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Interelement relationships and age-related variation of trace element concentrations in liver of striped dolphins (*Stenella coeruleoalba*) from Japanese coastal waters

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Abstract

Concentrations of 19 trace elements (V, Cr, Mn, Fe, Co, Cu, Zn, Se, Rb, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, Hg, and Pb) were determined in the liver of the striped dolphins (*Stenella coeruleoalba*) collected around Japan during 1977–1982 to examine the sex difference, age dependence, and interrelationships among trace elements. Tissue distribution of trace elements was also investigated in one adult and one fetus specimens. Generally, concentrations of Se, Sr, Ag, Cd, Cs, Ba, Hg, and Pb were higher in the tissues of adult than those of fetus, whereas the opposite trend was observed for Cr and Tl. There were no significant sex differences in the trace element levels in the liver. Significant positive correlations between age (0–26.5 years) and hepatic concentrations were found for Ag, Se, Hg, V, Fe, Pb, and Sr, suggesting their age-dependent accumulation in the liver. In contrast, hepatic concentrations of Mn and Zn decreased with age. Significant positive relationships were observed between Se, and Hg, Ag, V, Fe, and Sr in the liver.

Keywords: Trace elements; Mercury; Selenium; Age; Striped dolphin (*Stenella coeruleoalba*)

1. Introduction

Increasing human population and activities on global scale have led to the release of various trace elements into the environment (Nriagu and Pacyna, 1988). Hence, contamination status and toxic effects of trace elements in wild animals are of great concern. Marine mammals, which have a long life span and occupy higher trophic levels in the marine ecosystem, could accumulate harmful contaminants including trace elements. To understand the contamination status and toxic impacts by exposure to trace elements in marine mammals, basal information on the biological factors influencing trace element accumulation (e.g., sex, body size, and age) are required. Our previous studies reported the body distribution of Fe, Mn, Zn, Cu, Pb, Ni, Cd, Hg, and Se and their variation with body size and age in striped dolphins (*Stenella coeruleoalba*) from the North Pacific Ocean (Itano et al., 1984a,b,c; Honda et al., 1982, 1983; Honda and Tatsukawa, 1983). However, little information is available on the other trace elements such as Ag and V in striped dolphins (Kunito et al., 2004; Ciesielski et al., 2006). Inductively coupled plasma-mass spectrometry (ICP-MS) is now widely used for analysis of trace elements, and this technique enables to determine multi elements in trace concentrations for marine mammals. Thus, concentrations of undetermined trace elements (V, Cr, Co, Rb, Sr, Mo, Ag, Sb, Cs, Ba, and Tl) were analyzed in the liver samples of the striped dolphins characterized by Itano et al. (1984a,b,c), Honda et al. (1982, 1983),

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and Honda and Tatsukawa (1983) for Fe, Mn, Zn, Cu, Pb, Ni, Cd, Hg, and Se, and these nine elements were also reanalyzed for comparison in the present study. Furthermore, tissue distribution of the trace elements was investigated in one adult and one fetus specimens.

2. Materials and methods

2.1. Samples

Thirty-one striped dolphins (*S. coeruleoalba*) (male: $n = 13$, female: $n = 17$, and unknown: $n = 1$) were collected at Taiji on Kii Peninsula, Japan in 1979. Liver tissue was collected from each specimen. Liver, kidney, muscle, blubber, pancreas, spleen, lung, heart, first and second stomachs, intestine, diaphragm, tongue, stomach, ovary, adrenal, diaphragm, brain, and sternum were also sampled from one adult (male) and one fetus (female) collected from Kawana on Izu Peninsula, Japan in 1977 and Taiji in 1982, respectively. All the specimens were caught for commercial and scientific purposes under an appropriate permission and appeared to be in a good healthy condition without macroscopic pathological symptoms. Age was determined using dentinal growth layers of mandibular teeth (Kasuya, 1976). Age of the specimen from which teeth was not collected was estimated from the regression equation between age and body length. The body length of the specimens ranged from 133 to 242 cm and the age from 0.3 to 26.5 years except the fetus. All the tissue samples were packed in clean polyethylene bags and were kept at −20 °C in the Environmental Specimen Bank for Global Monitoring (*es-BANK*), Center for Marine Environmental Studies (CMES), Ehime University, Japan (Tanabe, 2006) until chemical analyses. The samples analyzed were prepared by removing the surface part to exclude a possible effect of contamination in the field sampling.

