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Maternal transfer of arsenic to eggs of black-tailed gull (*Larus crassirostris*) from Rishiri Island, Japan†

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Concentrations of total arsenic and individual arsenic compounds were determined in several tissues and eggs of the black-tailed gull (*Larus crassirostris*) to characterize accumulation and maternal transfer to eggs. A relatively high concentration of arsenic was observed in the liver, kidney, pancreas, muscle and gonad. The transfer rate of arsenic to eggs of the black-tailed gull was about 10%. Chemical speciation analysis revealed that arsenobetaine was the major arsenic compound in all the tissues. Dimethylarsinic acid, methylarsonic acid, arsenocholine, and an unidentified arsenic compound were also detected as minor constituents. Like maternal tissues, egg also contained arsenobetaine as the major arsenic compound and dimethylarsinic acid as a minor compound. These results suggest that arsenobetaine and dimethylarsinic acid can transfer from the mother bird to the eggs. To our knowledge, this is the first report on the maternal transfer of arsenic species to eggs of seabirds. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: arsenic; arsenobetaine; chemical speciation; maternal transfer; black-tailed gull; seabird; egg; HPLC–ICP-MS

INTRODUCTION

Arsenic exists in various chemical forms in the environment and organisms.[1,2] Because toxicity and metabolism of the arsenicals depend on their chemical forms, an evaluation of the toxicity and metabolism of arsenic requires the identification and quantification of individual arsenic compounds. Many investigations have dealt with lower trophic marine animals, and various arsenic compounds, such as arsenobetaine, tetramethylarsonium ion, arsenocholine, trimethylarsine oxide, arsenosugars, inorganic arsenic, and unidentified arsenic compounds, have been detected in fish, shellfish, crustaceans, and cephalopods.[1] In contrast, little is known about arsenic compounds in the tissues of higher trophic marine animals. Some studies reported the presence of arsenobetaine, dimethylarsinic acid, arsenocholine, methylarsonic acid, and tetramethylarsonium ion in the liver of marine mammals[3–5] and arsenobetaine, dimethylarsinic acid, arsenocholine, tetramethylarsonium ion, and inorganic arsenic compounds in tissues of sea turtles.[5–7] However, no investigation has been conducted on the accumulation and chemical species of arsenic in wild birds. Also, research on the transfer of arsenic to the embryo and fetus is important, because the embryo and fetus are generally susceptible to chemicals. However, as far as we know, there are no data on the maternal transfer of arsenic species to the fetus or eggs in wildlife.

In this study, concentrations of total arsenic and individual arsenic compounds in several tissues and eggs of black-tailed gulls (*Larus crassirostris*) were determined to understand the chemical forms of arsenic in the tissues of seabirds and to characterize the maternal transfer of arsenic compounds to eggs.

MATERIALS AND METHODS

Samples

Mature females ($n = 6$), their eggs ($n = 12$), and chicks ($n = 2$) of the black-tailed gull were collected from Rishiri Island,

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Table 1. Biometry of black-tailed gulls obtained from Rishiri Island, Japan[a]

	Sample no.	Date	Sex	Body weight (g)	Mother bird
Adult	BTG-501	9 June 2001	F	497	–
	BTG-503	9 June 2001	F	532	–
	BTG-509	11 June 2001	F	501	–
	BTG-511	11 June 2001	F	556	–
	BTG-517	9 June 2001	F	591	–
	BTG-518	9 June 2001	F	509	–
Chick	BTG-310	14 June 2001	–	51.6	–
	BTG-326	15 June 2001	–	53.0	–
Egg I	BTG-102	9 June 2001	–	53.0	BTG-501
	BTG-116	11 June 2001	–	49.3	BTG-509
	BTG-119	11 June 2001	–	53.8	BTG-511
Egg II	BTG-129	12 June 2001	–	58.0	BTG-518
Egg III	BTG-101	9 June 2001	–	56.0	BTG-501
	BTG-104	9 June 2001	–	60.0	BTG-503
	BTG-105	9 June 2001	–	54.0	BTG-503
Egg IV	BTG-126	12 June 2001	–	52.7	BTG-517
	BTG-127	12 June 2001	–	54.3	BTG-518
	BTG-128	12 June 2001	–	56.2	BTG-518
	BTG-147	14 June 2001	–	50.1	BTG-527
	BTG-148	14 June 2001	–	51.1	BTG-527

[a] Egg I: Not developed; egg II: formation of blood vessel; egg III: small embryo; egg IV: large embryo.

