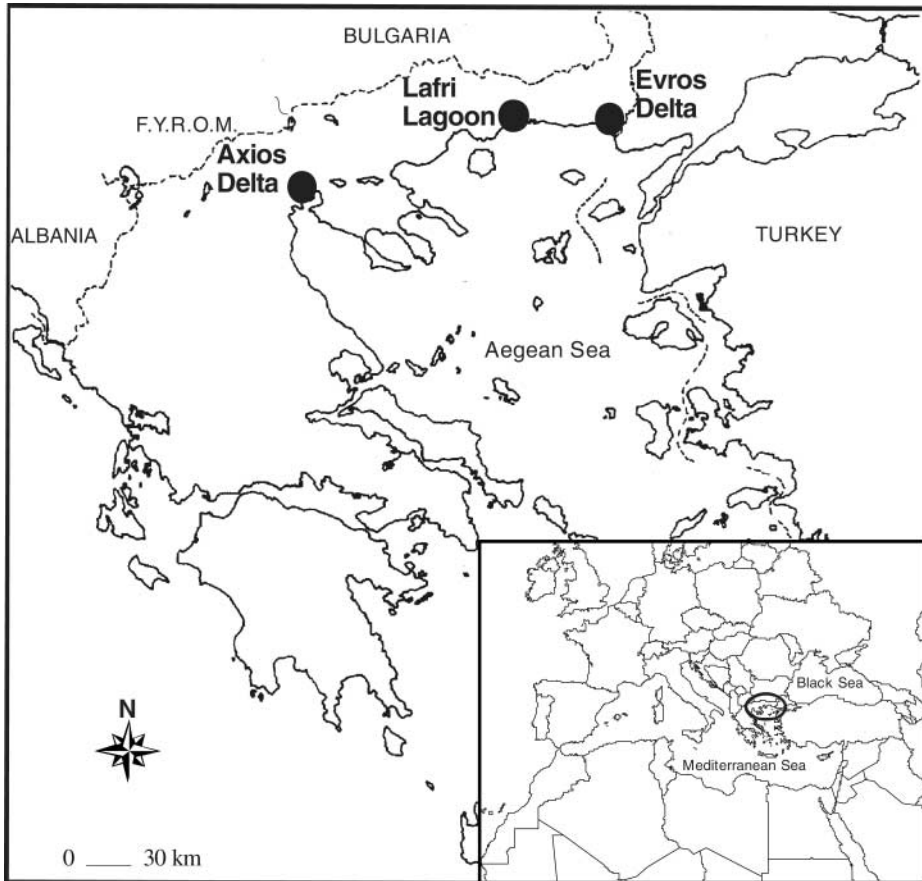


Figure 1. Map of Greece showing the location of the three Mediterranean gull breeding colonies, from where chick feathers were collected. (Inset) The central position of the studied colonies within the Mediterranean and Black Seas, the species' most significant historical breeding area.

the other two areas the gulls bred only in the years sampled. The gull colonies were located on either natural coastal islets (Axios and Evros Deltas) or an artificial islet (Lafri Lagoon). The feathers were stored in Hg-free plastic bags until analysis. Chick body mass was calculated to the nearest 0.1 g using a portable electronic balance and tarsus length was measured to the nearest 0.01 mm using digital calipers (Axios and Evros Deltas in 2003). The effect of measurement error in morphological characters can be substantial [30,31] and therefore, all visits were made early in the morning to avoid diurnal variations in body mass and are taken by the same person (VG) to eliminate variation among investigators. In addition, two repeated measurements of each chick were averaged to increase accuracy [30].

