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Comparison of Trace Element Concentrations Between Chick and Adult Black-Tailed Gulls (*Larus crassirostris*)

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Abstract Trace elements were measured in the feathers of black-tailed gull chicks (n = 10) and adults (n = 10) collected at Chilsando Is., Jeollanam-do, Korea, in June 2011. Pb, Mn and Fe were significantly greater in adult (arithmetic mean 2.02, 3.81, 92.1 µg/g dw, respectively) than chick (0.74, 2.14, 68.7 µg/g dw) gulls. In contrast, Zn was greater in chicks (74.9 µg/g dw) than adults (46.5 µg/g dw). Cd, Pb and Cr in all chicks and adults were lower than an approximate threshold level for toxic effects. Cd, Pb and Cr were comparable or lower than reported in other gull studies worldwide. Essential elements including Al, Cu, Mn, Zn and Fe were within the background and normal physiological levels reported earlier in other gull species including black-tailed gulls.

Keywords Black-tailed Gulls · Feathers · Trace elements · Threshold level

Trace elements have been gained attention from the public and scientific community because of their persistence in the environment, bioaccumulation and toxicity to birds and ultimate effect on human health (Shahbaz et al. 2013). Trace elements are persistent, ubiquitous and non-biodegradable with long biological half-lives and they are accumulated in various tissues of birds through the food chain (Burger et al. 2008). In aquatic and terrestrial ecosystems, trace elements can bioaccumulate and biomagnify and Cd, Pb, Cr, Cu, Mn and Zn can be toxicants even at low concentrations in some birds (Burger and Gochfeld 2009; Scheuhammer 1987).

Elevated Cd and Pb concentrations can cause teratogenic, mutagenic and carcinogenic effects in biological organisms including birds (Hofer et al. 2010). Cd and Pb also have been associated with reduced breeding success, behavioral abnormalities of chicks, reduced body mass and delayed fledging in some birds (Hofer et al. 2010). Bird species differ in the bioaccumulation and excretion of trace elements (Burger and Gochfeld 2009; Hofer et al. 2010). This could be associated with different diets and trophic levels, or physiological and ecological species-specific trace element requirements. Age of birds is also an important factor for trace element bioaccumulation. Cd and Pb bioaccumulate over time, resulting in increasing bioaccumulation with age, though growing chicks may also bioaccumulate higher concentrations than adults (Orłowski et al. 2007). Age-dependent bioaccumulation of Cr was found in feathers in adult and juvenile black-tailed gulls (*Larus crassirostris*) from Japan, but Zn, Cu and Pb concentrations were decreased with age. Mn and Cd levels did not differ between adult and juvenile black-tailed gulls (Agusa et al. 2005).

Cd, Pb and Cr concentrations (2, 4, 2.8 µg/g dw, respectively) in feathers were associated with adverse effects in birds (Burger and Gochfeld 2000c). In recent Korean studies, mean Cd concentrations in feathers of wild birds did not exceed the background level (2 µg/g dw) and included reports on black-tailed gull chicks (0.05–0.03 µg/g dw, Kim et al. 2013; 0.07 µg/g dw, Kim and Oh 2014a), heron and egret chicks (0.48–0.88 µg/g dw; Kim and Oh 2014b) and waterfowl species with and without embedded shot (1.26–1.51 µg/g dw, Kim and Oh 2014c). Most Korean

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birds were within the range of the background level for Pb (4 μg/g dw) which included black-tailed gull chicks (2.47–2.52 μg/g dw, Kim et al. 2013; 2.56 μg/g dw, Kim and Oh 2014a), heron and egret chicks (1.72–1.53 μg/g dw; Kim and Oh 2014b) and waterfowl species with and without embedded shot (1.63–2.53 μg/g dw, Kim and Oh 2014c). In contrast, black-tailed gull chicks from Hongdo Is. (10.8 μg/g dw, Kim et al. 2013) and mallards with embedded shot exceeded the background level for Pb (Kim and Oh 2014c). Average Cr concentrations in black-tailed gull chicks were 3.15–5.94 μg/g dw (Kim et al. 2013) and exceeded the background level for Cr (2.8 μg/g dw), but waterfowl species (0.51–0.72 μg/g dw) had lower than the background levels (Kim and Oh 2014c).

The objectives of this study were to compare trace element concentrations in breast feathers between black-tailed gull chicks and adults, and to examine the relationship among trace element concentrations in feathers.

