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Comparison of trace element concentrations in grey heron and black-crowned night heron chicks

Jungsoo Kim · Jong-Min Oh

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Abstract Cadmium (Cd), lead (Pb), manganese (Mn), zinc (Zn), and iron (Fe) concentrations were measured in the prey and liver of grey heron (*Ardea cinerea*) and black-crowned night heron (*Nycticorax nycticorax*) chicks (24–26 days after hatching) at the Pyeongtaek colony, Korea in 2001 ($n=10$, respectively) and 2008 ($n=11$ and $n=10$). Cadmium and Pb concentrations in livers of grey heron (Cd geomean 0.06, Pb 3.90 μg/g dw) and black-crowned night heron (Cd 0.20, Pb 4.24 μg/g dw) chicks were increased with diet concentrations of grey heron (Cd 0.18, Pb 1.76 μg/g dw) and black-crowned night heron (Cd 0.20, Pb 3.96 μg/g dw) chicks. Cadmium and Pb concentrations in prey items of grey heron and black-crowned night heron chicks were a good predictor of chick liver concentrations. Cadmium concentrations in livers of both heron species collected at the Pyeongtaek heronry were relatively low and within the background level (<3 μg/g dw) for birds. Five of 20 (25.0 %) grey heron and 4 of 18 (22.2 %) black-crowned night heron chicks were higher than the background level for lead (>6 μg/g dw). Prey Cd and Pb concentrations were within the range of other heron and egret studies. Manganese, Zn, and Fe concentrations in grey heron and black-crowned night heron chicks were within the background or normal physiological levels reported earlier in other birds including herons and egrets.

Keywords Trace elements · Lead and cadmium · Heron chicks · Livers and preys · Background level

Introduction

Herons and egrets have been well known as bio-indicators for monitoring trace element contaminations in aquatic ecosystems and local pollution around their colonies (Ullah et al. 2014; Hashmi et al. 2013; Padula et al. 2010; Custer et al. 2007). They forage mainly on aquatic organisms such as invertebrates, small fish, and amphibians (Kim and Koo 2007; Choi et al. 2010). Herons and egrets including grey herons (*Ardea cinerea*) and black-crowned night herons (*Nycticorax nycticorax*) are considered useful bio-indicators of environmental contamination because they are at high trophic level in the food web, and they can reflect information about ecological changes happening at lower trophic levels (Kwok et al. 2014; Ullah et al. 2014; Hashmi et al. 2013; Abdennadher et al. 2011; Padula et al. 2010; Ayaş 2007; Lam et al. 2005).

Trace elements can enter into the aquatic ecosystem by atmospheric deposition, weathering of the geological matrix, and through anthropogenic sources including industrial discharge, sewage, and agricultural and mining wastes (Ebrahimpour and Mushrifah 2010).

J. Kim · J.-M. Oh (✉)
Department of Environmental Science and Engineering,
Kyung Hee University,
1 Seocheon-dong, Giheung-gu, Yongin-si, Gyeonggi-do
446-701, Republic of Korea
e-mail: jmoh@khu.ac.kr

J. Kim
e-mail: herons@khu.ac.kr

Concentrations of trace element in the chick of birds can originate from the egg and from the diet (Becker and Sperveslage 1989; Shahbaz et al. 2013). Because diet is the main source of non-essential elements such as Cd and Pb in birds, previous knowledge of their diet and foraging habitats is essential to a sound interpretation of the contamination level (Burger and Gochfeld 2009). Relationship between trace elements in tissues or eggs and in the prey of birds has been reported on various birds including herons and egrets (Ullah et al. 2014; Kim and Oh 2014; Shahbaz et al. 2013; Custer et al. 2012).

Contamination of trace elements have been associated with a variety of problems such as smaller clutch size and nestling mortality (Berglund et al. 2010; Bel'skii et al. 2005), lesions in the kidney or intestinal organs, induction of reactive oxygen (Erdogan et al. 2005; Berzina et al. 2007), and reduced breeding success, behavioral abnormalities of chicks, reduced body mass, and delayed fledging in some birds (Jansenns et al. 2003; Hofer et al. 2010). Also, elevated Cd and Pb concentrations can cause teratogenic, mutagenic, and carcinogenic effects in biological organisms including birds (Hofer et al. 2010).