2.2. Chemical analysis of trace elements

Nineteen trace elements (V, Cr, Mn, Fe, Co, Cu, Zn, Se, Rb, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Hg, Tl, and Pb) were measured according to Ikemoto et al. (2004a) with slight modifications. All tissues were dried at 80 °C for 12 h and uniformly homogenized. After weighing about 0.10 g of the dried sample into a Teflon vial, 1.5 ml of HNO_3 was added and pre-digested at room temperature for 12 h. The sample was then digested in a closed microwave digestion system. Sixteen trace elements (V, Cr, Mn, Co, Cu, Zn, Rb, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, and Pb) were measured by ICP-MS (HP4500, Hewlett – Packard, Avondale, PA, USA). For Fe analysis, a flame-atomic absorption spectrometer (AAS; AA680, Shimadzu, Kyoto, Japan) was used. Concentrations of Se and Hg were determined with an AAS coupled with a hydride generation system (Model HFS-3, Hitachi, Tokyo, Japan) and a cold vapor system (Model HG-3000, Sanso, Tsukuba, Japan), respectively. Standard reference materials, SRM1577b (bovine liver; National Institute of Standards and Technology, Gaithersburg, MD, USA) and DORM2 (dogfish muscle; National Research Council Canada, Ottawa, ON, Canada), were used to assess the accuracy of the analysis. Recoveries of all the elements ranged from 88% to 130% of the certified values.

2.3. Statistical analyses

Statistical analyses were executed using StatView (version 5.0, SAS® Institute, Cary, NC, USA) and SPSS (version 12.0, SPSS Inc., Chicago, IL, USA). One half of the value of the respective limit of detection was substituted for those values below the limit of detection and then used in statistical analyses. Since concentrations of all the elements except Cr, Cu, Rb, and Tl were normally distributed, parametric analyses were used. Analysis of covariance (ANCOVA) was performed to examine the sexual differences in trace elements accumulation in liver with age as covariate. To understand the relationship between hepatic concentrations of trace elements and age and the interelement relationships in striped dolphins, Pearson's correlation coefficient and linear regression analysis were used. Data of Cr, Cu, Rb, and Tl, in which outliers were observed by Smirnov–Grubbs' outlier test, were not included in the correlation and regression analyses. A probability value of less than 0.05 was considered to indicate statistical significance.

3. Results and discussion

3.1. Tissue distribution of trace elements

Concentrations of trace elements in various tissues of one mature and one fetal striped dolphins are shown in Table 1. Iron concentrations were highest among the trace elements examined, while concentrations of Sb were below the detection limit (0.01 μg/g dry wt.) in all the tissues except for the liver of the mature specimen. Generally, concentrations of Se, Sr, Ag, Cd, Cs, Ba, Hg, and Pb were higher in the tissues of adult than those of fetus, whereas the opposite trend was observed for Cr and Tl (Table 1). Placental transfer of Tl has been reported for rats (Sabbioni et al., 1982) and high concentration of Tl was also observed in a fetus of the finless porpoise from Ise-Mikawa Bay, Japan (Furukawa et al., unpublished data). It should be noted that Cu level was remarkably high (about 13 times) in the liver of fetus compared with the mature (Table 1). Yang et al. (2004) also reported that hepatic Cu concentration in a fetal Dall's porpoise was about six times higher than that of mother. Both mature and fetus showed the highest levels of Mn, Cu, Se, Ag, and Hg in the liver, Cr in lung, and Fe in spleen (Table 1). In contrast, distributions of Rb and Cs were quite uniform except blubber, which is also observed in sea turtles (Anan et al., 2001) and seabirds (Agusa et al., 2005). This may be due to the fact that these elements are present as free ions in the body.

Table 1
Concentrations (ug/g dry wt.) of trace elements in various tissues of one mature and one fetus striped dolphins