Methods and Materials

Black-tailed gulls (*L. crassirostris*) are permanent residents and breed on uninhabitated islands in Korea. Gull chicks (n = 10) and adults (n = 10) were collected at Chilsando Island (35°19′ N latitude, E 126°16′ E longitude), Jeollanam-do, Korea, in June 2011. The chicks were marked with plastic rings 1–3 days after hatching and were recaptured 20–22 days after hatching from randomly selected nests (n = 10). Adults were caught in mist nets. Breast feathers (>10 feathers per sample) were plucked and the placed in an envelope and the adults were released. Among feather tracts in birds, breast feathers are the best indicator of whole-body burdens because they are considered to be more representative of exposure to trace elements (Burger and Gochfeld 2009).

For chicks and adults, body mass (0.1 g), culmen (0.1 mm) and tarsus (0.1 mm) length were measured. Chicks were euthanized by thoracic compression and frozen at −20°C until necropsy. These birds were later thawed and the breast feathers carefully removed from the body and weighed (±0.1 g). The feathers were washed vigorously in deionized water alternated with (1 mol/L) acetone (95 %) to remove external contamination (Burger and Gochfeld 2009). This procedure was repeated two times. Breast feathers were dried in an oven for 24 h at 105°C and weighed (±0.1 g) (Kim and Oh 2014a). The remaining carcass was used in another study.

Approximately 1–1.5 g of each sample was digested in the presence of a mixture of concentrated nitric, perchloric and/or sulfuric acids in Kjeldahl flasks. Al, Cu, Mn, Zn and Fe concentrations were determined by flame atomic absorption spectrophotometry (AAS; Hitachi Z-6100), after mineralization of samples. Cd, Pb and Cr concentrations were measured by AAS following treatment with DDTC (sodium *N*,*N*-diethyldithio-carbamate trihydrate ($(C_2H_5)_2NCS_2Na{\cdot}3H_2O$)-MIBK (methyl isobutyl ketone ($CH_3COCH_2CH(CH_3)_2$) (Kim and Oh 2014a). Seven or more spikes and blanks were included in the analysis (about 20 % of the total number of samples). A spike, a blank, a standard and a sample were run in triplicate in each analytical run. Spikes recoveries ranged from 94 % to 106 %. Recovered concentrations of the samples were within 5 % of the certified values. Detection limits were 1.0 μg/g dw for Al, Zn and Fe, 0.1 μg/g for Pb, Cu and Mn, and 0.01 μg/g for Cd. All element concentrations (μg/g) in feathers were estimated on a dry weight (dw) basis.

We tested trace element concentrations for differences between chicks and adults using *t* tests. Data were log transformed to obtain a normal distribution that satisfied the homogeneity of variance assumptions of *t* tests. Correlations between trace elements were examined using Pearson correlations (r). We present geometric means, 95 % confidence intervals, arithmetic means and standard deviations in tables and text. Statistical analyses were carried out using SPSS 12.0 version.

Results

Distribution in feathers varied among metals in adults and chicks, and decreased in the order: chicks, Zn > Fe > Al > Cu > Mn > Pb > Cr > Cd; adults, Fe > Zn > Al > Cu > Mn > Pb > Cr > Cd (Table 1).

Mean concentrations in all examined birds (adults + chicks) of Cd, Pb, Cr, Al, Cu, Mn, Zn and Fe were 0.14, 1.11, 0.61, 8.71, 4.05, 2.78, 58.4 and 77.4 μg/g dw, respectively. Pb ($p = 0.002$), Mn ($p = 0.001$) and Fe ($p = 0.004$) were significantly greater in feathers of adult than chick gulls, but Zn were significantly greater in chicks than in adults ($p = 0.001$; Table 1). Cd, Al, Cr and Cu did not differ between age groups. Although not significantly different the mean concentrations of Cd, Al, Cr and Cu were greater in adult than chick gulls. Pb, Mn and Fe in gull adults were approximately 2.8, 1.8 and 1.4 times greater than in chicks, but Zn in chicks were 1.6 times greater than in those of adults.

In feathers of chicks, Cd were positively correlated with Cr; Fe with Al and Mn. Pb were negatively correlated with Cr. In adults, Cd were significantly correlated with Cr and Fe; Fe was positively correlated with Al and Cr. For chicks and adults combined, Cd was significantly correlated with Al, Cr, Mn, Zn and Fe; Pb was positively correlated with

Table 1 Trace elements concentrations (geometric mean, 95 % confidence intervals (CIs) and mean ± SD, μg/g dw) in feathers of black-tailed gull adults and chicks

