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NOTE

Contamination status and accumulation characteristics of metals and a metalloid in birds on Teuri Island, Hokkaido, Japan

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Abstract

Teuri Island, Hokkaido in Japan is an important place for seabirds breeding. We measured the concentrations of heavy metals (Hg, Cd, Cr, Co, Ni, Cu, Zn, and Pb) and a metalloid (As) in rhinoceros auklet (*Cerorhinca monocerata*) (n = 7), thick-billed murre (*Uria lomvia*) (n = 2), spectacled guillemot (*Cepphus carbo*) (n = 6), slaty-backed gull (*Larus schistisagus*) (n = 15), jungle crow (*Corvus macrorhynchos*) (n = 3), Japanese anchovy (*Engraulis japonica*) (n = 6) and Atka mackerel (*Pleurogrammus azonus*) (n = 2). Spectacled guillemot had high As concentrations, with its source being their feeding habitat. Concentration of Hg in kidney of jungle crow was higher than other seabird species at Teuri.

Key Words: metals, seabirds

Teuri Island, Hokkaido in northern Japan is an important breeding ground for many seabird species. The island, where approximately one million birds across eight species breed[18)], has been designated a national wildlife protection area since 1982. It is well known as the largest breeding ground of rhinoceros auklet (*Cerorhinca monocerata*) in the world during their migration from spring to summer. It is also an important breeding ground for the common murre (*Uria aalge*), which is rare in Japan. Both species have been under protection by the Ministry of the Environment of Japan since 2001. Populations of spectacled guillemot (*Cepphus carbo*), which have been falling due to unknown causes, are also present on Teuri Island. The importance of Teuri

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Island for several seabird species is thus quite significant.

Recent international reports of increasing accumulation of metals such as mercury (Hg) and cadmium (Cd) in seabirds have led to concern[4,7,11,16,21]. The collection and provision of basic data concerning the marine environment of Japan is essential for monitoring these levels. There are some previous reports on this topic; however, organized research focusing on metals and metalloids pollution has been suspended. Therefore, environmental pollution studies for bird species on Teuri Island are sorely needed.

In the present study, the concentrations of several metals—Hg, Cd, chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), and lead (Pb)—and a metalloid—total arsenic (As)—in seabirds were analyzed to determine the contamination status and accumulation characteristics of seabirds in Teuri Island. Fish samples were also analyzed to estimate their exposure levels to the metals and metalloid from food. Japanese anchovy (*Engraulis japonica*) and Atka mackerel (*Pleurogrammus azonus*) were analyzed. These fish species are food of seabirds and especially Japanese anchovy is the principal diet of rhinoceros auklet[5]. Concentrations of these elements in stomach contents of slaty-backed gulls (*Larus schistisagus*) were also measured to know the pollution levels in their feed.

Thirty-three birds across five species were captured in Teuri Island (44°25´N, 141°52´E) in the Japan Sea of Hokkaido from May to July in 2012 and 2013. Liver and kidney samples from rhinoceros auklet (*Cerorhinca monocerata*) (n = 7), thick-billed murre (*Uria lomvia*) (n = 2), spectacled guillemot (*Cepphus carbo*) (n = 6), slaty-backed gull (*Larus schistisagus*) (n = 15) and jungle crow (*Corvus macrorhynchos*) (n = 3) were collected. Stomach contents (n = 6) and muscle (n = 15) of slaty-backed gull, as well as two fish species, Japanese anchovy (*Engraulis japonica*) (n = 6) and Atka mackerel (*Pleurogrammus azonus*) (n = 2), were also collected. The lengths of Japanese anchovy and Atka mackerel which were dropped by rhinoceros auklet on the land were around 15 cm. We also collected stomach contents of slaty-backed gull (n = 6). All samples were kept at −20°C in a deep freezer until the time of metal analysis. The five rhinoceros auklets were caught for sampling in accordance with the guidelines of the Ministry of the Environment. The slaty-backed gull and jungle crow specimens were culled to conserve the population of the common murre (*Uria aalge*). Two rhinoceros auklet and all thick-billed murre and spectacled guillemot were acquired via bycatch of net fishing.