Bird species including herons and egrets differ in the accumulation and excretion of trace elements, and these differences might be associated with different dietary items and trophic levels, or physiological and ecological species-specific trace element requirements (Ullah et al. 2014; Custer et al. 2012; Hofer et al. 2010). Grey herons and black-crowned night herons are summer residents in Korea. They forage within 4–7 km of their breeding sites, where they take the most abundant prey such as invertebrates, amphibians, reptiles, and fishes (Kim and Koo 2007). The objectives of our study were to (1) evaluate levels of trace elements (Cd, Pb, Cu, Mn, Zn, and Fe) in the liver and the prey of chicks of two heron species, (2) examine the relationship of trace elements between the liver and the prey of two heron species, and (3) compare trace element concentrations in heron livers with concentrations reported in other studies. Based on relationships between trace element concentrations in tissues and dietary items of various bird species (Ullah et al. 2014; Fritsch et al. 2012; Custer et al. 2012), we hypothesized that element concentrations in livers of grey heron and black-crowned night heron chicks would be positively correlated with element concentrations in the dietary items.

Materials and methods

Study site and sampling

This study was conducted from April to June in 2001 and 2008, at a breeding colony of herons and egrets, Pyeongtaek city (37° 02′ N, 127° 02′ E), Korea. The Pyeongtaek colony was surrounded by agricultural land. Rice paddy fields surround the colony where many herons and egrets foraged. The distance from the breeding site to additional foraging sites was 4–7 km.

Grey heron (2001, n=10; 2008, n=11) and black-crowned night heron chicks (2001, n=10; 2008, n=10) were marked with plastic rings 1–3 days after hatching. Chicks were recaptured in 24–26 days after hatching. We collected one chick sample from each nest. Prey samples were collected from stomach contents and regurgitated diets. Heron chicks were weighed (0.1 g), and the bill (0.1 mm), wing (0.1 mm), and tarsus (0.1 mm) length were measured. Chicks were euthanized by thoracic compressions, stored in chemically clean plastic bags, and frozen at −20 °C until they were dissected and analyzed.

These birds were later thawed and the liver carefully removed from the body and weighed (±0.1 g). The liver and prey of grey heron and black-crowned night heron chicks were dried in an oven for 24 h at 105 °C and weighed (±0.1 g). All trace element concentrations (μg/g) in livers and prey were estimated on dry weight (dw) basis.

Analysis of trace elements

Copper, Mn, Zn, and Fe concentrations were determined by flame atomic absorption (AA) spectrophotometry (Hitachi Z-6100), after mineralization of samples with nitric, sulfuric, and perchloric acid in Kjeldahl flasks. The livers and the prey with low Cd and Pb concentrations were measured by AA spectrophotometry, after treatment with DDTC (Sodium N, N-Diethyldithiocarbamate trihydrate ($(C_2H_5)_2NCS_2Na \cdot 3H_2O$)-MIBK (Methyl Isobutyl Ketone ($CH_3COCH_2CH(CH_3)_2$) (Kim and Oh 2014). 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 Mn, Zn, and Fe; 0.1 μg/g for Cu and Pb; and 0.01 μg/g for Cd.

Statistical analysis

We statistically tested for differences in trace element concentrations in livers and prey between species (grey heron and black-crowned night heron chicks) and year (2001 and 2008) using two-way analysis of variance (ANOVA) with interaction (species, year, species*year). If the interaction was significant ($P<0.05$) then a one-way ANOVA was run among all four groups. Bonferroni mean separation tests were performed to determine difference among means. Data were log transformed to obtain a normal distribution that satisfied the homogeneity of variance assumptions (Kim and Oh 2014). We present arithmetic geometric means and 95 % confidence intervals in tables and texts. Correlations between trace element concentrations in livers and preys were assessed using Pearson correlations (r). Statistical analyses were carried out using SPSS 12.0 version.