Tissue	V	Cr	Mn	Fe	Co	Cu	Zn	Se	Rb	Sr	Mo	Ag	Cd	Sb	Cs	Ba	Hg	Tl	Pb
Mature (male)																			
Blubber	0.033	4.2	0.174	70	0.012	0.677	76.4	16	0.508	0.435	0.029	0.018	0.141	<0.01	0.02	0.007	10	0.003	0.005
Diaphragm	0.014	0.54	0.415	590	0.012	4.34	186	7.7	3.51	0.883	0.017	0.006	0.358	<0.01	0.21	0.011	25	0.001	0.042
Heart	0.016	0.80	1.92	500	0.051	13.5	94.5	8.3	4.47	0.780	0.058	0.005	0.452	<0.01	0.16	0.007	19	0.003	0.009
Intestine	0.018	2.0	2.44	120	0.019	6.20	130	8.8	6.84	0.706	0.052	0.017	1.77	<0.01	0.19	0.006	19	0.002	0.013
Kidney	0.030	0.75	2.99	640	0.065	14.0	149	23	5.30	0.773	0.205	0.24	104	<0.01	0.13	0.007	30	0.012	0.044
Liver	0.17	0.30	10.5	610	0.037	25.1	147	250	4.90	0.242	2.49	7.6	20.4	0.01	0.11	0.002	830	0.011	0.067
Lung	0.10	9.0	0.811	850	0.22	4.13	88.2	24	2.24	16.6	0.037	0.008	2.24	<0.01	0.05	0.139	41	0.001	0.038
Muscle	0.009	0.63	1.04	830	0.017	7.53	31.8	12	4.56	0.885	0.016	0.004	0.367	<0.01	0.23	0.010	43	0.001	0.023
Pancreas	0.010	0.39	4.32	160	0.034	5.17	166	20	5.01	0.290	0.113	0.042	4.31	<0.01	0.08	0.009	38	0.005	0.021
Spleen	0.010	0.30	1.09	1200	0.018	4.34	108	29	6.16	0.667	0.034	0.007	1.75	<0.01	0.14	0.059	54	0.002	0.015
Stomach (First)	0.010	0.62	0.628	140	0.025	2.92	127	8.4	5.76	1.13	0.070	0.012	1.71	<0.01	0.18	0.010	24	0.004	0.019
Stomach (Second)	0.084	10	4.25	450	0.098	17.6	111	16	5.15	0.657	0.246	0.023	6.98	<0.01	0.21	0.011	25	0.008	0.012
Tongue	0.024	0.92	2.69	160	0.028	4.79	96.9	10	5.94	1.03	0.058	0.020	3.05	<0.01	0.16	0.010	21	0.008	0.014
Fetus (female)																			
Adrenal	0.042	4.9	2.33	110	0.010	8.11	66.2	3.6	3.47	0.070	0.249	0.004	0.004	<0.01	0.09	0.002	0.70	0.067	<0.001
Blubber	0.044	6.4	0.269	90	0.007	0.670	26.7	3.2	1.00	0.010	0.012	<0.001	<0.001	<0.01	0.02	<0.001	0.22	0.003	0.002
Brain	0.002	0.24	1.51	180	0.007	7.44	62.5	5.1	5.40	0.063	0.014	0.002	<0.001	<0.01	0.02	<0.001	2.3	0.030	0.001
Diaphragm	0.007	0.50	0.671	320	0.005	8.51	105	5.5	3.61	0.120	0.021	0.009	0.002	<0.01	0.08	0.005	2.7	0.021	0.014
Heart	0.035	4.3	1.09	560	0.010	12.3	110	1.1	4.75	0.056	0.115	0.004	<0.001	<0.01	0.11	<0.001	3.2	0.050	0.003
Intestine	0.029	3.1	1.27	280	0.013	8.26	101	6.5	5.10	0.152	0.086	0.006	0.002	<0.01	0.11	<0.001	2.5	0.034	0.009
Kidney	0.019	1.3	1.32	370	0.011	9.31	70.6	0.76	5.11	0.051	0.140	0.004	0.003	<0.01	0.13	<0.001	2.4	0.072	0.001
Liver	0.010	0.63	2.81	580	0.025	326	175	16	4.27	0.039	0.155	0.99	0.007	<0.01	0.07	<0.001	9.3	0.093	<0.001
Lung	0.096	13	0.798	1030	0.018	3.04	69.1	5.3	4.17	0.164	0.050	<0.001	<0.001	<0.01	0.07	<0.001	1.6	0.021	<0.001
Muscle	0.004	0.60	0.501	230	0.002	3.72	94.6	4.5	3.32	0.024	0.016	<0.001	<0.001	<0.01	0.10	<0.001	2.7	0.026	0.004
Ovary	0.053	6.5	1.04	390	0.012	5.54	71.2	4.6	3.60	0.077	0.067	0.005	<0.001	<0.01	0.09	<0.001	1.8	0.035	0.003
Spleen	0.002	0.25	0.750	1100	0.005	4.01	59.6	4.9	4.28	0.045	0.031	0.002	<0.001	<0.01	0.08	<0.001	2.1	0.029	<0.001
Sternum	0.014	1.2	1.29	310	0.044	1.20	107	3.3	3.29	13.6	0.011	<0.001	<0.001	<0.01	0.07	0.018	0.36	0.027	0.009
Stomach	0.006	0.77	2.70	220	0.007	10.5	155	4.8	6.34	0.111	0.097	0.003	0.002	<0.01	0.12	<0.001	2.0	0.045	0.006
Tongue	0.027	3.5	0.496	260	0.005	3.24	105	7.0	3.17	0.041	0.016	<0.001	<0.001	<0.01	0.08	<0.001	4.4	0.023	<0.001

3.2. Regional difference in hepatic trace element concentrations of striped dolphins

Concentrations of trace elements in liver of the striped dolphins used in this study are shown in Table 2. The concentrations were in the following order: Fe > Hg > Se, Zn > Cu > Cd > Mn, Ag > Mo > Sr > Cr > V, Rb > Cs, Pb > Co> Tl > Ba > Sb. Antimony was not detected in the most liver samples.

