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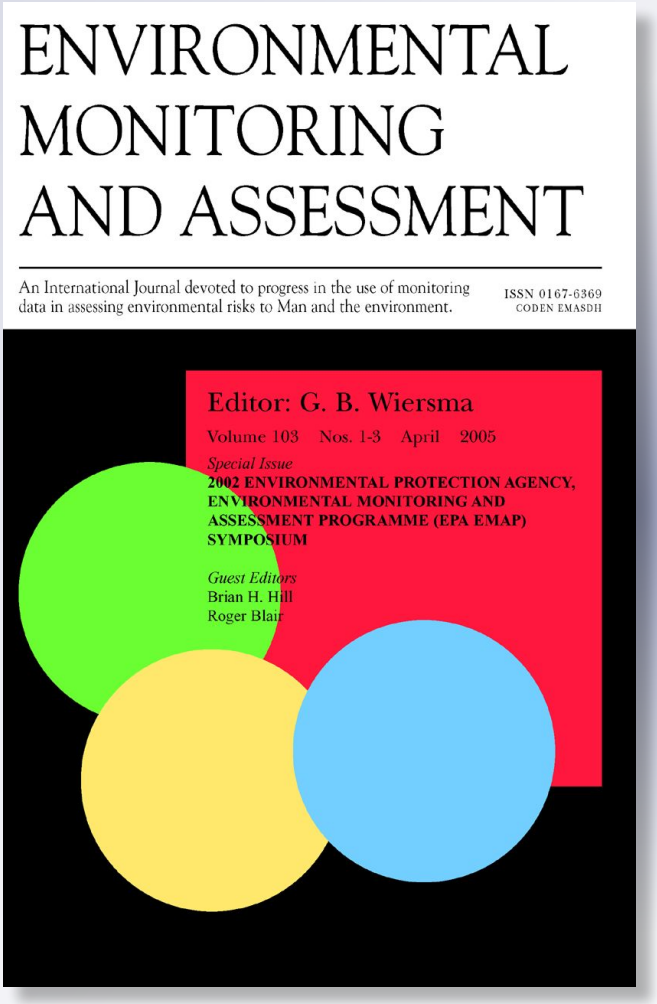

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Mercury levels and health parameters in the threatened Olrog's Gull (*Larus atlanticus*) from Argentina

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Abstract Mercury (Hg) exposure was investigated through feathers of Olrog's Gull and related to health parameters in adults (hematocrit, total plasma proteins, morphometric measures, sex) and chicks (hematocrit, total plasma proteins, immunoglobulins G and M) from a colony located in estuary of Bahía Blanca, Argentina. Mercury concentrations were 5.50 ± 2.59 μg g^{-1} ($n = 44$) in live adults, 1.85 ± 0.45 μg g^{-1} ($n = 45$) in live chicks and 1.81 ± 0.41 μg g^{-1} ($n = 41$) in dead chicks. Large differences were observed between live adults and live or dead chicks and small differences between live and dead chicks. In the adults, the sex of the birds was the variable that best explained Hg concentrations. Male birds had higher concentrations than females; this suggests that the clutch provides a sink for mercury during egg laying. Hg concentrations in both adults and live chicks were associated with higher hematocrits. This could be associated with upregulated erythropoiesis to compensate for increased rate of destruction of prematurely senescent, Hg-contaminated erythrocytes. Based on our results, on the levels of Hg pollution in the past in the study area, and on the dietary specialization of Olrog's Gull, we must be vigilant about potential

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negative effects of Hg pollution on this population and recommend continued monitoring on this threatened species.

Keywords Olrog's Gull · *Larus atlanticus* · Mercury pollution · Health · Feathers · Bahía Blanca estuary

Introduction

The Olrog's Gull (*Larus atlanticus*) is a vulnerable species (IUCN 2010) endemic to the Atlantic coast of Argentina, Uruguay and Southern Brazil (Yorio et al. 2005). Few Olrog's Gull breeding sites have been identified, all of them located in Argentina (Yorio et al. 2005). About 80% of the total breeding population is concentrated in the estuary of Bahía Blanca (henceforth EBB), with the largest colony of approximately 3,800 breeding pairs being on Isla del Puerto (Delhey et al. 2001a). After breeding, Olrog's Gull disperses throughout its geographical range (Burger and Gochfeld 2004).