Japan, in June 2001 (Table 1). Thirteen tissues (brain, feather, femur, gallbladder, gonad, heart, kidney, liver, lung, muscle, pancreas, skin, and spleen) and the intestine contents of a mature female ($n = 1$), the liver, kidney, and muscle of mature females ($n = 5$), and the liver of chicks ($n = 2$) were used for analysis in this study. Feathers were washed by sonication with 3% polyoxyethylene lauryl ether. After sonication, feathers were rinsed vigorously with MilliQ water. All samples were stored in a deep-freezer at −20 °C before being freeze-dried.

Chemical analyses

Total arsenic concentration was measured for feather and femur. For other tissues, intestine contents and eggs, chemical speciation was conducted and the sum of the concentration of each arsenic compound was regarded as the total arsenic concentration. The analytical procedure for total arsenic was described previously.[8] In brief, freeze-dried tissue was digested by an acid mixture (HNO_3, $HClO_4$, and H_2SO_4) by heating at over 300 °C. Total arsenic concentrations were measured using a hydride generator coupled to an atomic absorption spectrometer. Total arsenic concentrations were expressed on a dry weight basis (μg g^{-1} dry wt).

Chemical speciation of arsenic was performed according to the method of Goessler *et al.*[9] with slight modifications.[4] Arsenic compounds were extracted with a mixture of methanol/MilliQ water (9:1 v/v). Arsenic compounds were identified and quantified by high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP-MS). A Hamilton PRP-X100 anion-exchange column ($NH_4H_2PO_4$ buffer) and Supelcosil LC-SCX cation-exchange column (pyridine buffer) were used for the separation of each arsenic compound. Rubidium was added to both mobile phases as an internal standard. Arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, arsenobetaine, arsenocholine, trimethylarsine oxide, and tetramethylarsonium iodide were used as standard substances for qualitative and quantitative analyses in this study. The ion intensities were monitored at *m/z* 75, 77, and 87. Because the peak of arsenobetaine was split,[10,11] tenfold-diluted extracts were employed for measurement of arsenobetaine, whereas undiluted extracts were used for other arsenic compounds.[4] Concentrations of the arsenic compounds were expressed as arsenic content on a dry weight basis (μg g^{-1} dry wt). Moisture content was 75.7% for the brain, 63.4% for the gallbladder, 70.1% for the gonad, 67.7% for the heart, 69.1% for the kidney, 62.6% for the liver, 73.8% for the lung, 63.8% for the muscle, 56.5% for the pancreas, 45.6% for the skin, 63.3% for the spleen and 73.2% for the intestine contents. The accuracy of the methods for total arsenic and arsenic speciation was assessed using a certified reference material (CRM) DORM-2 (National Research Council of Canada). The values obtained were in fair agreement with the certified value and those of other studies.[9,12,13]

RESULTS AND DISCUSSION

Arsenic accumulation in tissues

Concentrations of total arsenic and arsenic compounds in tissues, intestine contents, and eggs of black-tailed gull are shown in Table 2. Arsenic was detected in all the tissues, intestine contents, and eggs examined, ranging from 0.05 to 2.91 μg g^{-1} dry wt. The highest arsenic concentration was observed in the liver of the black-tailed gull. The total arsenic concentrations in liver (1.59 ± 0.77 μg g^{-1}) were significantly higher than those of kidney (paired *t*-test, $p < 0.05$) and muscle ($p < 0.05$).

Arsenic burdens in each tissue and egg of a black-tailed gull were calculated from the weight of individual tissue and the concentration on a wet weight basis (Fig. 1). Arsenic burden of muscle (27.2%) was highest among all the tissues and egg, followed by bone (22.0%) and feather (19.8%). Although total arsenic concentrations in muscle (1.81 ± 0.62 μg g^{-1} dry wt), bone (0.35 μg g^{-1} dry wt), and feather (0.18 μg g^{-1} dry wt) were similar to or lower than those of other tissues, these tissues showed a high percentage of arsenic burden as a result of their large mass (26.1% for muscle, 13.1% for bone and 15.0% for feather).