The levels in this study were compared with those of striped dolphins from other locations (Table 3). Concentrations on a wet weight basis reported in other studies (Cardellicchio et al., 2002a,b; Itano et al., 1984a; Honda et al., 1983; Roditi-Elasar et al., 2003) were converted to those on a dry weight basis assuming that the moisture content was similar to that of striped dolphins in the present study (mean, 65%). In general, concentrations of essential elements such as Cr, Mn, Fe, Co, Cu, Zn, and Mo in this study were within the range of the previous studies. Cadmium concentrations in the liver of striped dolphins from the North Pacific Ocean, the North Atlantic Ocean, and the French Channel were higher than those from the Mediterranean Sea, the Baltic Sea, and the Brazilian Coast, while the opposite trends were observed for Hg (Table 3). The difference in the food habits of the different population of striped dolphins from these locations might be partly responsible for such results. Watanabe et al. (2002) suggested that Cd-accumulating marine mammals feed mainly on invertebrates, whereas the preferred diet of Hg accumulators is fish. Lead levels in this study were lower than those from other locations (Table 3). The Pb levels were also lower than those reported by Honda et al. (1983), although the samples used in the present study were part of the samples of Honda et al. (1983). This may be due to that the samples analyzed in Honda et al. (1983) but not in the present study had higher Pb levels and/or that the methodology was different between the studies (ICP-MS in the present study vs. AAS in Honda et al. (1983)). The regional difference in V, Rb, Sr, and Ag (Table 3) might be also associated with the difference in food habits and ambient levels of trace elements. It should be noted that the hepatic Ag concentrations in the striped dolphins were considerably higher than those of other marine mammals (Saeki et al., 2001; Ikemoto et al., 2004a; Kunito et al., 2004; Yang et al., 2006) except for the beluga whales (*Delphinapterus leucas*) from Alaska (Becker et al., 1995), in which extremely high concentrations of Ag (28.6–306.9 μg/g dry wt. [concentrations based on a wet wt. basis (10.0–107.4 μg/g wet wt.) were converted to those on a dry wt. basis assuming 65% water content in liver]) were observed in the liver.

3.3. Age-dependent accumulation of trace elements

To understand influences of sex (males, $n = 14$; females, $n = 18$) and age (0–26.5 years) on trace elements accumulation in the liver of the striped dolphins, ANCOVA was per-

Table 2
Concentrations (ug/g dry wt.) of trace elements in liver of fetus, immature, and mature striped dolphins

Growth stage	V	Cr	Mn	Fe	Co	Cu	Zn	Se	Rb	Sr	Mo	Ag	Cd	Sb	Cs	Ba	Hg	Tl	Pb
Fetus (n = 1)	0.010	0.63	2.81	580	0.025	326	175	16	4.27	0.039	0.155	0.99	0.007	<0.01	0.07	<0.001	9.3	0.093	<0.001
Immature (n = 10)																			
Mean	0.12	0.37	10.7	480	0.038	24.7	153	27	0.288	1.91	5.48	2.0	15.7	<0.01	0.07	0.007	34	0.005	0.036
S.D.	0.083	0.11	1.89	220	0.012	3.12	37.8	32	0.145	0.423	0.900	2.4	6.94	0.01	0.01	0.003	44	0.005	0.024
Min	0.012	0.23	8.00	240	0.008	19.0	96.6	3.5	0.104	1.00	3.89	0.28	0.149	<0.01	0.05	0.003	5.1	<0.001	0.010
Max	0.28	0.55	14.1	940	0.051	30.8	203	87	0.600	2.63	6.64	7.0	25.6	0.02	0.09	0.012	120	0.017	0.080
Median	0.092	0.35	11.0	420	0.041	24.2	152	7.1	0.245	1.91	5.61	0.89	17.1	<0.01	0.08	0.007	8.4	0.003	0.026
Mature (n = 22)																			
Mean	0.33	0.59	8.11	770	0.044	24.6	116	190	0.412	2.32	4.46	10	18.4	<0.01	0.09	0.005	630	0.010	0.102
S.D.	0.14	0.94	1.77	210	0.016	5.10	20.9	66	1.01	0.655	0.821	3.6	6.64	0.00	0.02	0.002	290	0.004	0.067
Min	0.12	0.20	4.42	370	0.024	18.0	78.0	74	0.022	0.242	2.49	5.4	7.77	<0.01	0.06	<0.001	230	0.004	0.047
Max	0.81	4.7	10.8	1200	0.10	35.3	149	300	4.90	3.18	5.93	16	33.9	0.02	0.15	0.008	1300	0.023	0.360
Median	0.31	0.33	7.89	770	0.040	23.9	116	210	0.183	2.26	4.52	9.4	17.0	0.01	0.08	0.004	540	0.009	0.078
All (n = 33)																			
Mean	0.26	0.52	8.74	670	0.041	33.8	129	140	0.492	2.13	4.64	7.6	17.0	<0.01	0.08	0.005	430	0.011	0.079
S.D.	0.16	0.77	2.38	250	0.015	52.7	32.5	97	1.07	0.713	1.24	5.2	7.31	0.00	0.02	0.003	370	0.016	0.065
Min	0.010	0.20	2.81	240	0.008	18.0	78.0	3.5	0.022	0.039	0.155	0.28	0.007	<0.01	0.05	<0.001	5.1	<0.001	<0.001
Max	0.81	4.7	14.1	1200	0.10	326	203	300	4.90	3.18	6.64	16	33.9	0.02	0.15	0.012	1300	0.093	0.360
Median	0.27	0.33	8.82	680	0.040	24.1	126	130	0.221	2.15	4.73	7.8	16.8	<0.01	0.08	0.005	440	0.009	0.071