The EBB is a mesotidal coastal plain of 2,300 km^2 which supports moderate biodiversity. Freshwater inputs originate from a drainage basin of 19,000 km^2 with freshwater tributaries and the injection of a significant volume of raw sewage with only pre-treatment (Freije and Marcovecchio 2004). Recreational and artisanal fishing activities are very important in the area. Various ports, towns (with a population of over 400,000 people), oil refineries, petrochemical and chlor-alkali plants, among other industries, are located along the northern shore of the estuary and have been identified elsewhere as important sources of pollutants. Among these contaminants, mercury (Hg) has been in the focus of several studies of natural and anthropogenic input and distribution of heavy metals in this area (Marcovecchio et al. 1986, 1988, 1991). Long-term monitoring in the EBB has shown decreasing trends in the levels of Hg since 1983, which would be attributable to the implementation of better practices in some industrial processes (De Marco et al. 2006).

The atmospheric deposition of Hg, which has increased considerably in the northern hemisphere over the past 100 years, and in the southern hemisphere in more recent decades (Dommergue et al. 2010), is an important contributor to declining bird populations both locally and globally (Frederick et al. 2004; Braune et al. 2006). Waterborne Hg from legacy sources such as chlor-alkali plants (Barr 1986) and manufacturing facilities (Brasso and Cristo 2008) have been reported to contribute to lowered productivity and breeding population sinks (Burgess and Meyer 2008; Evers et al. 2008).

Some physiological indices are useful in capturing subtle effects of environmental stress on birds (Fox et al. 2007a, b). Experimental studies with sublethal doses of Hg indicate that histological, immunological, and biochemical endpoints are sensitive to adverse effects of Hg in juvenile piscivorous birds (Spalding et al. 2000; Kenow et al. 2003, 2007; Hoffman et al. 2005). Among the metrics frequently used as indicators of overall health in wild avian populations are the hematocrit (defined as the packed cell volume of erythrocytes in the blood expressed as a percentage of the total blood volume) and the level of plasma proteins (Dawson and Bortolotti 1997a, b, c).

Seabirds in general, and pre-fledged chicks in particular, offer a number of advantages over other organisms as indicators of mercury contamination in the marine environment (Burger and Gochfeld 2004), but baseline ecological and physiological metrics, contaminant levels across species' ranges and reference intervals for bioindicators of health are lacking in most species (Mallory et al. 2010).

Despite decreasing trends detected in Hg levels in the EBB, this monitoring has been conducted using inorganic compartments and species of pelagic fish (De Marco et al. 2006). These fish have vast distributional ranges along the Atlantic coast of Argentina and use the EBB as nursery/spawning grounds, thus making any spatial interpretation of Hg uptake difficult and underlining the need to complement these studies with other biota to implement a more robust monitoring system of Hg pollution in the EBB.

During their breeding season in the EBB, Olrog's Gull adults prey mainly on the resident, grapsid crabs *Neohelice granulata* (Delhey et al. 2001b) and *Cyrtograpsus angulatus* (Petracci pers.

comm.) which they feed to their young. During the non-breeding season, alternative food resources such as fish discards (Martinez et al. 2000), molluscs *Mytilus* sp. (Escalante 1966), and barnacles *Balanus glandula* (Delhey et al. 2001b) are eaten by Olrog's Gull adults and juveniles. Crabs accumulate Hg in all tissues including the exoskeleton (Bianchini and Gilles 1996). Similarly, bernacles (Fialkowski and Newman 1998), molluscs (Chase et al. 2001) and fish (Marcovecchio et al. 1988) accumulate this metal in their tissues. This suggests that Olrog's gulls (1) experience substantial, year-round exposure to Hg through a variety of prey at different trophic levels and (2) have great potential as biomonitors for levels of this metal in the EBB.