2.2. Analytical procedures

Feather samples were analysed by the Institute of Avian Research in the laboratory of the Institute for Chemistry and Biology of the Marine Environment (ICBM-Terramare) in Wilhelmshaven, Germany. Feathers were handled using a polythene pincers cleaned between samples with laboratory detergent and water, 10% nitric acid and bidistilled water. Cleaning tubes were also washed with detergent and water and were left overnight in 10% nitric acid (p.a. grade), then washed

with bidistilled water and air-dried in a ventilated fume cupboard. Prior to analysis, each feather sample was also placed into a glass container and separately washed three times with bidistilled water and acetone (p.a. grade) in an ultrasonic bath. The Hg measurement was accomplished with a DMA-80 Direct Mercury Analyser. This analyser has the potential for sample analysis in a short time without sample chemical preparation and no waste disposal [32,33]. Each sample analysed comprised 2–3 feathers, weighed, wrapped in aluminum foil and introduced into the sample boat of the analyser. The sample was initially dried and burned at 750 °C in an oxygen current. The high temperature decomposes Hg compounds and elemental Hg is set free. Hg vapours are collected on a gold amalgamation trap and subsequently desorbed for quantification. Hg content is determined using atomic absorption spectrometry at a wavelength of 254 nm.

All samples were analysed in duplicate. For each series of 20 samples, a reference measurement was made. The certified reference material (CRM) was bought at National Research Council Canada (NRC-CNRC) and was DORM2 (dog fish muscle CRM for trace metals, 4640 ng g⁻¹) and DORM3 (fish protein CRM for trace metals, 409 ng g⁻¹). The measured values and analytical variance for DORM2 were 4385 ± 567 ng g⁻¹, coefficient of variance (CV) = 10.5%, and for DORM3 414 ± 43 ng g⁻¹, CV = 12.9% (*N* = 10 replicates). The laboratory takes part in international routine intercalibrations for mercury (i.e. Quasimeme) with satisfactory results. A batch of 20 feather samples was assessed only if blanks with empty sample boats and empty packets of foil were in the range of detection limit (see below).

Linear regression of log-transformed Hg concentrations in the two duplicates analysed suggested adequate analytic repeatability ($a = 0.08$, $p = 0.350$, $b = 0.97$, $p < 0.001$, $df = 1, 203$). The detection limit is 0.003 ng total Hg, the concentration is given in parts per billion on a dry weight basis (ng g⁻¹ dw).

2.3. Data analysis and statistical procedures

Hg concentrations deviated significantly from normality in all samples (Kolmogorov–Smirnov test, $p < 0.01$) and they were therefore log-transformed before further analyses [34]. Subsequently, chicks' tarsus length and body mass were used as age surrogates to assess the relationship of Hg concentration with age [35]. Chicks' tarsus length is a good predictor of age because it grows linearly with age and shows high growth rates even when food is scarce [35–37]. Body mass can also be used as an indication of age, although with less accuracy because heavy, well-fed chicks may be assigned to a higher age than lighter, poorly fed ones, and also Mediterranean gull chicks tend to lose weight before fledging, after the age of ~15 days [35–37]. However, the effect of weight on pollutant concentration can still be assessed.

Samples in different colonies were, in most cases, collected in different years and could not therefore be used for among-colony comparisons. Consequently, within-site temporal trends were assessed with one-way analysis of variance (ANOVA) in colonies for which multiple year samples were available (i.e. in the Axios and Evros Deltas), whereas between-site comparisons in the same year were made using the *t*-test (2000: Evros vs. Lafri; 2003: Axios vs. Evros). If differences were significant multiple pairwise comparisons were made using Tukey's HSD test for unequal sample sizes [34]. Geometric means are also given to facilitate comparison with other studies in the literature. All statistical analyses were performed in Statistica, version 6.0 (StatSoft, Tulsa, OK, USA).

3. Results

Hg concentrations in back feathers from Mediterranean gull chicks ranged from 229 ng g⁻¹ dw (Axios Delta in 2008) to 2845 ng g⁻¹ dw (Evros Delta in 1999) (Table 1). Linear regressions of log-transformed Hg contents with age surrogates were performed and slopes were found to be not

Table 1. Significant interyear differences within sites and differences among sites within the same year were found in mean total mercury concentrations (ng g^{-1} dw); (\pm SD, sample size, range) in back feathers of Mediterranean gull nestlings taken from three Greek breeding colonies.