Table 2. Concentrations of various arsenic compounds (mean ± SD) in tissues and eggs of black-tailed gulls[a]

	Tissue	*n*	Arsenic concentration (μg g^{-1} dry wt)				
			AB	DMA	AC	MMA	Total
Mother bird	Liver	6	1.55 ± 0.76	0.02 ± 0.01	0.05 ± 0.01 (*n* = 3), ND (*n* = 3)	ND	1.59 ± 0.77
			(0.79–2.83)	(0.01–0.05)	(0.03–0.06)	–	(0.81–2.91)
	Kidney	6	1.04 ± 0.41	0.07 ± 0.02	0.05 ± 0.02 (*n* = 5), ND (*n* = 1)	0.08 (*n* = 1), ND (*n* = 5)	1.17 ± 0.43
			(0.57–1.68)	(0.05–0.11)	(0.04–0.08)	–	(0.63–1.79)
	Muscle	6	0.82 ± 0.47	0.08 ± 0.01	ND	ND	0.90 ± 0.47
			(0.33–1.73)	(0.06–0.09)	–	–	(0.40–1.80)
	Pancreas	1	0.93	0.02	0.04	ND	0.99
	Gonad	1	0.67	0.11	0.03	ND	0.81
	Gallbladder	1	0.39	0.13	ND	ND	0.52
	Lung	1	0.65	0.02	ND	ND	0.67
	Spleen	1	0.27	0.09	ND	ND	0.36
	Skin	1	0.20	0.01	ND	ND	0.21
	Heart	1	0.27	0.08	ND	ND	0.35
	Brain	1	0.21	0.01	ND	ND	0.22
	Bone	1	NA	NA	NA	NA	0.35
	Feather	1	NA	NA	NA	NA	0.18
	Intestine content (front)	1	0.31	0.02	0.18	ND	0.51
	Intestine content (middle)	1	0.46	0.01	0.12	ND	0.59
	Intestine content (rear)	1	0.48	0.08	0.18	ND	0.74
Chick	Liver	2	0.67, 1.07	0.03, 0.04	0.07, 0.03	ND (*n* = 2)	0.76, 1.14
Egg I	Whole homogenate	3	0.18 ± 0.06	0.01 (*n* = 2), ND	ND (*n* = 3)	ND (*n* = 3)	0.19 ± 0.07
			(0.13–0.25)	–	–	–	(0.13–0.26)
Egg II	Whole homogenate	1	0.10	0.01	ND	ND	0.11
Egg III	Whole homogenate	3	0.14 ± 0.09	0.01, 0.02, ND	ND	ND	0.15 ± 0.10
			(0.04–0.21)	–	–	–	(0.05–0.22)
Egg IV	Whole homogenate	5	0.23 ± 0.04	0.03 ± 0.01	ND	ND	0.26 ± 0.05
			(0.19–0.28)	(0.01–0.04)	–	–	(0.21–0.32)

[a] AB: arsenobetaine; DMA: dimethylarsinic acid; AC: arsenocholine; MMA: methylarsonic acid; ND: not detected; NA: not analyzed.

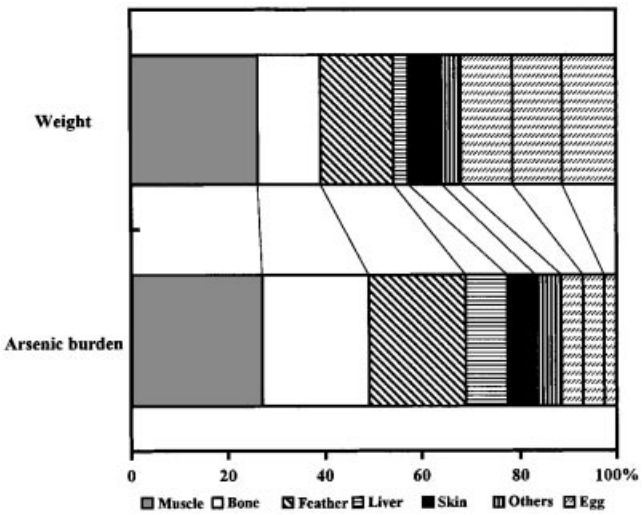

Figure 1. Distribution of arsenic in tissues and eggs of the black-tailed gull.