Table 3
Concentrations (ug/g dry wt.) of trace elements in liver of striped dolphins from various locations

Location	Year	*n*		V	Cr	Mn	Fe	Co	Cu	Zn	Se	Rb	Sr	Mo	Ag	Cd	Sb	Cs	Ba	Hg	Tl	Pb	References
North Pacific Ocean, Japan	1977–1982	33	Mean	0.26	0.52	8.74	670	0.041	33.8	129	140	0.492	2.13	4.64	7.6	17.0	<0.01	0.08	0.005	430	0.011	0.079	This study
North Pacific Ocean, Japan	1977–1980	57	Mean[a]			9.09	843		23.1	127						17.9				586		0.63	Honda et al. (1983)
North Pacific Ocean, Japan	1977–1980	15	Mean[a]								139									586			Itano et al. (1984a)
Ligurian Sea, Italy	1986–1990	15	Mean			8.6	1243		31.7	138	231									605			Capelli et al. (2000)
Apulian coasts, Italy	1991	10	Mean[a]								181									488			Cardellicchio et al. (2002a)
Apulian coasts, Italy	1991	10	Mean[a]		0.43		1084		28.5	158						4.3						0.63	Cardellicchio et al. (2002b)
Tyrrhenian and Ligurian Seas, Italy	1987–1994	<46	Median						22.0	111	266					4.43				593			Monaci et al. (1998)
Mediterranean coast, Spain	1987–1994	<24	Median						39.2	162	101					3.95				1043			Monaci et al. (1998)
Mediterranean coast, Israel	1994–2001	6	Mean[a]			6.3	997		28	137						11				517			Roditi-Elasar et al. (2003)
Corsican coast, France	1993–1998	3	Mean						21	97						3.7						7.8	Frodello and Marchand (2001)
Mediterranean coast, France	1989–1991	12	Mean						27.71	136						7.91				1653		0.49	Augier et al. (2001)
Mediterranean coast, France	1997–1998	9	Mean						17.63	87.27						3.14				332		0.05	Augier et al. (2001)
Tuscany and Latium coast, Italy	1987–1989	19	Median							225	106					7.33				324		0.05	Leonzio et al. (1992)
Baltic Sea, Poland	1998, 1999	2	Median	<0.3		7.3	291		11.6	111	4.38		0.13	1.14		3.39			0.18	16.2	<1.0	<0.8	Ciesielski et al. (2006)
Bay of Biscay, North-east Atlantic	1993	22	Mean				974		43	167						17							Das et al. (2000)
French Channel coast	1998–2001	3	Mean						26	140						35				37			Das et al. (2003)
Irish coast	1989–1993	<3	Mean						39	185						38				41			Das et al. (2003)
French Atlantic coast	1990, unknown	2	Mean		0				19	113						25				37			Holsbeek et al. (1998)
Sao Paulo and Parana States, Brazil	1997–1999	1		0.061	0.23	12.3	1810	0.041	33.4	287	190	4.31	0.299	2.34	3.2	7.83	<0.01	0.08	0.005	290	0.015	0.074	Kunito et al. (2004)

[a] Concentrations based on wet wt. were converted to dry wt. assuming 65% water content in liver.

formed. No significant differences between males and females were observed for all the trace elements examined. It is known that, in general, no remarkable sex-dependent accumulation of trace elements is observed for marine mammals (O'Shea, 1999). Therefore, data from all the samples were included for analysis of the age-dependent accumulation of trace elements.