Metals and a metalloid analysis followed the method of Yabe et al.[22]. For the metal analysis, 0.3 g per sample of bird liver and kidneys, 0.3 g per sample of stomach contents in slaty-backed gull, and whole fish were dried for 15 h at 50°C, and then digested with 6 mL of 60% nitric acid (Kanto Chemical Corporation, Tokyo, Japan) and 1 mL of 30% hydrogen peroxide (Kanto Chemical Corporation) in a microwave digestion system (Speedwave Two, Berghof, Germany). Fish were minced, homogenized, and dried before analysis. Digestion was performed under the following conditions: 180°C for 15 min, 200°C for 20 min, and 100°C for 20 min. After the samples were cooled, they were transferred to plastic tubes to which 0.1 mL of lanthanum chloride (Wako Pure Chemical Industries, Osaka, Japan) was added. The volume was then brought to 10 mL with 2% nitric acid. Concentrations of the metals and a metalloid were measured by using inductively coupled plasma-mass spectrometry (ICP-MS; 7700 series, Agilent Technology, Tokyo, Japan) for the samples collected in 2013, and by using an atomic absorption spectrophotometer (AAS; Z-2010, Hitachi High-Technologies Corporation, Tokyo, Japan) with acetylene flame or by argon non-flame method for those collected in 2012. The instrument was calibrated by using standard solutions of the respective metals to establish standard curves before analysis. All chemicals and standard stock solutions were of analytical reagent grade (Wako Pure Chemicals Industries). Water was distilled and deionized (Milli-Q, Merck Millipore, Billerica, Massachusetts). Analytical quality control was performed by using DOLT-4 (dogfish liver) and DORM-3 (fish protein) certified reference material (National Research Council of Canada).

For analysis using the AAS, concentrations of Cu and Zn were determined through the flame method with acetylene gas, whereas concentrations of Cd, Cr, Ni, Pb, and As were determined by using a graphite furnace with argon gas. Recovery rates (%) of all elements were acceptable: Cd (91–108), Cr (91–108), Co (96–111), Ni (98–111), Cu (88–90), Zn (78–83), and Pb (89–98), although As had lower recovery rates (%) (50–67). Detection limits (μg/kg) for Cd, Cr, Co, Ni, Pb, and As were 0.1, 1.1, 2.3, 3.0, 1.9, and 2.0, respectively. Detection limits (mg/kg) for Cu and Zn were 0.5 and 0.1, respectively.

For analysis using ICP-MS, recovery rates (%) of all elements were acceptable: Cd (83), Cr (79), Co (NA), Ni (87), Cu (83), Zn (91), Pb (83), and As (103). The detection limits (μg/kg) for Cd, Cr, Co, Ni, Cu, Zn, Pb, and As were 0.02, 0.06, 0.08, 0.48, 0.14, 4.52, 0.02, and 0.04, respectively.

The concentrations of total Hg in the liver and kidneys were measured by thermal decomposition, gold amalgamation, and atomic absorption spectrophotometry using a mercury analyzer (MA-3000; Nippon Instruments Corporation, Tokyo, Japan), after preparation of the calibration standard. Recovery rates of Hg for the DOLT-4 and DORM-3 ranged from 92% to 103%. The concentrations of Hg were converted from mg/kg wet weight to mg/kg dry weight by subtracting the calculated water content.

Stable isotope ratio analysis followed the method of Nakayama et al.[14]. Briefly, muscles were washed with distilled water to remove blood and then dried at 45°C for 48 h. Samples were then ground into a homogeneous powder and treated with a 2 : 1 chloroform–methanol solution (Kanto Chemical Corporation) to remove lipids, and the residue was dried. After each sample was weighed (0.5–1.0 mg) into a tin capsule (Säntis Analytical AG, Teufen, Switzerland), stable isotope ratios were determined by the flow injection method using a Finnigan MAT-252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) connected to a Fisons NA1500 elemental analyzer (Fisons Instruments SpA, Strada Rivoltana, Italy).

Stable isotope ratios were expressed in δ notation (as deviation from standards in parts per thousand (‰)) according to the following formula: $\delta X = [(R_{sample}/R_{standard} - 1)] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$[13]. Data are presented as values based on the international standard of v-PDB (Vienna Peedee Belemnite; fossilized shells from the PeeDee Formation in South Carolina) for C and based on atmospheric N_2 for N[13]. Replicate errors were within 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ analyses.