Chemical speciation of arsenic

The concentrations of several arsenic compounds in 11 tissues (liver, kidney, muscle, pancreas, gonad, gallbladder, lung, spleen, skin, heart, and brain) and intestine contents of the black-tailed gull are shown in Table 2. Arsenobetaine and dimethylarsinic acid were found in all the tissues and intestine contents examined. Moreover, arsenocholine was detected in the liver, kidney, pancreas, gonad, and intestine contents. At least one unidentified arsenic compound was also detected in some tissues and the intestine contents of black-tailed gulls in this study (data not shown). Arsenobetaine was the predominant arsenic compound in all the tissues, and the percentage of arsenobetaine to total arsenic ranged from 74.7 to 97.4%. On the other hand, the percentages of dimethylarsinic acid and arsenocholine were only 2.1–25.3% and 3.0–4.5% respectively. The percentage of arsenocholine (20.9–35.1%) in the intestine contents was much higher than those in all other tissues. It was reported that marine mammals and sea turtles contained arsenobetaine as a major arsenic compound.[3–7] Hence, it seems likely that higher trophic marine animals generally accumulate arsenobetaine as the major arsenic compound in their tissues.

For both total arsenic and arsenobetaine, significant positive correlations between liver and kidney (regression analysis, $r = 0.968$, $p < 0.01$ for total arsenic and $r = 0.980$, $p < 0.001$ for arsenobetaine) and between liver and muscle ($r = 0.835$, $p < 0.05$ for total arsenic and $r = 0.840$, $p < 0.05$ for arsenobetaine) were observed in the black-tailed gull (Fig. 2). There was a positive correlation also between kidney and muscle, but it was not statistically significant ($p > 0.05$). It should be noted that no significant correlation was found between tissues for dimethylarsinic acid.

The relationship of total arsenic concentration to concentration of arsenic compounds was examined using all samples ($n = 43$). Total arsenic showed a significant positive correlation with arsenobetaine (Pearson's correlation coefficient test, $r = 0.996$, $p < 0.0001$). Furthermore, a positive correlation was also observed for liver ($r = 0.999$, $n = 6$, $p < 0.0001$), kidney ($r = 0.993$, $n = 6$, $p < 0.0001$), muscle

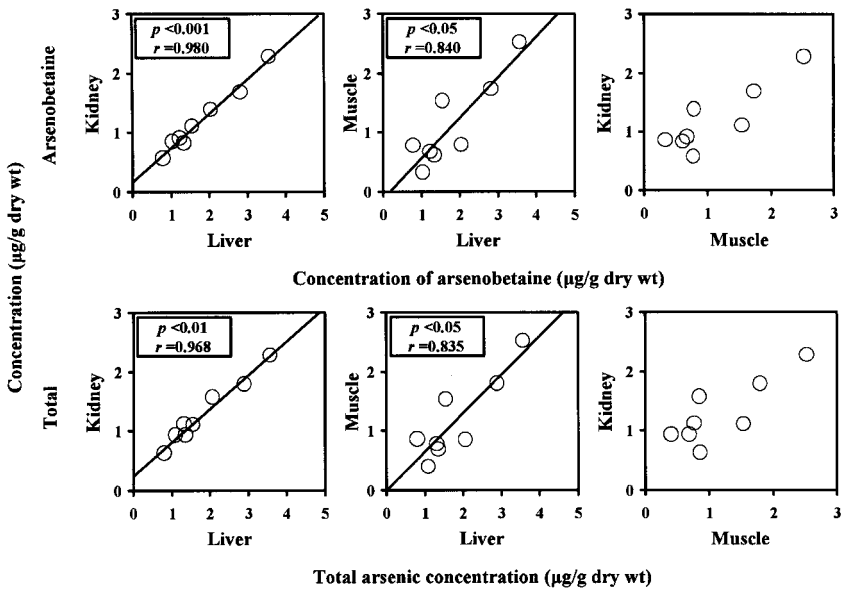

Figure 2. Relationship between tissues for total arsenic and arsenobetaine concentrations.