Despite this potential, the species' vulnerable status and its most important breeding area having been long impacted by Hg pollution since the early 1980s, no study has evaluated Hg levels and their possible effects on the health of Olrog's Gull.

With this background, the objectives of our work were (1) to assess the presence and levels of Hg in the world's largest Olrog's Gull colony, (2) to study possible associations between Hg levels and health indicators in Olrog's Gull and (3) to lay the foundation for future ecotoxiological monitoring throughout the species' breeding range.

Materials and methods

Fieldwork was conducted during the breeding season 2003 on the Isla del Puerto breeding colony (38°49′ S, 62°16′ W), EBB, Argentina. Adult Olrog's gulls ($n = 44$) were captured during late incubation period using funnel traps placed above active nests containing at least one egg. Live pre-fledged chicks ($n = 53$) were captured using fishing nets, and dead pre-fledged chicks ($n = 41$) were salvaged. All the chicks were approximately 30–45 days old. Live adults and pre-fledged chicks were weighed (nearest g) using a spring scale, and the following measurements were made: length of the wing chord (nearest 0.5 mm), tarsometatarsus length, bill length and total-head length (nearest 0.01 mm). A blood sample was collected by venipuncture of the brachial vein using 5-cm^3 heparinized syringes and 23G $\times 1''$ needles. A few drops of blood from each bird were placed on a small piece of commercial filter paper which was air-dried and stored in a paper envelope for molecular sex determination. A small volume of blood was transferred into a microcapillary tube coated with sodium heparin for hematocrit determination on a micro-hematocrit reader following centrifugation at 10,000 $\times$ g for 5 min. The remaining blood was placed in serum separation tubes (BD Vacutainer® SST™ Serum Separation Tubes) and centrifuged. The plasma was harvested and kept frozen (−30°C) until processed for determination of immunoglobulin G and M levels (hereafter Ig; determined only in live chicks) and total plasma protein levels.

The bird's plumage is a major pathway for elimination of Hg accumulated during the inter-molt period (Monteiro and Furness 1995). Therefore, Hg levels in adults, live and dead chicks were determined using feathers. Upon capture of adults, the birds were examined for molting remiges, rectrices and mantle feathers. All the body and flight feathers from each dead chick were plucked, and 10–12 body feathers were clipped from live adults and chicks. Feather samples were individually stored in sealed polyethylene bags and kept frozen. Details of mercury analysis are described in Bearhop et al. (2000) and concentrations are reported as micrograms per gram on a dry weight basis. The sex of each adult bird was determined by molecular means (Quintana et al. 2008). Immunoglobulin levels were determined in plasma from live chicks using an indirect enzyme-linked immunosorbent assay following (Müller et al. 2004) with slight modifications for plasma samples. Total plasma protein levels were determined in adults and chicks using commercial kits (Quick Start Bradford Protein Assay kit 3, Bio-Rad 5000203).

Due to logistical constraints, blood samples could not be collected from some of the birds. Hematocrit, total protein concentration, Ig level and sex could not be determined in those birds, thus resulting in unbalanced data sets and low sample sizes for these variables. Therefore, different models were built to maximize the use of available data. The association between the dependent variable "Hg concentration"

(continuous) and the independent variables "bill length", "total-head length", "wing length", "tarsometatarsus length" and "weight" (all continuous) in the adult birds was assessed in a multivariable linear regression model. To avoid collinearity problems, highly correlated (>70%) explanatory variables were not included together in models but examined separately. Based on preliminary analyses, "sex" was suspected a priori to be important in determining the Hg levels detected. However, this variable was not included initially in the first model because of sample size restrictions (i.e. many adults of unknown sex). After selecting the best model, "sex" was included in the final model to control for possible confounding phenomena by this variable (i.e. a relationship between Hg levels and size different for each sex).