Thick-billed murre and Atka mackerel were excluded from statistical analyses because of their small sample size (n = 2). Differences in metal and metalloid concentrations and isotope ratio values between species were tested through Tukey's HSD test ($p < 0.05$). Scatter plot for stable isotope ratio ($\delta^{13}C$ and $\delta^{15}N$, ‰) in muscle of slaty-backed gull (n = 15) was used bivariate analysis ($r = 0.585$, $p = 0.02$). Statistical analyses were performed in JMP Pro 10.0.2 (SAS Institute, Cary, NC, USA).

Spectacled guillemot had significantly higher total As concentrations in the liver and kidneys than any other species did (Table 1, 2). This bird species feeds on fish such as small right-eye flounder, crustaceans, and invertebrates. Right-eye flounder and crustaceans can accumulate high levels of As than other species in Japan[19], suggesting that feeding habits are the main source of As exposure among individuals. High total As levels would not normally be considered as a problem for seabirds, as this metalloid mainly accumulates in organic forms such as arsenobetaine[8] that are less toxic than inorganic forms and can be easily excreted[15]. However, exposure to high levels of As through the food supply has been shown to cause decreased growth in chickens[5]. An assessment of specific toxicity levels for seabirds is therefore needed. One spectacled guillemot specimen was found to have a nephritic Cd level (170.2 mg/kg dry weight). Although benchmark values for Cd concentrations in seabirds have not yet been established[4,16], the possibility of kidney damage caused by Cd pollution exists.

Concentration of Hg in kidney of jungle crow was higher than other seabird species at Teuri, Hg is bioaccumulated through the food web[1,3]. Slaty-backed gull and jungle crow would be the top predator around Teuri Island, therefore they had high accumulations.

The remains of various animals were found in stomach contents of slaty-backed gull; samples of No.S3 and S21 included small birds while other samples included fish and concentrations of Hg, Cd and As in stomach contents had a wide range (Fig. 1). It is reported that marine animals accumulate high levels of Hg, Cd and As[7,8]. In this study, concentrations of these metals were low in the stomach contents of No.S3 and S21, it would be reflected that they mainly ate bird, not fish at that time. There were no correlations between the concentrations of meals in stomach contents and bird tissues because stomach contents only reflect the accumulation of those days. Stable isotope ratios in slaty-backed gull were 12.3 ± 0.55 (‰, δ^{15}N) and -19.3 ± 0.45 (‰, δ^{13}C) (Fig. 2). The mean trophic shift for C (which denotes the changes in isotope ratio between diet and consumer) has been reported to be about +1‰, and the mean for N to be about +3‰[12]. In this study, the range of δ^{13}C were approximately +1‰ (-19.3 ± 0.45‰). Source of many metals and metalloids accumulation is derived from their food[2], therefore the results of δ^{13}C value and various accumulation patterns of metals and a metalloid in stomach contents suggest that slaty-backed gull feeds over various habitats.

Among alcid species (rhinoceros auklet, thick-billed murre and spectacled guillemot), rhinoceros auklet had high Hg contents (Table 1 and 2). rhinoceros auklet accumulated higher levels of Hg than did thick-billed murre, short-tailed shearwater (*Puffinus tenuirostris*), tufted puffin (*Fratercula cirrhata*), and horned puffin (*Fratercula corniculata*) (our study, unpublished data), This might be correlated with the fact that this species mainly feed on Japanese anchovy[10,17] and there was a tendency that Japanese anchovy accumulated higher concentrations of Hg than did Atka mackerel (Table 3). Hg concentrations in the liver (mg/kg dry weight) of rhinoceros auklet from the Canadian Pacific coast were 3.55 ± 1.31 in Lucy Island and 5.39 ± 1.17 in Storm Island, and Cd levels in the liver were 43.5 ± 11.3 in Lucy Island, 22.9 ± 6.91 in Storm Island, and 20.5 ± 6.38 in Cleland Island[6]. The results of this study showed comparable Hg accumulation (5.25 ± 1.50), but relatively low Cd levels (4.06 ± 1.89). Therefore, Teuri Island appears to be less polluted by Cd compared with the Canadian Pacific coast. There are no previous data on metal pollution in Teuri Island, and other countries have collected little data on rhinoceros auklet and spectacled guillemot in recent years. So, further research of these species, including resampling around Teuri Island, would be needed for more robust knowledge on metal pollution in seabirds.