Site	Year	Arithmetic mean	Geometric mean	<i>N</i>	Range
Axios Delta	2003	825 \pm 304	779	20	418–1618
	2006	931 \pm 238	903	22	574–1489
	2007	1130 \pm 201	1113	19	787–1511
	2008	1013 \pm 310	953	21	229–1655
	2009	1013 \pm 326	971	20	613–1981
Evros Delta	1999	944 \pm 429	877	40	349–2845
	2000	1264 \pm 305	1229	13	783–1684
	2003	1142 \pm 430	1080	20	552–2562
Lafri Lagoon	2000	815 \pm 281	768	30	311–1432

Table 2. Linear regressions of log-transformed Hg contents (ng g^{-1}) in back feathers with tarsus length or body mass (used as age surrogates) of Mediterranean gull nestlings did not reveal slopes significantly different from zero, suggesting that Hg concentrations varied independent of age.

Site	Year	<i>a</i>	<i>b</i>	<i>R</i> ²	<i>F</i>	<i>p</i>	<i>df</i>	Morphometric measurement
Axios Delta	2003	3.296	−0.008	0.020	0.369	0.551	1,18	Tarsus length (mm)
	2006	2.884	0.0002	0.006	0.123	0.729	1,20	Body mass (g)
	2007	2.983	0.00002	0.008	0.138	0.714	1,17	Body mass (g)
	2008	3.167	−0.0006	0.016	0.306	0.586	1,19	Body mass (g)
	2009	3.218	−0.0008	0.044	0.821	0.376	1,18	Body mass (g)
Evros Delta	1999	2.776	0.0006	0.014	0.556	0.460	1,38	Body mass (g)
	2000	3.089	0.00002	0.000	0.000002	0.999	1,11	Body mass (g)
	2003	3.902	−0.0185	0.054	1.029	0.754	1,18	Tarsus length (mm)
Lafri Lagoon	2000	2.910	−0.00008	0.000	0.009	0.927	1,28	Body mass (g)

significantly different from zero (Table 2), suggesting that Hg concentrations varied independent of age. Among-year comparisons revealed significant differences in Hg concentrations both in the Axios ($F = 3.55$, $df = 4, 97$, $p = 0.009$) and Evros Deltas ($F = 5.79$, $df = 2, 70$, $p = 0.005$; Table 1). Post-hoc pairwise comparisons with Tukey's HSD tests revealed significant differences in mean Hg concentrations in the Axios Delta between the years 2003 and 2007, being higher in the latter ($p = 0.004$; Table 1), and in the Evros Delta between the years 1999 and 2000, being higher in the latter ($p = 0.037$; Table 1). All other comparisons were not significant ($p > 0.137$). Because significant inter-annual variation was detected in both the Axios and Evros Deltas, among-site differences within the same year were only assessed. Mean Hg concentrations were significantly higher in the Evros Delta than at Lafri Lagoon ($t = -4.28$, $df = 41$, $p = 0.0001$; Table 1) in 2000 and significantly higher in the Evros Delta than in the Axios Delta ($t = -3.07$, $df = 38$, $p = 0.004$; Table 1) in 2003.

4. Discussion

This study presents the first data on Hg contamination in the Mediterranean gull. Mean Hg concentration in Mediterranean gull chick feathers varied from 815 to 1264 ng g^{-1} dw in the studied colonies and were similar to or, mostly, lower than those reported in other studies for both adult and chick feathers of several gull species (Table 3). Mediterranean gulls move >30 km

away from the colony to feed and can thus exploit a great variety of habitats (open water, coastal, inland) and consequently prey types [38,39]. However, it has been found that Mediterranean gulls mostly feed on terrestrial prey during the breeding season, a pattern contrasting that of other gull species such as the herring *L. argentatus* and Audouin's gulls *L. audouinii* [25,38,40]. Goutner [40] also found that Mediterranean gulls in northern Greece mainly fed their chicks with terrestrial prey, in varying proportions, depending on year and sampling method (insects: mainly Coleoptera, Orthoptera, Hymenoptera, 46.5–77.4% numbers, 12.7–46.7% biomass; molluscs: mainly Helicellinae gastropods, 8.9–38.3% numbers, 2.9–16.6% biomass; plant material: mainly wheat, 1.7–5.7% numbers, 45.8% biomass). However, consumption of aquatic prey (fish) has also been observed [39,41].