The associations between "Hg concentration" and the independent variables "hematocrit" (continuous) and "total proteins" (continuous) in the adults were studied using two different linear regression models. The association between "Hg concentration" and "hematocrit", "total proteins" and "Ig level" in live chicks was studied in a multivariable regression model, while the association between "Hg concentration" and "weight" was evaluated separately using linear regression.

In multivariable model building, independent variables were eliminated from a maximum model to achieve a simpler one that retained the significant main effects and interactions using the Akaike information criterion (AIC; Akaike 1974). Variables were eliminated if they did not reduce the AIC value by more than two units when included in the model (Burnham and Anderson 2002).

Differences in Hg concentration between adults, live, and dead chicks were evaluated using one-way ANOVA. To avoid a reduction in statistical power associated with Bonferroni correction, post hoc comparisons were made using effect size measures and their 95% CI (Nakagawa 2004). Statistical analyses were conducted using the statistical package R (R Development Core Team 2009). Cohen's d values and their 95% CI were calculated using Microsoft Excel (2003). The level of significance for statistical analyses was defined as $p < 0.05$.

Results

Mercury concentrations were 5.50 ± 2.59 μg g^{-1} (2.20–16.97, $n = 44$) in adults, 1.85 ± 0.45 μg g^{-1} (0.77–3.02, $n = 45$) in live chicks and 1.81 ± 0.41 μg g^{-1} (1.12–3.00, $n = 41$) in dead chicks (Fig. 1). Difference in Hg concentration between groups was significant (ANOVA: $F = 81.55$, $df = 2$, $P < 0.001$). Post hoc comparisons showed large differences between adults and dead chicks (Cohen's $d = 2.4$; 95% CI 1.63–2.52) and between adults and live chicks (Cohen's $d = 2.42$; 95% CI 1.66–2.55). Differences between live and dead chicks were small (Cohen's $d = 0.09$; 95% CI -0.04–0.22).

The variable "total-head length" was strongly correlated (>70%) with "bill length", "weight" and "wing length". Therefore, the contribution of each of these variables to the model evaluating the association between morphometric measures and Hg concentration was assessed separately. In the final model (Table 1), "total-head length" was the variable that best explained Hg levels: for each unit (mm) increase in size, the Hg concentration increased by 0.140 μg g^{-1}. Based on pre-

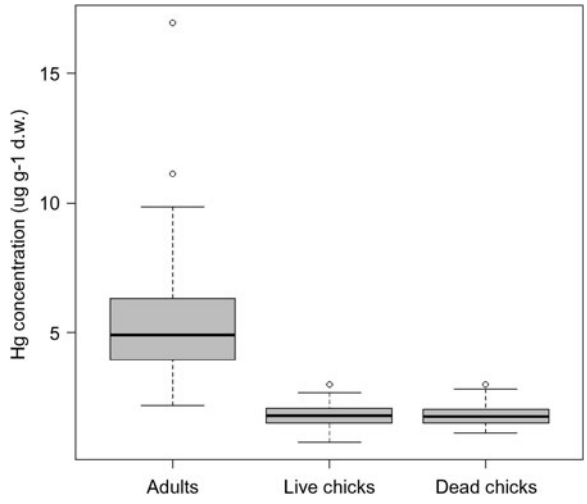

Fig. 1 Mercury concentrations feather tissue of live adults, live chicks and dead Olrog's Gull chicks. The middle 50% of the data (*box*), 25th and 75th percentiles (*lower and upper hinges*), median (*line*) and minimum and maximum (*whiskers*) are displayed for each group. *Points outside the ends of the whiskers* represent potential outliers

Table 1 Model describing the association between Hg concentration and size in adult Olrog's Gull

Model = Hg (μg g^{-1}) ~ Total-head length				
$n = 42$	Adj. $R^2 = 0.092$	F statistic = 5.15	AIC = 197.74	
Term	Coefficients	Std. error	p value	Δ AIC[a]
Intercept	−9.783	6.716	0.1531	–
Total-head (mm)	0.136	0.060	0.0287	13.99