	Cd	Pb	Cr	Al	Cu	Mn	Zn	Fe
Adults (n = 10)								
Geomean	0.14	1.84	0.72	9.70	4.13	3.74	45.7	90.3
CIs	0.13–0.16	1.42–2.27	0.66–0.78	7.06–12.1	3.78–4.48	3.27–4.22	39.7–51.6	78.2–102
Mean ± SD	0.14 ± 0.03	2.02 ± 0.69	0.73 ± 0.10	10.4 ± 3.92	4.16 ± 0.57	3.81 ± 0.77	46.5 ± 9.57	92.1 ± 19.5
Min–max	0.11–0.19	0.45–2.87	0.56–0.91	5.34–18.2	3.35–5.19	2.53–5.01	33.4–69.6	64.3–123
Chicks (n = 10)								
Geomean	0.13	0.66	0.51	7.83	3.98	2.06	74.7	66.4
CIs	0.11–0.14	0.45–0.88	0.37–0.65	4.51–11.1	3.61–4.35	1.60–2.52	71.0–78.4	53.8–79.1
Mean ± SD	0.13 ± 0.02	0.74 ± 0.35	0.61 ± 0.23	8.87 ± 5.35	4.02 ± 0.60	2.14 ± 0.74	74.9 ± 5.94	68.7 ± 20.4
Min–max	0.10–0.17	0.20–1.55	0.05–0.96	4.34–21.1	3.27–5.20	1.58–4.10	65.4–82.8	52.2–107
p value[a]	NS	0.002	NS	NS	NS	0.001	0.001	0.004

NS not significant

[a] *t* test results between adults and chicks

Table 2 Correlation coefficient (r) between trace element concentrations in feathers of black-tailed gulls

	Pb	Al	Cr	Cu	Mn	Zn	Fe
Chick							
Cd	NS	NS	0.749*	NS	NS	NS	NS
Pb		NS	−0.680*	NS	NS	NS	NS
Al			NS	NS	NS	NS	0.954**
Cr				NS	NS	NS	NS
Cu					NS	NS	NS
Mn						NS	0.732*
Zn							NS
Adult							
Cd	NS	NS	0.801**	NS	NS	NS	0.722*
Pb		NS	NS	NS	NS	NS	NS
Al			NS	NS	NS	NS	0.631*
Cr				NS	NS	NS	0.640*
Cu					NS	NS	NS
Mn						NS	NS
Zn							NS
Overall							
Cd	NS	0.473*	0.717**	NS	0.445*	0.459**	0.616**
Pb		NS	NS	NS	0.823**	NS	0.587*
Al			0.474*	NS	NS	NS	0.770**
Cr				NS	0.460*	NS	0.570**
Cu					NS	NS	NS
Mn						−0.616**	0.733**
Zn							−0.519*

NS not significant

* $p < 0.05$; ** $p < 0.01$

Mn and Fe; Al was correlated with Cr and Fe; Cr was positively correlated with Mn and Fe; Mn was correlated with Fe. Zn was negatively correlated with Mn and Fe (Table 2).

Discussion

Results of age-related differences for Cd and Cr in avian tissues are mixed. Adults had higher Cd than chicks or juveniles (Burger et al. 2009; Barbieri et al. 2010; Malinga et al. 2010). However, there were no differences in Cd feather concentrations between adult and chick black-tailed gulls (this study; Agusa et al. 2005) and laysan albatross (*Diomedea immutabilis*) (Burger and Gochfeld 2000a). In kelp gulls (*Larus dominicus*), Cr was elevated with age (chicks, subadults and adults) (Barbieri et al. 2010), but black-footed albatross (*Phoebastria nigripes*) chicks had higher Cr than adults (Burger and Gochfeld 2009). No statistical differences for Cr in feathers were found between adults and juveniles or chicks/chicks of gull (this study; Agusa et al. 2005) and albatross (Burger and Gochfeld 2000a), and known-aged adults of black-legged kittiwakes (*Rissa tridactyla*) (Burger et al. 2008). Pb concentrations increased with age in feathers of glaucous-winged gulls (*Larus glaucescens*), kelp gulls (Barbieri et al. 2010), but not in feathers of black-headed gulls (*Larus ridubundus*) (Orłowski et al. 2007) and black-legged kittiwakes (Burger et al. 2008). Pb in black-tailed gulls from this study was higher in adults than in chicks and the greater bioaccumulation of Pb in adults might be a consequence of the longer exposure time than for chicks. Pb in the feathers of chicks come from the female (exposure from the egg) and from their food, which is derived locally. Pb in feathers of adult birds comes from exposure during feather formation and the molt period. Because feather formation and the molt period occur away from the breeding colony (Burger et al. 2008), Pb in adult black-tailed gull is associated with the contamination during winter or migration.