Hg pollution studies are rare in Greece and were only conducted in several northern Greek water bodies in the 1980s [42], when Axios River was reported as the most Hg-polluted water body (mean concentration 800 ng g⁻¹, range 480–1190 ng g⁻¹; non-filtrated samples). Skoulikidis [43] contended that the highest levels of heavy metal pollution occur in transboundary rivers entering Greece, transported from neighbouring Serbia, Bulgaria and Turkey. Ferro-alloys, smelter and fertiliser plants disposing of their solid waste near the river beds, untreated industrial and domestic waste water discharging into the rivers, agricultural run-off and illegal landfills contaminating surface and ground water are the main remote sources of Hg pollution in Axios and Evros areas [43–45]. By contrast, Haidouti et al. [46] reported low Hg contents in profiles from 11 terrestrial (agricultural) areas ranging from 25 to 98 ng g⁻¹, being within the ranges found in uncontaminated soils elsewhere in the world.

Because aquatic habitats in northern Greece are exposed to higher Hg contamination than terrestrial habitats and as Hg bioaccumulates in organisms, it is expected aquatic prey to be more contaminated than terrestrial prey. This trend has been confirmed by Goutner and Furness [47] and Goutner et al. [48], when they found that white stork *Ciconia ciconia* chicks from terrestrial areas in northern Greece were significantly less exposed to Hg contamination than those from aquatic areas. The lower Hg concentration found in Mediterranean gulls compared with other gull species may therefore be mostly attributed to the prevalence of terrestrial prey in the chicks' diet, as suggested by earlier research.

As Hg bioaccumulates in organisms, concentrations increase with trophic level (biomagnification) and, in the same habitat or habitats with similar Hg exposure, among-species variations are mostly a function of trophic position [21,49–51]. In fact, it has been found that Hg concentrations in chick feathers were higher in the herring than in the black-headed gull *Larus (Croicocephalus) ridibundus*, with the former feeding in a higher trophic level than the latter [13]. What is more, the within-species differences in Hg concentrations among years and colonies found in our study could also be related to temporal and spatial differences in trophic ecology [5] and environmental conditions, although larger sample sizes and the examination of all possible factors involved are needed to draw safer conclusions.

The Hg threshold of adverse effects has not been determined for the Mediterranean gull. However, a concentration of 5000 ng g⁻¹ in feathers has been proposed and is currently used as a threshold of adverse effects for some bird species [7,18,22]. The Hg concentrations found in Mediterranean gull chicks in our study fell well below this threshold, suggesting that they should not pose a significant health threat for this species, or be considered responsible for its recent population decline in Greece.

Hg concentrations in Forster's tern *Sterna forsteri*, black-necked stilt *Himantopus mexicanus* and American avocet *Recurvirostra americana* chick feathers from San Francisco Bay, California, were highly variable and declined linearly with age [17]. An inverse relationship between body size and Hg concentration was also detected in black-headed, common *L. canus* and little gulls *L. minutus* from the south coast of the Caspian Sea in Iran [23]. In our study, Hg concentrations in Mediterranean gull feathers were generally independent of chick age, however, several sources

Table 3. Arithmetic mean concentrations of Hg (ng g⁻¹ dw) detected in feathers of gulls from various geographical areas.