[a]AIC value increment if the single term is dropped

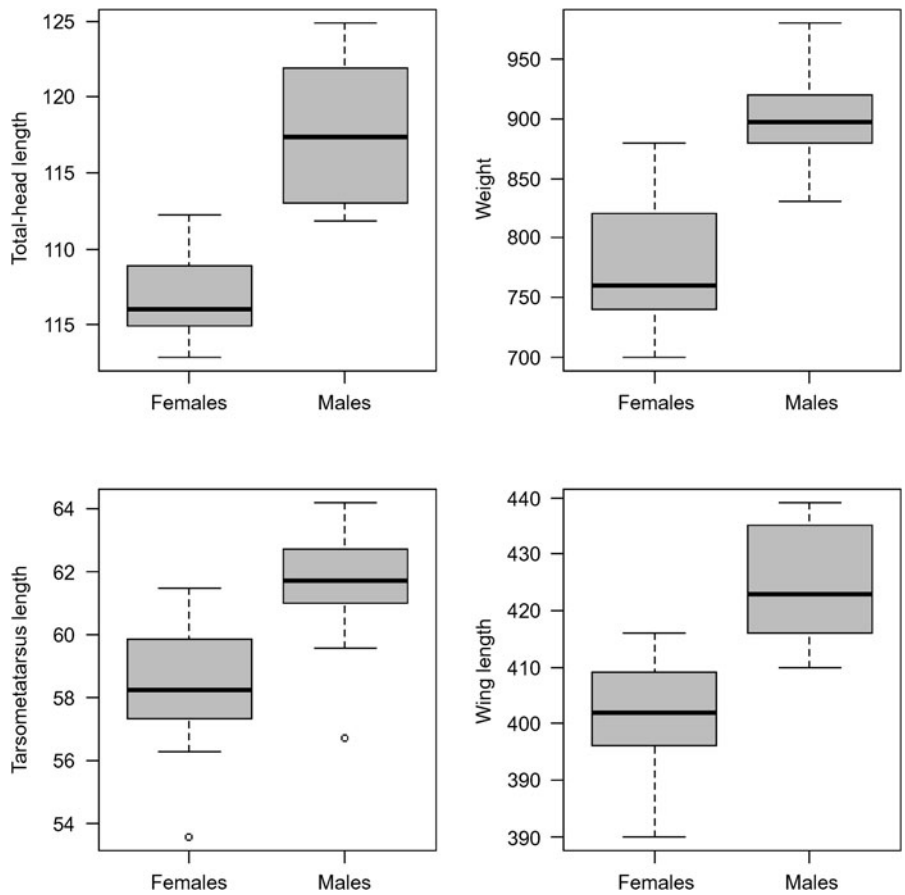

Fig. 2 Morphometrics of adult Olrog's Gull stratified by sex. Total-head length (*upper left*), weight (*upper right*), tarsometatarsus length (*bottom left*) and wing length (*bottom right*) are represented as middle 50% of the data (*box*), 25th and 75th percentiles (*lower and upper hinges*), median (*line*) and minimum and maximum (*whiskers*) for each sex

Table 2 Model describing the association between Hg concentration and sex in adult Olrog's Gull

Model = Hg (μg g^{-1}) ~ Sex				
$n = 22$	Adj. $R^2 = 0.226$	F statistic = 7.13	AIC = 79.5	
Term	Coefficient	Std. error	p value	Δ AIC[a]
Intercept	4.684	0.389	< 0.0001	–
Sex (ref. = females)				
Males	1.542	0.578	0.0147	4.7