In marine ecosystems, Cd and Pb generally are associated with environmental contamination (Jerez et al. 2011). Levels of both toxic elements in seabirds fluctuate widely among different studies, according to their feeding ecology, intensity and time of exposure in foraging areas, and the physiological and biochemical characteristics of Cd and Pb (Burger and Gochfeld 2009). The highest Cd level from this study was 0.19 µg/g dw, less than one-tenth of the concentration (>2 µg/g dw) in feathers of birds associated with adverse effect on the kidney (Burger and Gochfeld 2000c). Observed Cd levels in other gull studies were also considerably lower than the threshold level (Kim et al. 2013; Burger and Gochfeld 2009; Burger et al. 2009; Agusa et al. 2005). For Pb, adverse effects in feathers of birds occur at levels of 4 µg/g dw (Burger and Gochfeld 2000b; Custer and Hohman 1994), although seabirds can often tolerate higher levels (Burger and Gochfeld 2000b). The Pb concentrations in black-tailed gulls from this study were all below the threshold level. Average Pb concentrations of black-tailed gull chicks in Dokdo Is., Korea (Kim et al. 2013), Siberian gulls (*Larus heuglini*) in Iran (Mansouri et al. 2012) and kelp gull adults in Brazil (Barbieri et al. 2010) were greater than the threshold level. Elevated Pb (>4 µg/g dw) was associated with decrease of breeding success in birds (Brahmia et al. 2013; Eeva et al. 2009). At Hongdo Is., Korea, the highest mean Pb in gull chicks might be associated with fishing weights (Kim et al. 2013).

Cr in this study was not found at a level toxic to birds. Elevated Cr in birds was associated with anthropogenic activities such as mining, refining and intense use of Cr (Jerez et al. 2011) and oil contamination (Caccia et al. 2003). Cr from this study was greater than in the same species at Japan (Agusa et al. 2005), but far below reports from Korea (Kim et al. 2013). In feathers, 2.80 µg/g dw Cr might be associated with adverse effects (Burger and Gochfeld 2000c) including decreased embryo development, hatching success and viability of chicks (Kertész and Fáncsi 2003). In gull studies, some Korean black-tailed gull chicks (Kim et al. 2013), Spanish yellow-legged gull (*Larus michahellis*) chicks (Moreno et al. 2011) and Brazilian kelp gull adults (Barbieri et al. 2010) exceeded the levels of adverse effects. In general, elevated Cr in seabirds might be associated with oil contamination in the ocean (Caccia et al. 2003).

Significant differences between chick and adult black-tailed gulls were found for Mn, Fe (adults > chicks) and Zn (adults < chicks), but Cu did not differ between age groups. These trends have been reported for kelp gulls (Barbieri et al. 2010), bald eagles (*Haliaeetus leucocephalus*) (Burger and Gochfeld 2009) and black-tailed gulls (Agusa et al. 2005). No difference for Cu has been reported in feathers of gull species (Barbieri et al. 2010; Agusa et al. 2005). The absorption of Cu, Mn, Zn and Fe is regulated by homeostatic control in birds and elevated Zn levels in chicks might be associated with bone development (Agusa et al. 2005).

Al concentrations of black-tailed gulls were lower than the mean value reported from the same species (Kim et al. 2013) and waterbirds (Custer et al. 2007). Al is an essential element for organism, but elevated Al has been associated with the inhibition of bone formation, an increase in bone fracture rates, increased mortality, reduced reproductive success and growth of fledglings in some birds (Scheuhammer 1987). Excesses of essential elements such as Cu, Mn, Zn and Fe may be an additional source of stress to birds exposed to environmental contamination (Moreno et al. 2011). In the present study, none of these elements present in black-tailed gulls were found at toxic levels. Cu, Mn, Zn and Fe levels in this study were comparable or lower than collections from other gull studies and these elements probably are maintained by a normal homeostatic mechanism and are below health risk effect levels (Agusa et al. 2005; Moreno et al. 2011; Kim et al. 2013; Kim and Oh 2014a).

Antagonistic and synergistic effects between essential and toxic elements have been reported in birds (Custer and Custer 2000; Braune and Scheuhammer 2008; Schummer et al. 2011). Interactions of essential metals including Cu, Mn, Zn and Fe and non-essential metals, including Cd and Pb can serve as a defensive mechanism in birds. Correlations between essential and non-essential elements are associated with similar metabolic pathways involving metal binding proteins, such as metallothionein (Elliott et al. 1992; Roesijadi 1992). Zn, Cu and metallothionein are often positively correlated with Cd and may protect against the uptake of Cd in some bird species (Ek et al. 2004; Levengood and Skowron 2007; Schummer et al. 2011). In this study, a positive correlation between Zn and Cd concentrations was found in feathers of adult and chick black-tailed gulls combined.

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