In this study, some species are migratory birds. Therefore their accumulations of metals would include the pollutants of other areas. Rhinoceros auklet come Teuri Island from the northern Pacific or other parts of Japan from March to August. After their migration to Teuri Island, they are suggested to move to around the Sea of Okhotsk to take more amounts of fishes for breeding. Spectacled guillemot comes from Honshu, main island of Japan, during April to November. Both of these two bird species might be affected by contaminations at other areas. However, considering the amounts of feeding of these species at Teuri Island, their accumulated metals might be reflected the situation of

Table 1. Metal and metalloid concentrations in the liver of birds (mg/kg dry weight) and sample size (N). Different letters indicate significant differences among species (Tukey's HSD test, $p < 0.05$). Thick-billed Murre was excluded from statistics because of their sample size (n = 2).

Species	N	Hg	Cd	Cr	Co	Ni	Cu	Zn	As	Pb
Slaty-backed Gull	15	6.04 ± 3.51	4.52 ± 2.42	0.07 ± 0.06	0.08 ± 0.04	0.90 ± 1.46	10.3 ± 1.78^{b}	81.0 ± 26.1	0.19 ± 0.12^{b}	0.08 ± 0.10^{b}
Rhinoceros Auklet	7	5.25 ± 1.50	4.06 ± 1.89	0.12 ± 0.06	0.09 ± 0.02	0.30 ± 0.24	19.3 ± 3.63^{a}	81.7 ± 16.6	2.44 ± 1.17^{b}	0.14 ± 0.10^{b}
Thick-billed Murre	2	2.63 ± 1.65	3.47 ± 1.89	0.12 ± 0.04	0.06 ± 0.01	0.24 ± 0.12	15.9 ± 1.18	71.4 ± 8.74	2.72 ± 1.55	0.06 ± 0.02
Spectacled Guillemot	6	4.14 ± 1.52	7.01 ± 7.80	0.10 ± 0.02	0.07 ± 0.01	0.25 ± 0.06	19.8 ± 2.92^{a}	61.2 ± 7.94	15.2 ± 6.74^{a}	0.11 ± 0.14^{b}
Jungle Crow	3	10.8 ± 6.17	1.70 ± 0.55	0.11 ± 0.03	0.15 ± 0.09	*ND	20.7 ± 7.14^{a}	94.9 ± 21.7	2.96 ± 2.49^{b}	0.47 ± 0.15^{a}

*ND = not detectable

Table 2. Metal and metalloid concentrations in the kidney of birds (mg/kg dry weight). Different letters indicate significant differences among species (Tukey's HSD test, $p < 0.05$). Thick-billed Murre was excluded from statistics because of their sample size (n = 2).

Species	N	Hg	Cd	Cr	Co	Ni	Cu	Zn	As	Pb
Slaty-backed Gull	15	6.07 ± 3.41^{b}	31.8 ± 10.3	0.14 ± 0.03	0.21 ± 0.08^{a}	0.09 ± 0.10^{b}	8.79 ± 1.62^{c}	94.3 ± 21.9^{a}	0.30 ± 0.29^{b}	0.12 ± 0.06^{b}
Rhinoceros Auklet	7	4.55 ± 1.08^{b}	28.6 ± 7.73	0.35 ± 0.38	0.13 ± 0.01^{bc}	0.42 ± 0.13^{a}	15.2 ± 1.24^{a}	88.7 ± 9.40^{ab}	1.39 ± 0.50^{b}	0.08 ± 0.05^{b}
Thick-billed Murre	2	1.74 ± 0.69	16.5 ± 13.8	0.14 ± 0.02	0.05 ± 0.02	0.24 ± 0.04	12.6 ± 3.72	85.1 ± 15.7	2.94 ± 2.24	0.05 ± 0.02
Spectacled Guillemot	6	3.65 ± 1.10^{b}	48.5 ± 56.3	0.05 ± 0.02	0.08 ± 0.01^{c}	0.27 ± 0.07^{a}	12.9 ± 0.84^{b}	78.3 ± 12.4^{ab}	9.14 ± 4.15^{a}	0.08 ± 0.05^{b}
Jungle Crow	3	20.3 ± 14.3^{a}	4.99 ± 2.17	0.06 ± 0.01	0.25 ± 0.09^{ab}	*ND	15.4 ± 1.31^{ab}	61.5 ± 5.10^{b}	1.95 ± 1.44^{b}	0.88 ± 0.43^{a}