[a]AIC value increment if the single term is dropped

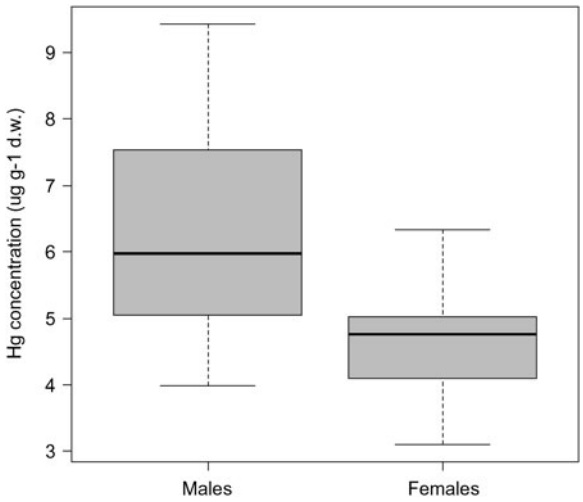

Fig. 3 Mercury concentrations in males and females of Olrog's Gull. The middle 50% of the data (*box*), 25th and 75th percentiles (*lower and upper hinges*), median (*line*) and minimum and maximum (*whiskers*) are displayed for each group

vious exploratory analyses, "sex" was suspected a priori to be important in determining the Hg levels detected because: (1) adult males had a mean Hg concentration significantly higher than females and (2) adult males were larger ($P < 0.01$ for all measurements) and heavier ($P < 0.001$) than adult females (Fig. 2). The sex of the adults was then added to the first model to control analytically for possible confounding phenomena. In the resulting model (Table 2), "total-head length" was no longer significant, and "sex" remained the variable that best explained Hg concentrations in the adults. Adult males had a mean Hg concentration 1.542 μg g^{-1} higher than adult females (females 4.69 ± 0.91 μg g^{-1} [3.10–6.33], $n = 12$; males 6.23 ± 1.74 μg g^{-1} [3.98–9.43], $n = 10$, $P < 0.05$; Fig. 3). These results indicate that the initial association observed between "Hg concentration" and "total-head length" was confounded by the sex of the birds.

The model evaluating the association between "hematocrit" and "Hg concentration" in the adults (Table 3) showed that for each unit increase in the hematocrit, the Hg concentration increased by 0.331 μg g^{-1}. When "sex" was included in the model, the effective sample size dropped considerably (i.e. unknown sex for many adults), thus precluding the analytic control of possible confounding by this variable. Despite this, on a separate analyses, no significant difference was observed in hematocric mean values between the sexes (females = 43.8; males = 42.5; $df = 10.05$; $P > 0.05$).

The model exploring the association between explanatory variables and Hg concentration in live chicks (Table 4) showed an increase of 0.024 μg g^{-1} in Hg concentration for each unit increase in the hematocrit.

Neither the concentration of total proteins and weight in the adult birds, nor the total proteins and Ig levels in the live chicks, were significantly associated with Hg concentrations.

Discussion

This work is the first comprehensive study of Hg levels in Olrog's Gull in the peer reviewed literature. Also, it provides the first examination of factors influencing Hg concentration and the possible negative effects of this metal on the health of breeding adults and chicks of Olrog's Gull in the EBB.

Mercury levels in tissues of marine birds and mammals are usually high (Thompson 1990) and oftentimes above the thresholds for toxicity determined in humans or captive animals. Under laboratory conditions, the levels associated with

Table 3 Model describing the association between hematocrit and Hg concentration in adult Olrog's Gull

$n = 30$	Adj. $R^2 = 0.1536$	F statistic = 6.26	AIC = 144.2	
Term	Coefficient	Std. error	p value	Δ AIC[a]
Intercept	−8.908	5.747	0.1324	–
Hematocrit	0.3310	0.132	0.0184	67.52

[a] AIC value increment if the single term is dropped

Table 4 Model describing the association between hematocrit and Hg concentration in live prefledged chicks of Olrog's Gull

$n = 18$	Adj. $R^2 = 0.1924$	F statistic = 5.05	AIC = 5.70	
Term	Coefficient	Std. error	p value	Δ AIC[a]
Intercept	0.920	0.414	0.0409	–
Hematocrit	0.024	0.011	0.0391	2.94

[a] AIC value increment if the single term is dropped

toxic effects in birds are 5–40 μg g^{-1} (dry weight) in feathers (Schreiber and Burger 2002).