Results

Comparison of trace element concentrations

Cadmium, Mn, and Cu concentrations in livers were significantly higher in black-crowned night herons in 2001 than in 2008 and higher in grey herons in both 2001 and 2008 (one-way ANOVA, Table 1). The lowest concentration of Cd and Mn in heron livers was measured in grey herons in 2001 and the lowest concentration of Cu was measured in grey herons in 2008 (one-way ANOVA, Table 1). Lead and Zn concentrations in livers did not differ between species (geomean Pb in black-crowned night heron=2.38 μg/g dw, in grey heron=2.62 μg/g dw; Zn in black-crowned night heron=73.2 μg/g dw, in grey heron=82.7 μg/g dw) but were higher for Pb in 2001 and higher for Zn in 2008 (two-way ANOVA, Table 1). Iron concentrations in heron livers did not differ between species (black-crowned night heron=697 μg/g dw, grey heron=628 μg/g dw) or between years (2001=605 μg/g dw, 2008=720 μg/g dw (two-way ANOVA, Table 1).

Cadmium concentrations in prey were higher in black-crowned night herons in 2008 than 2001 and grey herons in both 2001 and 2008 (one-way ANOVA, Table 2). Lead concentrations in prey were higher in black-crowned night herons (geomean 3.45 μg/g dw) than grey herons (1.68 μg/g dw) and higher in 2008 (6.25 μg/g dw) than 2001 (0.92 μg/g dw) (two-way ANOVA, Table 2). Copper concentrations were higher in black-crowned night herons (17.9 μg/g dw) than grey herons (6.90 μg/g dw) and higher in 2001 (29.2 μg/g dw) than 2008 (3.68 μg/g dw) (two-way ANOVA, Table 2). Zinc concentrations in prey were higher in 2008 (161 μg/g dw) than 2001 (81.7 μg/g dw) (two-

Table 1 Concentrations (geometric mean, 95 % confidence intervals (CIs), μg/g dw) of trace elements in the liver of heron chicks at the Pyeongtaek colony

	Grey herons		Black-crowned night herons		ANOVA *P* values		
	2001 (*n*=10)	2008 (*n*=11)	2001 (*n*=10)	2008 (*n*=10)	Species	Year	Species *year
Cadmium	0.03C* 0.03–0.03	0.24B 0.19–0.29	0.40A 0.35–0.45	0.22B 0.15–0.30	<0.001	0.223	<0.001
Manganese	4.23C 3.10–5.36	7.30B 6.66–7.95	11.3A 4.89–17.7	8.80B 7.52–10.2	<0.001	0.182	0.001
Copper	94.3B 63.9–125	16.1C 3.67–28.5	144A 74.1–213	70.1B 31.0–145	0.008	0.006	0.016
Lead	5.20 3.04–7.37	3.16 1.92–4.41	4.91 3.49–6.33	3.78 2.20–5.36	0.680	0.034	0.462
Zinc	103 59.6–146	137 100–174	106 85.7–127	176 136–216	0.264	0.005	0.378
Iron	595 228–962	816 584–1046	616 260–971	640 405–874	0.609	0.361	0.483

Means among species and years not sharing the same letter are significantly different

[*] $P<0.05$ one-way ANOVA

Table 2 Concentrations (geometric mean, 95 % confidence intervals (CIs), μg/g dw) of trace elements in the prey of heron chicks at the Pyeongtaek colony

	Grey herons		Black-crowned night herons		ANOVA P values		
	2001 (n=10)	2008 (n=11)	2001 (n=10)	2008 (n=10)	Species	Year	Species *year
Cadmium	0.51B[*] 0.44–0.58	0.45B 0.37–0.54	0.51B 0.30–0.71	1.38A 0.37–2.39	0.002	0.016	0.002
Lead	0.67 0.19–1.37	3.93 1.26–6.59	1.19 0.30–2.08	13.2 3.93–22.5	0.003	< 0.001	0.246
Copper	22.3 13.1–31.6	3.51 0.78–6.26	41.3 30.2–52.4	6.75 5.13–8.38	0.022	< 0.001	0.617
Zinc	84.1 19.9–148	132 54.4–209	73.5 15.1–132	213 140–298	0.431	0.002	0.175
Iron	605 150–1058	587 92.4–1081	494 86.3–902	1,599 1110–2086	0.144	0.313	0.052
Manganese	25.7 3.80–47.6	29.5 12.5–46.4	23.2 1.52–45.1	28.9 11.5–46.4	0.923	0.584	0.779