Significant positive correlations were observed between age and hepatic V, Fe, Se, Sr, Ag, Hg, and Pb concentrations in the liver of the striped dolphins (Table 4). Regression analysis also showed that the concentrations of Ag ($R^2 = 0.679$, $p < 0.001$), Se ($R^2 = 0.653$, $p < 0.001$), Hg ($R^2 = 0.634$, $p < 0.001$), V ($R^2 = 0.389$, $p < 0.001$), Fe ($R^2 = 0.284$, $p < 0.01$), Pb ($R^2 = 0.225$, $p < 0.01$), and Sr ($R^2 = 0.201$, $p < 0.01$) increased with age (Fig. 1), suggesting age-dependent accumulation of these elements in the liver of the striped dolphins. Concentrations of these element in the mature (>8 years) specimens were also significantly higher than those of the fetus and immature dolphins (Table 2). It is well known that Hg accumulates with age, accompanied by an increase in Se levels via formation of Hg–Se complex, in marine mammals (Itano et al., 1984a; Ikemoto et al., 2004a; Kunito et al., 2004). Age-dependent accumulations of V and Ag were also observed in other marine mammals (Saeki et al., 1999, 2001; Watanabe et al., 2002; Ikemoto et al., 2004a; Kunito et al., 2004).

On the contrary, negative correlations between age and hepatic Zn ($R^2 = 0.457$, $p < 0.001$) and Mn ($R^2 = 0.210$, $p < 0.01$) concentrations were observed (Fig. 1). Higher concentrations of Mn and Zn were also observed in the immature specimens (Table 2). Although Honda et al. (1983) found that hepatic Mn, Zn, and Cu concentrations decreased with increasing age in the striped dolphins, this relationship was not observed for Cu in the present study. This is attributable to the fact that the Cu concentration was considerably higher in the liver of fetus (Table 2) and also only one sample of the fetus specimens employed by Honda et al. (1983) was analyzed in the present study.

3.4. Relationships among trace element concentrations

As shown in Table 4, many significant positive correlations among trace elements were observed, with high correlation coefficients being observed for V–Se ($r = 0.692$, $p < 0.001$), V–Sr ($r = 0.655$, $p < 0.001$), V–Ag ($r = 0.637$, $p < 0.001$), V–Hg ($r = 0.611$, $p < 0.001$), Mn–Mo ($r = 0.580$, $p < 0.001$), Se–Ag ($r = 0.779$, $p < 0.001$), Se–Hg ($r = 0.837$, $p < 0.001$), Ag–Hg ($r = 0.824$, $p < 0.001$), Ag–Pb ($r = 0.664$, $p < 0.001$), Cs–Hg ($r = 0.553$, $p < 0.001$), Cs–Pb ($r = 0.743$, $p < 0.001$), and Hg–Pb ($r = 0.704$, $p < 0.001$) pairs. Positive correlations among V, Se, Ag, and Hg concentrations were also reported in the liver of marine mammals (Ikemoto et al., 2004a; Kunito et al., 2004; Mackey et al., 1996; Saeki et al., 1999, 2001). Interaction of Se with Hg, Cd, and Ag is well known (Whanger,

Table 4
Peason's correlation coefficients among age and concentrations of trace elements in liver of striped dolphins

	Age	V	Mn	Fe	Co	Zn	Se	Sr	Mo	Ag	Cd	Cs	Ba	Hg	Pb
Age	1.000														
V	0.624***	1.000													
Mn	−0.458**	−0.234	1.000												
Fe	0.533**	0.344*	−0.547***	1.000											
Co	0.256	0.371*	−0.022	0.324	1.000										
Zn	−0.676***	−0.567***	0.505**	−0.446**	−0.054	1.000									
Se	0.809***	0.692***	−0.383*	0.444**	0.236	−0.646***	1.000								
Sr	0.448**	0.655***	0.025	0.223	0.441*	−0.412*	0.355*	1.000							
Mo	−0.208	0.004	0.580***	−0.136	0.138	0.130	−0.229	0.511**	1.000						
Ag	0.824***	0.637***	−0.362*	0.431*	0.359*	−0.524**	0.779***	0.447**	−0.212	1.000					
Cd	0.229	0.473**	0.211	0.087	0.506**	−0.070	0.345*	0.452**	0.335	0.371*	1.000				
Cs	0.311	0.249	−0.117	0.323	0.498**	−0.050	0.322	0.170	0.001	0.509**	0.463**	1.000			
Ba	−0.236	−0.079	0.138	−0.020	0.120	0.229	−0.346*	0.174	0.390*	−0.298	−0.062	−0.217	1.000		
Hg	0.796***	0.611***	−0.334	0.396*	0.394*	−0.408*	0.837***	0.362*	−0.226	0.824***	0.405*	0.553***	−0.271	1.000	
Pb	0.475**	0.496**	−0.242	0.449**	0.445**	−0.320	0.470**	0.491**	0.007	0.664***	0.331	0.743***	−0.100	0.704***	1.000