*ND = not detectable

Table 3. Metal and metalloid concentrations in whole fish (mg/kg dry weight) and sample size (N)

Species	N	Hg	Cd	Cr	Co	Ni	Cu	Zn	As	Pb
Atka Mackerel	2	0.03 ± 0.00	0.20 ± 0.05	0.60 ± 0.05	0.22 ± 0.07	0.54 ± 0.09	4.58 ± 0.64	66.4 ± 1.87	5.18 ± 0.82	0.28 ± 0.07
Japanese Anchovy	6	0.14 ± 0.03	0.30 ± 0.17	0.41 ± 0.02	0.16 ± 0.05	0.65 ± 0.31	7.11 ± 2.08	121.4 ± 30.34	8.24 ± 6.33	0.22 ± 0.04

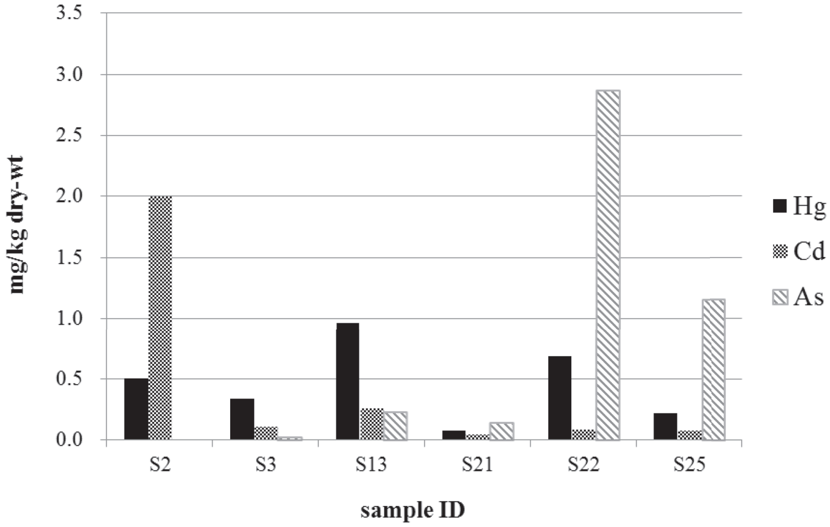

Fig. 1. Concentrations of Hg, Cd, and As in the stomach contents of slaty-backed gull (N = 6). The numbers under the bars indicate the sample ID.

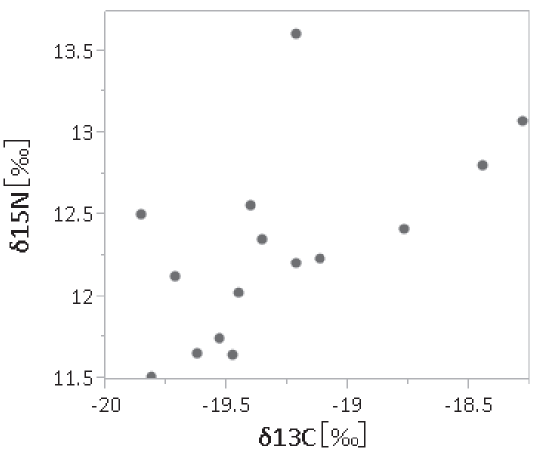

Fig. 2. Scatter plot for stable isotope ratio (δ^{13}C and δ^{15}N, ‰) in muscle of slaty-backed gull (n = 15) was used bivariate analysis ($r = 0.585$, $p = 0.02$). Statistical analyses were performed in JMP Pro 10.0.2 (SAS Institute, Cary, NC, USA).

pollution in northern areas of Hokkaido. Thick-billed murre migrates from the Arctic Ocean to pass the winter. Therefore most of their accumulation of pollution would reflect the contamination statues around Teuri Island. Present study showed that Teuri Island is less polluted than other many areas around the world. However, according to a report of the United Nations Environment Programme[20], concentrations of Hg in arctic marine animals are generally about 10–12 times higher than they were in pre-industrial times. There is a possibility that Teuri Island will become quite polluted by Hg. Therefore, continued monitoring of metal pollution is needed for the island. The results of the present study serve as useful basic data against which to compare future data.

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