Means among species and years not sharing the same letter are significantly different

[*] P<0.05 one-way ANOVA

way ANOVA, Table 2). Finally, iron concentrations did not differ between heron species or between years (two-way ANOVA, Table 2).

Correlation between livers and prey for trace elements

For Cd concentrations (r=0.684, P<0.01) in 2001, and Cu (r=0.818, P<0.01), Mn (r=0.811, P<0.01), and Zn (r=0.853, P<0.01) concentrations in 2008, there was a significant correlation between the liver and prey for black-crowned night heron chicks (Table 3). All remaining element and year combinations for black-crowned night herons were not significant. Also, there were no significant liver and prey correlations for any element measured in grey heron chicks.

Discussion

Cadmium

Cadmium concentrations in livers were increased with diet concentrations between grey heron (geomean 0.06, 0.18 μg/g dw, respectively) and black-crowned night heron (0.20, 0.20 μg/g dw) chicks. Food chain accumulation of Cd has been reported for many birds including herons and egrets (Ullah et al. 2014; Shahbaz et al. 2013; Fritsch et al. 2012; Roodbergen et al. 2008). In black-tailed godwits (*Limosa limosa*), Cd was transferred from soil to the bird, the concentrations might be elevated in soils, earthworms, eggs, and feathers (Roodbergen et al. 2008). In particular, elevated Cd concentrations in birds showed a high transfer of these

Table 3 Relationship of trace element concentrations between livers and preys of heron chicks

Species	Year	Regression equation	Correlation coefficient (r^2)	P value
Cd				
Black-crowned night herons (n=10)	2001	y=0.01x+0.13	0.469	<0.05
Cu				
Black-crowned night herons (n=10)	2008	y=−0.05x+10.3	0.669	<0.01
Mn				
Black-crowned night herons (n=10)	2008	y=−0.05x+10.2	0.669	<0.01
Zn				
Black-crowned night herons (n=10)	2008	y=1.36x−23.1	0.728	<0.001

elements from environmental substances such as soil and air through the food chain in contaminated areas (Fritsch et al. 2012; Hofer et al. 2010). A comparison between grey heron and black-crowned night heron chicks at the Pyeongtaek colony suggested species-specific accumulations in Cd concentrations. Species-specific accumulations of Cd in tissues and eggs of birds have also been reported in the same habitat for heron and egret species (Kwok et al. 2014; Shahbaz et al. 2013; Hashmi et al. 2013; Ayaş 2007), penguin species (Jerez et al. 2011) and passerine species (Berglund et al. 2011; Deng et al. 2007). Species-species variation may be associated with concentrations of Cd in prey and the environment (water and air). In addition, Cd exposure of grey heron and black-crowned night heron chicks may be related to processes relying on habitat-specific properties that determine element environmental availability, prey availability, and/or contamination and their behavior (Ullah et al. 2014; Fritsch et al. 2012; Jerez et al. 2011).

Cadmium concentrations in livers of both heron species collected at the Pyeongtaek heronry during this study were low. The highest Cd concentration in liver was 0.50 μg/g dw, less than one sixth of the concentration considered elevated (>3 μg/g dw; Scheuhammer 1987). Prey Cd concentrations in grey heron and black-crowned night heron chicks at the Pyeongtaek colony were comparable to the range of cattle egrets (*Bubulcus ibis*) (1.92 μg/g dw; Bostan et al. 2007), little egrets (*Egretta garzetta*) and cattle egret (1.09, 1.01 μg/g dw, respectively; Shahbaz et al. 2013), and cattle egrets (1.01–1.54 μg/g dw; Ullah et al. 2014) from Pakistan.