Species	Mean or range of means	Ranges	Area	Feather type	Collection year	Ref
<i>Larus argentatus</i>	1270	470–2890	Wadden Sea, Germany	back	1991	[13]
	1799	–	Long Island, N.Y., USA	breast	1989–1993	[55]
	761–2833	–	Massachusetts to Delaware	breast	1990	[56]
<i>Larus ridibundus</i> ¹	6410 (m), 4870 (f) (a)	2150–10940	Wadden Sea, Germany	body	1990	[10]
	880	140–2110	Wadden Sea, Germany	back	1991	[13]
<i>Larus pipixcan</i> ²	5100–7100	–	South coast, Caspian Sea, Iran	breast, tail	2008	[23]
	400(b)	–	Agassiz, USA	body	1999	[57]
<i>Larus glaucescens</i>	842 (m), 768 (f) (a)	–	Agassiz, USA	breast	1994	[58]
	1180–3480	–	Aleutians, N. Pacific, USA	breast	?	[59]
<i>Larus michahellis</i>	2100 (a)	750–4360	Ebro Delta, Spain	mantle	1997–1999	[60]
<i>Larus michahellis atlantis</i>	1300–3600	–	Eastern Iberian coast, Spain	scapular	2004	[54]
	4000	2700–6500	Azores Archipelago	body	1990–1992	[61]
<i>Larus canus</i>	2900–11300 (a)	–	Shiranui Sea, Japan	body	1965–1979	[62]
	4290 (a)	2740–8470	Iran (Khuzestan to Persian Gulf)	tail	1991–1996	[63]
<i>Larus novaehollandiae scopulinus</i> ³	2020	–	Kaikoura Peninsula, New Zealand	body	1988	[64]
<i>Larus audouinii</i> ⁴	1270 (a)	290–5180	Ebro Delta, Spain	mantle	1997–1999	[60]
	940–2020	320–2580	Aegean Sea, Greece	body	1997–1998	[65]
<i>Larus atlanticus</i>	3165–5090	2305–5536	Ebro/Alboran/Chafarinas, Spain	body	2004	[66]
	1850	770–3020	Bahía Blanca, Argentina	body	2003	[24]
	6230 (m), 4690 (f) (a)	3100–9430	Bahía Blanca, Argentina	body, mantle, rectrices, remiges	2003	[24]
<i>Larus minutus</i> ⁵	5660–7180	–	South coast, Caspian Sea, Iran	breast, tail	2008	[23]
<i>Larus canus</i>	2090–2880	–	South coast, Caspian Sea, Iran	breast, tail	2008	[23]
<i>Larus melanocephalus</i> ⁶	815–1264	229–2845	Axios, Evros, Lafri wetlands	body	1999–2009	This study

Notes: m, male; f, female; a, other than chick; b, geometric mean. Bird names according to <http://www.birdlife.org>. Synonyms: ¹*Chroicocephalus ridibundus*; ²*Leucophaeus pipixcan*; ³*Chroicocephalus scopulinus*; ⁴*Ichthyaeetus audouinii*; ⁵*Hydrocoloeus minutus*; ⁶*Ichthyaeetus melanocephalus* (<http://www.worldbirdnames.org/n-shorebirds.html>).

of bias were possibly involved such as small differences in chick age and low sample size [52,53] that did not allow us to draw safe conclusions and further research is needed to resolve this issue.

The ever-increasing environmental pollution due to human activities renders the monitoring of wildlife exposure to pollutants critical, making it necessary to evaluate and understand their impact on both local populations and the wider ecosystem. Greek colonies hold a central place within the Mediterranean and Black Seas, the Mediterranean gull's most significant historical breeding area. Results from our study revealed generally low Hg concentration in chick feathers, being lower than the level associated with deleterious effects in other birds and also mostly lower than those found in other gull species elsewhere in the world. Differences in trophic position [13] and also in pollutant availability in foraging habitat [54] might be responsible for the observed variability at both inter- and intra-specific levels. Overall, this study provided critical information on the Hg contamination of a seabird and highlighted the need for regular monitoring to fully understand pollution patterns and sources of variability.

Acknowledgements

We are indebted to Mrs U. Pijanowska (ICBM-Terramare) for laboratory technical support. We are thankful to all colleagues who contributed to the collection of feathers, particularly Dr S. Kazantzidis, M. Panagiotopoulou and G. Ifantis. During laboratory analyses V. Goutner was supported with a sabbatical from the University of Thessaloniki.

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