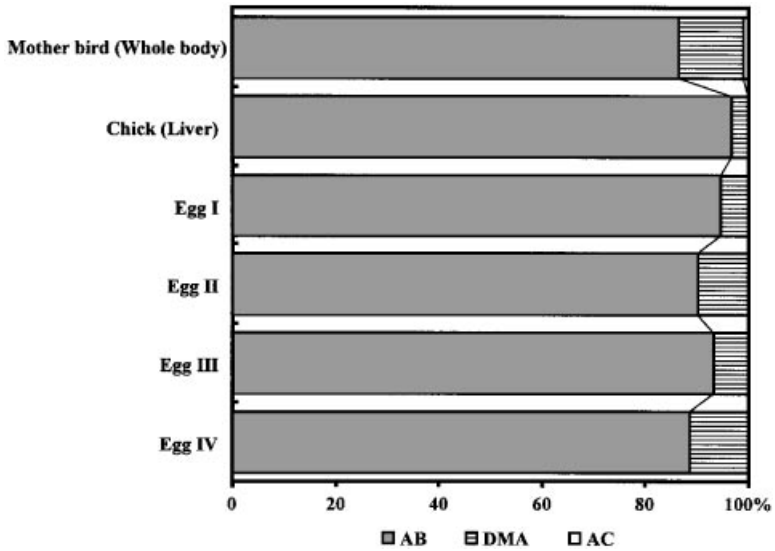

Figure 3. Composition of arsenic compounds in the whole body of the mother bird, the liver of the chick, and the egg contents of the black-tailed gull.

($r = 1.000$, $n = 6$, $p < 0.0001$), and egg content ($r = 0.996$, $n = 12$, $p < 0.0001$). A similar tendency was also obtained using the hepatic concentrations in the long-finned pilot whale, ringed seal, beluga, and bearded seal[3] ($r = 0.998$, $n = 14$, $p < 0.0001$). Hence, it is assumed that this relationship is common for higher trophic marine animals and that arsenobetaine plays an important role in the accumulation of arsenic in their tissues. A high retention of arsenobetaine was reported for fish,[14,15] mussel,[16,17] and shrimp.[18] In contrast, it is generally accepted that arsenobetaine is rapidly excreted into urine in humans and experimental mammals.[19,20] Hence, accumulation and excretion systems for arsenobetaine might be different between marine (or aquatic) animals and terrestrial animals. Further studies are needed to verify this hypothesis.

Maternal transfer of arsenic to eggs

Total arsenic concentrations in egg contents were significantly lower than those of liver, kidney, and muscle of the mother birds (Mann–Whitney's U test, $p < 0.001$). Similar results were also reported for heavy metals. It was reported that concentrations of heavy metals in eggs were significantly lower than those of livers of adult birds for the common tern (*Sterna hirundo*)[21] and Adélie penguin (*Pygoscelis adeliae*).[22] No significant correlation was found between total arsenic concentrations in eggs and in tissues (liver, kidney, and muscle) of the mother birds. For a mother bird (BTG-518) which laid three eggs, the eggs comprised 11.3% of the arsenic burden and this value was lower than their weight percentage (32.0%) (Fig. 1). Hence, it seems that transfer of arsenic to eggs might be limited in some manner in black-tailed gulls.

The composition of the arsenic compounds in the whole body of the mother bird, the liver of the chick, and the egg contents is shown in Fig. 3. Arsenobetaine was the major arsenic compound in the mother bird and in the liver of the chick and accounted for 86.5% and 96.7% of total arsenic respectively. Dimethylarsinic acid also occupied 12.6% of total arsenic in the mother bird and 3.3% in the liver of the chick. Arsenocholine was detected only in the mother bird and comprised less than 1% of the total arsenic. Like the mother bird and chick, all egg contents contained arsenobetaine as the major arsenic compound and dimethylarsinic acid as the minor constituent (Fig. 3). The percentages of arsenobetaine and dimethylarsinic acid ranged from 88.5 to 94.7% and from 5.3 to 11.5% of total arsenic in eggs respectively. The composition of arsenic compounds was similar during the growth of eggs (Fig. 3). These results suggest that arsenobetaine and dimethylarsinic acid are transferable from mother bird to eggs and the compositions of arsenic compounds in eggs are less variable according to the development of embryo. To our knowledge, this is the first report on the maternal transfer of arsenic species to the eggs of seabirds.

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