Lead

In this study, Pb concentrations in livers and prey items of black-crowned night heron chicks (geomean 4.24, 3.96 μg/g dw, respectively) were higher than in grey heron chicks (geomean 3.90, 1.76 μg/g dw, respectively). Lead concentrations were higher in various birds collected at contaminated areas compared to rural or uncontaminated areas, suggesting transfer of this element in the food web (Ullah et al. 2014; Shahbaz et al. 2013; Fritsch et al. 2012; Berglund et al. 2010; Roodbergen et al. 2008). One of most essential route of Pb exposure in birds was diet concentration (Scheifler et al. 2006). In earlier studies, strong interspecific variance (Kwok et al. 2014; Shahbaz et al. 2013; Hashmi et al. 2013; Berglund et al. 2011; Deng et al. 2007; Ayaş 2007) and regional difference (Padula et al. 2010) of Pb concentrations in tissues and eggs of birds has been reported. In contrast, Pb concentrations in this study did not differ between species.

Lead concentrations in livers of both heron species collected at the Pyeongtaek heronry during this study were higher compared to other herons and egrets (Tiller et al. 2005; Alleva et al. 2006). Average lead concentrations were lower than the concentration considered elevated, an approximate threshold level for over toxic effects (>6 μg/g dw; Franson 1996; Clark and Scheuhammer 2003), but 5 of 20 (25.0 %) grey heron and 4 of 18 (22.2 %) black-crowned night heron chicks were higher than the background level. Elevated Pb concentrations were associated with reduced clutch size and increased nestling mortality of birds, and lead contamination might contribute to the high mortality and health effects of bird nestlings (Berglund et al. 2010; Bel'skii et al. 2005). At the Pyeongtaek colony, clutch size, growth rate of chicks, and breeding success of grey herons and black-crowned night herons were not less than those of other heron and egret studies conducted worldwide (Kim and Koo 2007, 2009).

Liver Pb concentrations of both heron species at the Pyeongtaek colony are much higher than other herons and egrets (Horai et al. 2007; Alleva et al. 2006; Tiller et al. 2005). Prey Pb concentrations of black-crowned night heron chicks in 2008 (geomean 13.2 μg/g dw) were greater than in those of little egrets and cattle egrets (1.41, 1.06 μg/g dw, respectively; Shahbaz et al. 2013) and similar to prey of cattle egrets (11.1-16.9 μg/g dw; Ullah et al. 2014), but much lower than those of cattle egrets (76.2 μg/g dw; Bostan et al. 2007) from Pakistan.

Copper

Copper is an essential element for all known living organisms, particularly in cell physiology, and plays a vital role in function and structure of proteins (Janssens et al. 2009). Because many herons and egrets forage on agricultural fields for the breeding season, they can be exposed to Cu contamination combined with Cu sulfate of agrochemicals such as pesticides and fungicides (Eijsackers et al. 2005), and Cu concentrations in birds were elevated with environmental Cu level (Custer et al. 2008; Horai et al. 2007; Tiller et al. 2005). In the present study, Cu concentrations in the prey and the liver of black-crowned night heron were higher than in those of grey heron chicks. In the cattle egret, Cu concentrations were fluctuated by diet concentrations (Ullah et al. 2014). In contrast, black-tailed godwits did not demonstrate accumulation (Roodbergen et al. 2008). These different accumulation trends of Cu might be in part because Cu is regulated by homeostatic processes in organisms as an essential element (Pascoe et al. 1996; Nyholm 1995). Species-specific accumulation of Cu between grey heron and black-crowned night heron chicks was found. Similar species-specific difference in various tissues and eggs of birds has been reported (Kwok et al. 2014; Mansouri et al. 2012; Jerez et al. 2011; Lucia et al. 2010; Custer et al. 2007; Ayaş 2007).