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

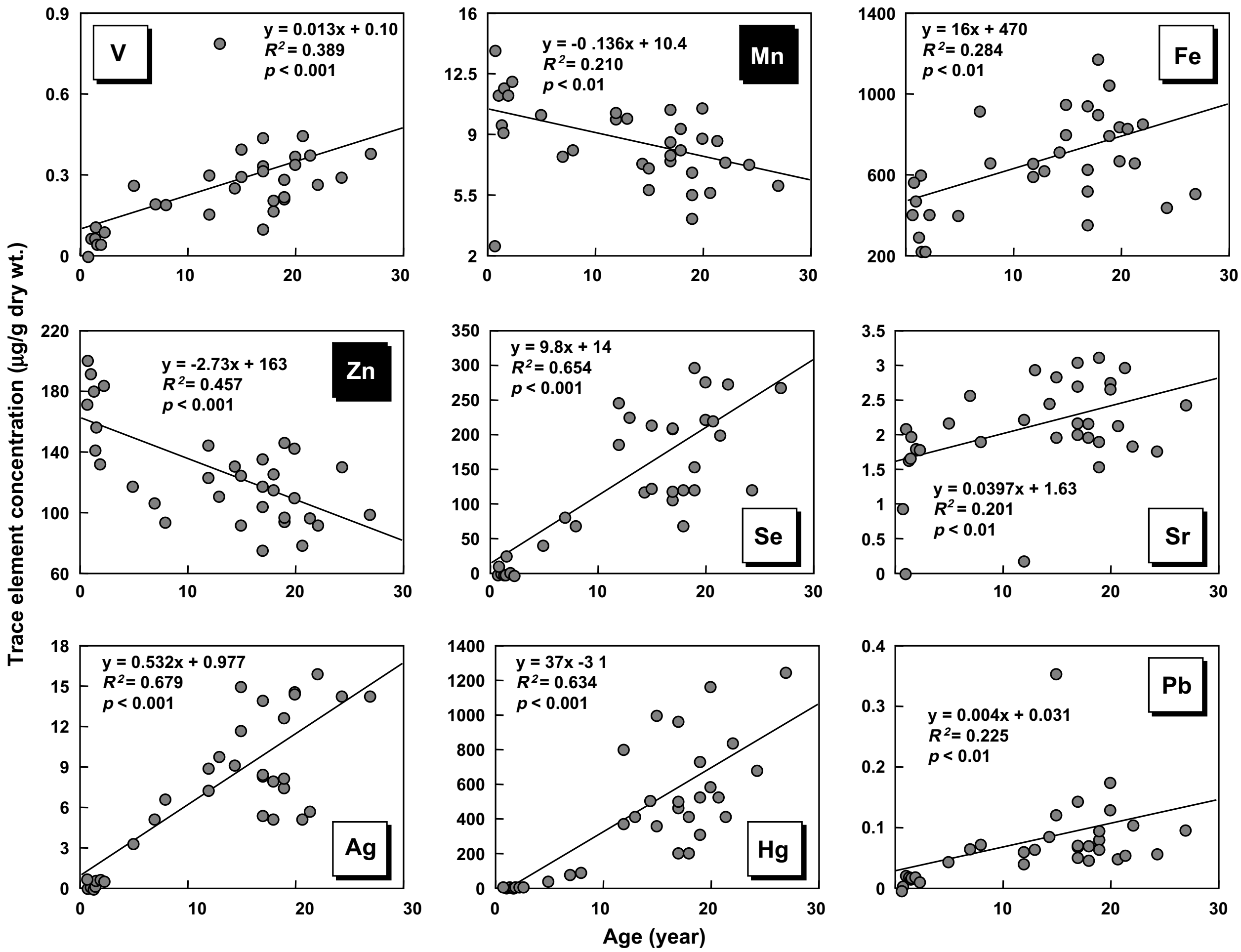

Fig. 1. Age-dependent variation of trace element concentrations in liver of striped dolphins from the North Pacific Ocean.