The plumage of gulls is renewed usually every year after breeding, and a considerable amount of the dietary Hg accumulated in soft tissues between molts is assimilated into the growing feathers (Furness et al. 1986; Braune and Gaskin 1987). Most species of gull do not molt many feathers during breeding, although a few species may renew two or three inner primaries and some head or body feathers. Most molt occurs immediately after breeding, following a well-known simple pattern of sequential replacement of flight feathers and progressive renewal of mantle feathers (Furness et al. 1986).

In the present study, adults were beginning to molt their mantle feathers by the time of sampling (Petracci pers. comm.), and special care was taken to collect feathers that were 1 year old. Therefore, the Hg burden detected in the adults would reflect uptake of metal between breeding seasons and across the species' wintering range. As previously mentioned, Olrog's Gull adults exploit a broader range of food resources during the non-breeding season, which makes it difficult to suggest specific trophic or geographical sources of the Hg detected.

With this background, and based on our finding of Hg concentrations ranging between 2.20 and 16.97 μg g^{-1} in breeding adults, we must be concerned about long-term effects of Hg pollution on this population, since these values largely fall within the range that is considered toxic (Schreiber and Burger 2002).

Our results show that Hg concentrations are lower and much less variable in the chicks than in the adults (Fig. 1). This could be explained by (1) a fairly simultaneous growth of all feather types in pre-fledglings, as was suggested by Lewis et al. (1993), (2) Hg concentrations in adult feathers reflecting metal input throughout a vast geographical area and a substantially longer accumulation period and (3) a wider array of prey items during the non-breeding season which might be exposing the adults to higher Hg concentrations from other food resources such as fish discards.

Some studies reported a lack of sex-specific differences in Hg concentrations in feathers of species such as red-billed gulls *Larus novaehollandiae* (Furness et al. 1990) and great skuas *Catharacta skua* (Thompson et al. 1991). However, other research showed that excretion of mercury into the eggs can be substantial especially in species with multi-egg clutches (Becker 1992), thus leading to lower Hg concentrations among laying females of species such as common murres *Uria aalge* (Stewart et al. 1994), Bonaparte's gulls *Larus philadelphia* (Braune and Gaskin 1987) and herring gulls *Larus argentatus* (Lewis et al. 1993).

In our study, an initially significant relationship was identified between Hg concentration and adult size (total-head length), but this association was later found to be confounded by the sex of the birds. Adults were sampled during the late laying period. Therefore, we suggest that the considerably lower Hg concentrations in females of Olrog's Gull could be attributable to the clutch providing a sink for mercury during egg laying, which is also supported by some of the above-mentioned studies. Additional research addressing Hg levels in laying females and in tissues of their chicks which correlate strongly with egg levels (i.e. down feathers in chicks < 10 days old (Ackerman et al. 2008)) are necessary to investigate this hypothesis.

Red blood cells (RBC) are the primary transport mechanism of Hg in the body (Magos 1987), and exposure to this metal leads to apoptosis-like (programmed cell death) signals in the cell membrane and subsequent phagocytosis by

macrophages (Eisele et al. 2006; Föller et al. 2008). To our knowledge, there is one other recent study reporting a positive correlation between Hg concentration in a tissue (blood) and hematocrit in birds (Hoffman et al. 2009).

At first glance, the positive association observed between hematocrit values and Hg concentrations both in adults and chicks from our study could be attributed to an overstimulated erythropoiesis to compensate for death or decreased oxygen carrying capacity by RBC, as was suggested by Gill and Pant (1985) in their study of Hg poisoning in freshwater fish. However, the relationship observed between sex and Hg concentration in the adults raised suspicion of confounding by this variable (i.e. males having higher Hg levels and also higher hematocrits). For example, male adult birds are thought to have higher hematocrit values than females, partly due to the erythropoietic effect of androgens (Sturkie and Griminger 1976), although more recent studies failed to detect significant differences in hematocrits between the sexes in breeding adults (Dawson and Bortolotti 1997a, and references therein) and prefledged chicks of American kestrels *Falco sparverius* (Dawson and Bortolotti 1997c).