In Japan, liver Cu concentrations of grey herons (mean 791 μg/g dw) and intermediate egrets (*Egretta intermedia*) (787 μg/g dw) were much higher than in great white egrets (*Egretta alba modesta*) (173 μg/g dw) (Horai et al. 2007) and these concentrations were greater than in those reported for black-crowned night heron chicks (geomean 20.5 μg/g dw; Custer et al. 2007), great blue heron (*Ardea herodias*) nestlings (mean 44.3–83.9 μg/g dw; Tiller et al. 2005) from America, and this study (geomean 16.1–144 μg/g dw). Elevated Cu concentrations of grey herons and intermediate egrets were attributed to aquatic Cu contaminations in their foraging sites (Horai et al. 2007). Copper concentrations in the prey of grey heron and black-crowned night heron chicks were higher than in two egret species (mean 0.98–2.10 μg/g dw; Shahbaz et al. 2013) from Pakistan and black-tailed godwits (1.94–3.19 μg/g dw; Roodbergen et al. 2008) from the Netherlands, but lower than in cattle egrets (29.6–45.8 μg/g dw; Ullah et al. 2014) from Pakistan.

Other essential elements

Manganese concentrations in bird tissues can be influenced by contaminated air and food (Hui 2002). Diesel fuel combustion and leaded gasoline also contribute to atmospheric Mn contamination (Zayed et al. 1999). At the Pyeongtaek colony, liver Mn concentrations were not related to prey concentrations for two heron species, and no relationship of Mn concentrations between cattle egrets and their prey has been reported (Ullah et al. 2014). Manganese concentrations (geomean 4.23–11.3 μg/g dw) in livers at the Pyeongtaek colony were within the range reported from three heron and egret species (9.85–13.4 μg/g dw; Horai et al. 2007). In this study, diet Mn concentrations were far greater than those in little egrets (2.13 μg/g dw) and cattle egrets from Pakistan (6.01 μg/g dw; Shahbaz et al. 2013). Dietary Mn concentrations were also greater than in cattle egrets from Pakistan (6.01–14.4 μg/g dw; Ullah et al. 2014).

Pesticides and fungicides containing Zn sulfate are a main source of Zn contamination in an agricultural land (Eijsackers et al. 2005). Zinc concentrations in livers were increased with diet concentrations in black-crowned night heron (geomean 139, 127 μg/g dw, respectively) and grey heron (120, 108 μg/g dw) chicks. Also, this increase has been reported in black-tailed godwits (Roodbergen et al. 2008), but not cattle egrets (Ullah et al. 2014). The increase of Zn concentrations in excrements and livers were reported for great blue herons (Tiller et al. 2005). Observed Zn levels in this study were similar or lower than the mean value reported from multiple heron and egret studies conducted worldwide (Horai et al. 2007; Tiller et al. 2005). Prey Mn concentrations in this study were far greater than those in little egrets (19.3 μg/g dw Shahbaz et al. 2013), but lower than those in cattle egrets (127–171 μg/g dw; Ullah et al. 2014; Shahbaz et al. 2013) and black-tailed godwits (416–508 μg/g dw; Roodbergen et al. 2008).

Iron is an essential element required for many metabolic functions. However, Fe is often not biologically available and high concentration of Fe may lead to anemia (Jager et al. 1996; Esselink et al. 1995). Iron concentrations in livers of grey heron and black-crowned night heron chicks were not associated with prey concentrations. Iron concentrations in this study were similar or lower than the mean value reported from tree swallow (*Tachycineta bicolor*) nestlings (Custer et al. 2012). Prey concentrations of Fe in grey heron chicks were within the range of other birds, but were far greater in black-crowned night heron chicks than in others (Ullah et al. 2014; Custer et al. 2012; Shahbaz et al. 2013).

Manganese, Zn, and Fe concentrations in grey heron and black-crowned night heron chicks were within the background or normal physiological levels reported earlier in other birds including herons and egrets (Ullah et al. 2014; Custer et al. 2012; Shahbaz et al. 2013; Roodbergen et al. 2008; Horai et al. 2007; Tiller et al. 2005).

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