1985), and thus Se might detoxify Hg and Ag via formation of insoluble complexes in the liver of the striped dolphins, because very strong positive correlations were found between Se and Hg, and Se and Ag (Table 4). Indeed, HgSe was identified in the liver of some marine mammals (Martoja and Berry, 1980; Nigro and Leonzio, 1996; Arai et al., 2004). Also, formation of Ag_2Se was expected in the liver of Franciscana dolphins (Kunito et al., 2004). According to Naganuma et al. (1983), Se also interacts with Pb *in vivo* and *in vitro*. The positive correlation between Se and Pb (Table 4) might in part reflect the interaction between these elements. Some of the other interelement correlations may be an indication of a relationship between the element and another parameter rather than a direct relationship between the two elements (Kunito et al., 2002). For example, a positive correlation between Hg and V may be due to their positive correlations with age (Table 4). It is well known that Hg have strong affinity to SH group in cysteine (Mason and Jenkins, 1995). Like Hg, V as vanadyl (VO^{2+}) also binds covalently to amino acids such as cysteine and histidine (Baran, 1998; Rehder and Jantzen, 1998). Hence, biological half-life of the two elements would be rather long, leading to the age-dependent accumulation of both elements in liver of marine mammals (Ikemoto et al., 2004a).

A significant positive correlation was found between Se and Hg concentrations in this study ($R^2 = 0.677$, $p < 0.001$; [Hg] = 1.2 × [Se] − 0.022 on a molar basis), and the Hg/Se molar ratios increased with Hg levels ($R^2 = 0.601$, $p < 0.001$; [Hg/Se] = 0.26 × [Hg] + 0.50) and age ($R^2 = 0.387$, $p < 0.001$; [Hg/Se] = 0.048 × [Age] + 0.46) in the liver of striped dolphins. According to Ikemoto et al. (2004b,c), like Hg, Ag would be detoxified primarily by formation of complex with Se (Ag_2Se), but not by binding to metallothionein in liver of marine mammals and seabirds. Also, the striped dolphins showed high concentration of Ag in the liver, as mentioned above. Thus, (Hg + 0.5Ag) concentration on a molar basis was calculated, and compared with Se (Fig. 2). A positive correlation was observed between concentrations of (Hg + 0.5Ag) and (Hg + 0.5Ag)/Se molar ratios ($R^2 = 0.586$, $p < 0.001$; [(Hg + 0.5Ag)/Se] = 0.26 × [(Hg + 0.5Ag)] + 0.53). Mercury levels were much higher than those of Ag, so including Ag did not greatly affect the relationship between Hg and Se in the present study (not shown). However, evaluation of Ag in the risk assessment of Hg might be important for

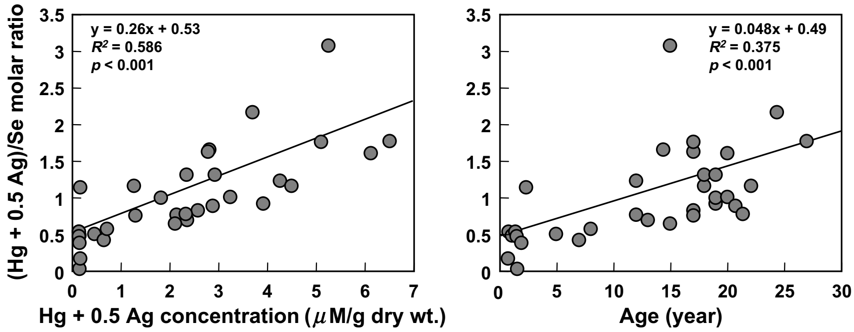

Fig. 2. Relationships between molar concentrations of (Hg + 0.5Ag), and (Hg + 0.5Ag)/Se in liver and age of striped dolphins from the North Pacific Ocean.

the Ag-accumulating species like a striped dolphin, especially for individuals with low Hg levels.

The (Hg + 0.5Ag)/Se molar ratio was larger than unity for about a half of the striped dolphins, especially the older specimens (Fig. 2). This result might indicate that Se-dependent detoxification process was not fully accomplished in the liver of these older striped dolphins with high hepatic concentrations of Hg. Alternatively, S as well as Se might be involved in the detoxification of Hg and Ag (Ikemoto et al., 2004b). Ng et al. (2001) and Arai et al. (2004) found a solid solution Hg(Se, S) in liver of a striped dolphin and a black-footed albatross, respectively. However, in the both studies, only one specimen was employed, and effects of age and Hg levels on the formation of Hg(Se, S) have been uncertain. Therefore, structural investigations on the Hg, Se, and Ag in many more samples and elucidation of the influence of age and Hg levels on the properties are necessary in future studies aimed at assessing the toxicological impacts by Hg and Ag in the striped dolphins.

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