In the present study, we failed to find any sex-related difference in hematocrit in adult birds. Although future analysis using larger sample sizes are desirable, these results indirectly rule out the presence of a confounding phenomenon by sex. As well, the strong, positive relationship observed between Hg level and hematocrit in pre-fledged chicks strengthens a causal relationship between Hg and hematological alterations in adults and chicks of Olrog's Gull.

Dehydration is a commonly reported cause of increased hematocrit in birds. In our study, all the captured birds were in good condition with free access to food and water prior to capture and not showing clinical signs of dehydration, which rules that out as possible confounding factor.

Chick mortality associated with Hg contamination often occurs during the first week after hatching (Ackerman et al. 2008) indicating that the effects of in ovo Hg exposure can be particularly evident during early post-hatch (Ackerman et al. 2008). After hatching, barring especially high levels of mercury in their diet, the chicks' Hg concentrations rapidly decline as they age and dilute their body burden through growth and depuration into growing feathers (Monteiro and Furness 2001). Consequently, Hg levels in the down feathers of chicks during the first 10 days post-hatch are strongly correlated with levels in the egg (Ackerman et al. 2008), while most of the Hg assimilated into fully grown feathers during later stages represents dietary intake (Monteiro and Furness 1995).

During the chick-rearing period, Olrog's Gull adults prey mainly on the crabs *N. granulata* and *C. angulatus*, which they feed to their young. Although there are no studies of Hg accumulation in these crustaceans from the EBB, crabs are known for their potential to bioconcentrate heavy metals (Falusi and Olanipekun 2007; Gbaruko and Friday 2007) thus making Olrog's Gull adults, and especially small chicks, susceptible to the effects of Hg.

The Hg concentrations in fully grown feathers of hatchlings from this study would reflect, to a large extent, metal levels in the prey they are fed by their parents and would be attributable to intake during a clearly defined time window (i.e. breeding period) and from a limited area (i.e. parental foraging area near the study colony). The Hg levels found in chicks of our study do not seem to represent a toxicological risk, which is supported by a lack of association between Hg concentrations and two of the three proxies of health measured (i.e. plasma proteins and immunoglobulin levels). However, the slight hematocrit alteration observed as a function of Hg concentration warrants further investigation. In that regard, it is worth noting that feathers were sampled when chicks were 30–45 days old. This suggests that chicks may have been exposed to considerably higher Hg levels both in ovo and during early post-hatch and, arguably, that these early levels were diluted into simultaneously growing feathers by the time the samplings took place.

Other research conducted on chicks considerably younger than those of this study reported depressed total plasma proteins (Hoffman et al. 2005) and total antibody production (Kenow et al. 2007) in chicks with higher Hg concentration. Then, the lack of association in our study between

these health parameters and metal concentration could be explained by (1) a late timing of sampling, which may have caused the early effects of higher Hg concentrations to be missed, (2) a true lack of effects of Hg on these health parameters or (3) a low sample size and power to detect significant differences.

Together, based on our results, on the diet specialization of Olrog's Gull parents during the breeding season, and the dynamics of Hg in seabirds, we suggest that monitoring Hg levels in fully grown mantle feathers of pre-fledged chicks older than 10 days offers a sensible, minimally invasive means of monitoring Hg pollution in the EBB. Also, this work presents novel data and indirect evidence about the possible effects of Hg on the health of Olrog's Gull and lays the foundations for future ecotoxicological studies in this vulnerable species.

Long-term comprehensive research reaching across relevant disciplines is needed to evaluate the effects of this and other contaminants on the population health of Olrog's Gull.

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